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Catalytic Prebiotic Formation of Glycerol Phosphate Esters and an Estimation of Their Steady State Abundance under Plausible Early Earth Conditions

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Abstract: The emergence of biological phosphate esters of glycerol could have been a crucial step in the origin and evolution of life on the early Earth as glycerol phosphates today play a central role in biochemistry. We investigate here the formation of the glycerol phosphates by employing various rock samples, salts, and minerals as potential catalysts to aid the phosphorylation process. We report the synthesis of various phosphate esters of glycerol including glycerol-1-phosphate, glycerol-2-phosphate, cyclic glycerol-monophosphate as well as various diphosphate esters. Furthermore, the decomposition rates of glycerol phosphate under mild heating were also studied while keeping the pH constant. It was observed that glycerol phosphate starts decomposing quickly under mild heating conditions into inorganic orthophosphate and pyrophosphate, and a steady state concentration of ~0.5 M of glycerol phosphate may have been reasonable in ponds with abundant glycerol, phosphate, urea, and catalytic minerals.

Keywords: phosphorus; phosphate esters; esterification; minerals; catalysis; origin of life; glycerol phosphates; phosphorylation; condensation; chemical evolution; decomposition

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1. Introduction

Glycerol phosphates (GP) play a central role in modern biochemistry. These compounds are directly associated with crucial life processes, such as cellular respiration and cell structure [1]. For a better understanding of the origin and evolution of early membranes, it is essential to understand the prebiotic syntheses of GP, which also are critical to the synthesis of phospholipids, an essential component of cell membranes in almost all organisms [2–4]. GP links via a phosphate diester bond to form a ‘head group’ that is the polar/hydrophilic part of the phospholipid molecule [4]. The roles of GP in the origin and evolution of cell membranes in various organisms have been discussed previously [4]. In addition, GP and its derivatives also play central roles in several other metabolic pathways such as respiration.

Prebiotic syntheses of GP have been reported previously by using ammonium phosphates to phosphorylate glycerol with condensation agents at 85 °C [5], under simulated hydrothermal conditions and by using various minerals and clays as catalysts [6], by employing various non-aqueous solvents [7–9], by using high energy phosphates such as amidophosphates [10], and by the formation of activated phosphate, e.g., imidazole phosphate, which then reacts with the organic compounds [11].

In addition, the plausible syntheses of GP from the meteoritic mineral schreibersite (Fe₂NiP, or its synthetic analog Fe₃P) have been reported [12]. Recently, another pathway of GP synthesis has also been reported in phosphine-doped interstellar analog ices of methanol (CH₃OH), carbon dioxide (CO₂), or water (H₂O) exposed to high energy radiation [13]. Both studies [12,13] highlight extraterrestrial, abiotic pathways leading to the formation of GP.

The above-mentioned methods have challenges such as the use of non-aqueous solvents that may not be prebiotically prominent [7–9], high energy conditions that may degrade

organic substrates [5,6,13] and use of high energy phosphates, which are uncommon in the rock record [10]. Furthermore, since water was the most realistic solvent on the Hadean earth [14,15], the most plausible prebiotic scenario for formation of GP would utilize water as a solvent, under mild heating conditions and the condensation would possibly be facilitated by the utilization of prebiotically relevant condensation agents such as urea [5,16–18]. Moreover, minerals could have further facilitated the catalysis of the prebiotic synthesis of GP [6]. Minerals such as kaolinite and quartz are prebiotically relevant silicates that have been shown to catalyze various prebiotic reactions [7,18]. The significance of silicates has also been discussed previously [19] (also see Tables 2 and 3 of [19]). Hematite has also been used in the prebiotic synthesis of GP [6]. Similarly, borates (including ulexite) have been considered to have played an important role in the chemical origin of biological molecules, particularly in the plausible stability of sugars [20–22], even though their presence on the Hadean Earth is somewhat questionable [23]. Olivines ($[\text{Fe,Mg}]_2\text{SiO}_4$) are considered to be one of the most ubiquitous and primitive minerals on the Earth [24–26]. These react with water to produce a rock type known as a serpentinite, and liberates molecular hydrogen [27,28]. Iron hydroxides, olivine, serpentinites and volcanic glasses are deemed to be of high prebiotic relevance [26,29] (also see Table 2 of [26]). In addition to the above-mentioned minerals, basaltic rocks were likely ubiquitous and are among the oldest known rocks [30].

In the present study, we attempt to phosphorylate glycerol under mild heating conditions. We have mimicked a prebiotically plausible drying hot pool of water containing a dissolved organic (glycerol), a condensation agent (urea), a phosphate (ammonium phosphate), along with various minerals including kaolinite, quartz, hematite, magnetite, olivine, ulexite, and salts (NH_4Cl , NaCl , Na_2CO_3) and some relevant rock samples (serpentinite, obsidian, and basalt). These substances were investigated for their role in the formation of GP. We also investigated GP decomposition under similar conditions in order to constrain a plausible steady-state concentration of GP on the early earth.

2. Results and Discussions

In our model of a drying hot pool containing glycerol, phosphates, urea, and combined with minerals, salts, or rock samples, we observed synthesis of various phosphate esters of glycerol (Table 1 and Figures 1–4, Scheme 1). In addition to the expected phosphate esters, e.g., glycerol-1-phosphate, glycerol-2-phosphate, we also observed cyclic mono-glycerol phosphate, and various isomers of glycerol diphosphate (two orthophosphates linked up to a glycerol molecule on different carbons not as a pyrophosphate linkage). The ^{31}P -NMR yields of the phosphorylated products were calculated based on the peak integration methods as previously reported [7–9,12,18].

Table 1. Yields ¹ (%) of various phosphates species detected in the phosphorylation of glycerol.

Sample	Catalyst	a	b	c	d	e	f	g	h	Net Org. PO ₄
1	None	3	2	ND	95	ND	ND	ND	ND	5
2	Na_2CO_3	26	21	ND	13	18	8	7	7	80
3	Serpentine	21	8.5	1	33	25	ND	ND	11.5	55.5
4	Hematite	18	10	1	8	50	ND	ND	13	79
5	Obsidian	22	14	1	9	42	2	2	8	83
6	NaCl	4	3	ND	93	ND	ND	ND	ND	7
7	Basalt	30	5	4	8	40	2	2	9	83
8	Magnetite	31	7	6	5	42	2	ND	7	88
9	NH_4Cl	22	15	ND	20	20	13	ND	10	70
10	Ulexite	57	17	4	20	ND	ND	ND	2	78
11	Quartz	31	23	ND	12	12	5	ND	17	71
12	Olivine	21	6	5	1	32	14	13	8	91
13	Kaolinite	65	15	3	11	ND	ND	ND	6	83

¹ The yields of the phosphorylated products as well as other inorganic phosphates were calculated based on the total phosphorus dissolved and by the peak integration method as previously reported [18]. The various compounds are represented as follows; glycerol-1-phosphate (a), glycerol-2-phosphate (b), cyclic GP (c), orthophosphate (d), various isomers of glycerol-diphosphate (e, f, and g) and inorganic P species (h), respectively. ND is “not detected”.

Figure 1. ^{31}P -NMR of GP formation in the presence of olivine. In addition to all the peaks already discussed. This sample also had some unidentified inorganic phosphate species labeled as peaks 'h' and 'j'.

Figure 2. Formation of GP in the presence of basalt. Peak 'g' is also a plausible isomer of GP diphosphate.

Figure 3. Comparable spectra of GP formation in the presence of serpentine as a catalyst. The unsiked spectrum (a) and same reaction spiked with standard GP (b).

Figure 4. The comparable yields (%) of mono GP (glycerol-1-phosphate, glycerol-2-phosphate and cyclic mono GP) and di GP in the presence of various catalysts.

Scheme 1. The structures of various compounds discussed in the text. The labeling of the structures is also similar to that mentioned in the NMR figures and Tables 1 and 2. Glycerol monophosphates (red), inorganic phosphates (black), glycerol diphosphates (blue) and glycerol triphosphates (green), respectively.

Table 2. Yields ¹ (%) of the decomposition products of GP.

Sample	a	b	c	d	e	f	k	Net	Org. PO ₄
Set 1	1	46	35	ND	ND	ND	19	ND	100
Set 2	2	49.50	30	ND	3.5	0.5	14	2.5	94
Set 3	3	39	19	ND	5	1	17	19	76
Set 4	4	28.50	12	ND	4.7	0.8	13	41	54.3
Set 5	5	46	35	ND	ND	1	18	ND	100
Set 6	6	46	32	ND	0.5	0.5	21	ND	99.5
Set 7	7	36	20	ND	4	0.5	15.5	24	72
Set 8	8	45	14	ND	7	1	5	28	65

¹ As mentioned in the description of Table 1, the yields of the decomposition products as well as other inorganic phosphates were calculated based on the total phosphorus dissolved and by the peak integration method as previously reported [18]. In the Table 2, various compounds are represented as follows; glycerol-1-phosphate (a), glycerol-2-phosphate (b), cyclic GP (c), inorganic orthophosphate (d), various isomers of glycerol-diphosphate (e and f), and inorganic pyrophosphate (k). ND means “not detected”.

In a typical reaction, heating glycerol with ammonium phosphate, urea, and with the added catalyst (mineral, salt, and rock sample), the overall yield of the GP was remarkably improved. No phosphorylated products were observed without urea. When the reaction was carried out in the presence of urea but in the absence of the catalysts, phosphorylation was observed but the yields were relatively very low (Table 1). Various phosphorylated derivatives were identified and confirmed by both ³¹P-NMR and MS [7–9,12,18,31].

The molecular weight of GP [C₃H₉O₆P-H] was confirmed at m/z 171 in the negative ion mode of MS. The other MS peaks confirmed were cyclic-glycerol-monophosphate [C₃H₆O₅P-H] at m/z 152 and glycerol-diphosphate [C₃H₁₀O₉P₂-H] at m/z 251.0, respectively. Glycerol-1-phosphate and glycerol-2-phosphate were identified by observing the proton-coupled spectra. The NMR peaks were identified by comparing peak multiplicity

and shift with both GP standards and with expectations of glycerol-P substances. For example, a triplet results from $\text{CH}_2\text{-O-P}$ on the terminal end of glycerol and a doublet results from a CH-O-P interaction on the second carbon of glycerol [12]. Cyclic GP was identified by a multiplet around 19 ppm and glycerol diphosphates were identified by a triplet and a doublet [31]. Chemical shifts can change as a result of pH differences caused by various catalysts used in the reactions.

Figures 1–3 show the ^{31}P -NMR spectra of some of the reactions of glycerol phosphorylation. In the figures, the letters are referenced in Scheme 1. As mentioned above, the glycerol-diphosphates observed were two individual phosphate groups attached to the same glycerol which mainly include: glycerol-1,2-diphosphate and glycerol-1,3-diphosphate. Although, glycerol-1,2,3-triphosphate was also observed, its yield was below 1%.

The other series of reactions studied the decomposition rate of the GP (Figure 5). The decomposition products of GP were phosphate and pyrophosphate. GP degraded into orthophosphate and to pyrophosphate with a half-life of about 7–8 days (Table 2). The unsealed reaction vials heated at 65–70 °C for 1 week showed the highest decomposition rate, i.e., 46% lost after 7 days (set 4) (Table 2). The decomposition follows a first order rate law for the unsealed experiment (Figure 5). The ^{31}P -NMR spectra of the phosphorylation reactions of glycerol in the presence of hematite and obsidian (Figures S1 and S2), the ^{31}P -NMR spectrum of the standard GP (Figure S3) and the decomposition reactions of GP (Figure S4a–h are given in the Supplementary Materials Section (SM)).

Figure 5. The decomposition rate of GP follows a first order rate law with a hydrolysis half-life of 7.8 days ($R^2 = 0.997$).

The phosphate esters of glycerol were successfully synthesized under simplistic conditions and mild heating, conditions that may be considered “prebiotic”. In previous studies, GP have been successfully synthesized but in general either yields were low (when water was used as a solvent) [6,12] or the reactions were carried out in non-aqueous solvents such as deep eutectic solvents or formamide [7–9]. This is the first report of diphosphate derivatives of glycerol. The overall yields of the organophosphates ranged from 5–91%, respectively. The lowest yields were observed only when urea was used as a condensation agent and no catalyst was used. The reactions were also tried at lower temperature windows (i.e., 50 °C) but the yields were low compared to the reactions performed at 70 °C

even though the same catalysts were used. The overall formation of GP seemed to be much more dependent on temperature and prolonged heating rather than catalysts.

Figure 4 shows the relationship between catalysts and GP forms. In our studies, we found that various catalysts provide certain ‘selectivity’ towards the formation of various phosphate esters of glycerol. It was observed that the highest yields of monophosphate (GP) were achieved in the presence of kaolinite and ulexite, and the formation of glycerol diphosphate was mostly favored by olivine and hematite (Figure 4). At present, the reason for this selectivity is unknown. However, all minerals and powdered rocks samples favored the formation of GP, as also shown in the previous work that the minerals (regardless of the type) double the yield of the GP [6] with silicates among the best possible catalysts [7]. One possibility is the preferential physical adsorption or chemisorption of the reactants onto various mineral surfaces.

The present work was a mildly hot aqueous reaction conditions mimicking a hot evaporating pool of water or other ‘evaporitic environments’ as suggested by Cleaves and colleagues [32] in the Hadean. The environment most similar to this work is a mildly hot evaporating pool of water, around 65–70 °C that has dissolved organics, condensing agents, and salts and minerals. Alternatively, a small hot body of water in volcanic olivine, basalt, or obsidian may also be conducive to GP formation. Urea is widely accepted to be of prebiotic relevance as it has been identified in Miller-Urey’s gas discharge tube experiments [33], and it was vital to the condensation reactions reported here.

A warm hot drying pool of water—as also suggested in earlier studies [18]—with dissolved glycerol, an inorganic source of phosphorus (phosphate or reduced phosphorus compounds) [34–36], and minerals could generate cell membrane-forming glycerol phosphates and hence such sites could potentially provide the GP for the emergence of phospholipids for cell membrane formation. The minerals utilized in the present work are also prebiotically relevant [37,38] while oxides of iron, such as hematite and magnetite, are also significant (with hematite being ubiquitous on the surface of Mars [39,40]). Furthermore, serpentinization reactions of olivine- and pyroxene-rich rocks with water produces magnetite [26–29] and would have existed on the Hadean Earth [27,41–44].

The study also shows that phosphorylation is favored at mildly hot temperatures, but prolonged heating can also cause decomposition of the organophosphates [45]. The present study on the rates of decomposition of the GP at mildly high temperatures reveals that GP formation would be balanced by its decomposition. At steady state conditions, the rate of generation of GP (Φ_{GP}) would be balanced by its hydrolytic loss ($k[GP]$):

$$\Phi_{GP} = k[GP]$$

We find a rate of generation of GP to be between 55 and 91% over the course of three days for mineral-catalyzed reactions. This would correspond to 0.05 M/day as a rate of formation. With a decay constant k of 0.089 (Figure 5), the steady state concentration of GP (as $[GP]$) would be about 0.5 M, assuming no limiting reagents. Such a high steady state concentration implicates this pathway as being prebiotically relevant.

The formation of GP in such a circumstance would be balanced by further modification, either to generate phospholipids or to incorporate GP into protometabolic processes. Each of those would alter the steady state GP concentration. This is consistent with the idea suggested by Clark and Kolb [46] who discussed the possibility of various stages from abiotic synthesis to the possible origin of life to have happened in separate ponds or other sites that might be interconnected, and thus various multi-pot processes could potentially be mixed [47–50]. Hence, the GP produced under hot conditions was transferred to the water bodies with lower temperatures fairly quickly [46,51–54] where it could then become incorporated into other prebiotic processes.

3. Materials and Methods

Ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), urea, ammonium chloride (NH_4Cl), sodium carbonate (Na_2CO_3) sodium chloride (NaCl) and glycerol were purchased from

Fischer Scientific (Pittsburgh, PA, USA). Deuterium oxide (D_2O) and standard glycerol phosphate disodium salt hydrate (isomeric mixture) were obtained from Fair Lawn (NJ, USA). All chemicals utilized in the study were used as received. The deionized (DI) water was obtained by using a Barnstead (Dubuque, IA, USA) NANO pure®Diamond Analytical combined reverse osmosis-deionization system [18].

Ulexite ($NaCaB_5O_9 \cdot 8H_2O$) was purchased from eBay and was crushed to fine powder (30–40 μm grain size) before further proceeding. Kaolinite ($(Al_2Si_2O_5(OH)_4)$ 25–35 μm grain size) and white quartz (SiO_2) as sand (200–800 μm grain size) were purchased from MP Biomedicals (Santa Ana, CA, USA). Magnetite (Fe_3O_4) and hematite (Fe_2O_3) (both having a grain size around 100–800 μm) were purchased from Fisher Scientific. Antigorite (a serpentinite mineral, $Mg,Fe^{2+}_3Si_2O_5(OH)$) was collected from Klamath mountain, OR (USA), while forsterite (Mg_2SiO_4) (olivine) sample was obtained from Jackson, NC (USA). Basalt sample was collected from the Hawaiian Islands, HI, (USA) while obsidian was from Newberry crater, OR (USA).

3.1. Phosphorylation of Glycerol

In a typical reaction, 0.15 g (1.3 mmoles) ammonium dihydrogen phosphate $NH_4H_2PO_4$, 0.2 g (3.3 mmoles) urea (used as a condensation agent), 1.2 g (13 mmoles) glycerol were added to a clean glass vial (15 mL capacity) with 5 mL DI water. The mixture was stirred at room temperature until a clear solution was formed. To this solution 0.5 g of rock, mineral, or salt was added. Each catalyst was tried in a separate reaction (e.g., one at a time). The initial pH of the reaction was 8–8.5. The reaction mixture was then heated at 65–70 °C for 3 days. The reaction vials were kept unsealed to allow for the evaporation of water under heating and to mimic a hot drying pool of water containing the reactants as reported previously [18].

3.2. Decomposition Reaction Studies of the Glycerol Phosphates

The decomposition reactions of GP were performed to quantitatively as well as qualitatively observe their decomposed products. To study the decomposition reactions of GP, 0.21 g of standard GP was added to 10 mL of DI water and was analyzed at 1 day (set 2), 3 days (set 3), and 7 days (set 4), 65–70 °C open (or unsealed conditions, exactly like the synthesis of GP mentioned in Section 3.1) and 65–70 °C closed (the reactions vials were tightly sealed) at 1 day (set 6), 3 days (set 7), and 7 days (set 8), respectively. Both unsealed (set 1) and sealed reactions (set 5) were also observed at room temperature for about 7 days.

3.3. ^{31}P -NMR and Mass Spectrometry Analyses of GP Reactions

The ^{31}P -NMR studies were performed on unity INOVA spectrometer. The instrumental details and all the optimized conditions of the analyses have been discussed in our previous studies [7–9,12,18].

The reaction samples as a consequence of continuous heating were dried to completion under room temperature with the exception of unreacted liquid glycerol left in each sample (if any). If the reaction sample had minerals/rock samples such as magnetite, hematite, basalt, obsidian, serpentinite, olivine, it was rehydrated with about 3 mL of 1M NaOH solution to precipitate out iron as water insoluble iron hydroxide. This was done for the better analysis of NMR spectra which would be negatively impacted by the presence of Fe^{2+} ions in solution. The reaction samples were then centrifuged, and the clear supernatant was transferred to a watch glass and let dry at room temperature. The dried samples in the watch glass were rehydrated with D_2O and their ^{31}P -NMR studies were performed. The signal was averaged from 3000 transients.

For the confirmation and matching of the target products (glycerol-1-phosphate and glycerol-2-phosphate), the reaction samples were spiked with the standard GP. Spiking was performed by transferring equal volumes of the reaction solutions in two clean NMR tubes. One NMR tube was labeled as A while the other was labeled as B. The ^{31}P -NMR spectrum of the tube A was recorded. To the NMR tube B, around 0.018 g (solid) standard GP

(isomeric mixture) was slowly added. Tube B was shaken gently until the added standard GP was completely dissolved. The ^{31}P -NMR of the tube B “spiked” with the standard was also recorded. The ^{31}P -NMR spectra of the tubes A and B were compared and studied. The results showed that the peaks of glycerol-1-phosphate and glycerol-2-phosphates exactly matched with that of the peaks in the reaction samples (Figure 3). This step confirmed the formation of the target GP.

Mass spectrometry (MS) analyses (negative ion mode) were performed on a 6130 Single Quadrupole Mass Spectrometer (Agilent, Santa Clara, CA, USA) attached to an Agilent 1200 HPLC by direct injection, and DI water was used as a solvent [18].

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/catal11111384/s1>, Figure S1: ^{31}P -NMR figure of GP formation in the presence of hematite, Figure S2: ^{31}P -NMR of GP formation in the presence of obsidian, Figure S3: ^{31}P -NMR of the standard glycerol phosphate, Figure S4a–h: ^{31}P -NMR of the decomposition reactions of the GP

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