Biological nucleic substances sensor using a PMP complex (Cu)

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In living system, cells respond to chemical and physical stimulus, and release various signals, *i.e*. production of specific substances and proteins, throughout their life cycle within specific tissue and organs [1]. In the cell, biological phosphoester is essential compound, *e.g.*, ATP and phosphoenolpyruvate for biological energy donator, and biological nucleic substances for genetic information. Therefore, if the properly sensing of phosphoester can be employed as a new cellular biosensor to evaluate cellular status, it will give molecular information to proof biological safety, drug efficacy profiling *in vitro*. However, *in situ* assay of biological phosphoester has not been well developed [2, 3].

In this study, we present an artificial enzyme and its application for biological nucleic substances sensor. We have designed and synthesized a peculiar catalytic molecule polymer-metal-polymer complex (PMP complex), which possesses both specific catalytic function and molecular-transduceability [4]. A basic design of the PMP complex (Cu) was synthesized with metal coordinative polymer, copper ion, and counter polymer(s) with functional residues as illustrated in Fig. 1 [4]. The synthesized PMP (Cu) was formed a thin membrane to drop and dry on a glassy carbon electrode surface, which can be used sensor device. Electrochemical evaluation was performed in a three electrode system.

Dephosphorylation activity of phosphoester of PMP(Cu) was evaluated by HPLC analysis. Due to HPLC analysis, phosphoester was dephosphorized successfully. The PMP(Cu) is coated on a GC disc electrode surface and is characterized electrochemically. Fig.2 shows Cyclic Voltammetry measurement characterized the stability of the PMP (Cu). Addition pyrophosphate (PPi), the oxidation and reduction current peaks were observed clearly. This redox peak was attributed to phosphate anion that was hydrolyzed PPi by PMP (Cu). Biological nucleic substances (ATP, ADP, AMP and PPi) were measured by the PMP (Cu) coated electrode with a potential application at –250 mV vs. Ag/AgCl. Fig. 3 shows the dependence of current value as a concentration of ATP in 0.1M HEPES buffer solution (pH 7.4, containing 0.1 M KCl). The increment current response was depended on ATP concentration. ATP concentration from 1.0 μM to 20 mM could be evaluated with the present sensor device. In the case of ADP, AMP and PPi, there ware also measured by response current in proportion to phosphate number, respectively.

The designed PMP (Cu) can catalyze diphosphoriration of nucleic substance. The produced free phosphate anion will be reduced electrochemically on electrode. The PMP (Cu) is successfully employed as an artificial enzyme to fabricate the sensor device. In conclusion, PMP (Cu) is novel artificial enzyme and is a molecular transducer on biosensor. PMP (Cu) can be applied to accurate sensing of nucleic substances that may promise cellular signals biosensing, *e.g.* pyroseqenceing sensor device.

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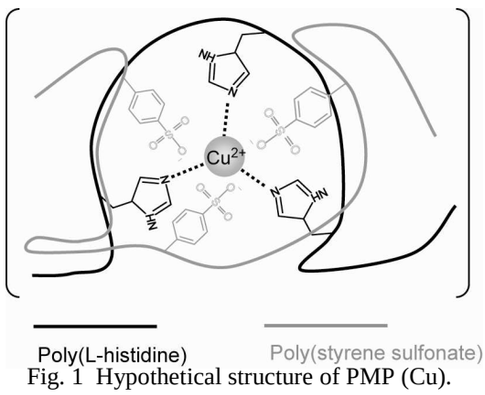

Fig. 1 Hypothetical structure of PMP (Cu).

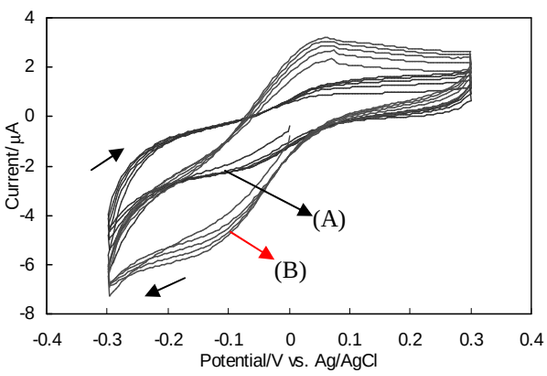

Fig.2 Cyclic voltammograms of PMP (Cu) coated electrode in (A) 0.1M HEPES buffer (pH 7.4, containing 0.1M KCl) (B) 10mM PPi. The number of cycles is 5.

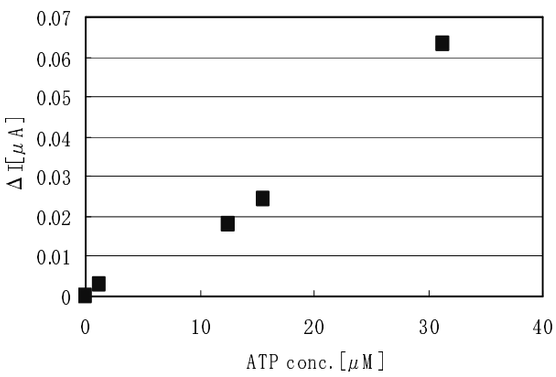

Fig. 3 Response current of PMP(Cu) coated electrode on ATP application. -250 mV vs. Ag/AgCl constant potential is applied.