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Direct Electrical Communication between D-Amino Acid Oxidase and Electrodes via a Conductive Polymer Chain

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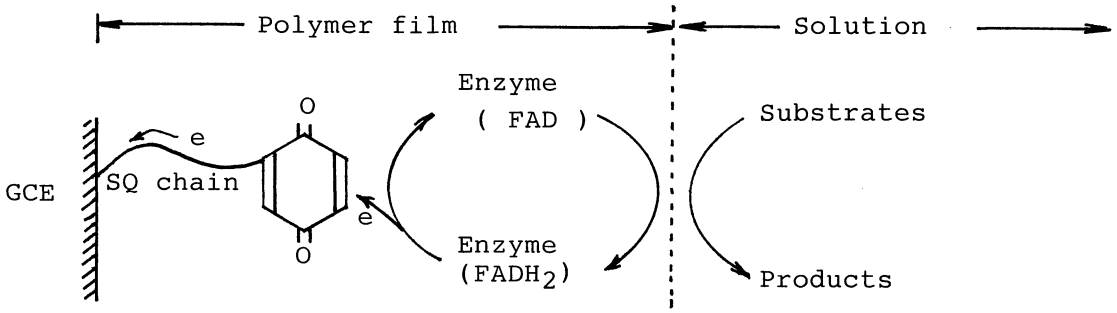
D-amino acid oxidase, a flavoprotein, was immobilized in a conductive redox polymer prepared on Au-electroplated glassy carbon electrode by an electrochemical polymerization of mercaptohydroquinone. The enzyme - modified electrode possessed of an excellent selectivity for D-amino acids and showed fast response and good stability with the substrates.

Electrochemical immobilization of enzymes in various conducting films on electrodes has been examined by several groups.[1-4] In the previous paper,[5] we reported immobilization of glucose oxidase(GOD) in a conductive redox polymer, poly(mercapto-p-benzoquinone/mercaptohydroquinone) film (SQ film) where the polymer chain served as an effective electron - transfer chain between the redox center ($FAD/FADH_2$) of the enzyme and an electrode material(Scheme 1). Judging from the two points revealed in the previous work ; i) the electron transfer between the redox center in GOD and quinone moiety in the polymer proceeds at sufficient rates, and ii) the polymer chain can penetrate thick insulating protein around FAD without lowering of enzyme activity, this direct response observed on the GOD immobilized in the polymer may be expected for other oxidases having FAD as a coenzyme by means of fixation in the polymer. In order to gain further understanding of electrical relationship between flavoprotein and the polymer chain, we

attemped to immobilize D-amino acid oxidase (DAAO), a flavoprotein, in the polymer film and investigated its direct response to substrates. The immobilization of DAAO in the polymer film was performed by electropolymerization of mercaptohydroquinone in the presence of DAAO. The electropolymerization was accomplished at 0.5 V vs. Ag/AgCl for 2 h in an 1 cm^{-3} phosphate buffer solution (pH 6.4) containing 0.1 M NaCl (1 M = 1 mol dm^{-3}), 0.5 mM mercaptohydroquinone and 0.2 cm^3 DAAO(Porcine Kidney, 12 U / mg) using an Au-electroplated glassy carbon electrode(GCE) as a substrate electrode. The resulting electrode(DAAO/SQ/GCE) is depicted in Fig. 1.

Electrochemical measurements were performed at 20 ^{o}C in N_2 atmosphere using a three-compartment cell equipped with the DAAO/SQ/GCE working electrode, a Pt wire counter electrode and an Ag/AgCl reference electrode.



Scheme 1. Electron transfer between FAD/$FADH_2$ in enzyme and quinone moiety in SQ film through a conductive polymer chain

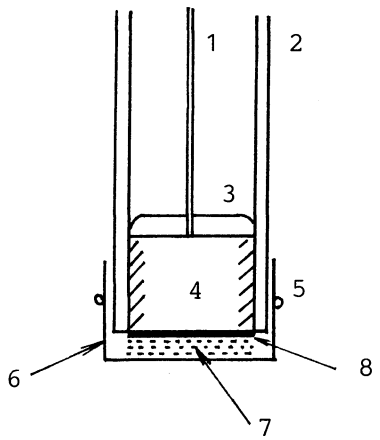


Fig. 1. Constitution of DAAO/SQ/GCE

1. Cu wire, 2. Glass tube,
3. Hg, 4. GCE,
5. O ring,
6. Dialysis membrane,
7. SQ film containing DAAO,
8. Electroplated Au.

The phosphate buffer solution containing 0.1 M NaCl was used for the preparation of stock substrate solutions. In the amperometric measurement, the stock substrate solution was added to the deaerated buffer solution under slow stirring in the cell. Amperometric responses of the DAAO/SQ/GCE were shown in Fig. 2. An addition of 10 mM D-proline caused a rapid increase in oxidation current, whereas no increase in current was observed by an addition of 10 mM L-proline. This shows that the DAAO/SQ/GCE exhibited the excellent selectivity for D-proline. Similar selectivity was observed for almost all the D-amino acids examined here such as D-valine, D-leucine, D-alanine, D-lysine, and D-phenylalanine without D-glutamic acid. These results indicate that the DAAO immobilized in the polymer film may maintain its physiological activity.

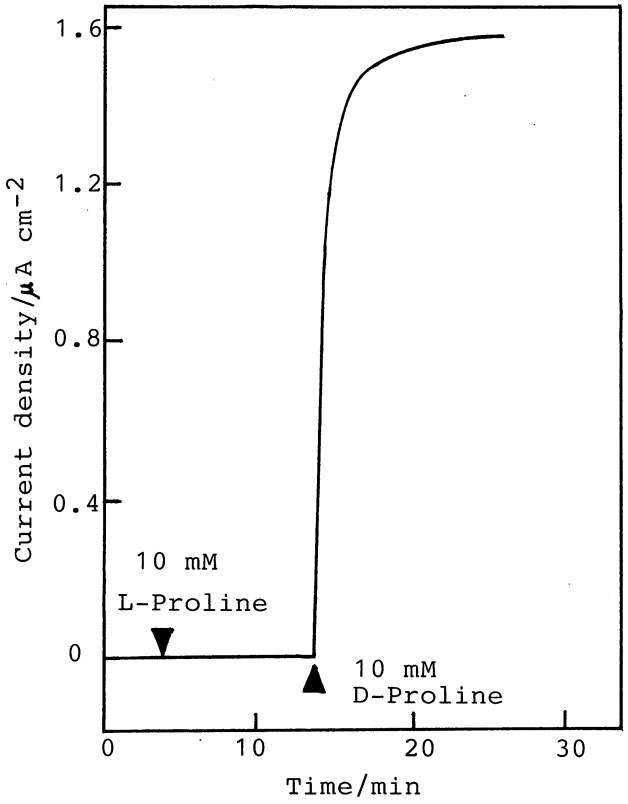


Fig. 2. Typical current response of DAAO/SQ/GCE to addition of L-and D-proline at 0.3 V vs. Ag/AgCl. Each triangle represents an increase in D-and/or L-proline concentration in phosphate buffer solution.

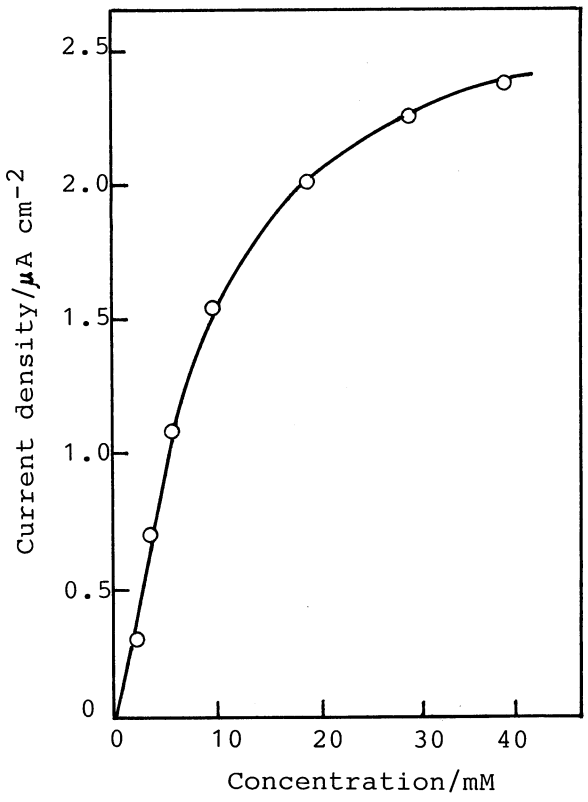


Fig. 3. Variation of steady-state current densities with concentration of D-proline. Experimental conditions were the same as in Fig. 2.

Steady-state current densities at 0.3 V vs. Ag/AgCl based upon the oxidation of D-amino acids by DAAO showed the concentration dependence of the substrates. A typical calibration curve of D-proline was shown in Fig. 3. Although the calibration curve is not linear, D-proline concentration up to ca. 20 mM may be detected by the DAAO/SQ/GCE. Charlwood[6] found that the bond between protein and FAD is very week in DAAO and FAD can be easily removed, therefore, by dialysis from the enzyme. To determine the stability of the DAAO/SQ/GCE, we measured the decrease in the oxidation current for a 12 h period using a large amount of 10 mM D-proline solution where the substrate concentration scarcely changes during the long period of electrolysis. The decrease in current was found to be only less than 5 % after the period. The result shows that the SQ film[7] around the DAAO hinders the enzyme from releasing FAD and suggests that this polymer chain may function as an excellent electron-transfer wire between active centers of the flavoprotein and substrate electrodes without lowering enzymatic activity. Further works on other flavoproteins were currently under way.

References

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7) The thickness of the SQ polymer film prepared in this work was ca. 2 μm in a dry state.

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