Immobilization of Glucose Oxidase in Ferrocene-Modified Pyrrole Polymers

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A novel approach to an amperometric enzyme electrode for the analysis of glucose is described. The technique entails the electrochemical codeposition of the redox enzyme, glucose oxidase, in the conducting organic polymer, polypyrrole. Reagentless glucose electrodes have been generated by the synthesis of N-substituted pyrrole monomers containing redox-active side chains designed to accept electrons from the reduced form of the enzyme. Ferrocene–pyrrole conjugates were found to be efficient oxidants of reduced glucose oxidase, which on anodic polymerization form redox-active films. Preliminary results suggest that the enzyme may be entrapped in ferrocene-containing polypyrrole films to construct a reagentless glucose electrode.

The development of analytical devices that combine the specificity and sensitivity of biological systems with appropriate electrochemical techniques is likely to have a significant impact on the health care, agricultural, environmental, security, food, pharmaceutical, and fermentation industries (*1*, *2*). Enzymatic redox reactions are particularly amenable to interfacing with electrochemical transducers since electron exchange is a key step in their natural cycle. However, direct electron transfer from the active center of enzymes to electrode surfaces, although claimed in some cases (*3*, *4*), is often inhibited by steric constraints. Thus, considerable effort has been directed toward devising more effective means for promoting electron transfer between the redox catalyst and the electrode. Such approaches center on the development of soluble redox mediators as electron shuttles (*5*), chemically modified electrodes that promote orientation of the enzyme on the electrode surface (*6*) and on electron relays covalently bound to the surface of the enzyme (*7*, *8*).

The entrapment of enzymes in polypyrrole films provides a simple method of enzyme immobilization with potential for use in biosensor construction (*9*). Because the immobilization procedure only involves the application of a suitable potential to an electrode in an appropriate aqueous solution of monomers and enzyme, the technique is particularly amenable to the localization of enzymes to small or defined electrode geometries. Preliminary studies have provided evidence that glucose oxidase may be incorporated into polypyrrole (*9*, *10*) or poly(*N*-methylpyrrole) (*11*) films electrochemically deposited on platinum electrodes. Polypyrrole-entrapped glucose oxidase electrodes respond rapidly to glucose and reach a steady state within 20–40 s (*9*). In these preliminary studies the enzyme-catalyzed oxidation of glucose has been followed by the electrochemical detection of hydrogen peroxide either at the surface of the underlying electrode or, conceivably, on the conducting polymer itself (*9–11*). However, in the clinical world of blood glucose determination, there is an increasing trend toward whole blood tests, rather than prediluted samples, which for an electrochemical test necessitates the use of less anodic potentials than those required to oxidize hydrogen peroxide. Ferrocene and its derivatives exhibit redox potentials in a range where little interference from blood components is noted (+100 to +400 mV vs SCE) and are known to mediate electron exchange between the reduced form of glucose oxidase and electrode surfaces (*5*, *11–14*).

Since it has been established that glucose oxidase can be successfully immobilized in polypyrrole films (*8*) and that an electron acceptor to the enzyme, ferrocene, can be easily modified by chemical substitution (*5*, *12–14*), it was hoped that pyrrole–ferrocene conjugates could be synthesized and used in place of pyrrole for the immobilization of the enzyme. This, it was hoped, would result in the construction of a reagentless glucose electrode that would operate by the reduced form of glucose oxidase interacting directly with the ferrocene moieties in the polymer.

This paper reports a preliminary investigation of the interaction of glucose oxidase with ferrocene-containing polymers of pyrrole and the use of this system as a first step in the construction of a reagentless glucose sensor.

EXPERIMENTAL SECTION

Materials. *N*-(2-Cyanoethyl)pyrrole (99+%), lithium aluminum hydride, ferrocenecarboxylic acid (95%), and *n*-pentane (98%) were obtained from Aldrich Chemical Co., Ltd. (Gillingham, Dorset, UK). Glucose oxidase (β-D-glucose, oxygen 1-oxidoreductase, EC 1.1.3.4) (125 $U \cdot mg^{-1}$ type VII, *Aspergillus niger*), 6-aminohexanoic acid, and 1,1′-carbonyldiimidazole were from Sigma Chemical Co., Ltd. (Poole, Dorset, UK). Nitrogen (O_2-free grade) was obtained from BOC, Ltd. (Guildford, Surrey, UK). All other reagents and solvents were of the highest grade available from BDH Chemicals, Ltd. (Poole, Dorset, UK), or Fisons Scientific Equipment (Loughborough, Leicestershire, UK) and were used without further purification.

Synthesis. *N*-(2-Cyanoethyl)pyrrole was reduced to *N*-(3-aminopropyl)pyrrole with $LiAlH_4$ in dry diethyl ether. Excess $LiAlH_4$ was destroyed with water and the product collected as an oil in a yield of 89% after rotary evaporation of the ether phase. The compound was characterized by infrared (IR), nuclear, magnetic resonance (NMR), and mass spectrometry (MS).

[(Ferrocenyl)amidopropyl]pyrrole (FAPP) (I) was synthesized by coupling ferrocenecarboxylic acid to *N*-(3-aminopropyl)pyrrole using 1,1′-carbonyldiimidazole in dry methylene chloride. The product was recrystallized from a minimum volume of boiling methanol and was obtained in an overall yield of 24% from *N*-(2-cyanoethyl)pyrrole and characterized by melting point (136–137 °C), IR, NMR, and MS.

Ferrocenoyl chloride was prepared according to a previously published procedure (*15*). Ferrocenylamidopentanoic acid was prepared by reacting 5-aminopentanoic acid with ferrocenoyl chloride in dry pyridine. ([(Ferrocenyl)amidopentyl]amidopropyl)pyrrole (FAPAPP) (II) was synthesized by coupling ferrocenoyl amidopentanoic acid to *N*-(3-aminopropyl)pyrrole with 1,1′-carbonyldiimidazole in dry methylene chloride. The product was recrystallized from a boiling mixture of diethyl ether/chloroform (1:1, by vol) and obtained in an overall yield of 16% from ferrocene 1-carboxylic acid. Characterization was by melting point (115–116 °C), IR, NMR, and MS.

Electrochemical Measurements and Electrode Design. Direct current (dc) cyclic voltammetry, linear sweep voltammetry,

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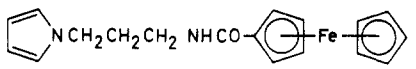

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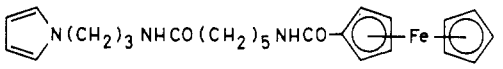

II

and chronoamperometric measurements were performed with an EG&G Princeton Applied Research Model 273 potentiostat–galvanostat. Experiments were performed by use of a three-electrode, water-jacketed cell with a volume of 10 mL. Temperature regulation was provided by a Julabo F10 water bath and circulator, and unless otherwise stated, all experiments were performed at 25 ± 0.1 °C. Output from the potentiostat was recorded on a Gould series 6000 X-Y-t recorder or by interfacing to an Apple IIe microcomputer. The reference electrodes were flat-tip sintered Ag/AgCl probes with a sensor tip of 2-mm diameter (Clark Electromedical Instruments, Reading, UK). The Ag/AgCl electrodes were equilibrated in the appropriate electrolyte for 2 h prior to use. The counter electrode was constructed from Pt wire (1-mm diameter). Working electrodes (G.E.C. Hirst Research Centre, Wembley, UK) comprised a Pt–ink layer printed onto a ceramic base (8.5 mm × 50 mm) with a silica masking layer defining the electrode (4 mm × 4 mm) and contact areas.

The reference, working, and counter electrodes were secured in position in the thermostated cell with a Teflon lid. All experiments were performed under nitrogen.

Electrode Characterization. Working electrode areas were determined electrochemically by cyclic voltammetry of the hexacyanoferrate redox couple (*17*). The morphology of the electrode surface was investigated by scanning electron microscopy (SEM) and the elemental composition determined by an attached energy dispersive analysis of X-rays (EDAX) system.

Modified Electrodes. Polypyrrole-modified electrodes were prepared by electrooxidation of unstirred deaerated aqueous solutions of pyrrole (0.1 M, Aldrich, redistilled) at +0.8 V (vs. Ag/AgCl) (*9*). Electrodeposition of ferrocene-modified polypyrrole was effected by cycling the electrode between 0 and +1.0 V at 100 mV·s^{-1} in aqueous perchlorate containing specified ratios of pyrrole, FAPP (I) and FAPAPP (II) added to the electrolyte in small volumes of acetonitrile. All polymer films were washed exhaustively with buffered electrolyte and cyclic voltammograms recorded between 0 and +0.5 V at scan rates in the range 5–100 mV·s^{-1} in the presence and absence of soluble glucose oxidase (6.3 μM) and D-glucose (50 mM).

Entrapment of enzyme in the redox copolymer (FAPP/pyrrole) was effected by cycling the working electrode between 0 and +0.8 V at 5 mV·s^{-1} in sodium phosphate buffer pH 5.6 (10 mM) containing sodium perchlorate (0.1 M), pyrrole (1 mM), FAPP (1 mM), and glucose oxidase (200 U·mL^{-1}; 1.6 mg·mL^{-1}). The resulting electrode was thoroughly washed in fresh electrolyte and cycled between 0 and +0.5 V at scan speeds 5–100 mV·s^{-1} in the presence and absence of D-glucose (50 mM). The steady-state response of the redox polymer-entrapped enzyme electrode was monitored at +0.3 V in phosphate-buffered perchlorate solution following the addition of glucose to final concentrations of 1–100 mM.

RESULTS AND DISCUSSION

Ferrocene–Pyrrole Conjugates as Oxidants for Glucose Oxidase. A number of ferrocene derivatives with redox potentials in the range −50 to +400 mV (vs SCE) have been shown to act as oxidants for reduced glucose oxidase (*12–14, 18*). Ferrocenecarboxylic acid and the pyrrole conjugates FAPP (I) and FAPAPP (II) were tested as potential oxidants for glucose oxidase by dc cyclic voltammetry in N_2-purged quiescent electrolyte comprising 50 mM potassium perchlorate buffered to pH 7.0 with 30 mM potassium phosphate and containing 50 mM glucose. Under the experimental conditions selected and over the range of potential scan (−50 to +400 mV) and scan rates (1–100 mV·s^{-1}) FAPP, FAPAPP, and ferrocenecarboxylic acid generated voltammograms consistent with

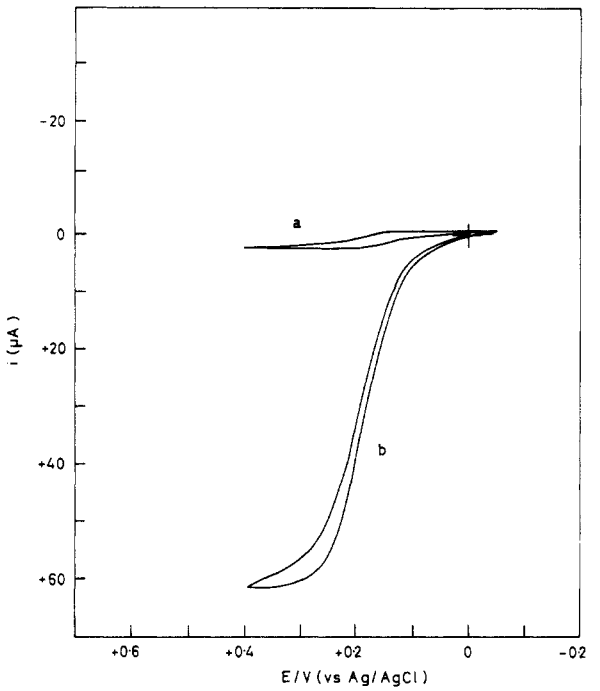

Figure 1. Oxidation of reduced glucose oxidase using FAPAPP: (a) cyclic voltammogram recorded in 50 mM potassium phosphate buffer, pH 7.0, containing 50 mM potassium perchlorate, 50 mM FAPAPP at a scan rate of 5 mV·s^{-1}; (b) cyclic voltammogram recorded under the above conditions with the addition of 12.6 μM glucose oxidase to the electrolyte and at a scan rate of 5 mV·s^{-1}.

a reversible one-electron redox agent (ΔE_p = 60 mV (25 °C); $ip/\nu_{1/2}$ constant). Under the same conditions neither glucose nor glucose oxidase exhibited any observable electrochemistry.

Figure 1 shows cyclic voltammograms of FAPAPP (0.5 mM) at 5 mV·s^{-1} in phosphate-buffered perchlorate containing 50 mM glucose in the absence (a) and presence (b) of glucose oxidase (12.6 μM). The absence of discernible peaks and the large current flowing at oxidizing potentials observed in the cyclic voltammograms on addition of enzyme is particularly apparent at the slower scan rates and is indicative of the catalytic regeneration of ferrocene from the ferricinium ion by the reduced enzyme.

The rates of reaction between the reduced form of the enzyme and the oxidized ferrocene analogues can be derived from an analysis of the cyclic voltammograms provided that the heterogeneous electron transfer reaction (ferrocene → ferricinium$^+$ e$^-$) is fast (diffusion controlled) compared to the rate of homogeneous reaction between ferricinium ion and enzyme and that the latter is fully reduced by the presence of excess glucose. Under these conditions the concentration of reduced glucose oxidase will effectively remain unchanged throughout the experiment and the reaction can be treated as pseudo first order (*19*)

$$O + Z \xrightarrow{k_f} R;\ R \rightleftharpoons O + e^-$$

where O and R refer to the oxidized and reduced forms of ferrocene, Z is the reduced enzyme, and k_f (=k[Z]) is the pseudo-first-order rate constant. Cyclic voltammograms were recorded at scan rates in the range 1–100 mV·s^{-1} in the presence and absence of 6.3, 12.6, 18.9, and 25.2 μM glucose oxidase and the data analyzed by equating the experimentally derived ratio of catalytic to diffusion controlled current (i_k/i_d) to the kinetic parameter $(k_f/a)^{1/2}$, where $a = nF\nu/RT$ (*15, 19*). A scan-rate-independent pseudo-first-order rate constant was obtained for each glucose oxidase concentration from the slopes of the plots of k_f/a versus $1/\nu$. The second-order homogeneous rate constants for the reaction of the ferricinium analogues and the enzyme was deduced from the slope of the

Table I. Second-Order Homogeneous Rate Constants (k_s) for Oxidation of Reduced Glucose Oxidase with Ferricinium Derivatives at pH 7.0 and 25 °C

ferrocene derivative	$10^{-5}k_s$, L·mol^{-1}·s^{-1}
ferrocenecarboxylic acid	2.2
FAPP (I)	6.7
FAPAPP (II)	21.0

linear plot of pseudo-first-order rate constant (k_f) as a function of glucose oxidase concentration. Kinetic data calculated according to this procedure are presented in Table I and show that all three ferrocene analogues act as rapid oxidants for reduced glucose oxidase. The second-order homogeneous rate constant (k_s) for ferrocenecarboxylic acid quoted in Table I is consistent with literature values of 2.01×10^5 L·mol^{-1}·s^{-1} (*15*) and 1.8×10^5 L·mol^{-1}·s^{-1} (*5*). The observed rates for ferrocenecarboxylic acid and FAPP are both lower than that for the reaction of reduced glucose oxidase with its natural electron acceptor, oxygen, 1.5×10^6 L·mol^{-1}·s^{-1} (*20*). In contrast, the rate of reoxidation of the reduced enzyme by FAPAPP is faster than with oxygen and may reflect features of the overall polarity or molecular conformation of the mediator (*5*).

Electropolymerization of Ferrocene–Pyrrole Conjugates. The anodic electrodeposition of films of FAPP and FAPAPP from predominantly aqueous electrolyte was investigated in order to optimize conditions for the growth of the polymers and, ultimately, to permit entrapment of glucose oxidase within the polymer films (*8*). Constant potential electrodeposition (*20*, *21*) of FAPP and FAPAPP from aqueous electrolytes yielded inferior films with poorly defined redox couples for the ferrocene species. On the other hand, successive potential scans of the pyrrole monomers in aqueous perchlorate between 0 and +1.0 V at 100 mV·s^{-1} yielded a growing film of the polymeric ferrocene species. Figure 2 illustrates the cyclic voltammograms recorded during the anodic electropolymerization of FAPAPP. The inside cyclic voltammogram shows the reversible one-electron redox couple of the solution species of FAPAPP at 100 mV·s^{-1} between 0 and +0.45 V. Extension of the anodic scan limit to +1.0 V leads to an increase in current on the forward scan due to oxidation of the pyrrole moiety. With successive scans, growth of the film containing the ferrocene redox species can be monitored by observing the peaks attributable to immobilized ferrocene. Control of the film thickness can be achieved simply by limiting the number of cyclic potential scans. The resulting films varied in color depending on their thickness from brown/yellow through blue to dark blue/green.

The growth of pure polymer films of FAPP or FAPAPP from aqueous electrolytes is self-limiting and results in relatively thin films. This could be the consequence of low conductivity of the polymer film, which may be expected of polymerized N-substituted pyrroles (*22*, *23*).

Films of poly(FAPAPP) display smaller $\Delta E^{1/2}$ and ΔE_p values that approximate more closely to the theoretical values (*24*) than those for poly(FAPP). Differences in the length of the alkyl chain linking the ferrocene to the pyrrole and the additional amide bond in FAPAPP may cause differences in polymer packing densities and orientation and mobility of the ferrocene, thereby resulting in an overall enhancement of charge transfer rates for the FAPAPP derivative.

Growth of Copolymer Films of FAPP or FAPAPP with Pyrrole. Copolymers of FAPP and FAPAPP with pyrrole were formed by electropolymerization of mixtures of the monomers in aqueous electrolyte. With all molar ratios of FAPP–pyrrole a single oxidation peak for the monomer mixture was noted, which was located at positions on the potential axis intermediate between those of pyrrole (+0.85 V) and FAPP (+1.0 V) depending upon the composition. The existence of only one oxidation peak for the monomer mixtures suggests that a true copolymer is being formed rather than a mixture of polypyrrole and poly(FAPP).

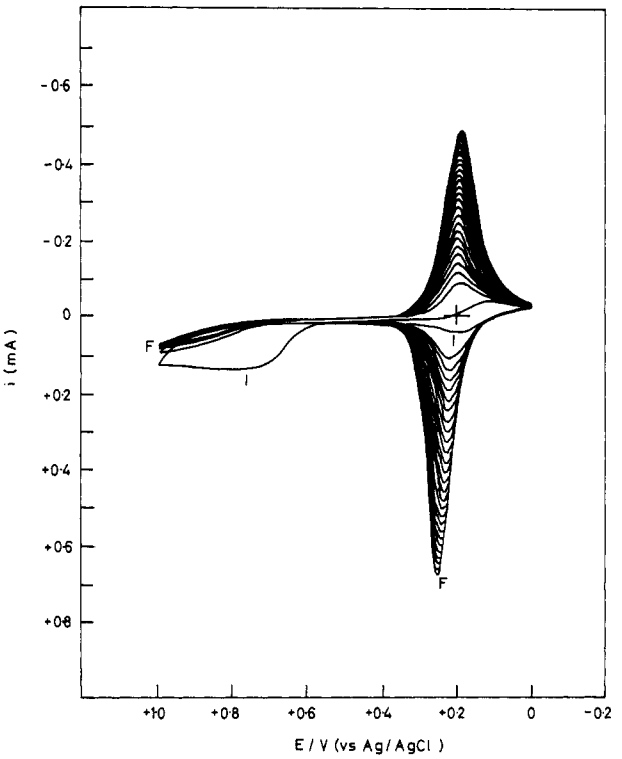

Figure 2. Anodic electropolymerization of FAPAPP. The anodic electropolymerization of FAPAPP was performed by cycling the electrode between 0 and +1.0 V at 100 mV·s^{-1} in a solution of 0.1 M lithium perchlorate containing 2 mM FAPAPP. The cycle labeled "I" was the initial cycle, which was started at +0.4 V, scanned back to 0 V, and then returned to +0.4 V to record a cyclic voltammogram of the ferrocene moiety of the monomer species. As the scan is continued toward +1 V a peak due to the oxidation of the pyrrole moiety can be seen that diminishes in height on sucessive scans. Also with successive scans the peak due to the immobilized ferrocene species can be seen to grow; the final scan is labeled F.

Characterization of the Polymer Films. Polymer films of FAPP and FAPAPP grown by the cycling technique exhibited apparent surface concentrations of ferrocene in the range 10^{-10}–10^{-8} mol·cm^{-2} as determined from the charge under the oxidation peak of the ferrocene (*21*). Analysis of the thicker copolymer films of FAPP/pyrrole with a surface coverage of $\sim 10^{-8}$ mol·cm^{-2} was performed by EDAX. Distinct peaks attributable to the Fe of the ferrocene and the Cl of the perchlorate anion used as the electrolyte for film synthesis were present in addition to Al, Si, and Pt of the underlying electrode. Cyclic voltammograms of FAPP and FAPAPP copolymer films with apparent surface coverages of $(1.4 \pm 0.3) \times 10^{-9}$ mol·cm^{-2} and $(1.3 \pm 2.5) \times 10^{-10}$ mol·cm^{-2}, respectively, reveal ΔE_p values that increase with increasing molar ratios of pyrrole–modified pyrrole in the polymerization medium. It is probable that these increases in peak sepration (ΔE_p) reflect the presence of a thicker polymer film exhibiting a slower rate of charge transfer.

Catalytic Mediation of Glucose Oxidase with Polymer Films. Cyclic voltammograms were recorded between 0 and +0.5 V for both homopolymer and heteropolymer films in 50 mM potassium phosphate buffer pH 5.6 containing 0.1 M potassium perchlorate and 50 mM glucose. Figure 3 illustrates the cyclic voltammograms recorded at several scan rates in the range 5–100 mV·s^{-1} in the presence and absence of 6.3 μM glucose oxidase for a copolymer film grown from a solution containing a 1:1 molar ratio of pyrrole to FAPP. The esti-

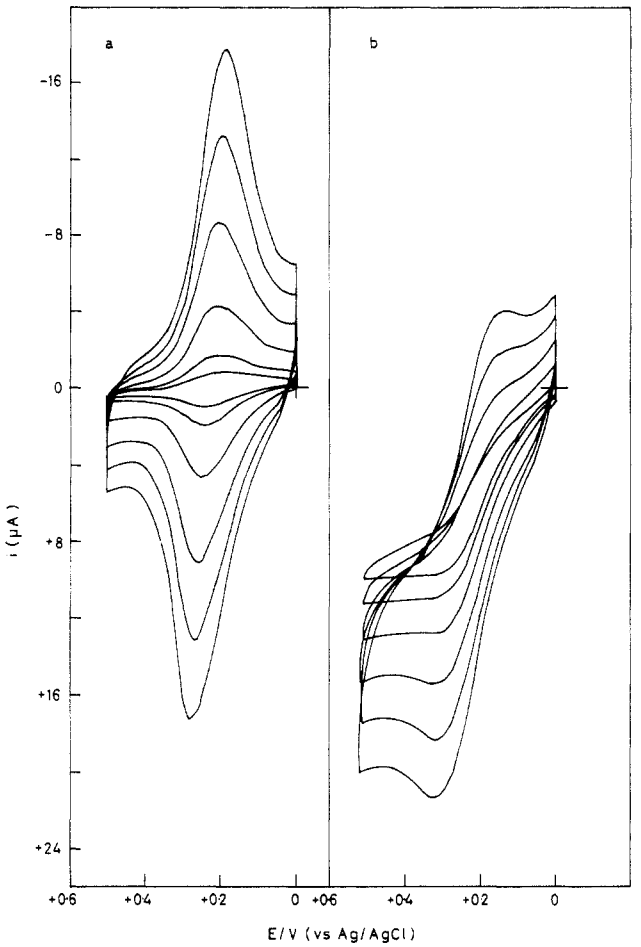

Figure 3. Mediation of the glucose oxidase reaction using a copolymer film of FAPP/pyrrole. Cyclic voltammograms were recorded for a copolymer film of FAPP/pyrrole in 0.1 M potassium phosphate buffer, pH 5.6, containing 0.1 M potassium perchlorate and 50 mM glucose between 0 and +0.5 V at scan speeds of 5, 10, 25, 50, 75, and 100 $mV \cdot s^{-1}$ in the absence (a) and presence (b) of 6.3 μM glucose oxidase.

mated surface coverage of ferrocene sites for the film was 1.14 $\times 10^{-9}$ $mol \cdot cm^2$. Addition of enzyme to the cell leads to an enhanced anodic current and a decreased cathodic current. At slow scan speeds (<10 $mV \cdot s^{-1}$) the cathodic peak disappears completely and the anodic peak appears as a plateau. Reduced glucose oxidase thus appears to regenerate ferrocene from the ferricinium ion within the polymer film. However, the rate of reduction of the immobilized ferricinium ion is markedly slower than that for the soluble ferrocene species presumably due to steric hindrance created by the reduced mobility of the redox groups in the immobilized state.

All four types of polymers (poly(FAPP), poly(FAPAPP), and copolymers of both with pyrrole) were catalytically active with glucose oxidase although with differing rates. Polymer films containing FAPP were enzymically regenerated faster that those with FAPAPP, although all films regained their initial cyclic voltammetric behavior after thorough rinsing and placing in enzyme-free electrolyte.

The experimentally derived ratio of anodic current in the presence of enzyme (i_k) to the peak anodic current in its absence (i_d) at a given scan rate may be taken as a measure of the catalytic efficiency of the modified electrodes (25). For scan speeds of 10 $mV \cdot s^{-1}$, and homopolymer films, the ratio (i_k/i_d) is relatively large (>7, poly(FAPP); ≈3, poly(FAPAPP)) for very thin films (surface coverage $\sim 10^{-10}$ $mol \cdot cm^{-2}$) but falls rapidly to ≪1 with consequential immeasurably low rates of enzymic regeneration of the immobilized ferrocene with thicker films (surface coverage $\sim 10^{-8}$ $mol \cdot cm^{-2}$). These results can be interpreted if it is assumed that the enzyme is too large

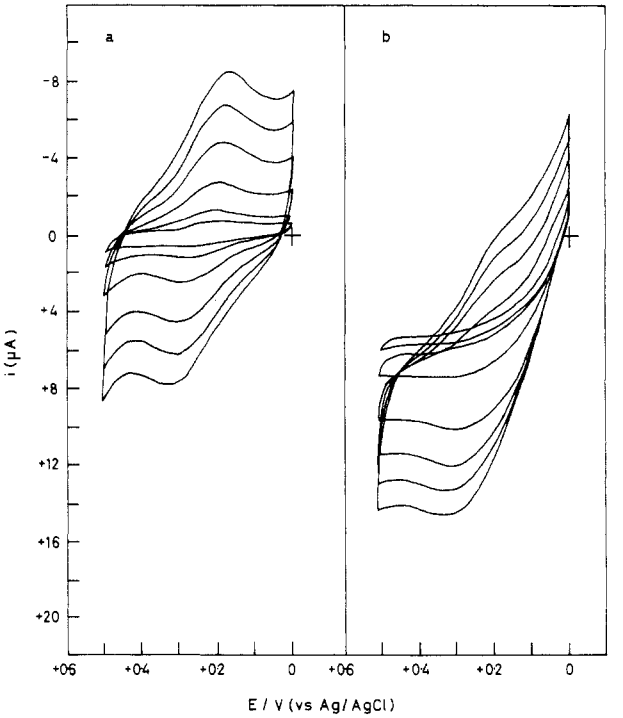

Figure 4. Mediation of the FAPP/pyrrole copolymer immobilized glucose oxidase electrode. The copolymer-immobilized glucose oxidase electrode was fabricated from a solution of 10 mM sodium phosphate buffer, pH 5.6, containing 0.1 M sodium perchlorate, 1 mM pyrrole, 1 mM FAPP, and 200 $U \cdot mL^{-1}$ of the enzyme, by cycling the electrode between 0 and +0.8 V at 5 $mV \cdot s^{-1}$. Cyclic voltammograms of the resulting electrode were recorded in 0.1 M sodium phosphate buffer, pH 5.6, containing 0.1 M sodium perchlorate, between 0 and +0.5 V at scan speeds of 5, 10, 25, 50, 75, and 100 $mV \cdot s^{-1}$ in the absence (a) and presence (b) of 50 mM glucose.

to diffuse into the films to any significant extent and that a decrease in catalytic efficiency with increasing film thickness must be a function of limitations due to charge propagation throughout the film. The catalytic efficiencies (i_k/i_d) for the homogeneous reactions of FAPP and FAPAPP with glucose oxidase, 16 and 18, respectively, at 10 $mV \cdot s^{-1}$, are considerably greater than those for even the most efficient polymeric films, with comparative values of 7.2 and 2.75, respectively.

Similar observations were noted for the catalytic efficiencies of the copolymer films of pyrrole and FAPP/FAPAPP. It is conceivable that the differences in the mobility or orientation of the ferrocenyl ligands in the polymers may explain why polymers of FAPP apparently mediate the glucose oxidase reaction more effectively than polymers of FAPAPP and why other polymeric forms of ferrocene were totally ineffective (26).

Electrodeposition of Glucose Oxidase in the Redox Copolymer FAPP/Pyrrole. The final stage of this study was to exploit electrochemical deposition and to entrap glucose oxidase in ferrocene-containing polypyrrole copolymers to create a reagentless glucose sensor in a single operation. Glucose oxidase was deposited in the redox copolymer FAPP/pyrrole from a solution containing 10 mM sodium phosphate buffer pH 5.6, 0.1 M sodium perchlorate, 1 mM pyrrole, 1 mM FAPP, and 200 U/mL enzyme by cycling the working electrode between 0 and +0.8 V at 5 $mV \cdot s^{-1}$. Cyclic voltammograms of the resulting electrode after thorough washing with fresh electrolyte were recorded in 50 mM sodium phosphate buffer, pH 5.6, containing 0.1 M sodium perchlorate. The electrode was cycled between 0 and +0.5 V at scan speeds in the range 5–100 $V \cdot s^{-1}$ in the presence and absence of 50 mM glucose. Figure 4 shows the increase in anodic, and decrease in cathodic currents, on addition of glucose are reminiscent of the effects observed with added soluble enzyme (Figure 3). At low scan speeds (<10 $mV \cdot s^{-1}$),

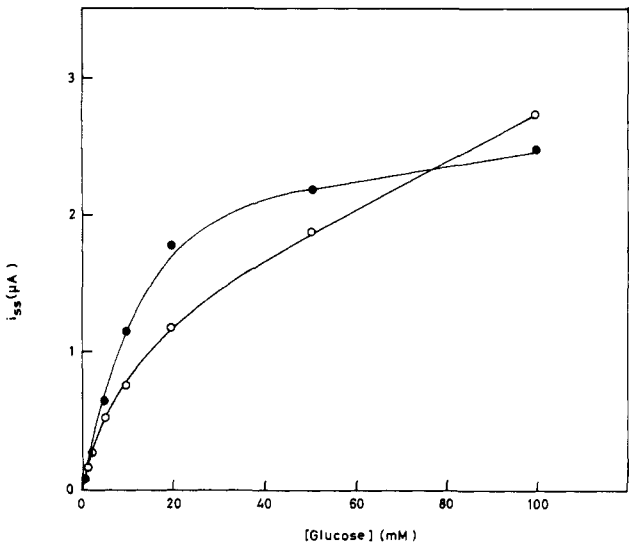

Figure 5. Steady-state current response of the FAPP/pyrrole copolymer entrapped glucose oxidase electrode to glucose. Steady-state current (i_{ss}) responses to additions of glucose (1–100 mM final cell concentration) recorded at an applied potential of +0.3 V (vs Ag/AgCl) in nitrogen-saturated (O) and air-saturated (●) 0.1 M sodium phosphate buffer, pH 5.6, containing 0.1 M sodium perchlorate.

the cathodic peak disappears altogether and the anodic peak is replaced by a broad plateau. These observations indicate regeneration of the polymer-immobilized ferrocene by the entrapped enzyme. The catalytic efficiency (i_k/i_d) of this coimmobilized system at the slow experimental time scale of 10 $mV \cdot s^{-1}$ was 5.3 compared to 7.2–7.3 for poly(FAPP) and poly(pyrrole/FAPP; 1:1 molar ratio) with soluble enzyme and 16 for the homogeneous reaction with soluble FAPP and soluble enzyme. The reduced catalytic efficiency of the coimmobilized enzyme/mediator system probably reflects the extra steric hindrance created by the matrix environment. Thus, in addition to the fact that the mediator may not be orientated in such a way as to promote efficient electron transfer, the entrapped enzyme probably has reduced degrees of diffusional mobility within the polymer matrix.

The redox polymer-entrapped glucose oxidase electrode was operated in the steady state at an applied potential of +0.3 V, the potential at which the enhanced anodic current reached a plateau. Figure 5 shows the steady-state current response of the electrode in nitrogen (O_2-free)- and air-saturated phosphate-buffered perchlorate solution to additions of glucose resulting in final cell concentrations of 1–100 mM. The response was dependent on the dissolved oxygen concentration with steady-state currents at 5, 10, 30, and 50 mM glucose in air-saturated phosphate-buffered perchlorate being 25%, 41%, 38%, and 17%, respectively, higher than that in nitrogen-saturated electrolyte. At glucose concentrations greater than 80 mM the steady-state current response was slightly less in air-saturated electrolyte than in nitrogen-saturated buffered perchlorate, presumably because of the inhibitory effects of high concentrations of H_2O_2 produced within the microenvironment of the entrapped enzyme.

The sensitivity of the redox polymer-entrapped electrode to oxygen contrasts with other ferrocene-mediated glucose electrodes (*12–15*) in which ferrocene was present as a noncovalently bound species, immobilized at the electrode surface by virtue of its low solubility in aqueous solution. Two features may explain why the present reagentless electrode exhibits a greater response in aerated buffer than in nitrogen-saturated buffer: first, the operating potential of +300 mV (vs Ag/AgCl) used in the present study to encourage electron exchange between the reduced enzyme and the immobilized ferricinium ion is sufficiently oxidizing to reoxidize H_2O_2 produced extraneously by the enzyme either at the underlying Pt electrode or, conceivably, on the polymer itself. In contrast, the operating potential of +160 mV (vs SCE) reported by Cass et al. (*15*) is too low to oxidize H_2O_2 on the underlying graphite electrode (*27*). A second factor that probably contributes to the oxygen dependence of the response to glucose of the present electrode is the fact that reaction of the reduced enzyme with a freely diffusing ferricinium species is much faster than the reaction of the enzyme with the polymeric ferrocene species. It is likely, therefore, that the oxygen dependence of the redox polymer-entrapped enzyme electrode is due partly to the slow reaction of glucose oxidase with the polymeric ferrocene with the consequent production of H_2O_2 due to competitive reduction of O_2 and the fact that enzymically generated H_2O_2 is readily oxidized at the anodic operating potential used. In principle, the sensitivity of the present electrode to oxygen could be reduced by synthesizing other ferrocene–pyrrole conjugates containing ferrocene analogues with less anodic redox potentials (*5*).

Another problem that would limit the utility of the present electrode is the lability of the mediation reaction. After two days of use, the electrodes failed to show any response to glucose in the absence of oxygen, although enzyme activity was still detectable by spectrophotometric assay and the electrodes could be operated in an amperometric mode in aerated buffer. It is likely that physical or chemical modifications occurring on aging of the polymer films may account for this instability.

CONCLUSIONS

The use of conducting organic polymers such as polypyrrole offers an elegant procedure for electrodepositing enzymes on an electrode surface (*9–11*). In addition, this report demonstrates that the simplicity of the anodic polymerization of pyrrole may be combined with the well-behaved electrochemistry of mediators such as ferrocene (*12–14, 18*) in order to electroentrap enzymes into "tailor-made" polypyrrole matrices bearing redox functionalities that will accept electrons from the reduced form of the enzyme. However, while this report provides "proof-of-concept", it has not addressed problems of oxygen sensitivity and long-term stability of the coimmobilized system. Nevertheless, the technique of enzyme deposition within redox modified polymers warrants further investigation as a means of producing prototype reagentless enzyme electrodes in one simple anodic electrodeposition step. Furthermore, this approach may circumvent the toxicity problems associated with leaching mediators when electrode formats in which the mediator is not covalently bound to the electrode surface are used in vivo (*15*). Finally, the approach should also display considerable potential for localizing enzyme systems on defined areas of electrode arrays and for exploiting multistep enzyme systems in predefined geometries.

ACKNOWLEDGMENT

The authors acknowledge the helpful advice of Dr. Bernadette Yon Hin throughout the course of this work.

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RECEIVED for review September 9, 1987. Accepted August 10, 1988. The authors acknowledge the Science and Engineering Research Council for financial support.

Micellar Induced Simultaneous Enhancement of Fluorescence and Thermal Lensing

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Micelles have been found to simultaneously enhance the fluorescence and thermal lens of solubilized substrates. From the studies of effects of various types of micelles on the fluorescence and thermal lens signals of the anthracene derivatives, it was found that the enhancement depends on the type of micelles and the structure of the substrate. Highest enhancement was achieved when there were strong interactions between them. For instance, the enhancement in the fluorescence and thermal lens of the 11-(9-anthroyloxy)undecanoic acid, whose long hydrocarbon tail renders strong interaction with micelles, specifically nonionic micelles, is about 5.5 and 3.7 times more than that for anthracene. An enhancement mechanism has been proposed in which the fluorescence is enhanced because micelles isolate and protect the analayte from quencher molecules as well as increase the viscosity of the medium. The thermal lens enhancement, on the other hand, is due to the modification of the thermooptical properties of water by micelles, namely, increase dn/dT and decrease thermal conductivity.

Fluorescence and thermal lens are two of the most sensitive techniques in trace chemical analysis. The fluorescence technique is based on the measurement of emitted photons of an excited analyte while the thermal lens technique is based on the measurement of the heat generated by the nonradiative relaxation. The two techniques are thus complementary and have been used to determine fluorescent as well as nonfluorescent substances.

Fluorescence can be enhanced by increasing the radiative processes of a molecule. In particular, isolation of the fluorescence molecule from quenching impurities and/or solvent molecules can be used to achieve this enhancement (*1*, *2*).

The thermal lens technique is based on the measurement of the temperature rise that is produced in an illuminated sample by nonradiative relaxation of the energy absorbed from a laser (*3*-*7*). Thus, its intensity can be enhanced by improving the nonradiative relaxation processes of the analyte and more significantly the thermooptical properties of the solvent. It has been shown that higher sensitivity can be achieved by choosing solvents with a high temperature coefficient of the index of refraction, dn/dT, and low thermal conductivity, k, value. Generally, water is the worst medium for the thermal lens technique owing to its low dn/dT and high k value. Nonpolar solvents such as carbon tetrachloride and hydrocarbons are good thermooptical solvents because they have high dn/dT and low k values. At the same excitation laser intensity, thermal lens measurements in *n*-pentane and CCl_4 are estimated to be 40 to 47 times more sensitive than those in water (*7*). It is thus possible to enhance the fluorescence as well as thermal lens signals of an analyte by improving its radiative relaxation processes and performing the thermal lens measurement in a solvent that has high dn/dT and low k values (*7*).

Surfactant organized assemblies such as micelles, reversed micelles, and microemulsions have been used extensively in recent years to enhance the fluorescence and to facilitate the measurement of phosphorescence at room temperature (*8*-*18*). These micellar effects are possible because of the unique characteristics of the surfactant-organized media such as their ability to compartmentalize the analyte. The radiative relaxation processes of the analyte are modified when it is solubilized in the micellar system because the micelles not only protect the analyte from quenching molecules but also modify the physical properties of the environment around it, i.e., viscosity, polarity, etc. It is possible to use micelles to simultaneously enhance the fluorescence as well as thermal lens of the analyte. The micelles enhance the fluorescence because they protect the analyte from quenching molecules. The thermal lens is enhanced because micelles modify the environment around the analyte from thermooptically poor water to thermooptically good hydrocarbon. In spite of its great potential, micelles have not been fully used to simultaneously enhance the sensitivity of these two techniques. All but one study has been reported but the reported work was based on the enhancement by reversed micelles (*7*).

Such considerations prompted the present study, which aims to investigate the effect of aqueous micelles on the fluorescence and thermal lens of solubilized analyte. It will be demonstrated in this communication that micelles can simultaneously enhance the fluorescence as well as the thermal

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