Direct Electrical Communication between the Redox Center of Glucose Oxidase and Electrode *via* Conductive Polypyrrole Matrix

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A glucose oxidase (GOD) modified polypyrrole electrode was prepared by coelectropolymerization with unmodified pyrrole. Direct electrical communication between the redox center of GOD and the metal electrode via the conductive polypyrrole matrix was demonstrated using the electrode.

A covalently immobilized GOD electrode was obtained by coelectropolymerization of the GOD-modified pyrrole with unmodified pyrrole, and it showed a higher activity and stability compared with the entrapped system.[1] Indirect glucose detection is achieved by the electrochemical oxidation of H_2O_2 on the electrode in most of the glucose sensors containing the GOD-modified polymer system. In such indirect detection of glucose, the oxidative current is affected by the O_2 concentration in the system. If polypyrrole (PPy) serves as the electron mediator for the GOD oxidation, amperometric glucose detection can be realized in the absence of O_2. Furthermore, after charge separation from the redox center of GOD, smooth charge migration to the metal electrode takes place in the conductive PPy matrix. However, for these PPy immobilized GOD electrodes except for a special case with a microporous membrane,[2] no distinct current response due to direct electrical communication between the redox center of GOD and the metal electrode with PPy as the electron mediator and the conducting matrix has been reported. It is assumed that the PPy rigid chains are not in contact with the redox center in the protein shell of GOD. Consequently, GOD is necessary for immobilization with a flexible spacer on the PPy chains. Although a GOD-modified PPy with a carboxymethyl spacer has already been applied to indirect glucose detection,[3] its flexible spacer is expected to induce electron transfer from the redox center of GOD to PPy.

In this study, direct glucose detection through the use of a GOD-modified PPy with a carboxymethyl spacer was demonstrated and discussed.

A GOD-modified pyrrole was obtained by coupling the surface lysyl residues of GOD (EC 1.1.3.4) covalently to 3-carboxymethylpyrrole. This synthesis was carried out by the procedure reported previously.[3] An enzyme film electrode was prepared by potentiostatic electro-oxidation at +1.1 V (vs. Ag-AgCl) on the Pt electrode (surface area: 0.785 cm^{-2}) from a polymerization solution containing the GOD-modified pyrrole (30 μM for GOD unit) and unmodified pyrrole (0.1 M). All electrochemical polymerizations and measurements were carried out in 0.1 M sodium phosphate buffer solution (pH 7.0) containing 0.1 M sodium chloride at 298 K.

The reaction product and unreacted material were separated by gel filtration. The pyrrole content and the protein content eluted from the reaction mixture were determined spectrophotometrically by a coupling procedure with p-(dimethylamino)benzaldehyde and Pierce Coomasie Protein Assay Reagent, respectively.[4] The results are outlined in Table 1. The fraction could be attributed to the GOD-modified pyrrole which reacted with both coupling reagents. The number of pendant pyrrole moieties per molecule of GOD was 27 or 28. The enzyme activity of the GOD-modified pyrrole was about 86 % for native GOD.

Table 1. GOD and pyrrole concentration of the enzyme modified pyrrole

Volume eluted(ml)	GOD conc.(μ M)	Pyrrole conc.(mM)
27～30	60.1	1.64
30～33	60.2	1.71

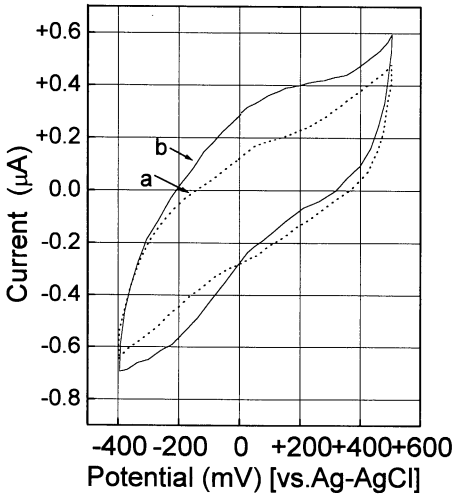

Figure 1. Cyclic voltammograms (10 mV/s) of the GOD-modified polypyrrole electrode in 0.1 M phosphate buffer solution (pH 7) containing 0.1 M NaCl and glucose (0 mM [a] and 100 mM [b]) under N_2 atmosphere.

An immobilized enzyme film electrode was obtained on the Pt electrode by the coelectropolymerization of the GOD-modified pyrrole and unmodified pyrrole at +1.1 V (vs. Ag-AgCl) for 600 s. Figure 1 shows cyclic voltammograms (10 mV/s) of the

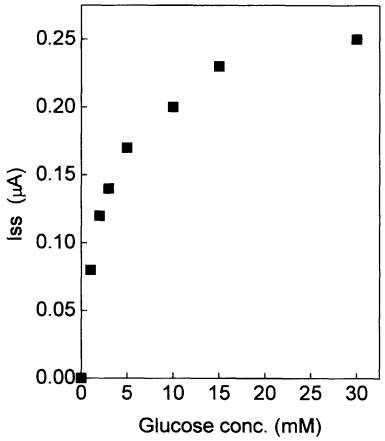

Figure 2. Calibration curve for the amperometric response of glucose at a GOD-modified polypyrrole electrode, the steady state current Iss was measured at +0.3 V (vs. Ag-AgCl) under N_2 atmosphere.

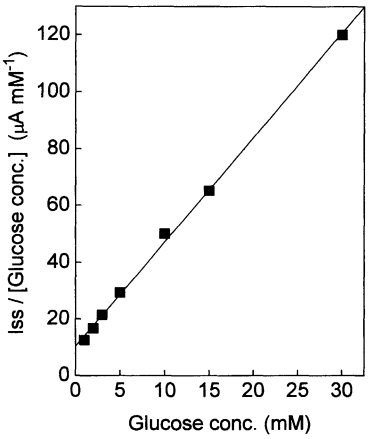

Figure 3. Hanes plots for the data given in Figure.2. The coefficient of correlation and the apparent Michaelis constant were 0.999 and 2.80 mM, respectively.

enzyme film electrode under N_2 atmosphere in 0.1 M phosphate buffer solution (pH 7) containing 0.1 M NaCl and glucose (0 mM [a], 100 mM [b]). Oxidative and reductive peaks were found at +0.1 V and 0 V (vs. Ag-AgCl), respectively (Figure 1a). Since the potential range just overlapped the redox wave of usual PPy film, the redox wave (Figure 1a) was attributed to the GOD-modified PPy film formed on the electrode. The broad redox peak may be due to the relatively low conductivity of the enzyme film electrode obtained. As shown in Figure 1b, the current for PPy oxidation is enhanced by the addition of 100 mM glucose to the electrolyte solution. This mechanism could be explained as follows. After enzyme reaction between the redox center of GOD and glucose took place, PPy was immediately reduced by the resulting redox center of GOD in the reduced state. Therefore, the current for PPy oxidation would be enhanced, in comparison with the original response by the addition of glucose. From the catalytic current, we concluded that electron transfer from the redox center of GOD to the PPy conductive matrix took place in the system. It was expected that the rotations and motions of flexible carboxymethyl spacer contributed to free access between the redox center of GOD and the conductive PPy chain. In order to obtain more detailed information on the bio-electrochemical reaction, we investigated the glucose concentration dependence of the steady state current at +0.3 V (vs. Ag-AgCl), which is attributed to PPy oxidation. The calibration curve (Figure 2) showed a linear increment of the oxidative current with an increase in glucose concentration even with the applied potential of +0.3 V (vs. Ag-AgCl) under N_2 atmosphere. The curve showed a typical enzyme-substrate reaction that is a linear increment of the amperometric response and saturation were observed. These current values are equivalent to that observed from a PPy electrode with a microporous membrane.[2] Furthermore, a linear relationship was observed in the Hanes plot (Figure 3) based on the above calibration curve, the coefficient of correlation was 0.999. The apparent Michaelis constant K'_M determined from the Hanes plot was 2.80 mM, which is similar to that observed for other enzyme film electrode.[4] The linear relationship of the Hanes plots indicates that a favorable enzyme reaction, as shown in the pseudo-homogeneous system, takes place even in the enzyme film electrode.[5] Such a relationship was rarely found in other PPy immobilized GOD electrodes. Furthermore, the absence of different kinetics for a wide range of glucose concentration (from 0 mM to 30 mM) suggested the homogeneous conformations and/or environments for the immobilized enzyme in the polymer film. These phenomena may be characteristic features of the GOD-modified PPy electrode. It can be concluded that the GOD-modified PPy electrode is also under enzyme kinetic control rather than diffusion control. Glucose detection in air is also possible at the applied potential of +0.3 V (vs. Ag-AgCl) with the GOD-modified PPy electrode. The oxidative current at +0.3 V (vs. Ag-AgCl) would be greater than that under N_2 atmosphere. According to rapid electron transfer from the redox center of GOD to O_2, the electron transfer to PPy is difficult to occur, and the contribution of PPy oxidation to the oxidative current in air is probably negligible. Since slight current due to H_2O_2 oxidation is observed even at +0.3 V (vs. Ag-AgCl), this may be the dominant component of the response in air. The amount of H_2O_2 depends on glucose concentration, therefore, the amperometric response related to glucose concentration is observed in air. Additional improvement of electron transfer from the redox center of GOD to PPy is required for realization of glucose detection independent of O_2 concentration.

In this study, direct glucose detection was achieved for the first time using the GOD-modified PPy electrode. This bio-electrochemical reaction without the use of an electron mediator such as ferrocene and quinone was due to the electron transfer from the redox center of GOD to the conducting PPy.

References and Notes

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