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Biological phosphate ester sensing using an artificial enzyme PMP complex

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Abstract

Biological phosphate ester (phosphoric ester) substances, e.g. ATP, ADP, AMP, pyrophosphate and deoxyribonucleic acid, play important roles as either energy-carrying substances or substances carrying biological information. The author has designed and synthesized an artificial enzyme PMP complex (Cu) which dephosphorylates phosphate ester substances. The synthesized PMP complex (Cu) showed a prominent dephosphorylating effect on biological phosphate ester substances, e.g. ATP, ADP, AMP and pyrophosphate. A PMP complex (Cu)-coated electrode was employed as a sensor device. A current response could be obtained as a reaction to the application of phosphate ester substances on the sensor when a constant potential of −250 mV versus Ag/AgCl was applied. In the case of ATP, the sensor could determine the ATP concentration within the range between 1 nM and 20 mM, and could also determine the presence of other biological phosphate ester substances.

Keywords: Sensor; Artificial enzyme; Biological phosphate ester; Dephosphorylation; PO_4^{3-}; Electrochemical detection; Polyion complex

1. Introduction

Biological phosphate ester substances, e.g. ATP, ADP, AMP, pyrophosphate and deoxyribonucleic acid, play important roles as either energy-carrying substances or substances carrying biological information. The concentration profiles of energy-carrying substances determine the viability of the living organism. They are also employed as stress markers, because cells leak ATP in response to extracellular stress [1]. The electrochemical detection of deoxyribonucleic acid will probably be important, because high-performance DNA sequencers with inbuilt electrochemical detectors may be constructed in the future, without the cost and bulk of current laser/PMT detectors.

However, electrochemical phosphate detection has not been developed yet [2,3]. In the case of neutral aqueous solutions, orthophosphates reach a stable $H_2PO_4^- \leftrightarrow HPO_4^{2-}$ equilibrium in the presence ratio of 49.9973:49.9973, according to the Henderson–Hasselbalch equation. Neither orthophosphate shows electrochemical activity on a bare electrode. It means that over 99.9946% of the orthophosphate is undetectable in neutral aqueous solutions with electrochemical techniques and other in situ analysis methods. However, the author has found that the product of dephosphorylation, i.e. PO_4^{3-}, can be reduced electrochemically. But the PO_4^{3-} form of orthophosphate is protonated immediately in neutral aqueous solutions, and reaches a stable $H_2PO_4^- \leftrightarrow HPO_4^{2-}$ equilibrium.

In the present study, the author has found that the dephosphorylation products of phosphate esters can be detected amperometrically. A polymer–metal–(functional) polymer complex (PMP complex) was used in this detection procedure. It is an artificial enzyme designed and synthesized by the author. Using this artificial enzyme, the dephosphorylation of phosphate esters could be achieved on the surface of the electrode.

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In previous studies on artificial enzymes, the chief aim was to mimic natural enzymes through molecular design [4–6]. The dephosphorylation of phosphate esters was successfully catalyzed through the hydrolysis reaction by metal complexes [7–9]. However, in this study, the author has designed a peculiar artificial enzyme (a PMP complex) as a molecular transducer for biosensing.

In this paper, the designed and synthesized PMP complex (Cu) is evaluated as both an artificial enzyme and a molecular transducer for phosphate–ester biosensing.

2. Materials and methods

2.1. Chemicals

ATP, ADP, AMP, pyrophosphate and copper(II) chloride dihydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, JAPAN). Poly-L-histidine hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Poly (sodium-4-styrene sulfonate) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

2.2. Molecular design of the PMP complex

The PMP complex was designed as an artificial enzyme to be used as a molecular transducer [10]. In the dephosphorylation of phosphate esters, it is known that the copper complex shows a prominent hydrolysis activity [11–13]. The author designed the overall structure of the densely packed nano cavities of the Cu complex within the PMP complex. As shown in Fig. 1, the PMP complex (Cu) consists of polyhistidine (coordinative polymer), polystyrene sulfonate (functional polymer) and Cu^{2+}. The coordinative polymer forms a shrunken structure through coordination with Cu^{2+}. At the same time, multiple Cu^{2+}-coordinated nano cavities are formed in the matrix, and the functional polymer binds to the coordinative polymer to form a polyion:polyion complex with the coordinative polymer. The functional polymer stabilizes the structure and provides functional residues to the inside of the nano cavities [10]. In the present case, styrene residues provide hydrophobic and electrophilic conditions within the nano cavities. The coordinative polymer–metal–(functional) polymer complex is very storage stable and can be spread on a solid substrate to form a thin membrane through a simple spinning and dry-coating process.

2.3. Synthesis of the PMP complex (Cu)

For the present molecular design of the PMP complex (Cu), poly-L-histidine hydrochloride (MW 39,200) was dissolved into ultrapure water and desalted by equilibrium dialysis (Slide-A-Lyzer 3.5 kD, PIERCE, Rockford, IL, USA). Copper(II) ions were added to the solution by small to small, in order to maintain neutral pH, and mixed. Then, poly (sodium-4-styrene sulfonate) (MW 70,000) was added into the coordinative mixture solution, and was allowed to form polyion complexes with the shrunken polyhistidine–Cu^{2+} complexes. The synthesized PMP complex (Cu) was collected by centrifugation and was repeatedly washed with ultrapure water in order to remove free copper(II) ions.

2.4. Preparation of the electrode coated with the PMP complex (sensor device)

For the disk-type sensor device, the synthesized PMP complex (Cu) was poured (8 μl) and dried on a glassy carbon electrode surface (3 mm in diameter, BAS, Tokyo, Japan). The glassy carbon-disk electrode coated with the PMP complex (Cu) was used as the disk-type electrode. A mesh-type sensor device was fabricated out of platinum mesh (150 mesh, 10 mm × 10 mm, Nilaco, Tokyo, Japan). The PMP complex (Cu) was poured onto the platinum mesh and dried. Then, the mesh coated with the PMP complex (Cu) was used as the mesh-type sensor device. The sensor electrode was immersed in a buffer solution (0.1 M HEPES, pH 7.4) until use. This way, it can be stored for over a month.

2.5. Electrochemical evaluation

Electrochemical evaluation of the phosphate esters was performed by the three electrode systems using an electrochemical analyzer (HZV-100, HOKUTODENKO, Tokyo, JAPAN). The buffer solution used for the electrochemical evaluation was 0.1 M HEPES (pH 7.4, containing 0.1 M KCl). Biological nucleic substances (ATP, ADP and pyrophosphate) were measured by the electrode coated with the PMP

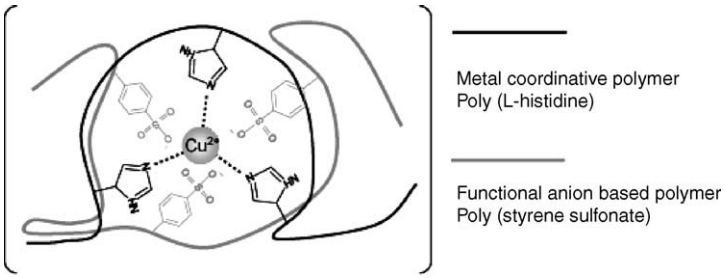

Fig. 1. Hypothetical molecular structure of the PMP complex (Cu).