Simultaneous Immobilization of Glucose Oxidase and a Mediator in Conducting Polymer Films

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Simultaneous immobilization of glucose oxidase and ferrocenecarboxylate as an electron relay in polypyrrole films was successfully accomplished to provide a simple biosensor capable of detecting glucose rapidly in a wide concentration range without use of any mediator in solution.

Electrochemical immobilization of glucose oxidase in conducting polymers such as polypyrrole and poly-*N*-methylpyrrole on a platinum or glassy carbon electrode has recently been reported by several groups;[1—3] the aim was to facilitate electron transfer between the enzyme and the electrode material. Such immobilization provides a simple method of controlling the amount and spatial distribution of enzyme in the polymer support. However, no evidence has yet been given for direct electrical communication between the enzyme and the electrode materials. All the results reported were obtained in the presence of oxygen or some other electron acceptor such as phenazine methosulphate or benzoquinone in solution. These substances serve as mediators in electron transport from the enzyme to the electrode material or to the conducting polymer matrix on reduction of the enzyme, which takes place in the presence of glucose. If we could entrap both a mediator and glucose oxidase in an electroconducting polymer film, electron transport from the reduced enzyme to the electrode material might be facilitated without the need for any mediator in solution, in the presence of glucose. Work relevant to such an idea has been reported by Ikeda *et al.*,[4] who immobilized glucose oxidase on the surface of a *p*-benzoquinone–carbon paste electrode by covering it with a nitrocellulose film. In the present study, we have investigated the simultaneous immobilization of glucose oxidase and a mediator in polypyrrole films, for application in amperometric glucose sensors. Ferrocenecarboxylic acid was used as the mediator because it is known to be one of the most effective mediators in solution.[5]

To immobilize both glucose oxidase (GO) and ferrocene-

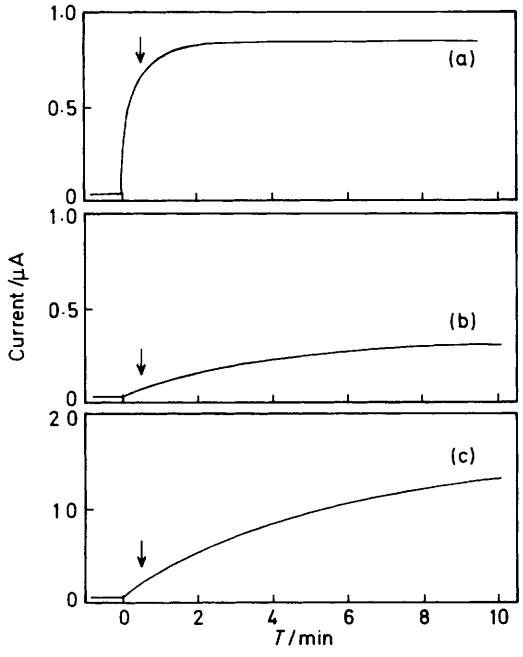

Figure 1. Typical changes of current with the passage of time after glucose addition: (a) GO/$FcCO_2H$/PPy electrode in base solution alone; (b) GO/PPy electrode in solution containing 1×10^{-4} mol dm^{-3} $FcCO_2H$; (c) GO/PPy electrode in O_2-saturated solution; the arrow shows the point at which stirring was stopped.

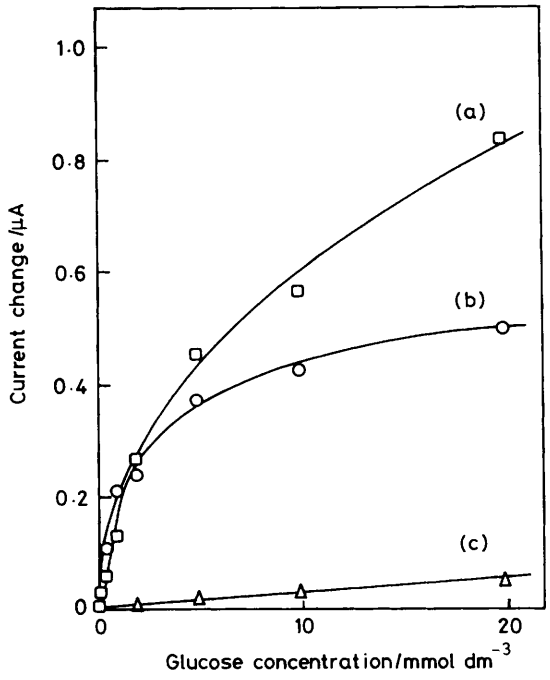

Figure 2. Calibration curves for glucose of GO/$FcCO_2H$/PPy electrodes having different thicknesses of polypyrrole film; quantities of electricity for deposition of the films: (a) 200, (b) 100, (c) 400 mC cm^{-2}.

carboxylic acid ($FcCO_2H$) simultaneously in a polypyrrole film (PPy), electropolymerization of pyrrole was carried out at 0.8 V [*vs.* saturated calomel electrode (s.c.e.)] from an aqueous solution (pH *ca.* 6.3) of glucose oxidase (1 g dm^{-3}), ferrocenecarboxylic acid (0.1 mmol dm^{-3}), and pyrrole (0.2 mol dm^{-3}). The amounts of immobilized glucose oxidase and ferrocenecarboxylic acid in the polypyrrole film were estimated to be *ca.* 1.9 mg (1.2×10^{-8} mol) and 6.2×10^{-9} mol, respectively, when the quantity of electricity used for deposition of the polypyrrole film was 200 mC cm^{-2}. The responses of the resulting electrodes (0.28 cm^2) to glucose addition were measured at 25 °C by the following procedure.[1] A constant potential was applied to the electrodes (0.4 V *vs.* s.c.e.), then the background current was allowed to decay to a constant value. Samples of stock glucose solution were added to a phosphate buffer solution (50 cm^3; pH 7), which was then stirred for 30 s. The current in the quiescent solution was recorded. All solutions were deoxygenated with nitrogen prior to use.

Figure 1 shows typical changes of current with time after glucose addition at the electrodes without any mediator in solution. For comparison, changes at polypyrrole films in which glucose oxidase alone was immobilized (GO/PPy) are also shown, with ferrocenecarboxylic acid or oxygen as mediator in solution. In the case of the GO/$FcCO_2H$/PPy electrodes, the steady-state current was reached a few minutes after glucose addition. In contrast, when the mediators were used in solution with the GO/PPy electrode, it took more than 10 minutes to reach a steady-state current. The results demonstrate the effectiveness of simultaneous immobilization of glucose oxidase and ferrocenecarboxylic acid in a polypyrrole film.

The steady-state currents are plotted in Figure 2 against glucose concentration for different thickness of polypyrrole film. The glucose responses depended markedly on film thickness, showing a maximum when the quantity of electricity for deposition was about 200 mC cm^{-2}. The observed optimal thickness seems to reflect a greater response at polypyrrole films containing greater amounts of both glucose oxidase and ferrocenecarboxylic acid, obtained by use of higher deposition charges. However, very thick polypyrrole films do not allow easy diffusion of glucose; thus the availability of glucose oxidase and ferrocenecarboxylic acid for the electron transfer is limited.

Thus, we have developed a simple and widely applicable method for simultaneous immobilization of enzyme and mediator in conducting polymer films.

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