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Feasibility Study of Introducing Redox Property by Modification of PMBN Polymer for Biofuel Cell Applications

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Abstract In this study, the feasibility of introducing redox property to an amphiphilic phospholipid polymer (PMBN) was investigated. The active ester group in the side chain of the polymer was used to react with pyrroloquinoline quinine (PQQ). Redox peaks that corresponded to PQQ redox potentials were observed after the modification. Glucose oxidase was immobilized to the modified polymer. Electrochemical oxidation of glucose was carried out with the polymer electrode. The oxidation current increased with elevating glucose concentration indicating electron transfer established between the electrode and enzyme. It suggests that by modification, PMBN is possible to use for enzyme electrode for bioelectronics.

Keywords PMBN polymer · PQQ · Redox property · Enzyme immobilization · Glucose oxidation · Enzymatic biofuel cells

Introduction

The growing aging population demands new healthcare systems. Developments in medical science have led to an increasing number of implantable devices for monitoring and diagnostic purposes. Enzyme-based bioelectronics could be used as in vivo biosensors for continuous monitoring, such as glucose sensors for continuous monitoring the blood sugar level for diabetes patients [1–3], as well as the power source, enzymatic biofuel cells, for implantable devices [4–8]. Two major challenges hinder the development of enzymatic

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bioelectronics. One is the efficient electron transfer between enzymes and the electrode surface, and the other is the long-term stability of enzymes.

For these applications, immobilization of enzymes at the electrode surface and rapid electron transfer between enzymes and electrodes are the most important factors. Redox polymers, which entrap enzymes in the matrices and act as mediator to shuttle electrons in the enzyme reactions, are widely used. Ferrocene derivatives and Os compounds are the most studied polymer mediators [9–14].

Os-based polymers showed high performance and attracted attention in the development of biosensors and biofuel cells [15, 16]. Os polymers have a wide potential window that enable them to be the mediators for both oxidation and reduction reactions; also, they have high electron transfer rate resulting in efficient electron transfer between enzymes and electrode surfaces [17]. However, the toxicity of Os compounds could limit their use for long-term in vivo system due to leaching out of Os derivatives. Although the toxicity of ferrocene derivatives is low and there is no indication of carcinogenic effect, the positive redox potential (ca. 0.3 V vs Ag/AgCl) of ferrocene is not favorable for biofuel cell applications. Also, for implantable applications, there are issues of biocompatibility and blood clotting on these materials.

A novel phospholipid polymer, (poly [2-methacryloyloxyethyl phosphorylcholine (MPC)–*co*-*n*-butyl methacrylate (BMA)-*co*-*p*- nitrophenyloxycarbonyl poly(ethylene glycol) methacrylate (MEONP)]) (PMBN) was originally developed for conjugation with biomolecules [18]. The chemical structure of PMBN is shown in Fig. 1. The phospholipid polymer with MPC unit has good biocompatibility and inhibits the adhesion and activation of blood cells, thus minimizing blood coagulation when it contacts blood [18, 19]. The molecular structure of the polymer is easily designed by changing monomer units and composition. PMBN consists of three segments of polymer chains. Each provides different functions to the polymer. MPC is the hydrophilic unit imitating biomembrane with excellent blood compatibility [20]. BMA is the hydrophobic unit, and the MEONP unit has an active ester group in the side chain. The MEONP unit can react with other functional groups or a specific biomolecule [21, 22]. This provides the possibility of functionalizing the polymer for specific applications. It has been used for developing highly sensitive microdiagnostic devices on recognition of antibody [23]. Another important property of PMBN polymer is its ability of maintaining enzyme activity [23, 24]. These properties are crucial for implantable devices.

Pyrroloquinoline quinine (PQQ) is an essential redox cofactor for dehydogenase. It has been used for NAD(P)H oxidation [25], as well as used as a mediator for glucose oxidase reconstituted on the PQQ-FAD monolayer for the development of biofuel cells [26]. The chemical structure of PQQ is shown in Fig. 2. Good electrochemical properties and electron

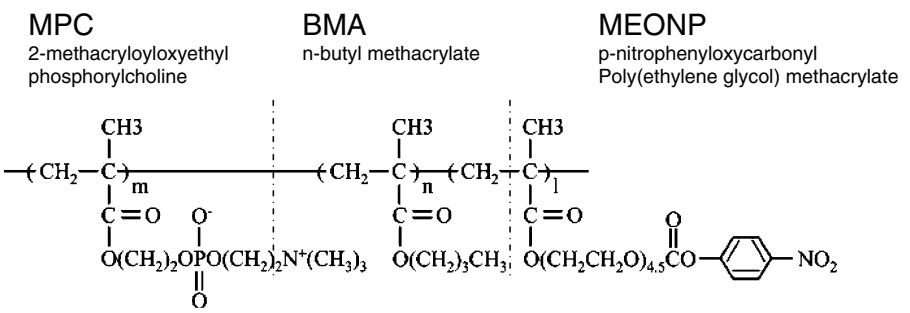


Fig. 1 Chemical structure of poly[MPC-*co*-BMA-MEONP]

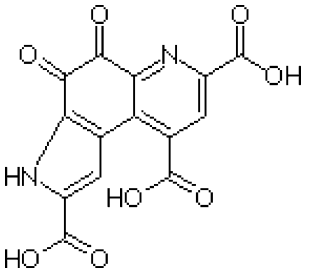
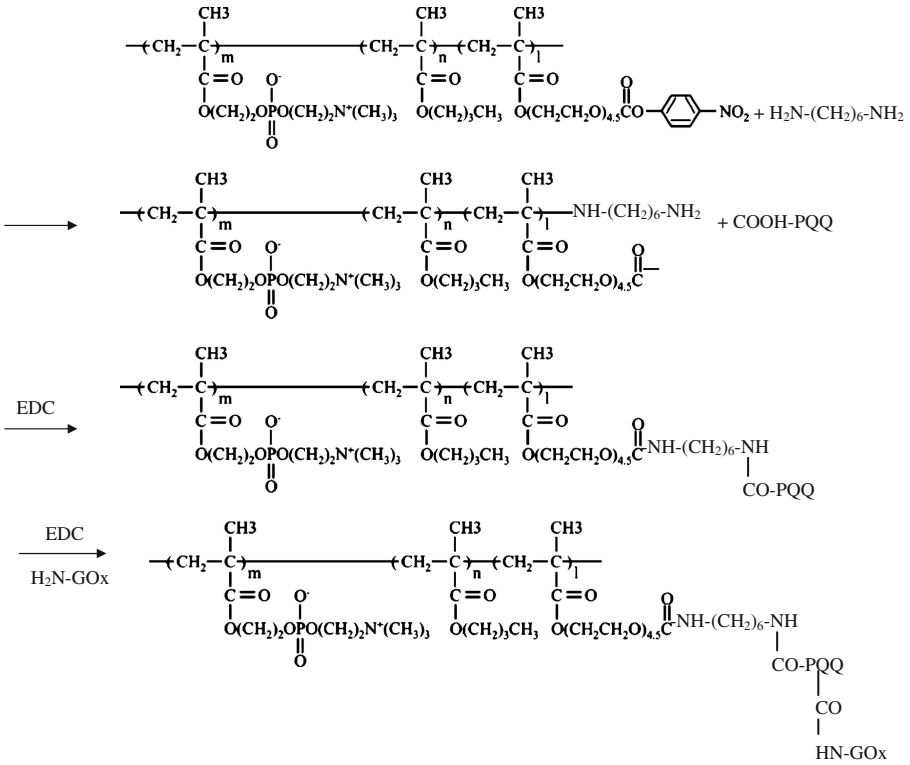


Fig. 2 Chemical structure of PQQ ($C_{14}H_6N_2O_8$)

transfer rate were observed from these studies. PQQ is considered as a new redox-cofactor vitamin for mammals [27]. It also has a relative negative redox potential (ca. −0.10 V vs Ag/AgCl), which is desirable for biofuel cell applications.

In this study, we focused on introducing redox property to PMBN by conjugating mediator molecules, PQQ, to the side chain via the active ester group. Enzymes were immobilized on PMBN polymer by covalent binding to the mediator. Redox property of the modified polymer and the activity of the enzyme electrode for glucose oxidation were examined by electrochemical methods.



Scheme 1 PMBN modification with PQQ and GOx

Experimental

Materials

PMBN polymer was synthesized following the procedures reported previously [18]. PQQ was from Sigma-Aldrich. Glucose oxidase (GOx) from *Aspergillus niger* was purchased from Fluka, and a solution containing 3,000 U/ml GOx was prepared using 0.1 M pH 7.0 phosphate buffer solution. *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Fluka) was used as the cross-linking agent; hexamethylenediamine (HMDA) was employed as a spacer and to react with the active ester on the PMBN polymer in order to carry out further modification.

Electrochemical Study

Electrochemical measurements were performed using a Hokuto Denko HA 150G potentiostat and a conventional three-electrode cell. A glassy carbon electrode with the

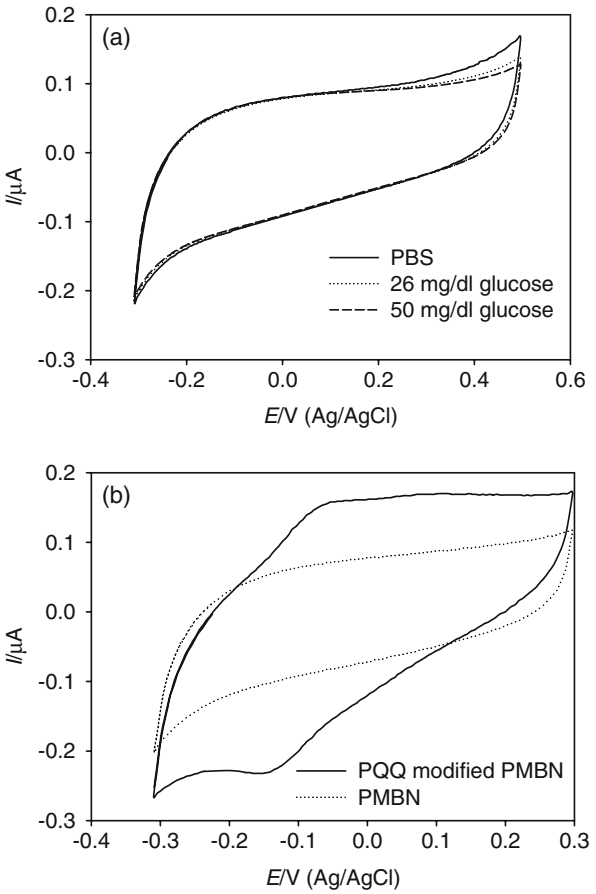


Fig. 3 Cyclic voltammograms of PMBN polymer modified electrode in PBS solutions (**a**) with different concentration of glucose (**b**) plain and PQQ modified PMBN, pH 7.0, scan rate 50 mV/s, A=1 mm^2

surface area of 1 mm^2 was the working electrode, and a platinum coil was the counter electrode. The reference electrode was Ag/AgCl (0.208 V vs NHE), and all electrode potentials given here are with reference to the Ag/AgCl electrode unless stated otherwise. Cyclic voltammetry and linear sweep voltammetry were used to examine the activity of the polymer enzyme electrode; 0.1 M, pH 7.0 phosphate buffer was used as the electrolyte.

Polymer Modification Scheme

The scheme of modifying PMBN polymer is demonstrated in Scheme 1. The process involved several steps. First, 2 μl of 1 wt.% PMBN ethanol solution was pipetted on top of the glassy carbon electrode and dried in air; 0.4 μl of 1 wt.% HMDA aqueous solution was added on the PMBN layer and left to dry in air. The electrode was washed to remove nitrobenzol from amine group that reacted with the active ester group on the MEONP unit of PMBN polymer; 2 μl of 3 mM PQQ and 10 mM EDC solution was pipetted on the polymer modified electrode and dried in air. The electrode was then washed thoroughly to

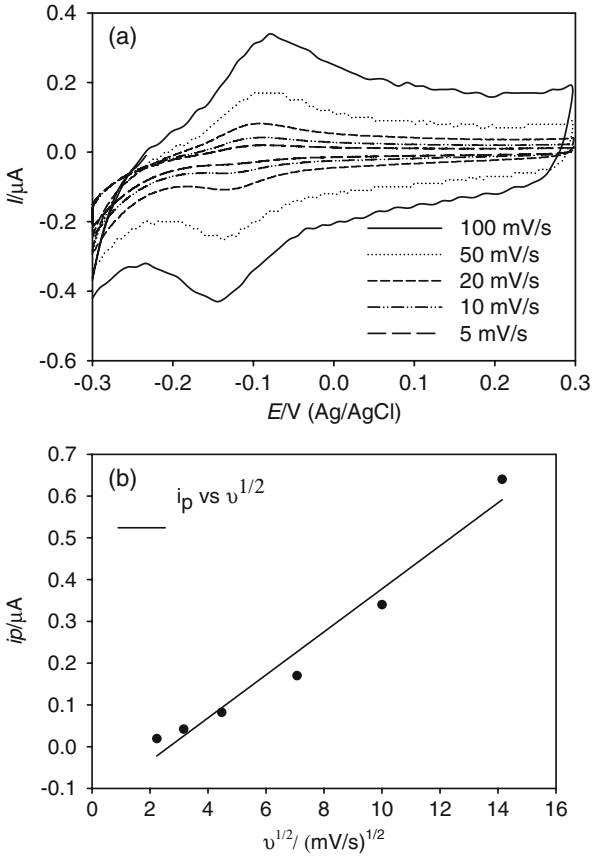


Fig. 4 **a** Cyclic voltammograms of PQQ modified PMBN polymer electrode in PBS solutions, pH 7.0 with various scan rates, A=1 mm^2. **b** Peak current vs square root of scan rate $\nu^{1/2}$

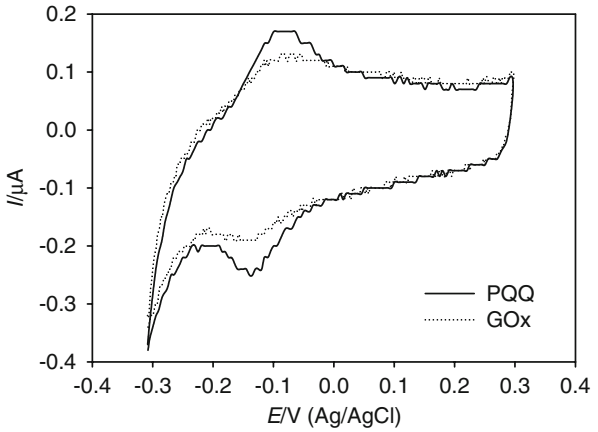


Fig. 5 Cyclic voltammograms of PQQ and GOx modified PMBN polymer in PBS, pH 7.0, scan rate 50 mV/s, A=1 mm^2

remove unattached PQQ before modified by 2 μl 0.1 M phosphate buffer solution containing 6 units of GOx. The GOx modified electrode was dried in air and stored at 4 °C before being tested.

Results and Discussion

Electrochemical Characterization of Modified PMBN Polymer

Figure 3a shows the cyclic voltammograms of PMBN modified glassy carbon in 0.1 M phosphate buffer (pH 7.0) and buffer with glucose. It is clear that apart from double-layer charges, there were no distinct reaction peaks observed, indicating that PMBN polymer by itself does not have the activity toward glucose oxidation, as well as redox property. After

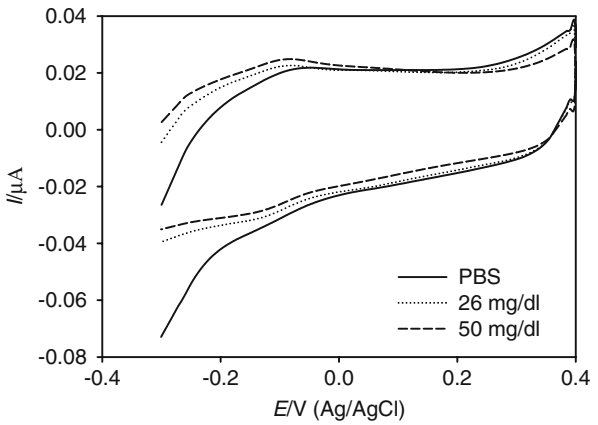


Fig. 6 Cyclic voltammogramms of GOx modified PMBN polymer in PBS and glucose solutions, pH 7.0, scan rate 5 mV/s, A=1 mm^2

being modified by PQQ, the CV compared to bare PMBN polymer in phosphate buffer in Fig. 3b showed a distinct redox peak at the potential around −0.09 V, which is in the range of PQQ/$PQQH_2$ redox potential. This suggested that redox property was introduced to PMBN polymer by PQQ modification.

Cyclic voltammograms, with various scan rates, for PQQ modified PMBN polymer electrode in phosphate buffer are shown in Fig. 4a. It can be observed that the redox peak potential E_p was independent of scan rates, with the oxidation peak around −0.09 V and the potential difference between oxidation and reduction peaks from 0.051 to 0.056 V. These features indicate a reversible reaction from PQQ redox process. Also, the peak oxidation current i_p was proportional to the square root of scan rate $\nu^{1/2}$ (Fig. 4b), suggesting a diffusion-controlled PQQ/$PQQH_2$ redox process.

Immobilization of Enzyme and Glucose Oxidation

After immobilization of glucose oxidase on PQQ modified PMBN polymer, the PQQ redox peaks were suppressed, which is demonstrated in Fig. 5. This is due to the presence of GOx partially blocked electrode surface and PQQ site. However, the redox potential was still the same, at around −0.09 V even after being immobilized with GOx. Figure 6 showed the cyclic voltammograms for PQQ–GOx modified PMBN electrode in phosphate buffer solution with various glucose concentrations. There was a small increase of peak oxidation current from blank phosphate buffer solution of up to 50 mg/dl glucose. Although the difference between peak currents was small, the oxidation current difference was greater for more negative potentials. This behavior indicated that GOx enzyme immobilized on PMBN was active toward glucose oxidation, and with the PQQ as the mediator, electron communication between GOx and the electrode was also obtained. The oxidation currents at −0.2 V with phosphate buffer and 26 and 50 mg/dl glucose solutions are 0.0069, 0.0146, and 0.0171 μA, respectively. However, there seemed to have a limitation of 50 mg/dl for glucose concentration on this electrode, probably due to the low loading of enzyme immobilized on the polymer electrode. It is possible to increase the enzyme loading by increasing the composition of MEONP segment. Further optimization of the polymer content for enzyme immobilization will be studied.

Conclusions

In this study, an enzyme electrode was fabricated by immobilizing glucose oxidase on a novel biocompatible phospholipid polymer PMBN. Redox property was introduced by modifying the polymer with pyrroloquinoline quinine through the active ester group on the side chain of MEONP segment. PQQ was used as the electron transfer redox mediator for glucose oxidase. The GOx immobilized electrode showed activity toward glucose oxidation, \and electron transfer between the enzyme and the electrode was also obtained through the mediator, PQQ. It indicates the feasibility of applying PMBN polymer for implantable bioelectronics applications. More research on the optimization of the polymer content and functional group are necessary for further developments.

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