chemistry proceedings

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Metal–Peptide Complexes—A Novel Class of Molecular Receptors for Electrochemical Phosphate Sensing †

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† Presented at the 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, 1–15 July 2021; Available online: https://csac2021.sciforum.net/.

Abstract: Amyloid-β (Aβ) peptides are crucial in the pathology of Alzheimer's disease. On the other hand, their metal complexes possess distinctive coordination properties that could be of great importance in the selective recognition of (bio)analytes, such as anions. Here, we report a novel group of molecular receptors for phosphate anions recognition: metal–peptide complexes of Aβ peptides, which combine features of synthetic inorganic ligands and naturally occurring binding proteins. The influence of the change in the metal ion center on the coordination and redox properties of binary Cu(II)/Ni(II)-Aβ complexes, as well as the affinity of these complexes towards phosphate species, were analyzed. This approach offers the possibility of fine-tuning the receptor affinity for desired applications.

Keywords: metal–peptide complexes; voltammetry; molecular receptors

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Citation: Tobolska, A.; Wezynfeld, N.E.; Wawrzyniak, U.E.; Bal, W.; Wróblewski, W. Metal–Peptide Complexes—A Novel Class of Molecular Receptors for Electrochemical Phosphate Sensing. *Chem. Proc.* **2021**, *5*, 39. https://doi.org/10.3390/CSAC2021-10449

Academic Editor: Nicole Jaffrezic-Renault

Published: 30 June 2021

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1. Introduction

The determination of phosphate anions in body fluids provides information about various disorders such as hyperparathyroidism or vitamin D deficiency [1]. Therefore, the monitoring of phosphate levels is of interest for human health. Chemical sensors are an ideal alternative to classic analytical methods, but their construction requires the synthesis of appropriate receptors, selectively binding to the analyte.

Amyloid β peptides (Aβ) related to Alzheimer's disease are well known for their neurotoxic properties [2]. However, metal complexes, with their N-terminally truncated analogs, have unique coordination properties that could be employed in the design of potential receptors for biorelevant anionic species [3,4]. The $A\beta_{5-9}$ (Arg-His-Asp-Ser-Gly-NH_2) peptide possesses a His-2 binding motif, and thus forms stable complexes with transition metal ions such as Cu(II) or Ni(II). At pH 7.4, the Cu(II) or Ni(II) ion is bound by three nitrogen atoms (3N) from the His residue, the N-terminal amine, and the peptide backbone amide [5]. The resulting chelates also exhibit a labile coordination site, enabling ternary interactions. Hence, metal–peptide complexes offer the possibility of fine-tuning their affinity for desired applications by altering the amino acid sequence and the metal ion center.

The present work explores and compares the coordination and redox properties of Cu(II) and Ni(II) complexes of the $A\beta_{5-9}$ peptide, followed by their ability to interact with biologically relevant phosphate anions and nucleotides.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals were purchased from Merck and Sigma Aldrich. All solutions were prepared daily with deionized water (18 MΩ·cm). In order to avoid Cu(II)/Ni(II) contamination, the glassware was raised with 6 M HNO_3 followed by deionized water. AMP and ATP stock solutions were adjusted to pH 7.0–7.4 and kept on ice during measurements to prevent nucleotides hydrolysis.

2.2. Peptide Synthesis

Synthesis of the A$\beta_{5\text{-}9}$ peptide was performed according to the Fmoc/tBu strategy [6] on a Prelude™ peptide synthesizer (Protein Technologies, Inc., Tucson, AZ, USA). The crude was purified by HPLC with the UV detection (Waters, Milford, MA, USA) at 220 nm. The purity of the peptide was verified by ESI-MS (Waters, Milford, MA, USA).

2.3. Voltammetry

Electrochemical measurements (CV, DPV) were performed using the CHI 1030 potentiostat (CH Instrument, Austin, TX, USA) in a three-electrode arrangement with a GCE (BASi, 3 mm diameter) as a working electrode, an Ag/AgCl electrode (Mineral, Warsaw, Poland) as a reference, and a platinum wire as an auxiliary electrode. The GCE was sequentially polished with the alumina powder (1.0 μm and 0.3 μm) on a Buehler polishing cloth. Then, the working electrode was sonicated for 1 min and rinsed thoroughly with deionized water. All voltametric experiments were carried out in 100 mM KNO_3 at pH 7.4 under argon. The pH was adjusted with small aliquots of concentrated KOH or HNO_3 solutions. The peptide-to-metal(II) ratio was 1.0:0.9.

3. Results and Discussion

The electrochemical response for the A$\beta_{5\text{-}9}$ metal complexes recorded at pH 7.4 is depicted in Figure 1. The binary Cu(II)-A$\beta_{5\text{-}9}$ complex enabled both the reduction (Figure 1A, blue line) and oxidation of Cu(II) ions (Figure 1B, blue line). The exchange of the metal center complex to Ni(II) caused significant differences in the redox behavior, with a decrease in metal center oxidation by 188 mV compared to Cu(II) complexes (Figure 1B, green line). We did not observe any signals associated with Ni(II) reduction (Figure 1A, green line).

Distinct electrochemical properties are likely caused by the differences in geometry and the stabilities of the A$\beta_{5\text{-}9}$ complexes. Under studied conditions, Cu(II) complex is square-planar, while Ni(II) complexes are mostly octahedral. Additionally, the conditional stability constant of the Cu(II)-A$\beta_{5\text{-}9}$ (5.8×10^{12} M^{-1}) is about five orders magnitude higher than for Ni(II)-A$\beta_{5\text{-}9}$ (1.7×10^{6} M^{-1}) [3,5].

Considering the application of the metal complexes of A$\beta_{5\text{-}9}$ as a recognition element, we studied their response to selected anionic species. Observed changes in the oxidation potentials of the metal center upon the addition of 10 mM phosphates were similar (~150–160 mV) for Cu(II) and Ni(II) complexes of A$\beta_{5\text{-}9}$. Comparable sensitivity of chelates is probably related to a similar Lewis acidity of the metal centers. Furthermore, both complexes exhibit a good selectivity towards phosphates in the presence of chlorides and sulfates (Table 1). Aside from phosphates, only acetates, among other tested analytes, interacted with the metal–peptide complexes, causing changes in redox activity. Nevertheless, the voltametric signals for acetates occurred at different potentials than for phosphates.

Since the intercellular level of organic phosphates can be 20 times higher than inorganic phosphates, we decided to investigate the affinities of the studied metal–peptide complexes for selected nucleotides (AMP, ATP). Similar to phosphate anions, the presence of nucleotides shifted the oxidation peak to less positive values. However, in contrast to Cu(II)-A$\beta_{5\text{-}9}$, the signal of the metal center oxidation for Ni(II)-A$\beta_{5\text{-}9}$ occurs at different potentials for mono- and triphosphates (see Table 1). We suggest that this is due to the ability of the octahedral nickel complex to interact with more than one phosphate group of ATP as a result of the chelate effect.

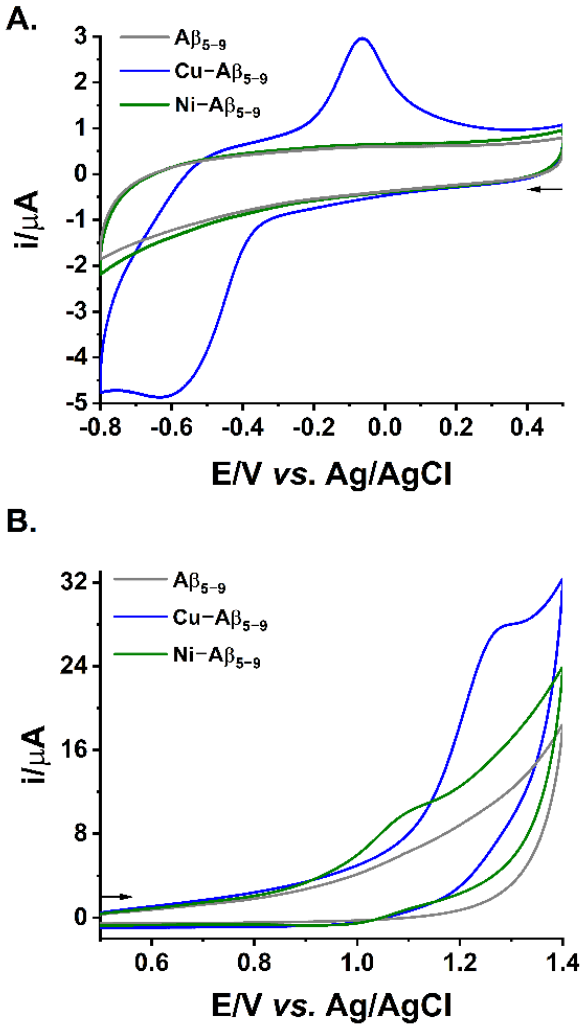


Figure 1. CV curves registered for 0.5 mM $A\beta_{5-9}$ in absence (grey line) and in the presence of 0.45 mM Cu(II) (blue line), or 0.45 mM Ni(II) (green line) scanned towards negative (**A**) and positive (**B**) potentials and recorded in 100 mM KNO_3 at pH 7.4, scan rate v = 0.1 V/s. Analyzed based on data published in the literature [3–5].

Table 1. Comparison of the affinity towards selected anions and nucleotides of the Cu(II)-$A\beta_{5-9}$ and Ni(II)-$A\beta_{5-9}$ complexes. $\Delta E_{M(II)/M(III)}$ is the difference of the potential values of Cu(II) or Ni(II) oxidation of the respective ternary system and the binary complex. Calculated based on results published previously [3,4].

Anion	$\Delta E_{Cu(II)/Cu(III)}$ (mV)	$\Delta E_{Ni(II)/Ni(III)}$ (mV)
Cl^-	−2	−8
SO_4^{2-}	−8	−21
CH_3COO^-	−84	−68
$H_2PO_4^-/HPO_4^{2-}$	−150	−156
AMP	−136	−126
ATP	−152	−230

4. Conclusions

Metal–peptide complexes of peptides possessing the His-2 motif ensure there are labile coordination sites, enabling ternary interactions with phosphate anions and nucleotides. Such interactions lead to a strong electrochemical response, which could be valuable for designing a promising class of peptide-based molecular receptors with poten-

tial applications as recognition elements in electrochemical biosensors and in vitro clinical diagnostics.Our research proved that the change in the metallic center of the $A\beta_{5\text{-}9}$ complex significantly influences its coordination properties and redox activity. Nevertheless, altering the metal center from Cu(II) to Ni(II) does not change the sensitivity of the complex toward phosphate anions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/CSAC2021-10449/s1.

Author Contributions: Conceptualization, W.B., N.E.W. and U.E.W.; methodology, W.W., U.E.W. and N.E.W.; formal analysis, A.T., U.E.W. and N.E.W.; investigation, A.T.; resources, W.W. and W.B.; data curation, A.T., U.E.W. and N.E.W.; writing—original draft preparation, A.T.; writing—review and editing, A.T., N.E.W. and W.W.; visualization, A.T.; supervision, N.E.W., W.W. and W.B.; project administration, N.E.W. and A.T.; funding acquisition, N.E.W. and A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Warsaw University of Technology under the program Excellence Initiative, Research University (ID-UB), BIOTECHMED-1 project no. PSP 504/04496/1020/45.010407 (N.E.W.) and implemented as a part of the Operational Program Knowledge Education Development 2014–2020 (Project No POWR.03.02.00-00-I007/16-00) co-financed by the European Social Fund (A.T.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We gratefully acknowledge the assistance of Dawid Płonka (Institute of Biochemistry and Biophysics, Polish Academy of Sciences) in peptide synthesis.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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