

Hypothesis

# A Few Experimental Suggestions Using Minerals to Obtain Peptides with a High Concentration of L-Amino Acids and Protein Amino Acids

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Abstract: The peptides/proteins of all living beings on our planet are mostly made up of 19 L-amino acids and glycine, an achiral amino acid. Arising from endogenous and exogenous sources, the seas of the prebiotic Earth could have contained a huge diversity of biomolecules (including amino acids), and precursors of biomolecules. Thus, how were these amino acids selected from the huge number of available amino acids and other molecules? What were the peptides of prebiotic Earth made up of? How were these peptides synthesized? Minerals have been considered for this task, since they can preconcentrate amino acids from dilute solutions, catalyze their polymerization, and even make the chiral selection of them. However, until now, this problem has only been studied in compartmentalized experiments. There are separate experiments showing that minerals preconcentrate amino acids by adsorption or catalyze their polymerization, or separate L-amino acids from D-amino acids. Based on the [GADV]-protein world hypothesis, as well as the relative abundance of amino acids on prebiotic Earth obtained by Zaia, several experiments are suggested. The main goal of these experiments is to show that using minerals it is possible, at least, to obtain peptides whose composition includes a high quantity of L-amino acids and protein amino acids (PAAs). These experiments should be performed using hydrothermal environments and wet/dry cycles. In addition, for hydrothermal environment experiments, it is very important to use one of the suggested artificial seawaters, and for wet/dry environments, it is important to perform the experiments in distilled water and diluted salt solutions. Finally, from these experiments, we suggest that, without an RNA world or even a pre genetic world, a small peptide set could emerge that better resembles modern proteins.

Keywords: GADV-hypothesis; prebiotic chemistry; chiral selection; prebiotic environments

### 1. General Comments

We should ask ourselves, is it possible to think of a way for the origin of life to have occurred without minerals? Minerals have always existed on our planet; thus, it could be common sense that they played a role in the origin of life on our planet. Naturally, this is considering that life arose on Earth, but if not? Even if life arose elsewhere, we could wonder whether minerals played a role, be it a minor or major role. After the seminal work of Bernal, minerals were considered important for the preconcentration of molecules, catalysis of molecules and polymer syntheses, protection against degradation by UV radiation or hydrolysis, and even for a primitive genetic code [1]. For the first three suggestions, at least, there is wide experimental evidence that could support Bernal's hypothesis [2–6]. Bernal did not suggest in his book that minerals played any role in the origin of chirality [1].



Before we get onto the subject of this article, let us talk about one group of molecules that are important for today's living beings and are chiral, the amino acids. Naturally, there are several molecule groups that are chiral and important for living beings; therefore, amino acids are the most studied compounds in prebiotic chemistry. On the primitive Earth there were two sources of amino acids: (a) endogenous, meaning they were synthesized on Earth; and (b) exogenous, meaning they were delivered to Earth by meteorites, comets, and interplanetary dust particles [7–17]. Zaia et al. [11] carried out a survey of amino acids produced in experiments simulating prebiotic Earth environments and the interstellar medium, as well as amino acids found in meteorites. Therefore, we can certainly say that there were amino acids on the prebiotic Earth, as well as that minerals and amino acids probably interacted with each other on the prebiotic Earth.

#### 2. Amino Acids

In 1850, the German chemist Adolph Strecker synthesized an alanine racemic mixture from an aqueous solution with ammonia and hydrogen cyanide. Firstly, 2-amino propane nitrile was obtained, which, after acid hydrolysis, produced the racemic mixture of alanine (reaction 1). Nowadays, this reaction is known as the Strecker reaction. The Strecker reaction or even modified versions of it are widely used in industry to obtain amino acids as well as other compounds for applications in medicine and agriculture [18].

$$H_{3}CC(H)O + HCN + NH_{3} = H_{3}CC(H)(CN)NH_{2} + H_{3}O^{+} = H_{3}CC(H)(COOH)NH_{2}$$
(1)

In addition, there are several different reaction conditions to synthesize amino acids using the Strecker type reaction [18]. However, many of them could not be carried out under conditions that existed on prebiotic Earth or in the interstellar medium [19]. In experiments simulating the interstellar medium, the amino acid synthesis did not rely on a unique mechanism [20]. Thus, the formation of amino acids in the interstellar medium is a very robust reaction, meaning it did not depend on the initial mixture of gases [20]. In addition, there are a large number of ways to synthesize amino acids under conditions that existed on prebiotic Earth, such as hydrothermal vents; aqueous solution; mixture of gases, as in the Miller experiment; and the high impacts of bodies on Earth [10,11,14,19,21–26].

All amino acids, except glycine, have at least one carbon chiral, thus, they are optically active. The most commonly used system to classify amino acids is as L-amino acids or D-amino acids. L-amino acids deflect polarized light to the left so they are named as levogyre and D-amino acids deflect polarized light to the right, so they are classified as dextrogyre. However, the Cahn-Ingold-Prelog system is more complete, since some amino acids have more than one chiral carbon. In the Cahn-Ingold-Prelog system all L-protein-amino acids are classified as (S)-amino acids, with one exception L-cysteine is classified as (R)-amino acid. L-isoleucine and L-threonine, in the Cahn-Ingold-Prelog system are classified as (2S: 3S)-isoleucine and (2S: 3R)-threonine, respectively [27]. Although the L/D-system is an over simplification, we will use it throughout the text, because it is the best known and its simplification will not be a problem in the present work.

In all experiments simulating environments of the prebiotic Earth, a racemic mixture of amino acids was obtained [9–11,13,14,19,28,29]. However, analysis of meteorites showed that an enantiomeric excess occurred for some amino acids [12,15–17]. Among the meteorites analyzed, carbonaceous chondrites contain a large variety of organic molecules, making them the most interesting from the point the view of the origin of life [15–17]. The carbonaceous chondrites are subdivided into eight groups (CI type 1, CM type 1–2, CR type 1–3, CB, CH type 3, CO type 3, CV type 3, CK type 3–6), and their abundance and diversity of amino acids depend on the history of the parent body [15–17]. Several amino acids in the meteorites showed L-enantiomeric excess such as: alanine, serine, aspartic acid, threonine,  $\alpha$ -amino-n-butyric acid,  $\beta$ -amino-n-butyric acid, valine, glutamic acid, proline, norvaline, isovaline, leucine, isoleucine,  $\alpha$ -methynorvaline,  $\alpha$ -methyl-isoleucine,  $\alpha$ -methyl-alloisoleucine, and 2-amino-2-methylhexanoic acid [15–17]. Furthermore, several amino

acids in the meteorites showed D-enantiomeric excess, such as alanine, allo-isothreonine,  $\beta$ -aminoisobutyric acid, and allo-isoleucine [17]. Even if an L-enantiomeric excess occurred in meteorites, this does not explain why the proteins of today's living organisms are mostly made up of L-amino acids.

Minerals and amino acids have existed since the prebiotic Earth, and interactions between them have naturally occurred. From these interactions, two problems could be solved: (a) selection of the so-called "20-protein-amino acids" from a huge number of amino acids from endogenous/exogenous sources on the Earth; and (b) selection of the L-amino acids from the racemic mixture. We could wonder if the selection process of protein amino acids (PAAs) by minerals contributed to their chiral selection, or if it occurred the other way around, or even if both interacted with each other in a feedback process. Regarding these two problems, we must always remember the following: (a) non-PAAs play important roles in living organisms [30–33]; (b) D-amino acids also play several important roles in today's living beings [34–38]; and (c) among amino acids, glycine, an achiral amino acid, is synthesized in high amounts in experiments simulating exogenous and endogenous environments of Earth, and is also found in high amounts in meteorites [11].

Since Miller's classic experiment, non-PAAs and PAAs have been synthesized in experiments simulating terrestrial and non-terrestrial environments, and have been found in meteorites [10,11,21,26,39]. Thus, PAAs and non-PAAs existed on prebiotic Earth. We could draw several hypotheses as to why the PAAs were selected instead of non-PAAs: higher protein amino acid concentration compared to non-PAA concentration on the prebiotic Earth [11], PAAs condense more extensively than non-PAAs [40], some sequences of PAAs formed small peptides with catalytic activity [41], and more stable proteins are formed from PAAs [42]. In addition, we should point out the work of Ikehara and coworkers on the [GADV]-protein world hypothesis that primitive proteins were composed of four amino acids glycine/alanine/aspartic acid/valine-GADV [43-45]. Furthermore, the interaction between small peptides, with PAAs or without PAAs, with other biomolecules (amphiphilic molecules, amino acids, sugars) or biopolymers (small polynucleotide and polysaccharide) could provide important information about the increasing complexity of the systems [46]. There are several other hypotheses on why PAAs were chosen instead of non- PAAs [47–50]. Thus, in general, the data show that a few PAAs give the peptides/proteins some characteristics that make them advantageous over non-PAAs. However, how were these few PAAs selected over non-PAAs? and, were the minerals the right choice to do it?

Nowadays, it is well established that D-amino acids play important roles in living beings. However, were these amino acids incorporated/produced into the living beings during several stages of their evolution or are they "dinosaur amino acids" from the prebiotic world? We could assume, since the overwhelming majority of the amino acids of living beings are L-amino acids, that it is probable D-amino acids were incorporated into living beings in different stages of their evolution. In addition, as suggested by Higgs, the origin of homochirality could have partially occurred before the origin of life and have been completed at the same time as the origin of life [51]. Thus, the D-amino acids in living beings are a necessity for the physiological process. However, can we rule out the possibility that a small portion of D-amino acids, the number of hydrogen bonds is much smaller, which has an effect on the stability of the proteins. Could this lack in stability be a driving force for homochirality? However, the authors also showed that even proteins containing L/D-amino acids maintained some biochemical functions. It should be noted that D-amino acids were found in several peptides and proteins [36].

Among the PAAs, glycine is the only achiral, and it is obtained in high concentrations in experiments simulating endogenous or exogenous environments of the prebiotic Earth, as well as meteorite analyses [11]. However, can we assume that just because an amino acid had a high concentration on the prebiotic Earth that this would imply finding it in high amounts in primitive peptides/proteins?

In summary, PAAs gave some advantages over non-PAAs in peptides/proteins. D-amino acid decreases the stability of the protein, but does not have an effect on some biochemical functions. Glycine, an achiral amino acid, was probably found in higher concentrations on prebiotic Earth. How are all these connected with minerals? In the following paragraphs, we will try to give some insights into how we can connect these data with minerals.

#### 3. Biomolecules/Biomolecule Precursors and Adsorption on Minerals

As pointed out above, according to Bernal's suggestions, minerals probably played several important roles in the origin of life on Earth [1]. Biomolecule or biomolecule precursor concentrations on the prebiotic Earth were very low [11,53–56]. Thus, from all these suggestions, the most important is the adsorption of biomolecules or precursors of biomolecules. This is because, if the preconcentration of biomolecules or precursors of biomolecules. This is because, if the preconcentration of biomolecules or precursors of biomolecules did not occur, none of the other processes mentioned above would occur; there would not have been the formation of biomolecules from precursors, or polymers from monomers, meaning that the molecular evolution would not occur. Naturally, there are other ways to preconcentrate biomolecules or precursors of biomolecules such as: wet/dry cycles, freezing/sublimation, and sorption/precipitation with minerals, however, the environments needed for wet/dry cycles and freezing/sublimation to occur, were not as widespread as minerals on the prebiotic Earth [57].

As mentioned above, minerals could have participated in chiral amino acid selection, as well as in the selection of the PAAs. In general, these two problems are treated separately. However, on prebiotic Earth, there were endogenous and exogenous sources of amino acids. Amino acids synthesized on Earth or brought from outside the Earth fell into the sea that covered almost the entire planet [57]. Thus, the prebiotic seas contained L- and D-amino acids, and non-PAAs and PAAs, and minerals could adsorb all these molecules. In addition, besides amino acids, other molecules could be found in the primitive seas that competed for mineral adsorption sites. Here, we have a huge problem that could be a double-edged sword for prebiotic chemistry. On the one hand, this may lead to more complex prebiotic chemistry, due to the large variety of species, which could mean more possibilities for the formation of different and more complex molecules. On the other hand, this complex mixture of molecules may not lead to the formation of any important molecule or biopolymer in high concentration to be used for the molecular evolution. Schwartz [58], in his article *"Intractable mixtures and the origin of life"*, has already addressed this problem, denominating this mixture the *"gunk"*.

The early Earth's atmosphere could have been made up of  $CO_2$ ,  $H_2O$ ,  $SO_2$ , and  $N_2$  [59]. Thus, the main source of carbon on the prebiotic Earth was  $CO_2$  in the atmosphere. In experiments similar to Miller's using a mixture of  $CO_2/N_2$  the quantity of amino acids was too low. However, when  $Fe^{2+}$ was added, as anti-oxidant, and  $CaCO_3$  as buffering solution to aqueous solution, the following amino acids were obtained in high amounts: glycine, alanine, glutamic acid, and aspartic acid [29]. In experiments simulating hydrothermal vents, CO<sub>2</sub> with Fe<sup>2+</sup> produced acetate, pyruvate, malate, fumarate, succinate,  $\alpha$ -ketoglutarate, isocitrate, and aconitate [60]. When the experiments were performed with CO2 plus Fe° plus hydroxylamine, glycine, alanine, aspartic acid, and glutamic acid were produced [60]. The  $CO_2/N_2$  of the atmosphere could be converted to HCN, a more reactive molecule, by UV radiation [56]. Meteorites could be another source of cyanide on the prebiotic Earth [61,62]. After acidification, Murchison meteorite extracts presented approximately 400 nmolg<sup>-1</sup> of HCN [61]. The analysis of carbonaceous chondrite meteorites (Allan Hills 83100, Graves Nunataks 06100, Lewis Cliff 85311 and 90500, Lonewolf Nunatks, Murchison, Massif 04133) and one Martian meteorite (Allan Hills 84001) showed cyanide in the extracts in the range from 2472 nmolg<sup>-1</sup> (Lewis Cliff 85311) to 50 nmolg<sup>-1</sup> (Allan Hills 83100), with the exception of three meteorites (Massif 04133, Graves Nunataks 06100, Allan Hills 84001) where cyanide concentrations were below <0.1 nmolg<sup>-1</sup> [62]. These meteorites also presented iron cyanocarbonyl complexes  $\{[Fe^{2+}(CN)_5(CO)]^{3-}, [Fe^{2+}(CN)_4(CO)_2]^{2-}\}$  [62]. There are several small molecules that probably played important roles in the origin of life, such as cyanide, thiocyanate, ammonia, formaldehyde, and so on, of which cyanide is likely one the most important precursors of biomolecules [22,23]. However, for the formation of biomolecules the concentration of cyanide should be in the range from  $10^{-3}$  to 1.0 mol  $L^{-1}$  [24,63]. It is probable that in the seas of the prebiotic Earth the cyanide concentration was much lower, from  $10^{-8}$  to  $10^{-6}$  mol L<sup>-1</sup> [54,56]. Therefore, cyanide needed to be preconcentrated to be used for the formation of biomolecules. Could minerals adsorb cyanide? At very acidic pH (2.0), montmorillonite adsorbs cyanide [64] and this range of pH can be found in hydrothermal vents [65]. However, when the pH increases, the cyanide adsorption decreases [64]. In addition, Samulewski et al. [66] studied the cyanide adsorption onto several minerals (bentonite, ferrihydrite, goethite, hematite, kaolinite, montmorillonite, olivine), using a wide pH range (4.0–10.0), and the authors observed that less than 2% of cyanide adsorbed onto montmorillonite and bentonite, and the other minerals did not adsorb it. Thus, could this important molecule for prebiotic chemistry be preconcentrated by minerals at the pH of prebiotic seawater? Yes, cyanide can be preconcentrated by bentonite in a wide pH range when it reacts with Fe<sup>2+</sup> forming ferrocyanide [66]. In this case ferrocyanide enters the interlayer of bentonite, forming Prussian blue [66]. Thus, cyanide could be preconcentrated as ferrocyanide in the pH of prebiotic seawater or itself in hydrothermal vents. It should be noted that montmorillonite is one the most widely studied minerals in the prebiotic chemistry area [67].

Amino acids were delivered to prebiotic seas by endogenous and exogenous sources. Thus, in the prebiotic seas, non-PAAs, PAAs, L-amino acids, and D-amino acids could probably be found. As highlighted above, if an amino acid is delivered in high amounts to prebiotic seas, by endogenous or exogenous sources, should it be found in high amounts in the peptides/proteins? In several of the hypotheses discussed above, this is thought to be true [41–45,48,49]. It is inferred that if an amino acid is produced in large quantities, it must consequently be in large quantities in the peptides/proteins. However, these amino acids were in low concentrations in the prebiotic seas, thus, before the formation of peptides the amino acids need to be preconcentrated [11,28,55,68]. Several reviews show that minerals, especially montmorillonite, adsorb amino acids [2,4,5]. The adsorption of amino acids onto minerals depends on the properties of both and the medium used for the adsorption. Therefore, for adsorption studies, we cannot separate the properties of minerals from the properties of amino acids and the adsorption medium, meaning all three are connected. The following characteristics of minerals have an effect on the adsorption of amino acids: surface area, pore size, net superficial charge (pHpzc), transition metals, and cation exchange capacity. However, because minerals have a net charge, they adsorb more amino acids with charged-R-groups than amino acids with uncharged R-groups [4]. In addition, amino acids with uncharged R-groups are produced by endogenous and exogenous sources in higher amounts than amino acids with charged R-groups [11]. A few questions should be asked. Could minerals adsorb more PAAs than non-PAAs? Were the first peptides/proteins composed of more amino acids with charged R-groups than uncharged R-groups? Could minerals make chiral selection of amino acids?

In conclusion, on the prebiotic Earth, minerals preconcentrated biomolecules and precursors of biomolecules. However, the question here is with respect to selectivity. If minerals had no selectivity, the problem raised by Schwartz [58], in his article *"Intractable mixtures and the origin of life"* probably makes sense. In addition, if minerals were very selective, they would have no use for prebiotic chemistry; very selective minerals are useful for technology, but not for prebiotic chemistry. However, minerals have some selectivity that depends on their properties and the molecules that they will adsorb. In the next section we will discuss the selectivity of minerals together with the properties of amino acids.

#### 4. Minerals

Nowadays, more than 4300 species of minerals are known on our planet and approximately 50 new species of minerals are identified every year [69]. However, before life arose on our planet, there were approximately 350–500 species of minerals. The mineral evolution as suggested by Hazen could be divided into 10 stages: stage-1 (>4.56 Gy) occurred before the formation of the planets and

took place in stellar nebula, at this stage about 60 refractory minerals existed; stage-2 (>4.56 to 4.44 Gy) due to gravitational attraction, small bodies are formed, refractory minerals plus water and heat formed about 250 minerals; stage-3 (4.55 to 4.0 Gy) planetary differentiation occurred and due to seawater and atmosphere clay minerals are formed, there were about 350 to 500 minerals on Earth; stage-4 (4.0 to 3.5 Gy) first formation of granite due to partial melting of wet basalt and there were about 1000 minerals on the Earth; stage-5 (>>3.0 Gy) due to the global scale process of plate tectonics, the number of minerals probably increased to about 1500 on the Earth; stage-6 (3.9 to 2.5 Gy) anoxic biological process started to have an effect on surface minerals, formation of deposits of carbonates, sulfates, evaporites, and banded iron. There were about 1500 minerals on the Earth; stage-7 (2.5 to 1.9 Gy) "great oxidation event" due to rise in oxygen >1% than modern atmosphere; probably 70% of minerals are due to the changes in atmosphere and oceans. As a result of the new oxidation state of metals, hundreds of new minerals were formed. There were >4000 minerals on the Earth; stage-8 (1.9 to 1.0 Gy) because of changes in the oceans the production of banded iron formation ends, there were >4000 minerals on the Earth; stage-9 (1.0 to 0.542 Gy) during this stage, the diversity of minerals did not change, dramatic changes in the climate and several snowball events occurred; stage-10 (0.542 to present) due to living beings, shells, teeth, and bones, as well as organic minerals, are produced. There are >4300 minerals on the Earth [69,70]. Table 1 presents a few examples of minerals used in experiments for adsorption, polymerization, and chiral selection of amino acids. Among the minerals shown in Table 1, montmorillonite is the most commonly used in prebiotic experiments [2,4,5,67]. Montmorillonite belongs to the dioctahedral smectite group of phyllosilicates [71]. There are several reasons to use this mineral for prebiotic chemistry, such as being widespread on Earth, their high surface area and cationic exchange capacity-CEC, and their interlayer with cations and transition metals as contaminants ( $Fe^{2+}$ , Fe<sup>3+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, and so on). These characteristics give montmorillonite the necessary properties to be used as a preconcentrator of molecules and catalyst of polymer formation.

Adsorption				
Mineral Experiment		Reference		
akaganéite	Amino acids dissolved in water pH between 3.0 and 7.6 (Gly, Ala, Ser, His Phe)	Holm et al. [72]		
apatite	Amino acids dissolved in water or aqueous solution of Ca <sup>2+</sup> or PO <sub>4</sub> <sup>3–</sup> (Ala, Asp, Lys)	Tanaka et al. [73]		
bentonite/kaolinite	Amino acids dissolved in artificial seawater bentonite/kaolinite were tumbled for 24 h, at pH 3.0, 6.0 and 8.0. (Ala, Met, Gln, Cys, Lys, His)			
ferrihydrite	Aqueous solution of amino acids, at room temperature, pH 5–6, samples turned (35 rpm) for 24 h or 1 week (Gly, Ala, β-Ala, Aib, -aminobutyric acid, isovaline)	Matrajt and Blanot, [75]		
goethite	Amino acids dissolved in salt solutions (Gly, Ala, β-Ala)	Farias et al. [76]		
goethite	Amino acids dissolved in 0.01 and 0.1 mol L-1 of NaCl (sarcosine, H <sub>2</sub> MIDDA, H <sub>2</sub> EDDA)	Norén et al. [77]		
hematite	Amino acid dissolved in water (Ala)	Pandey et al. [78]		
Kaolinite/hectorite	Aqueous solutions (Ala)	Silva et al. [79]		
magnetite	Aqueous solution of amino acids, at room temperature, pH 6, shaking for 24 h (Gly, Ala, Cys, Glu, His, Lys, Ser)	Schwaminger et al. [80]		
montmorillonite	Amino acids dissolved in artificial seawater were tumbled for 24 h, at pH 3.0, 6.0 and 8.0. (Gly, Ala, β-Ala)	Farias et al. [81]		
montomorillonite	Amino acid dissolved in water at pH from 2.0 to 11.0 (Gly)	Ramos and Huertas, [82]		

Table 1. Minerals used for the adsorption, polymerization, and chiral selection of amino acids.

#### Adsorption Mineral Experiment Reference Amino acid dissolved in distille water, pH montmorillonite Kalra et al. [83] ranges 3.5-5.5 and 6.8-9.2 (Gly, Ala) Clays were modified with Na, Ca and Cu and montmorillonite/beidellite Benincasa et al. [84] amino acid was adsorbed and desorbed (Gly) Amino acids dissolved in water were mixed for 2, 4, 24 and 48 h, at pH 6.0, 6.9 and 8.4 (Gly, Ala, montmorillonite/kaolinite Hedges and Hare, [85] Asp, Glu, Thr, Ser, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, Arg). Aqueous solutions of amino acids (Gly, Ala, montmorillonite/illite Greenland et al. [86,87] β-Ala, Leu, Ser, Asp, Glu, Phe, Arg, His, Lys) Adsorption study using atomic force pyrite microscope (Gly, di-Gly, tri-Gly, penta-Gly, Lys, Afrin et al. [88] poly-Lys, Glu, poly-Glu) Amino acids were dissolved in 5.0 mmol L-1 of pyrite Bebié and Schoonen, [89] NaCl with and without Fe<sup>2+</sup> (Gly, Ala, Ser, Cys) Aqueous solutions of amino acid tumbled for sand (sea) Zaia et al. [90] 24 h at room temperature (Ala, Glu, Tyr, Lys) silica/alumina Adsorption study using RMN (Gly) Lopes et al. [91] Aqueous or saline solutions of amino acids zeolite Krohn and Tsapatis, [92] (Phe, Arg) Polymerization Mineral Experiment Reference Aqueous solution in the presence of CDI (Glu, apatite Hill Jr. and Orgel, [93] cGlu) Bentonite/pyrite/ Aqueous solutions heated at 170 °C, for 30, 90 aragonite/sphalerite/ Kuwamura et al. [94] and 240 min, at pH 7.0 (Ala) calcite/zeolite Aqueous solution or dry mixture heated at 95 ferrihydrite Matrajt and Blanot, [75] °C for 24 h or 1 week (Gly, Ala) Dry mixture of minerals and amino acids Goethite/akaganéite/hematite/zinc heated at 50, 90 or 120 °C, from 1 to 35 days Shanker et al. [95] oxide/titanium dioxide (Gly, Ala). Heating of dry mixtures of amino acid and Kaolinite/hectorite Silva et al. [79] mineral at 160 °C, 270 °C, 280 °C. (Ala) Aqueous solution heated (100-150 °C) for 9 to Kaolinite/zeolites Zamaraev et al. [96] 85 h (Gly) Wet/dry microwave cycles (Gly, Val, Leu, Ser, kaolinite Yanagawa et al. [97] Met, Phe, tri-Gly, hexa-Gly) Kaolinite/silica/aluminum Heating of dry mixtures of amino acid and White et al. [98] mineral at 145 °C (Gly) hydroxide Amino acid was pre adsorbed on iron oxides, maghemite/hematite/akaganéite Georgelin et al. [99] the material was dried and after heated (Gly) montmorillonite Heating of dry mixtures (Gly, Arg) Bu et al. [100] montmorillonite Wet/dry cycles (Arg, Glu) Jaber et al. [101] Wet/dry cycles (Gly) Bujdák et al. [102] montmorillonite Bujdák et al. [103] Wet/dry cycles (Gly, Ala) Montmorillonite/hectorite/ Wet/dry cycles (Ala, Ala + Gly, Ala + Gly2, Ala Bujdák and Rode, [104] silica/alumina + cyc-Gly<sub>2</sub>) Dry samples with Zn<sup>2+</sup>, Mg<sup>2+</sup>, ATP plus amino Montmorillonite/kaolinite Rishpon et al. [105] acid (Gly) Amino acids heated at 30-100 °C for 147 days Olivine plus orthpyroxene Takahagi et al. [106] at 200 bar (Gly, Ala, Glu, Val, Ser, Asp) Dry samples of amino acids heated at 175 °C, Rohlfing and McAhaney, [107] sand (sea) for 1.5 h (several mixtures of amino acids

mainly Gly)

### Table 1. Cont.

Polymerization				
Mineral	Experiment	Reference		
silica	Adsorption of amino acids onto silica and heating the solid at 160 °C, 30 min. (Leu, Glu)	Bedoin et al. [108]		
Silica	Amino acid was pre adsorbed on silica, the material was dried and after heated (Glu, Leu, Asp, Val)	Sakhano et al. [109]		
silica	Amino acid was pre adsorbed on silica, the material was dried and after heated (Gly)	Meng et al. [110]		
	Chiral Selection			
Mineral	Experiment	Reference		
allophane	Selective adsorption (Ala, Ala-Ala)	Hashizume et al. [111]		
apatite	Polymerization of NCA-amino acids (Glu) and adsorption of the polymers	Hitz and Luisi, [112]		
bentonite Selective adsorption (Leu, Asp)		Bondy and Harrington, [113]		
calcite	Molecular simulation of adsorption (Ala)	Asthagiri and Hazen, [114]		
calcite	Selective adsorption (Asp)	Hazen et al. [115]		
kaolinite	High rate of polymerization of L-amino acids (Asp, Ser)	Jackson, [116]		
montmorillonite	Selective adsorption and deamination (Asp, Glu)	Siffert and Naidja, [117]		
quartz	Molecular simulation of adsorption (Ala)	Pauzat et al. [118]		
quartz	Polymerization of NCA-amino acids (Trp, Leu, Ile) and adsorption of the polymers	Hitz and Luisi, [112,119]		
quartz	Adsorption of amino acids on L- and D-quartz (L-, D-Ala)	Bonner et al. [120]		
vermiculite (modified)	Selective adsorption (Ala, Lys, His)	Fraser et al. [121,122]		

Table 1. Cont.

CDI-1-1'-carbonyldiimidazole, cGlu-L-γ-carboxyglutamic acid, NCA-N-carboxyanhydride, Aib-aminoisobutyric acid.

The data shown in Table 1 leave no doubt that minerals can preconcentrate and polymerize amino acids, and can even make a chiral separation of some of them. However, a few remarks about the data shown in Table 1 are necessary. In general, all adsorption experiments were performed in distilled water or saline solution, thus, the effect of salts of seawater remains unknown. Since most amino acid adsorption experiments were performed separately, the interactions among amino acids and the effects of amino acid competition for mineral adsorption sites are also unknown. The polymerization experiments were mostly performed by heating solid mixtures or wet/dry cycles or with polymerizing agents. The effect of salts on the polymerization has not been studied. Thus, we do not know the effect of salts on the activity of the water and, consequently, on the polymerization of amino acids.

However, as pointed out before, the problem that we need to solve is: are minerals capable of forming peptides with all or at least with most amino acids being proteic and L-chiral? In addition, if these peptides have enzymatic activity, we will be halfway to fitting more pieces into the origin of life's jigsaw. Unfortunately, as pointed out above, this problem has always been thought of as a compartmentalized one, and we have no data on whether minerals can solve it. In the next section, we will discuss several suggestions for experiments that could better help us to understand the role played by minerals in the origin of peptides.

#### 5. A Few Suggestions for Experiments

In this section, we are going to make a few suggestions as a guide for experiments that could shed some light on how minerals participated in the formation of peptides with high amounts of L-amino acids and PAAs. Nowadays, we are used to seeing living beings as perfect "machines" where all "gears" fit and work perfectly. However, the first peptides did not only need to contain L-amino acids and PAAs, they also needed to show some advantages over randomly synthesized peptides. It is probable minerals are the right choice to do this job, because, as pointed out before, minerals are not very selective, but have some selectivity.

#### 5.1. Environments for Adsorption and Polymerization of Amino Acids

First, let us choose the locations where the amino acids could be preconcentrated and the polymers synthesized. Hydrothermal vents are widespread on today's Earth, and were probably more common 4.0 billion years ago. Hydrothermal vents or places near them seem to have several advantages over other environments: temperature and pH gradients, transition metals to catalyze reactions, protection against UV radiation, synthesis of several molecules including amino acids, and various minerals [14,53,60,65,72,123]. In addition, the sedimentary material between the oceanic crust and seawater could have played an important role in the preconcentration of biomolecules, synthesis of biomolecules, and increase in the complexity of the biomolecules (polymers) [124]. Thus, minerals in hydrothermal vents have all the conditions to preconcentrate and polymerize amino acids.

The pH of seawater on the prebiotic Earth was around 7.00 [125,126], and in hydrothermal vents the pH could reach 2.0–3.0 (black smokers) or 9.0–11.0 as in the Lost City hydrothermal field [65]. However, near hydrothermal vents, when the temperature is below 150 °C, the pH is close to 6.0 [127].

Table 2 shows the major cation and anion composition of several types of seawater used in prebiotic experiments. Based on the work of Halevy and Bachan, [125], Samulewski et al. [66], suggested seawater with high concentrations of  $Ca^{2+}$  and  $Cl^-$ . This seawater probably better resembles the composition of major cations and anions of seawater of Earth 4.0 billion years ago. Another seawater that could be used is that suggested by Zaia [67], based on the work of Izawa et al. [128]. This seawater has a high concentration of  $Mg^{2+}$  and  $SO_4^{2-}$ , although several doubts can be raised, mainly regarding the high concentrations of  $SO_4^{2-}$  on prebiotic Earth [129–131]. However, Mars has high concentrations of  $Mg^{2+}$ ,  $Ca^{2+}$ , and  $SO_4^{2-}$  in its soils [132]. Thus, these results could help us to understand the formation of polymers in ancient lakes of Mars. Why is it so important to add salts for experiments of adsorption and polymerization of amino acids? Water promotes the hydrolysis of organic molecules, and due to its high activity, the polymerization of amino acids is thermodynamically unfavorable [5]. However, salts of seawater change the water activity, lowering it. In the pores of minerals, water binds to salts and minerals, changing its activity [133]. Thus, salts change everything. Naturally, someone could suggest a different composition for seawater, or use a different concentration of salts to those shown in Table 2.

Another environment that we could choose is wet/dry cycles. Although most of prebiotic Earth was covered by water [57], this environment should not be ruled out because of the potential it demonstrated for amino acid polymerization, as shown in several works from the Rode group and others [97,101–104,134]. Campbell et al. [134] suggested performing wet/dry cycles using the deliquesce of the salts. According to these authors, using this property of salts, it would not depend on rains or flooding to supply the water to reactional system. Furthermore, one of the salts that show the highest deliquesce (CaCl<sub>2</sub>) appears in high concentrations in the seawater suggested by Samulewski et al. [66] (Table 2). In hydrothermal hot springs, wet/dry cycles occur, besides the polymerization of biomolecules, Damer and Deamer [135,136] pointed out the high potential of these environments for the formation of protocells from amphiphilic molecules. Thus, experiments involving amphiphilic molecules could be considered.

Hydrothermal vents and wet/dry cycles are very different from each other and could provide important insights into how L-amino acids and PAAs were selected from a wide variety of amino acids that could exist on prebiotic Earth.

Name	Composition of Seawater (g L <sup>-1</sup> )		
hydrothermal seawater	* NaCl (37.05 g), KBr (0.310 g), ** KI (0.010 g), ** NH <sub>4</sub> Cl (0.610 g), ** SrCl <sub>2</sub> 6H <sub>2</sub> O (0.040 g), CaCl <sub>2</sub> 2H <sub>2</sub> O (6.26 g), KOH (1.07 g), NaOH (0.200 g),		
◆ seawater	* CaCl <sub>2</sub> 2H <sub>2</sub> O (34.12 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (10.35 g), KBr (0.268 g), NaCl (19.90 g), ** KI (0.006 g), ** NH <sub>4</sub> Cl (0.273 g), ** SrCl <sub>2</sub> 6H <sub>2</sub> O (1.205 g), Na <sub>2</sub> SO <sub>4</sub> (0.333 g)		
♦ prebiotic seawater	* Na <sub>2</sub> SO <sub>4</sub> (0.271 g), MgCl <sub>2</sub> .6H <sub>2</sub> O (0.500 g), CaCl <sub>2</sub> 2H <sub>2</sub> O (2.50 g), KBr (0.050 g), K <sub>2</sub> SO <sub>4</sub> (0.400 g), MgSO <sub>4</sub> (15.00 g)		
*** seawater	NaCl (27.18 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (50.81 g), MgSO <sub>4</sub> (14.45g), K <sub>2</sub> SO <sub>4</sub> (1.01 g), Na <sub>2</sub> CO <sub>3</sub> (0.127 g),		
• ancient calcite seawater	NaCl (32.73 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (9.15 g), CaCl <sub>2</sub> 2H <sub>2</sub> O (8.08 g), Si (2.2 mmol L <sup>-1</sup> )		
• ancient aragonite seawater	NaCl (32.73 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (2.03 g), CaCl <sub>2</sub> 2H <sub>2</sub> O (3.53 g), Si (2.2 mmol L <sup>-1</sup> )		
** seawater	NaCl (29.23 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (10.16 g)		
seawater	CaCl <sub>2</sub> 2H <sub>2</sub> O (29.40 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (0.95 g), KCl (1.49 g), NaCl (1.17 g)		

Table 2. Suggested compositions for artificial seawaters.

Hydrothermal seawater/seawater and *to* prebiotic seawater were suggested by Zaia [67] and these recipes are based on the work of de Ronde et al. [137] and Izawa et al. [128], respectively. *\** Each salt should be added in the order as they are shown. *to* Seawater suggested by Winter and Zubay [138]. *Seawater* suggested by Jones et al. [139].
Seawater suggested by Pedreira-Segade et al. [140]. *Seawater* suggested by Samulewski et al. [66], based on the work of Halevy and Bachan [125]. *\*\** These substances should be added after experiments without them were carried out.

#### 5.2. Suggested Minerals for the Adsorption and Polymerization of Amino Acids

Second, let us choose the minerals—naturally, we have to choose minerals that existed before life arose on Earth. For reasons explained before, montmorillonite is a good choice [2,4,5,67]. The comparison of the results obtained in these experiments with montmorillonite with the existing results could provide several clues about how the amino acids were selected and polymerized.

Iron plays important roles in today's living beings and was also found on prebiotic Earth [69,141]. However, iron stands out not only for its importance for living beings, but mainly for being the fourth most abundant element in the crust of the Earth [69]. Before the "great oxidation event" meaning the increase in the oxygen level in the atmosphere, most of the iron on the Earth was as  $Fe^{\circ}$  or  $Fe^{2+}$  [69]. The most common minerals containing  $Fe^{\circ}/Fe^{2+}$  were olivine, pyroxine, Fe-Ni-metal, and pyrite [69]. In the seas of the prebiotic Earth, three main sinks of  $Fe^{2+}$ : carbonate ( $CO_3^{2-}$ ), dissolved silica ( $SiO_2$ ) and sulfide ( $S^{2-}$ ) existed, that produced siderite ( $FeCO_3$ ), greenalite ( $Fe_3Si_2O_5(OH)_4$ ), and pyrite (FeS), respectively [142].

As pointed out above, iron is an abundant element in the crust of the Earth, therefore, we can wonder whether minerals containing iron may have had an important role in the origin of life. Among the minerals containing iron, magnetite, an iron oxide that is synthesized in hydrothermal vents by serpentinization reaction [65], is a good candidate to be used in these experiments. There are several reasons to choose this mineral: widespread, high pH of point zero of charge-pH<sub>pzc</sub>, catalyst for chemical reactions, and iron forms complexes with amino acids [143].

There are other iron hydroxide-oxides that should be considered in these experiments, although most researchers do not consider them as prebiotic minerals. However, the Bassez group has shown that Fe<sup>2+</sup> could be oxidized to Fe<sup>3+</sup> without UV radiation or oxygen. According to Bassez, Fe<sup>3+</sup> could be formed under conditions using pressures ranging from 10 to 25 MPa, temperatures ranging from 300 to 350 °C, and pH values ranging from 9.5 to 14 (see references in Bassez [144]. Hydrothermal vents present these conditions [65,123]. Among the iron hydroxide-oxides, ferrihydrite, a precursor for several iron oxides, has the highest surface area. Thus, this property makes ferrihydrite an interesting mineral to test these hypotheses.

Zeolites are minerals that could probably have played important roles in the origin of life. Zeolites have a very interesting property; the hydrophilic/hydrophobic character of zeolites depends on Si/Al ratios [145]. This property could be used for the adsorption of amino acids with uncharged R-groups.

There are other minerals that could be used in these experiments, such as: quartz, silica, olivine, and pyrite.

Table 3 shows the surface area and pH at the point of zero charge ( $pH_{pzc}$ ) of the suggested minerals. These two parameters are very important for the following discussion.

Name	pH <sub>PZC</sub>	Surface Area (m <sup>2</sup> g <sup>-1</sup> )	References
ferrihydrite	6.4-8.5	200-400	Zaia et al. [146]; Cornell and Schwertmann [147]
magnetite	6.2–7.8	4–100	Samulewski et al. [66]; Schwaminger et al. [80]; Kosmulski [148]; Cornell and Schwertmann [147]
montmorillonite	1.8–3.0	38–500Theng [71]; Macht et al. [149]; Wang and Lee [ Hedges and Hare [85]; Greenland et al. [86]	
zeolite	negative	360–700	Carneiro et al. [151]; Lambert [5]; Krohn and Tsapatis [92]

Table 3. Surface area and pH at the point of zero charge (pH<sub>pzc</sub>) of minerals.

#### 5.3. Suggested Amino Acids for the Adsorption and Polymerization

Third, let us choose the amino acids. Among hundreds of amino acids, which criteria will we use to choose just a few of them? We are going to use a very simple criterion, to be chosen, the amino acid must have been synthesized in an experiment simulating a terrestrial or interstellar environment (ISM) or have been found in meteorites. Using the data collected by Zaia et al. [11], we can assemble a small set of amino acids to be used in the experiments (Table 4). The data presented in Table 4 will help to predict which amino acids will be more able to adsorb on a given mineral and provide us with a parameter to choose the proportion that each amino acid should have in the mixture.

It should be noted that experiments simulating Earth's environments presented more PAAs than non-PAAs (Table 4). In addition, in experiments simulating ISM environments or meteorite analyses, many more non-PAAs than PAAs were presented (Table 4). Could this mean that endogenous amino acids were more important than exogenous amino acids for the origin of life? In addition, could this mean that life originated on Earth?

<b>Table 4.</b> Amino acid dissociation constants, amino acids produced in experiments simulating Earth's
environments and interstellar environment (ISM) or found in meteorite analyses and relative abundance
of them.

Amino Acids Produced in Environments Simulating Those of Earth						
* Amino Acid	pKa of -COOH Group	pK <sub>b</sub> -NH <sub>3</sub> Group	pK <sub>x</sub> R-Group	pI		
Glycine [1.00]	2.31	9.24	-	5.77		
Alanine [0.300–2.00]	2.47	9.48	-	5.98		
β-Alanine [0.050–0.300]	4.08	10.31		6.95		
Isoleucine [0.050–0.300]	2.79	9.59	-	6.19		
Valine [0.050–0.300]	2.72	9.60	-	6.16		
Serine [0.020–0.150]	2.03	8.93	15.17	5.70		
Glutamic acid [0.020–0.150]	1.88	9.54	4.27	2.70		
Proline [0.020–0.150]	1.94	11.33	-	7.12		
Aspartic acid [0.020–0.150]	1.70	9.61	5.11	3.41		
α-Amino-n-butyric acid [0.020–0.150]	2.62	9.53	-	6.08		
Leucine [0.010–0.050]	2.79	9.52	-	6.15		
Phenylalanine [0.010–0.050]	2.47	9.45	-	5.96		
Threonine [0.010–0.050]	2.21	9.00	-	5.60		
Cysteine [0.010–0.050]	2.35	9.05	10.17	5.68		
Methionine [0.010–0.050]	2.53	9.50	-	6.02		
Arginine [0.010–0.050]	2.41	9.12	12.41	10.77		
Lysine [0.010–0.050]	2.74	9.44	10.29	9.82		
Histidine [0.010–0.050]	1.85	9.44	6.61	8.02		
γ-Amino-n-butyric acid [0.010–0.050]	4.53	10.22	_	7.09		

Amino Acids Produced in Environments Simulating ISM or Found in Meteorites						
** Amino Acid	pK <sub>a</sub> of -COOH Group	pK <sub>b</sub> -NH <sub>3</sub> Group	pK <sub>x</sub> R-Group	pI		
Glycine [1.00]	2.31	9.24	-	5.77		
Aminoisobutyric acid [0.500–3.00]	2.58	9.72	-	6.15		
β-Alanine [0.500–1.500]	4.08	10.31		6.95		
γ-Amino-n-butyric acid [0.500–1.500]	4.53	10.22	-	7.09		
Alanine [0.500–1.500]	2.47	9.48	-	5.98		
Glutamic acid [0.500–1.000]	1.88	9.54	4.27	2.70		
Serine [0.500–1.000]	2.03	8.93	15.17	5.70		
Isovaline [0.500–1.000]	2.68	9.78	-	6.23		
Aspartic acid [0.200–0.800]	1.70	9.61	5.11	3.41		
β-Amino-n-butyric acid [0.200–0.800]	4.22	10.53	-	7.22		
α-Amino-n-butyric acid [0.100–0.300]	2.62	9.53	-	6.08		
Valine [0.100–0.300]	2.72	9.60	_	6.16		

Table 4. Cont.

Non- protein amino acids (PAAs) are in red, PAAs in black. The  $pK_a$ ,  $pK_b$ ,  $pK_x$  and pI values of amino acids were obtained from Chemicalize site, [152]. \* Amino acids were placed in the same decreasing order of relative abundance obtained from experiments simulating atmospheres, hydrothermal and other environments as described by Zaia et al. [11], at Table 8. \*\* Amino acids were placed in the same decreasing order of relative abundance obtained from experiments simulating ISM and meteorite analyses as described by Zaia et al. [11], at Table 8. The values between [] are the relative abundances taking glycine as 1.00 (Zaia et al. [11]).

#### 5.4. Experiment

#### 5.4.1. General Comments

These experiments are suggested to test Ikehara's GADV-hypothesis [43–45]. Ikehara's GADV-hypothesis is an elegant and simple hypothesis that the first peptides were composed of four amino acids. This hypothesis takes for granted that the peptides on prebiotic Earth were composed of a high concentration of the following amino acids: glycine, alanine, aspartic acid, and valine. However, as pointed out several times before, the prebiotic seas could have contained a huge variety of molecules that could be adsorbed by minerals and a large variety of peptides that could be synthesized. We should never forget the problem raised by Schwartz [58], in his article *"Intractable mixtures and the origin of life"*.

A set of experiments was designed to verify if Ikehara's GADV-hypothesis stands with a mixture of amino acids (Table 5). There are four experiment sets, two named GADV and two named GADV plus amino acids. All experiments should be first performed with L-amino acids and, subsequently, with L-/D-amino acids. The main goal of GADV experiments is to test if the set of Ikehara's amino acids produces peptides and the size of these peptides. For aspartic acid, the relative abundance of amino acids from endogenous sources is five times less than from exogenous sources. The GADV plus

amino acids mixture, as well as the amino acids suggested by Ikehara, contains protein and non-PAAs (Table 5). For all experiments, the relative abundances of amino acids were the same as suggested by Zaia et al. [11].For the experiments with GADV plus amino acids set (Table 5), several amino acids that appear in Table 4 did not appear in the endogenous/exogenous amino acid mixtures (Table 5). For the endogenous mixture, from lysine, arginine, and histidine, lysine was chosen because of its high solubility when compared to the others [152]. In addition, other amino acids that appear in Table 4, did not appear in the mixture (Table 5) for two reasons: (1) to simplify the analysis of the obtained peptides; and (2) all R-groups are present in the mixtures.

	GADV		GADV Plus Amino Acids			
	Endogenous Sources	Exogeneous Sources	Endogenous Sources	Exogeneous Sources		
Amino acid proportion	Gly [1.00]/Ala [2.00]/Val [0.300]/Asp [0.150]	Gly [1.00]/Ala [1.50]/Val [0.300]/Asp [0.800]	Gly [1.00]/Ala [2.00]/β-Ala [0.300]/Val [0.300]/Asp [0.150]/α-ABA [0.150]/Phe [0.050]/Lys [0.050]/γ-ABA [0.050]	Gly [1.00]/AIB [3.00]/γ-ABA [1.50]/β-Ala [1.50]/Ala [1.50]/IsoVal [1.00]/Asp [0.800]/β-ABA [0.800]/Val [0.300]/α-ABA [0.300]		
Seawater composition						
Seawater (Zaia, [67]) Samulewski et al. [66]						
Na <sub>2</sub> SO <sub>4</sub> (0.271 g), MgCl <sub>2</sub> .6H <sub>2</sub> O (0.500 g), CaCl <sub>2</sub> 2H <sub>2</sub> O (2.50 g), KBr (0.050 g), K <sub>2</sub> SO <sub>4</sub> (0.400 g), MgSO <sub>4</sub> (15.00 g) CaCl <sub>2</sub> 2H <sub>2</sub> O (29.40 g), MgCl <sub>2</sub> . 6H <sub>2</sub> O (0.95 g), KCl (1.49 g), NaCl (1.17 g)						
		Hydro	othermal conditions			
The amino a	acids could be dissolve magnetite, ferrihyc	d in distilled water and trite, and zeolite Temp	d artificial seawater. The following mi erature100–150 °C, pH 6.00–7.00, and	nerals could be: montmorillonite, heating time 24 h.		
Wet/dry cycles conditions						
The amino a magnetite,	acids could be dissolve ferrihydrite, and zeolit	d in distilled water an e. Wet phase temperat	d artificial seawater. The following mi ure 60 °C and pH 6.00–7.00, dry phase	nerals could be: montmorillonite, e temperature 100–150 °C for 24 h		
		Wet/dry cycles condi	tions (using deliquesce of the salts)			
The amino acids could be mixed with the salts of artificial seawater [67] and seawater [128]. Wet phase temperature 60 °C for 4 h, dry phase temperature 100–150 °C for 20 h						
In rod .	an DAAa Chu alwa	ing Algelaning V	al valing Acrossmentic acid Dhe	nhonylalaning Iva lyaina		

Tal	ble	5.	Experime	nt-Testing	Ikehara's	s GAD	V-hypothesis
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In red non-PAAs, Gly-glycine, Ala-alanine, Val-valine, Asp-aspartic acid, Phe-phenylalanine, Lys-lysine, ABA-Amino-n-butyric acid, AIB-Aminoisobutyric acid, IsoVal-isovaline.

For all experiments, the amount of adsorbed amino acids on the surface of the minerals should be measured, because this could provide clues about the amino acid composition of the peptides.

As the experimental conditions suggested in this article could be considered narrow (Table 5), readers may want to use other reaction conditions: temperature, pH, different artificial seawater, and different minerals. However, it is important to use the set of amino acids described in Table 5. I would like to suggest the following conditions: cpH 4.0 or >pH 9.0, variation in the total concentrations of salts used in the artificial seawater (10%, 50%, and 150% of that suggested in Table 2), and the minerals olivine, pyrite, and silica.

#### 5.4.2. Hydrothermal Experiments

For hydrothermal experiments, a pH range from 6.0 to 7.0 is recommended (Table 5), because at this range of pH, the pH<sub>pzc</sub> of magnetite and ferrihydrite are positively charged and montmorillonite and zeolite are negatively charged (Table 3). In addition, this range of pH is above the ip (isoelectric point) of most of the amino acids (Table 4). Thus, magnetite and ferrihydrite could adsorb more aspartic acid and those amino acids whose ip is below the pH range. Montmorillonite and zeolite could adsorb more lysine and amino acids whose ip is above of the pH range [4]. Table 3 does not specify which type of zeolite could be used in the experiments. Using the property of zeolites that the hydrophilic/hydrophobic character depends on Si/Al ratios [145], it is possible to design experiments to obtain peptides with different proportions of R-groups of amino acids.

The temperature (100–150 °C) suggested for hydrothermal experiments should be enough for the peptide formations on the surface minerals, as well as in solution (Table 5) [106]. In addition, we should remember that the salts of seawater change the water activity, facilitating the formation of polymers [133]. However, the temperature could cause degradation of the amino acids and peptides. According to theoretical calculations performed by Kitadai [153] non-PAAs are more stable than PAAs. This could cause the obtention of peptides with a higher proportion of non-PAAs. However, Kitadai [153] did not take the role of salts and minerals into account in his calculations.

#### Suggestions for Experimental Procedures

The following experimental suggestions could be helpful: (a) determine the adsorption and desorption of individual amino acids and each set at room temperature, (b) polymerize only the adsorbed amino acids, (c) polymerize the adsorbed amino acids plus amino acids in solution, (d) determine the stability in solution of each amino acid and the sets of amino acids at the chosen temperature, (e) determine the stability of the adsorbed peptides and peptides in solution, (f) always use a seawater, and (g) add transition metals to the seawater (Cu<sup>2+</sup>, Fe<sup>3+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>).

#### 5.4.3. Wet/Dry Cycle Experiments

For wet/dry cycle experiments, the temperature of 60 °C is suggested for the wet phase of the cycle and for the dry phase of the cycle the same range used for hydrothermal experiments (Table 5). For wet/dry cycles, it is important to perform the experiments in distilled water and low salt concentrations because, probably, small lagoons of prebiotic Earth contained a low amount of dissolved salts. The peptides will not be synthesized in the wet phase of the cycle, because of the low temperature and time to dry the sample. However, the temperature range suggested for the dry phase of the cycle is more than enough to remove all the water, promoting the formation of the peptide bond. The pH range suggested is the same as for the hydrothermal experiments, and, thus, the observations made for the hydrothermal experiments will be valid for the wet/dry cycles. As described above, usually in wet/dry cycles, the water is supplied to the system by adding it. However as suggested by Campbell et al. [134] various salts can remove water from the atmosphere, forming a liquid solution. Thus, we also suggested a few experiments using this propriety of the salts (Table 5). The hypothesis of Damer and Deamer could be tested experimentally, so amphiphilic molecules could be added to a reactional system [135,136]. In Table 5, we do not suggest any experiment, but it would be interesting to verify if the protocells make a selection of some type of specific peptides.

#### Suggestions for Experimental Procedures

For using the traditional methodology of wet/dry cycles, the following experimental suggestions could be helpful: (a) perform wet/dry cycle experiments using a low amount of salts and distilled water, (b) adsorb each amino acid or amino acid set and heat the dry solids at the temperature recommended for the dry phase, (c) consider the possibility of working with mineral mixtures, and (d) add transition metals to the mixture ( $Cu^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Ni^{2+}$ ,  $Zn^{2+}$ ).

For using the deliquesce propriety of the salts, the following experimental suggestions could be helpful: (a) perform wet/dry cycles using a salt mixture that resembles the composition of the salts of seawater of the prebiotic Earth, (b) perform experiments using only dry samples, and (c) add transition metals to the mixture ( $Cu^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Ni^{2+}$ ,  $Zn^{2+}$ ).

#### 6. What Should We Expect from These Experiments?

Naturally, we expect to obtain peptides whose composition contains only PAAs and L-amino acids. However, we should always keep in our mind that these peptides do not need to be "perfect", meaning they would contain only PAAs and L-amino acids. They just need to show some advantages over peptides synthesized randomly. There are two environments of prebiotic Earth in which peptides can be synthesized: (a) hydrothermal vents and (b) solid state heating. In hydrothermal vents the formation of peptides occurs, because of the heat generated in these environments and because they also contain minerals and transition metals. When mixtures of amino acids plus minerals are heated in solid state, the formation of peptides occurs because of the elimination of water. Thus, using these two environments, we should figure out one way to obtain peptides, at least, with a high amount of PAAs and L-amino acids. In view of previous work that shows that minerals can adsorb amino acids and catalyze the formation of peptides and even make the chiral selection of some them (Table 1), there is a reasonable possibility that these experiments can at least obtain peptide sets with a high concentration of PAAs and L-amino acids. However, among several sets of peptides containing L-, D-amino acids, and non-PAAs and PAAs, how did molecular evolution select a few peptide sets containing high amounts of PAAs and L-amino acids? It may be that this selection occurred not for one reason, but for several reasons, such as: peptides with L-amino acids are more stable than peptides with D-amino acids [52]; PAAs condense more extensively than non-PAAs [40]; peptides with PAAs are more stable than peptides with non-PAAs [42]; hydrothermal vents with high salt concentrations need a specific set of amino acids for protein folding to occur [48]; the high concentration of glycine, a non-chiral amino acid on the prebiotic Earth [11]; the higher concentration of PAAs compared to non-PAAs on prebiotic Earth [11];  $\beta$ - or  $\gamma$ -amino acids cannot self-assemble to N-phosphopeptides [47]; a small enantiomeric excess, due to the contribution of amino acids from exogenous sources [15–17,61]; or even an unknown reason. Indeed, what we are suggesting is that without any RNA world or even a pre genetic world a combination of several of these properties of peptide formation could result in peptide sets with more similarity to modern proteins. Finally, a lot more experimental work is necessary to establish how this important step in molecular evolution occurred.

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#### References

- 1. Bernal, J.D. The Physical Basis of Life; Routledge and Paul: London, UK, 1951.
- 2. Lahav, N.; Chang, S. The possible role of solid surface area in condensation reactions during chemical evolution. *J. Mol. Evol.* **1976**, *8*, 357–380. [CrossRef] [PubMed]
- 3. Mosqueira, F.G.; Albarran, G.; Negrón-Mendoza, A. A review of conditions affecting the radiolysis due to40K on nucleic acid bases and their derivatives adsorbed on clay minerals: Implications in prebiotic chemistry. *Orig. Life Evol. Biosph.* **1996**, *26*, 75–94. [CrossRef] [PubMed]
- 4. Zaia, D.A.M. A review of adsorption of amino acids on minerals: Was it important for origin of life? *Amino Acids* **2004**, 27, 113–118. [CrossRef] [PubMed]
- 5. Lambert, J.-F. Adsorption and polymerization of amino acids on mineral surfaces: A review. *Orig. Life Evol. Biosph.* **2008**, *38*, 211–242. [CrossRef] [PubMed]
- Baú, J.P.T.; Villafañe-Barajas, S.A.; da Costa, A.C.S.; Negrón-Mendoza, A.; Colín-García, M.; Zaia, D.A.M. Adenine adsorbed onto montmorillonite exposed to ionizing radiation: Essays on prebiotic chemistry. *Astrobiology* 2020, 20, 26–38. [CrossRef]
- Basiuk, V.A.; Navarro-González, R. Dust in the universe: Implications for terrestrial prebiotic chemistry. Orig. Life Evol. Biosph. 1995, 25, 457–493. [CrossRef]
- 8. Engel, M.; Macko, S. The stereochemistry of amino acids in the Murchison meteorite. *Precambrian Res.* 2001, 106, 35–45. [CrossRef]
- 9. Fitz, D.; Reiner, H.; Rode, B.M. Chemical evolution toward the origin of life. *Pure Appl. Chem.* 2007, 79, 2101–2117. [CrossRef]
- 10. Johnson, A.P.; Cleaves, H.J.; Dworkin, J.P.; Glavin, D.P.; Lazcano, A.; Bada, J.L. The Miller volcanic spark discharge experiment. *Science* 2008, 322, 404. [CrossRef]

- 11. Zaia, D.A.M.; Zaia, C.T.B.; de Santana, H. Which amino acids should be used in prebiotic chemistry studies? *Orig. Life Evol. Biosph.* **2008**, *38*, 469–488. [CrossRef]
- Pizzarello, S.; Schrader, D.L.; Monroe, A.A.; Lauretta, D.S. Large enantiomeric excesses in primitive meteorites and the diverse effects of water in cosmochemical evolution. *Proc. Natl. Acad. Sci. USA* 2012, 109, 11949–11954. [CrossRef] [PubMed]
- 13. Ruiz-Mirazo, K.; Briones, C.; De La Escosura, A. Prebiotic systems chemistry: New perspectives for the origins of life. *Chem. Rev.* **2014**, *114*, 285–366. [CrossRef] [PubMed]
- Colín-García, M.; Heredia, A.; Cordero, G.; Camprubí, A.; Negrón-Mendoza, A.; Ortega-Guitiérrez, F.; Beraldi, H.; Ramos-Bernal, S. Hydrothermal vents and prebiotic chemistry: A review. *Bol. Soc. Geol. Mex.* 2016, *68*, 599–620. [CrossRef]
- Elsila, J.E.; Aponte, J.C.; Blackmond, D.G.; Burton, A.S.; Dworkin, J.P.; Glavin, D.P. Meteoritic amino acids: Diversity in compositions reflects parent body histories. *ACS Cent. Sci.* 2016, *2*, 370–379. [CrossRef] [PubMed]
- 16. Burton, A.S.; Berger, E.L. Insights into abiotically-generated amino acid enantiomeric excesses found in meteorites. *Life* **2018**, *8*, 14. [CrossRef] [PubMed]
- 17. Glavin, D.P.; Burton, A.S.; Elsila, J.E.; Aponte, J.C.; Dworkin, J.P. the search for chiral asymmetry as a potential biosignature in our solar system. *Chem. Rev.* **2020**, *120*, 4660–4689. [CrossRef]
- 18. Kouznestsov, V.V.; Galvis, C.E.P. Strecker reaction and α-amino nitriles: Recent advances in their chemistry, synthesis, and biological properties. *Tetrahedron* **2018**, *74*, 773–810. [CrossRef]
- 19. Kitadai, N.; Maruyama, S. Origins of building blocks of life: A review. *Geosci. Front.* **2018**, *9*, 1117–1153. [CrossRef]
- 20. Elsila, J.E.; Dworkin, J.P.; Bernstein, M.P.; Martin, M.P.; Sandford, S.A. Mechanisms of amino acid formation in interstellar ice analogs. *Astrophys. J.* **2007**, *660*, 911–918. [CrossRef]
- 21. Miller, S.L. A Production of amino acids under possible primitive Earth conditions. *Science* **1953**, *117*, 528–529. [CrossRef]
- 22. Lowe, C.U.; Rees, M.W.; Markham, R. Synthesis of complex organic compounds from simple precursors: Formation of amino acids, amino acid polymers, fatty acids and purines from ammonium cyanide. *Nature* **1963**, *199*, 219–222. [CrossRef] [PubMed]
- 23. Ferris, J.P.; Joshi, P.C.; Edelson, E.H.; Lawless, J.G. HCN: A plausible source of purines, pyrimidines and amino acids on the primitive earth. *J. Mol. Evol.* **1978**, *11*, 293–311. [CrossRef] [PubMed]
- 24. Ferris, J.P.; Donner, D.B.; Lotz, W. Chemical evolution. IX. Mechanism of the oligomerization of hydrogen cyanide and its possible role in the origins of life. *J. Am. Chem. Soc.* **1972**, *94*, 6968–6974. [CrossRef] [PubMed]
- 25. Ferus, M.; Rimmer, P.; Cassone, G.; Knížek, A.; Civiš, S.; Šponer, J.E.; Ivanek, O.; Šponer, J.; SaeidFirozeh, H.; Kubelík, P.; et al. One-pot hydrogen cyanide-based prebiotic synthesis of canonical nucleobases and glycine initiated by high-velocity impacts on early Earth. *Astrobiology* **2020**. [CrossRef] [PubMed]
- Sandford, S.A.; Nuevo, M.; Bera, P.P.; Lee, T.J. Prebiotic astrochemistry and the formation of molecules of astrobiological interest in interstellar clouds and protostellar disks. *Chem. Rev.* 2020, 120, 4616–4659. [CrossRef] [PubMed]
- 27. Belitz, H.D.; Grosch, W.; Schieberle, P. Amino Acids, Peptides, Proteins. In *Food Chemistry*, 4th ed.; Springer: Berlin/Heideberg, Germany, 2009.
- Kobayashi, K.; Masuda, H.; Ushio, K.-I.; Ohashi, A.; Yamanashi, H.; Kaneko, T.; Takahashi, J.-I.; Hosokawa, T.; Hashimoto, H.; Saito, T. Formation of bioorganic compounds in simulated planetary atmospheres by high energy particles or photons. *Adv. Space Res.* 2001, 27, 207–215. [CrossRef]
- 29. Cleaves, H.J.; Chalmers, J.H.; Lazcano, A.; Miller, S.L.; Bada, J.L. A reassessment of prebiotic organic synthesis in neutral planetary atmospheres. *Orig. Life Evol. Biosph.* **2008**, *38*, 105–115. [CrossRef]
- Rubenstein, E. Biologic Effects of and Clinical Disorders Caused by Nonprotein Amino Acids. *Medicine* 2000, 79, 80–89. [CrossRef]
- 31. Seebach, D.; Beck, A.K.; Bierbaum, D.J. The World ofβ- and gamma;-peptides comprised of homologated proteinogenic amino acids and other components. *Chem. Biodivers.* **2004**, *1*, 1111–1239. [CrossRef]
- 32. Rodgers, K.J.; Shiozawa, N. Misincorporation of amino acid analogues into proteins by biosynthesis. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 1452–1466. [CrossRef]
- 33. Soares, A.R.; Marchiosi, R.; Siqueira-Soares, R.D.C.; De Lima, R.B.; Dos Santos, W.D.; Ferrarese-Filho, O. The role of L-DOPA in plants. *Plant Signal. Behav.* **2014**, *9*, e28275. [CrossRef] [PubMed]

- 34. Corrigan, J.J. D-amino acids in animals. Science 1969, 164, 142–149. [CrossRef] [PubMed]
- 35. Fujii, N. D-amino acids in living higher organisms. *Orig. Life Evol. Biosph.* **2002**, *32*, 103–127. [CrossRef] [PubMed]
- 36. Fujii, N.; Saito, T. Homochirality and life. Chem. Rec. 2004, 4, 267–284. [CrossRef]
- 37. Bastings, J.J.A.J.; van Eijk, H.M.; Damink, S.W.O.; Rensen, S.S. D-amino acids in the health and disease: A focus on cancer. *Nutrients* **2019**, *11*, 2205. [CrossRef]
- 38. Sasabe, J.; Suzuki, M. Distinctive Roles of D-amino acids in the homochiral world: Chirality of amino acids modulates mammalian physiology and pathology. *Keio J. Med.* **2019**, *68*, 1–16. [CrossRef]
- 39. Burton, A.S.; Stern, J.C.; Elsila, J.E.; Glavin, D.P.; Dworkin, J.P. Understanding prebiotic chemistry through the analysis of extraterrestrial amino acids and nucleobases in meteorites. *Chem. Soc. Rev.* **2012**, *41*, 5459–5472. [CrossRef]
- Frenkel-Pinter, M.; Haynes, J.W.; Martin, C.; Petrov, A.S.; Burcar, B.T.; Krishnamurthy, R.; Hud, N.V.; Leman, L.J.; Williams, L.D. Selective incorporation of proteinaceous over nonproteinaceous cationic amino acids in model prebiotic oligomerization reactions. *Proc. Natl. Acad. Sci. USA* 2019, *116*, 16338–16346. [CrossRef]
- 41. Van Der Gulik, P.; Massar, S.; Gilis, D.; Buhrman, H.; Rooman, M. The first peptides: The evolutionary transition between prebiotic amino acids and early proteins. *J. Theor. Biol.* **2009**, *261*, 531–539. [CrossRef]
- Shibue, R.; Sasamoto, T.; Shimada, M.; Zhang, B.; Yamagishi, A.; Akanuma, S. Comprehensive reduction of amino acid set in a protein suggests the importance of prebiotic amino acids for stable proteins. *Sci. Rep.* 2018, *8*, 1–8. [CrossRef]
- 43. Ikehara, K.; Omori, Y.; Arai, R.; Hirose, A. A novel theory on the origin of the genetic code: A GNC-SNS hypothesis. *J. Mol. Evol.* **2002**, *54*, 530–538. [CrossRef] [PubMed]
- 44. Ikehara, K. Possible steps to the emergence of life: The [GADV]-protein world hypothesis. *Chem. Rec.* 2005, 5, 107–118. [CrossRef] [PubMed]
- 45. Ikehara, K. Pseudo-Replication of [GADV]-Proteins and origin of life. *Int. J. Mol. Sci.* **2009**, *10*, 1525–1537. [CrossRef] [PubMed]
- 46. Frenkel-Pinter, M.; Samanta, M.; Ashkenasy, G.; Leman, L.J. Prebiotic peptides: Molecular hubs in the origin of life. *Chem. Rev.* **2020**, *120*, 4707–4765. [CrossRef] [PubMed]
- 47. Jiang, Y.; Tan, B.; Chen, Z.Z.; Liu, T.; Zhong, R.G.; Li, Y.M.; Stewart, D.J.; Zhao, Y.F.; Jiang, H.L. Phosphoryl group differentiating α-amino acids from β- and γ-amino acids in the prebiotic peptide formation. *Int. J. Quantum Chem.* **2003**, *94*, 232–241. [CrossRef]
- 48. Longo, L.M.; Lee, J.; Blaber, M. Simplified protein design biased for prebiotic amino acids yields a foldable, halophilic protein. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 2135–2139. [CrossRef]
- 49. Cornell, C.E.; Black, R.A.; Williams, J.A.; Lee, K.K.; Drobny, G.P.; Keller, S.L.; Xue, M.; Litz, H.E.; Ramsay, A.; Gordon, M.; et al. Prebiotic amino acids bind to and stabilize prebiotic fatty acid membranes. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 17239–17244. [CrossRef]
- 50. Davidson, J.A. Amino Acids in life: A prebiotic division of labor. J. Mol. Evol. 2019, 87, 1–3. [CrossRef]
- 51. Wu, M.; Walker, S.I.; Higgs, P.G. Autocatalytic replication and homochirality in biopolymers: Is homochirality a requirement of life or a result of it? *Astrobiology* **2012**, *12*, 818–829. [CrossRef]
- 52. Skolnick, J.; Zhou, H.; Gao, M. On the possible origin of protein homochirality, structure, and biochemical function. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 26571–26579. [CrossRef]
- 53. Dowler, M.J.; Ingmanson, D.E. Thiocyanate in Red Sea brine and its implications. *Nat. Cell Biol.* **1979**, 279, 51–52. [CrossRef]
- 54. Stribling, R.; Miller, S.L. Energy yields for hydrogen cyanide and formaldehyde syntheses: The HCN and amino acid concentrations in the primitive ocean. *Orig. Life Evol. Biosph.* **1987**, *17*, 261–273. [CrossRef] [PubMed]
- 55. Brack, A. From interstellar amino acids to prebiotic catalytic peptides: A review. *Chem. Biodivers.* **2007**, *4*, 665–679. [CrossRef] [PubMed]
- 56. Tian, F.; Kasting, J.F.; Zahnle, K. Revisiting HCN formation in the Earth's early atmosphere. *Earth Planet. Sci. Lett.* **2011**, *308*, 417–423. [CrossRef]
- 57. Bada, J.L.; Korenaga, J. Exposed areas above sea level on Earth > 3.5 gyr ago: Implications for prebiotic and primitive biotic chemistry. *Life* **2018**, *8*, 55. [CrossRef]
- 58. Schwartz, A.W. Intractable mixtures and the origin of life. Chem. Biodivers. 2007, 4, 656–664. [CrossRef]

- 59. Camprubí, E.; De Leeuw, J.W.; House, C.H.; Raulin, F.; Russell, M.J.; Spang, A.; Tirumalai, M.R.; Westall, F. The emergence of life. *Space Sci. Rev.* **2019**, *215*, 56. [CrossRef]
- 60. Muchowska, K.B.; Varma, S.J.; Moran, J. Synthesis and breakdown of universal metabolic precursors promoted by iron. *Nature* **2019**, *569*, 104–107. [CrossRef]
- 61. Pizzarello, S. Hydrogen cyanide in the Murchison meteorite. *Astrophys. J. Lett.* 2012, 754, L27. [CrossRef]
- 62. Smith, K.E.; House, C.H.; Arevalo, R.D., Jr.; Dworkin, J.P.; Callahan, M.P. Oganometallic compounds as carriers of extraterrestrial cyanide in primitive meteorites. *Nat. Commun.* **2019**, *10*, 2777. [CrossRef]
- 63. Ferris, J.P.; Edelson, E.H. Chemical evolution. 31. Mechanism of the condensation of cyanide to hydrogen cyanide oligomers. *J. Org. Chem.* **1978**, *43*, 3989–3995. [CrossRef]
- Colín-García, M.; Heredia, A.; Negron-Mendoza, A.; Ortega, F.; Pi, T.; Ramos-Bernal, S. Adsorption of HCN onto sodium montmorillonite dependent on the pH as a component to chemical evolution. *Int. J. Astrobiol.* 2014, 13, 310–318. [CrossRef]
- Martin, W.F.; Baross, J.; Kelley, D.; Russell, M.J. Hydrothermal vents and the origin of life. *Nat. Rev. Genet.* 2008, *6*, 805–814. [CrossRef] [PubMed]
- 66. Samulewski, R.B.; Ivashita, F.F.; Paesano, A., Jr.; Zaia, D.A.M. Study of ferrocyanide adsorption onto different minerals as prebiotic chemistry essays. *Astrobiology* **2020**. article submitted.
- 67. Zaia, D.A.M. Adsorption of amino acids and nucleic acid bases onto minerals: A few suggestions for prebiotic chemistry experiments. *Int. J. Astrobiol.* **2012**, *11*, 229–234. [CrossRef]
- Whittet, D.C.B. Is extraterrestrial organic matter relevant to the origin of life on earth? *Orig. Life Evol. Biosph.* 1997, 27, 249–262. [CrossRef] [PubMed]
- 69. Hazen, R.M.; Papineau, D.; Bleeker, W.; Downs, R.T.; Ferry, J.M.; McCoy, T.J.; Sverjensky, D.A.; Yang, H. Mineral evolution. *Am. Mineral.* **2008**, *93*, 1693–1720. [CrossRef]
- 70. Hazen, R.M. The Initial Idea for Mineral Evolution. Available online: https://hazen.carnegiescience.edu/ research/mineral-evolution (accessed on 11 November 2020).
- 71. Theng, B.K.G. *Clay Mineral Catalysis of Organic Reactions*; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2018.
- 72. Holm, N.G.; Dowler, M.J.; Wadsten, T.; Arrhenius, G. β-FOOH.Cln (akaganéite) and Fe1-xO (wüstite) in hot brine from the Atlantis II Deep (Red Sea) and the uptake of amino acids by synthetic β-FOOH.Cln. *Geochim. Cosmochim. Acta* 1983, 47, 1465–1470. [CrossRef]
- 73. Tanaka, H.; Miyajima, K.; Nakagaki, M.; Shimabayashi, S. Interactions of aspartic acid, alanine and lysine with hydroxyapatite. *Chem. Pharm. Bull.* **1989**, *37*, 2897–2901. [CrossRef]
- 74. Benetoli, L.O.B.; de Souza, C.M.D.; da Silva, K.L.; de Souza, I.G., Jr.; de Santana, H.; Paesano, A., Jr.; da Costa, A.C.S.; Zaia, C.T.B.V.; Zaia, D.A.M. Amino acid interaction with and adsorption on clays: FT-IR and Mössbauer spectroscopy and X-ray diffractometry investigations. *Orig. Life Evol. Biosph.* 2007, 37, 479–493. [CrossRef]
- 75. Matrajt, G.; Blanot, D. Properties of synthetic ferrihydrite as an amino acid adsorbent and a promoter of peptide bond formation. *Amino Acids* 2004, *26*, 153–158. [CrossRef] [PubMed]
- 76. Farias, A.P.S.F.; Carneiro, C.E.A.; Fonseca, I.C.D.B.; Zaia, C.T.B.V.; Zaia, D.A.M. The adsorption of amino acids and cations onto goethite: A prebiotic chemistry experiment. *Orig. Life Evol. Biosph.* 2016, 48, 1401–1412. [CrossRef] [PubMed]
- 77. Norén, K.; Loring, J.S.; Persson, P. Adsorption of alpha amino acids at the water/goethite interface. *J. Colloid Interface Sci.* **2008**, *319*, 416–428. [CrossRef] [PubMed]
- Pandey, P.; Pant, C.K.; Gururani, K.; Arora, P.; Kumar, S.; Sharma, Y.; Pathak, H.D.; Mehata, M.S. Surface interaction of l-alanine on hematite: An astrobiological implication. *Orig. Life Evol. Biosph.* 2013, 43, 331–339. [CrossRef]
- Silva, F.D.C.; Lima, L.C.B.; Silva-Filho, E.C.; Fonseca, M.G.; Lambert, J.-F.; Jaber, M. A comparative study of alanine adsorption and condensation to peptides in two clay minerals. *Appl. Clay Sci.* 2020, 192, 105617. [CrossRef]
- Schwaminger, S.P.; García, P.F.; Merck, G.K.; Bodensteiner, F.A.; Heissler, S.; Günther, S.; Berensmeier, S. Nature of interactions of amino acids with bare magnetite nanoparticles. *J. Phys. Chem. C* 2015, *119*, 23032–23041. [CrossRef]

- Farias, A.P.S.F.; Tadayozzi, Y.S.; Carneiro, C.E.A.; Zaia, D.A.M. Salinity and pH effect Na+-montmorillonite dissolution and amino acid adsorption: A prebiotic chemistry study. *Int. J. Astrobiol.* 2014, *13*, 259–270. [CrossRef]
- Ramos, M.E.; Huertas, F.J. Adsorption of glycine on montmorillonite in aqueous solutions. *Appl. Clay Sci.* 2013, 10–17. [CrossRef]
- 83. Kalra, S.; Pant, C.K.; Pathak, H.D.; Mehta, M.S. Adsorption of glycine and alanine on montmorillonite with or without coordinated cations. *Indian J. Biochem. Biophys.* **2000**, *37*, 341–346.
- 84. Benincasa, E.; Brigatti, M.F.; Lugli, C.; Medici, L.; Poppi, L. Interaction between glycine and Na-, Ca- and Cu-rich smectites. *Clay Miner.* **2000**, *35*, 635–641. [CrossRef]
- 85. Hedges, J.I.; Hare, P. Amino acid adsorption by clay minerals in distilled water. *Geochim. Cosmochim. Acta* **1987**, *51*, 255–259. [CrossRef]
- 86. Greenland, D.J.; Laby, R.H.; Quirk, J.P. Adsorption of amino-acids and peptides by montmorillonite and illite. Part 1-Cation exchange and proton transfer. *Trans. Faraday Soc.* **1965**, *61*, 2013. [CrossRef]
- 87. Greenland, D.J.; Laby, R.H.; Quirk, J.P. Adsorption of amino-acids and peptides by montmorillonite and illite. Part 2-Physical adsorption. *Trans. Faraday Soc.* **1965**, *61*, 2024. [CrossRef]
- 88. Afrin, R.; Ganbaatar, N.; Aono, M.; Cleaves, H.J., II; Taka-Aki, Y.; Hara, M. Size-dependent affinity of glycine and its short oligomers to pyrite surface: A model for prebiotic accumulation of amino acid oligomers on a mineral surface. *Int. J. Mol. Sci.* **2018**, *19*, 365. [CrossRef] [PubMed]
- 89. Bebié, J.; Schoonen, M.A.A. Pyrite surface interaction with selected organic aqueous species under anoxic conditions. *Geochem. Trans.* **2000**, *8*, 1–8. [CrossRef]
- 90. Zaia, D.A.M.; Vieira, H.J.; Zaia, C.T.B.V. Adsorption of L-amino Acids on Sea Sand. J. Braz. Chem. Soc. 2002, 13, 679–681. [CrossRef]
- 91. Lopes, I.; Piao, L.; Stievano, L.; Lambert, J.F. Adsorption of amino acids on oxide supports: A solid NMR study of glycine adsorption on silica e alumina. *J. Phys. Chem. C* **2009**, *113*, 18163–18172. [CrossRef]
- 92. Krohn, J.E.; Tsapatsis, M. Amino acid adsorption on zeolite β. Langmuir 2005, 21, 8743–8750. [CrossRef]
- Hill, A.R., Jr.; Orgel, L.E. Oligomerization of L-γ-carboxyglutamic acid. Orig. Life Evol. Biosph. 1999, 29, 115–122. [CrossRef]
- 94. Kuwamura, K.; Konogaya, N.; Maruoka, Y. Enhancement and inhibitory activities of minerals for alanine oligopeptide elongation under hydrothermal vents. *Astrobiology* **2018**, *18*, 1403–1413. [CrossRef]
- Shanker, U.; Bhushan, B.; Bhattacharjee, G. Kamaluddin Oligomerization of glycine and alanine catalyzed by iron oxides: Implications for prebiotic chemistry. *Orig. Life Evol. Biosph.* 2012, 42, 31–45. [CrossRef] [PubMed]
- Zamaraev, K.I.; Romannikov, V.N.; Salganik, R.I.; Wlassoff, W.A.; Khramtsov, V.V.; Zamaraev, K.I.; Romannikov, V.N.; Salganik, R.I.; Wlassoff, W.A.; Khramtsov, V.V.; et al. Modelling of the prebiotic synthesis of oligopeptides: Silicate catalysts help to overcome the critical stage. *Orig.Llife Evol. Biosph.* 1997, 27, 325–337. [CrossRef] [PubMed]
- Yanagawa, H.; Kojima, K.; Ito, M.; Handa, N. Synthesis of polypeptides by microwave heating I. Formation of polypeptides during repeated hydration-dehydration cycles and their characterization. *J. Mol. Evol.* 1990, *31*, 180–186. [CrossRef] [PubMed]
- 98. White, D.H.; Kennedy, R.M.; Macklin, J. Acyl silicates and acyl aluminates as activated intermediates in peptide formation on clays. *Orig. Life Evol. Biosph.* **1984**, *14*, 273–278. [CrossRef] [PubMed]
- Georgelin, T.; Akouche, M.; Jaber, M.; Sakhno, Y.; Matheron, L.; Fournier, F.; Méthivier, C.; Martra, G.; Lambert, J.-F. Iron(III) Oxide Nanoparticles as Catalysts for the Formation of Linear Glycine Peptides. *Eur. J. Inorg. Chem.* 2017, 2017, 198–211. [CrossRef]
- 100. Bu, H.; Yuan, P.; Liu, H.; Liu, D.; Qin, Z.; Zhong, X.; Song, H.; Li, Y. Formation of macromolecules with peptide bonds via the thermal evolution of amino acids in the presence of montmorillonite: Insight into prebiotic geochemistry on the early Earth. *Chem. Geol.* **2019**, *510*, 72–83. [CrossRef]
- 101. Jaber, M.; Georgelin, T.; Bazzi, H.; Costa-Torro, F.; Lambert, J.-F.; Bolbach, G.; Clodic, G. Selectivities in Adsorption and Peptidic Condensation in the (arginine and glutamic acid)/montmorillonite clay system. *J. Phys. Chem. C* 2014, 118, 25447–25455. [CrossRef]
- 102. Bujdak, J.; Slosiarikova, H.; Texler, N.; Schwendinger, M.; Rode, B.M. On the possible role of montmorillonites in prebiotic peptide formation. *Mon. Chem.* **1994**, *125*, 1033–1039. [CrossRef]

- Bujdák, J.; Faybíková, K.; Eder, A.; Yongyai, Y.; Rode, B.M. Peptide chain elongation: A possible role of montmorillonite in prebiotic synthesis of protein precursors. *Orig. Life Evol. Biosph.* 1995, 25, 431–441. [CrossRef]
- 104. Bujdák, J.; Rode, B.M. Silica, alumina, and clay-catalyzed alanine peptide bond formation. *J. Mol. Evol.* **1997**, 45, 457–466. [CrossRef]
- Rishpon, J.; O'Hara, P.J.; Lahav, N.; Lawless, J.G. Interaction between ATP, metal ions, glycine and several minerals. J. Mol. Evol. 1982, 18, 179–184. [CrossRef] [PubMed]
- 106. Takahagi, W.; Seo, K.; Shibuya, T.; Takano, Y.; Fujishima, K.; Saitoh, M.; Shimamura, S.; Matsui, Y.; Tomita, M.; Takai, K. Peptide synthesis under the alkaline hydrothermal conditions on Enceladus. ACS Earth Space Chem. 2019, 3, 2559–2568. [CrossRef]
- 107. Rohlfing, D.L.; McAlhaney, W.W. The thermal polymerization of amino acids in the presence of sand. *Biosystems* **1976**, *8*, 139–145. [CrossRef]
- 108. Bedoin, L.; Alves, S.; Lambert, J.F. Origins of life and molecular information: Selectivity in mineral surface induced prebiotic amino acids polymerization. *ACS Earth Space Chem.* **2020**. [CrossRef]
- 109. Sakhno, Y.; Battistella, A.; Mezzetti, A.; Jaber, M.; Georgelin, T.; Michot, L.J.; Lambert, J.-F. One step up the ladder of prebiotic complexity: Formation of nonrandom linear polypeptides from binary systems of amino acids on silica. *Chem. Eur. J.* 2019, 25, 1275–1285. [CrossRef] [PubMed]
- 110. Meng, M.; Stievano, L.; Lambert, J.F. Adsorption and thermal condensation mechanism of amino acids on oxide supports 1. Glycine on silica. *Langmuir* **2004**, *20*, 914–923. [CrossRef]
- 111. Hashizume, H.; Theng, B.K.G.; Yamagishi, A. Adsorption and discrimination of alanine and alanyl-alanine enantiomers by allophane. *Clay Miner.* **2002**, *37*, 551–557. [CrossRef]
- 112. Hitz, T.; Luisi, P.L. Enhancement of homochirality in oligopeptides by quartz. *Helv. Chim. Acta* 2002, *85*, 3975–3983. [CrossRef]
- Bondy, S.; Harrington, M. L Amino acids and D-glucose bind stereospecifically to a colloidal clay. *Science* 1979, 203, 1243–1244. [CrossRef]
- Asthagiri, A.; Hazen, R.H. An ab initio study of adsorption of alanine on the chiral calcite (2131) surface. *Mol. Simul.* 2007, 33, 15–30. [CrossRef]
- 115. Hazen, R.M.; Filley, T.R.; Goodfriend, G.A. Selective adsorption of L- and D-amino acids on calcite: Implications for biochemical homochirality. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 5487–5490. [CrossRef] [PubMed]
- 116. Jackson, T.A. Preferential polymerization and adsorption of L-optical isomers of amino acids relative to D-optical isomers on kaolinite templates. *Chem. Geol.* **1971**, *7*, 295–306. [CrossRef]
- 117. Siffert, B.; Naidja, A. Stereoselectivity of montmorillonite in the adsorption and deamination of some amino acids. *Clay Miner.* **1992**, *27*, 109–118. [CrossRef]
- Pauzat, F.; Marloie, G.; Markovits, A.; Ellinger, Y. Global versus local adsorption selectivity. *Int. J. Astrobiol.* 2015, 14, 563–570. [CrossRef]
- 119. Hitz, T.H.; Luisi, P.L. Spontaneous onset of homochirality in oligopeptide chains generated in the polymerization of n-carboxyanhydride amino acids in water. *Orig. Life Evol. Biosph.* **2004**, *34*, 93–110. [CrossRef] [PubMed]
- 120. Bonner, W.A.; Kavasmaneck, P.R.; Martin, F.S.; Flores, J.J. Asymmetric adsorption by quartz: A model for the prebiotic origin of optical activity. *Orig. Life Evol. Biosph.* **1975**, *6*, 367–376. [CrossRef]
- Fraser, D.G.; Fitz, D.; Jakschitz, T.; Rode, B.M. Selective adsorption and chiral amplification of amino acids in vermiculite clay-implications for the origin of biochirality. *Phys. Chem. Chem. Phys.* 2011, 13, 831–838. [CrossRef]
- 122. Fraser, D.G.; Greenwell, H.C.; Skipper, N.T.; Smalley, M.V.; Wilkinson, M.A.; Demé, B.; Heenan, R.K. Chiral interactions of histidine in a hydrated vermiculite clay. *Phys. Chem. Chem. Phys.* 2011, 13, 825–830. [CrossRef]
- 123. Holm, N.G.; Andersson, E. Hydrothermal simulation experiments as a tool for studies of the origin of life on earth and other terrestrial planets: A review. *Astrobiology* **2005**, *5*, 444–460. [CrossRef]
- 124. Westall, F.; Hickman-Lewis, K.; Hinman, N.; Gautret, P.; Campbell, K.; Bréhéret, J.; Foucher, F.; Hubert, A.; Sorieul, S.; Dass, A.; et al. A Hydrothermal-sedimentary context for the origin of life. *Astrobiology* 2018, 18, 259–293. [CrossRef]
- 125. Halevy, I.; Bachan, A. The geologic history of seawater pH. Science 2017, 355, 1069–1071. [CrossRef] [PubMed]

- 126. Krissansen-Totton, J.; Arney, G.N.; Catling, D.C. Constraining the climate and ocean pH of the early Earth with a geological carbon cycle model. *Proc. Natl. Acad. Sci. USA* 2018, *115*, 4105–4110. [CrossRef] [PubMed]
- 127. Ding, K.; Seyfried, W.E., Jr.; Zhang, Z.; Tivey, M.K.; von Damm, K.L.; Bradley, A.M. The in-situ pH of hydrothermal fluids at mid-ocean ridges. *Earth Planet. Sci. Lett.* **2005**, 237, 167–174. [CrossRef]
- 128. Izawa, M.R.M.; Nesbit, H.W.; MacRae, N.D.; Hoffman, E.L. Composition and evolution of the early oceans: Evidence from Tagish Lake meteorite. *Earth Planet. Sci. Lett.* **2010**, *298*, 443–449. [CrossRef]
- 129. DeWitt, H.L.; Hasenkopf, C.A.; Trainer, M.G.; Farmer, D.K.; Jimenez, J.L.; McKay, C.P.; Toon, O.B.; Tolbert, M.A. The formation of sulfate and elemental sulfur aerosols under varying laboratory conditions: Implications for early Earth. *Astrobiology* 2010, *10*, 773–781. [CrossRef]
- 130. Gaillard, F.; Scaillet, B.; Arndt, N.T. Atmospheric oxygenation caused by a change in volcanic degassing pressure. *Nature* **2011**, *478*, 229–232. [CrossRef]
- 131. Kasting, J.F.; Catling, D.C.; Zahnle, K. Atmospheric oxygenation and volcanism. *Nature* **2012**, *487*, E1. [CrossRef]
- 132. Wänke, H.; Brückner, J.; Dreibus, G.; Rieder, R.; Ryabchikov, I. Chemical composition of rocks and soils at the pathfinder site. *Space Sci. Rev.* 2001, *96*, 317–330. [CrossRef]
- Vieira, A.D.N.; Kleinermanns, K.; Martin, W.F.; Preiner, M. The ambivalent role of water at the origins of life. FEBS Lett. 2020, 594, 2717–2733. [CrossRef]
- 134. Campbell, T.D.; Febrian, R.; McCarthy, J.T.; Kleinschmidt, H.E.; Forsythe, J.G.; Bracher, P.J. Prebiotic condensation through wet-dry cycling regulated by deliquescence. *Nat. Comm.* **2019**, *10*, 4508. [CrossRef]
- 135. Damer, B.; Deamer, D. The hot spring hypothesis for an origin of life. *Astrobiology* **2020**, *20*, 429–452. [CrossRef] [PubMed]
- Damer, B.; Deamer, D.; Damer, B.; Deamer, D. Coupled phases and combinatorial selection in fluctuating hydrothermal pools: A scenario to guide experimental approaches to the origin of cellular life. *Life* 2015, 5, 872–887. [CrossRef] [PubMed]
- Ronde, C.E.j.; Channer, D.M.R.; Faure, K.; Bray, C.J.; Spooner, E.T.C. Fluid Chemistry of Archean seafloor hydrothermal vents: Implications for the composition of circa 3.2 Ga seawater. *Geochim. Cosmochim. Acta* 1997, 61, 4025–4042. [CrossRef]
- 138. Winter, D.; Zubay, G. Binding of adenine and adenine-related compounds to the clay montmorillonite and the mineral hydroxylapatite. *Orig. Life Evol. Biosph.* **1995**, *25*, 61–85. [CrossRef] [PubMed]
- 139. Jones, C.; Nomosatryo, S.; Crowe, S.A.; Bjerrum, C.J.; Canfield, D.E. Iron oxides, divalent cations, silica and the early earth phosphorus. *Geology* **2015**, *43*, 135–138. [CrossRef]
- Pedreira-Segade, U.; Feuillie, C.; Pelletier, M.; Michot, L.J.; Daniel, I. Adsorption of nucleotides onto ferromagnesian phyllosilicates: Significance for the origin of life. *Geochim. Cosmochim. Acta* 2016, 176, 81–95. [CrossRef]
- 141. Curi, R.; Procopio, J. Fisiologia Básica, 2nd ed.; Editora Guanabara Koogan Ltda: Rio de Janeiro, Brazil, 2017.
- 142. Tosca, N.J.; Jiang, C.Z.; Rasmussen, B.; Muhling, J. Products of the iron cycle on the early Earth. *Free Radic. Biol. Med.* **2019**, *140*, 138–153. [CrossRef]
- 143. Schwertmann, U.; Cornell, R.M. Iron Oxides in Laboratory; John Wiley & Sons: Hoboken, NJ, USA, 2008.
- 144. Bassez, M.P. Water near its supercritical point and at alkaline pH for the production of ferric oxides and silicates in anoxic conditions. a new hypothesis for the synthesis of minerals observed in banded iron formations and for the related geobiotropic chemistry inside fluid inclusions. *Orig. Life Evol. Biosph.* 2018, 48, 289–320. [CrossRef]
- 145. Burton, A.W.; Zones, S.I. Organic molecules in zeolite synthesis: Their preparation and structure-directing effects. In *Introduction to Zeolite Science and Practice*, 3rd ed.; Čejka, J., van Bekkum, H., Corma, A., Schüth, F., Eds.; Elsevier: Amsterdam, The Netherlands, 2007; pp. 137–179.
- 146. Zaia, D.A.M.; Coutinho, M.A.; Mosca, D.H.; Da Costa, A.C.S.; Urbano, A. Study of the formation of ferrihydrite under prebiotic chemistry conditions: Artificial seawater 4.0 Gy and ammonium thiocyanate. *Int. J. Astrobiol.* 2020, 1–12. [CrossRef]
- 147. Cornell, R.M.; Schwertmann, U. *The Iron Oxides: Structure, Properties, Reactions, Occurences and Uses;* Wiley-VCH Verlag Gmbh & Co. KGaA: Weinheim, Germany, 2003.
- 148. Kosmulski, M. pH-dependent surface charging and points of zero charge. IV. Update and new approach. *J. Colloid Interface Sci.* **2009**, 337, 439–448. [CrossRef]

- 149. Macht, F.; Eusterhues, K.; Pronk, G.J.; Totsche, K.U. Specific surface area of clay minerals: Comparison between atomic force microscopy measurements and bulk-gas (N2) and -liquid (EGME) adsorption methods. *Appl. Clay Sci.* **2011**, *53*, 20–26. [CrossRef]
- 150. Wang, X.C.; Lee, C. Adsorption and desorption of aliphatic amines, amino acids and acetate by clay minerals and marine sediments. *Mar. Chem.* **1993**, *44*, 1–23. [CrossRef]
- Carneiro, C.E.; De Santana, H.; Casado, C.; Coronas, J.; Zaia, D.A.M. Adsorption of amino acids (Ala, Cys, His, Met) on zeolites: Fourier Transform Infrared and Raman Spectroscopy Investigations. *Astrobiology* 2011, 11, 409–418. [CrossRef] [PubMed]
- 152. Chemicalize. The pKa, pKb, pKx and pI Values of Amino Acids. Available online: https://chemicalize.com/ welcome#/ (accessed on 17 September 2020).
- 153. Kitadai, N. Predicting Thermodynamic behaviors of non-protein amino acids as a function of temperature and pH. *Orig. Life Evol. Biosph.* **2016**, *46*, 3–18. [CrossRef] [PubMed]

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