Electro-conductive Enzyme Membrane

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An electro-conductive enzyme membrane has been synthesized at a platinum electrode surface by electrochemical polymerization of pyrrole in the presence of glucose oxidase, while retaining enzyme activity and reversible electron transfer between the enzyme molecules and the electrode.

Electrochemical synthesis of conductive polymer membranes has gained an increasing interest as it is a simple process with many applications. Electropolymerized electrodes of polymer batteries have been extensively studied along with electrochromic displays.[1—4] Murray and his co-workers reported an ion-gate membrane which is made of electrochemically synthesized polypyrrole and is electrochemically controllable by ion permeability.[5] Electrochemical control of amino acid permeability has also been performed with an electrochemically synthesized polypyrrole membrane.[6] Shinohara *et al.* have assembled an artificial synapse using an electrochemically synthesized polypyrrole membrane, which electrically released neurotransmitter molecules in the cell.[7,8]

A technique, for the entrapment of an enzyme in a conductive polymer, was proposed.[9] However, the technique had difficulty with the electroconductivity of the enzyme-entrapped polymer and electron transfer between the enzyme and polymer matrix. A novel membrane, electro-conductive enzyme membrane, has been prepared by electrochemical polymerization of pyrrole in the presence of glucose oxidase. Electron transfer has been shown between glucose oxidase and polypyrrole matrix in the membrane.

Electrochemical polymerization was carried out in a glass vessel equipped with a platinum wire electrode (0.5 mm in diameter, 7.5 mm in length), a platinum plate electrode (10 × 5 mm^2), and a Ag/AgCl reference electrode. These electrodes were connected to a potentiostat (Hokuto Denko, Model HA-301) and a coulometer (Hokuto Denko, Model HF-201). Pyrrole was dissolved in 0.1 mol dm^{-3} KCl solution to make a 0.1 mol dm^{-3} solution which was deoxygenated before use. Glucose oxidase (GOD)(TOYOBO Co., grade II, 113 U mg^{-1}) was added to 1 cm^3 of the solution, which was transferred to the glass vessel. The electrode potential of the platinum wire electrode was set at 0.7 V *vs.* Ag/AgCl to initiate electrochemical polymerization, this was held at a fixed charge. A black membrane deposited on the electrode surface. The membranes were synthesized at various concentrations of GOD and at different charges, and were thoroughly washed with 0.1 mol dm^{-3} KCl solution.

The polypyrrole/GOD membrane was assayed for enzyme activity and characterized by electrochemical measurements. The membrane was immersed in a 0.1 mol dm^{-3} citrate buffer solution (pH 5.5) containing 0.17 mmol dm^{-3} 3,3'-dimethoxy benzidine, 93 mmol dm^{-3} glucose, and 7 mU cm^{-3} peroxidase (TOYOBO Co., grade III, 127 U mg^{-1}).[10] The solution was incubated at 25 °C and stirred. An aliquot was assayed for absorbance at 436 nm with a spectrophotometer (Japan Spectroscopic Co., Model UVIDEC-610C).

Each membrane, synthesized under different conditions, showed enzyme activity. The enzyme activity depended on the GOD concentration of the electrolyte as shown in Figure 1. Since the total charge for electrochemical polymerization was fixed at 850 mC cm^{-2}, the membranes obtained were of equal thickness. The enzyme activity sharply increased with increase in GOD concentration of the electrolyte, reaching a plateau around 30 mg cm^{-3}. This indicates that the total amount of entrapped GOD increased to a concentration of 30 mg cm^{-3}.

However, the enzyme activity reached its maximum and plateau at an electrolysis charge of about 100 mC cm^{-2}. The thickness of the membrane produced was proportional to the electrolysis charge. The enzyme activity was limited by the membrane thickness, probably due to the extent of glucose diffusion.

The polypyrrole/GOD membranes were then characterized by electrochemical measurements using a polarographic analyzer (Yanako, Model P-1100). Differential pulse voltammetry was carried out on the polypyrrole/GOD membrane which was in contact with the 0.1 mol dm^{-3} citrate buffer solution (pH 5.3) containing 1 mol dm^{-3} KCl. Entrapped GOD gave a distinct cathodic peak which could be attributed to electron transfer from the platinum electrode to the GOD molecules through the polypyrrole chains. Free GOD molecules in solution showed no appreciable cathodic peak on a platinum electrode owing to a large barrier against direct electron transfer between the protein molecules and the electrode surface.

Electrical conductivity was measured for the free-standing and dried polypyrrole/GOD membrane. The membrane showed electrical conductivity of approximately 10 S cm^{-1} at 25 °C. This may give rational for electron transfer from the platinum electrode to entrapped GOD molecules through polypyrrole chains.

Differential pulse voltammetry was also performed on the reduced form of membrane-bound GOD deposited on a platinum electrode. A distinct anodic peak appeared around −0.32 V *vs.* Ag/AgCl. This suggests electron transfer from the reduced GOD to the electrode. The anodic and cathodic peaks appeared in the gap of 60 mV, indicating that electron transfer between membrane-bound GOD and the electrode should be reversible through conductive polypyrrole chains.

The electro-conductive enzyme membrane has been synthesized by electrochemical polymerization of pyrrole in the

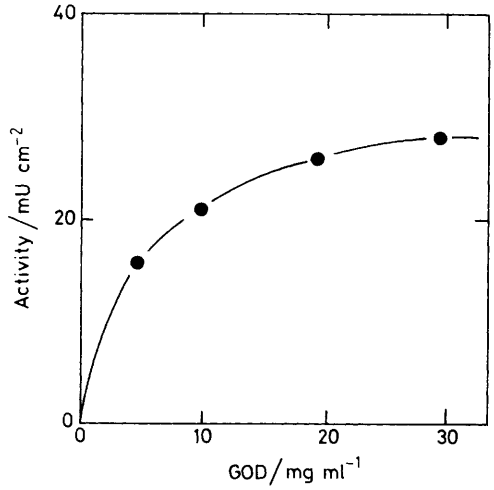

Figure 1. Enzyme activities of GOD/polypyrrole membranes synthesized at different GOD concentrations in electrolysis.

presence of enzyme molecules. The membrane will find application as a novel material for bioelectronics, *i.e.*, biosensors, bio-fuel cells, bioreactors, and other bioelectronic devices.

Received, 9th August 1988; Com. 8/03262I

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