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Ferrocene-Modified Siloxane Polymers as Electron Relay Systems in Amperometric Glucose Sensors

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This paper describes the design and testing of amperometric glucose sensors in which electrical communication between the flavin redox centers of glucose oxidase and an electrode is achieved via a network of donor-acceptor relays chemically bound to a flexible siloxane polymer backbone. Procedures are discussed for the synthesis of a variety of mediator(ferrocene)-containing siloxane copolymers which differ in flexibility and in the spacing between redox moieties, and for the optimization of the interaction between the flavin redox centers of glucose oxidase and the polymer-bound electron transfer relay system through investigation of these new flexible polymers in glucose sensors.

INTRODUCTION

Amperometric glucose electrodes based on glucose oxidase undergo several chemical or electrochemical steps which produce a measurable current that is related to the glucose concentration. In the initial step, glucose converts the oxidized flavin adenine dinucleotide (FAD) center of the enzyme into its reduced form ($FADH_2$). Because these redox centers are located well within the enzyme molecule, direct electron transfer to the surface of a conventional electrode does not occur to any measurable degree. A common method[1] of indirectly measuring the amount of

reduced glucose oxidase, and hence the amount of glucose present, relies on the natural enzymatic reaction:

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconolactone} + H_2O_2$$

where oxygen is the electron acceptor for glucose oxidase. The oxygen is reduced by the $FADH_2$ to hydrogen peroxide, which may then diffuse out of the enzyme and be detected electrochemically. The working potential of such a device is quite high (H_2O_2 is oxidized at approximately +0.7V vs. the normal hydrogen electrode), however, and the sensor is therefore highly sensitive to many interfering electroactive species; H_2O_2 is also known to have a detrimental effect on glucose oxidase activity. Alternatively, one could use the electrode to measure the change in oxygen concentration that occurs during the above reaction. In both of these measuring schemes, this type of sensor has the considerable disadvantage of being extremely sensitive to the ambient concentration of O_2.

In recent years, systems have been developed which use a non-physiological redox couple to shuttle electrons between the $FADH_2$ and the electrode by the following mechanism:

$$\text{glucose} + \text{GO(FAD)} \rightarrow \text{gluconolactone} + \text{GO}(FADH_2)$$

$$\text{GO}(FADH_2) + 2M_{ox} \rightarrow \text{GO(FAD)} + 2M_{red} + 2H^+$$

$$2M_{red} \rightarrow 2M_{ox} + 2e^- \text{ (at the electrode).}$$

In this scheme, GO(FAD) represents the oxidized form of glucose oxidase and $GO(FADH_2)$ refers to the reduced form. The mediating species M_{ox}/M_{red} is assumed to be a one-electron couple. Sensors based on derivatives of the ferrocene/ferricinium redox couple,[2–4] and on electrodes consisting of organic conducting salts such as TTF-TCNQ (tetrathiafulvalene-tetracyanoquinodimethane)[5–9] have recently been reported. In potential clinical applications, however, sensors based on electron-shuttling redox couples suffer from an inherent drawback: the soluble, or partially soluble, mediating species can diffuse away from the electrode surface into the bulk solution, which would preclude the use of these devices as implantable probes.

With this in mind, several research groups have been investigating systems where the mediating species is chemically bound in a manner which allows close contact between the $FAD/FADH_2$ centers of the enzyme and the mediator, yet prevents the latter from diffusing away from the electrode surface. For instance, Degani and Heller[10,11] have designed an electron transfer relay system where the mediating species are chemically attached to the enzyme itself. In this scheme, however, the chemical modification of the enzyme can cause a measurable decrease in its activity. More recently, these studies have been extended to include systems where the mediating redox moieties are covalently attached to polymers such as poly(pyrrole),[12] poly(vinylpyridine),[13] and poly(siloxane).[14–18] In this paper, we report the design

and response of amperometric glucose sensors based on this latter family of polymeric mediators.

EXPERIMENTAL

Reagents. Glucose oxidase (EC 1.1.3.4, type VII, 129 units/mg) was obtained from Sigma (St. Louis, MO). The methylhydrosiloxane homopolymer and methylhydro(dimethyl)siloxane copolymers were obtained from Petrarch Systems (Bristol, PA). Vinylferrocene was obtained from Aldrich (Milwaukee, WI). Graphite powder (product no. 50870) and paraffin oil (product no. 76235) were obtained from Fluka (Ronkonkoma, NY). Glucose (Sigma, cat. no. G-5250) solutions were prepared by dissolving appropriate amounts in 0.1M phosphate/0.1M KCl buffer (pH 7.0); the glucose was allowed to reach mutarotational equilibrium before use (ca. 24 h). All other chemicals were reagent grade and were used as received.

Polymer synthesis. Methyl,β-ferrocenylethyl-siloxane polymers were prepared by the hydrosilylation of vinylferrocene with the methylhydrosiloxane homopolymer or the methylhydrosiloxane dimethylsiloxane copolymers (m:n ratios of 1:1, 1:2, and 1:7.5; see Figure 1) in the presence of chloroplatinic acid as a catalyst.[16] Purification of the polymers was achieved by reprecipitation from chloroform solution, via dropwise addition into a large excess amount of acetonitrile at room temperature. This reprecipitation was repeated 2–3 times to ensure that no low molecular weight species (which could act as freely diffusing electron transfer mediators) were present. Thin layer chromatography and high-performance liquid chromatography showed that no oligomeric materials were present in the purified materials.

Electrode construction. The modified carbon paste for the sensors was made by thoroughly mixing 50mg of graphite powder with a measured amount of the ferrocene-containing polymer (the latter was first dissolved in chloroform); in the present work, the molar amount of the ferrocene moiety was the same for all electrodes (36μmole of ferrocene per gram of graphite powder). After evaporation of the solvent, 5mg of glucose oxidase (129 units/mg) and 10μl of paraffin oil were added, and the resulting mixture was blended into a paste. The paste was packed into a 1.0ml plastic syringe which had previously been partially filled with unmodified carbon paste, leaving approximately a 2mm deep well at the base of the syringe. The resulting surface area of the electrode was 0.025cm^2. Electrical contact was achieved by inserting a silver wire into the top of the carbon paste.

Electrochemical methods. Cyclic voltammetry and constant potential measurements were performed using a Princeton Applied Research Potentiostat (Model 173) and a Universal Programmer (Model 175). All experiments were carried out in a conventional electrochemical cell containing pH 7.0 phosphate buffer with 0.1M KCl at 23(±2)°C. All experimental solutions were deaerated by bubbling N_2 through the solution for at least 10 min; in the constant potential experiments, a gentle flow of N_2 was also used to facilitate stirring. In addition to the modified

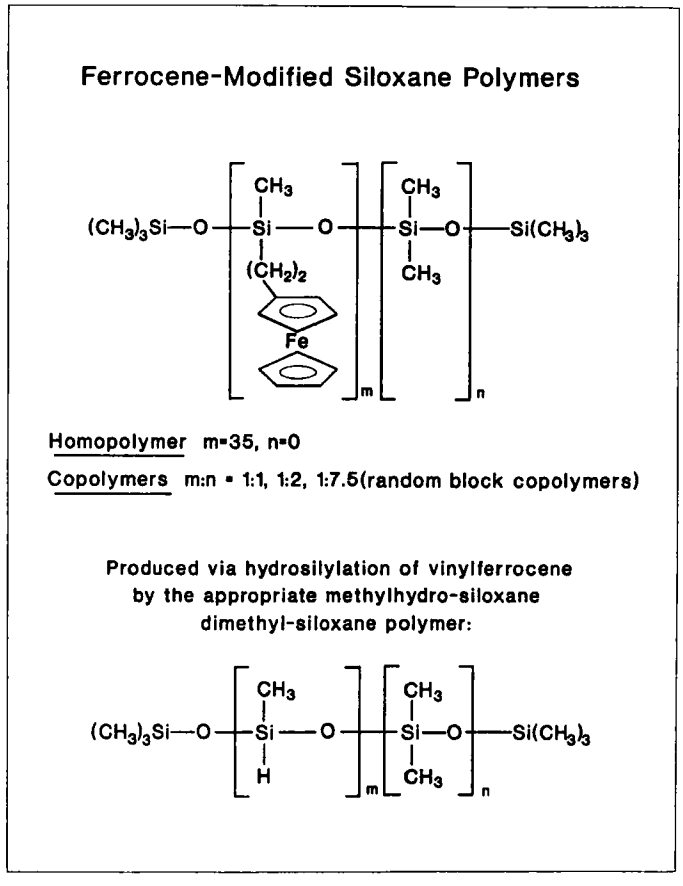

FIGURE 1 Schematic diagram of the ferrocene-containing siloxane polymers used as electron transfer mediators in glucose oxidase-modified carbon paste electrodes.

carbon paste working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode were employed. In the constant potential experiments, the background current was allowed to decay to a constant value before samples of a stock glucose solution were added to the buffer solution. A constant background current was attained approximately 10 min after application of the potential.

RESULTS AND DISCUSSION

Cyclic voltammetry. Figure 2 shows typical voltammetric results for a carbon paste electrode containing glucose oxidase and the ferrocene-modified siloxane 1:2 copolymer as the electron relay system. With no glucose present, the voltammogram displays very low anodic currents; that due to the oxidation of the polymer-bound ferrocene moieties is too small to be seen. Upon addition of glucose, however, the

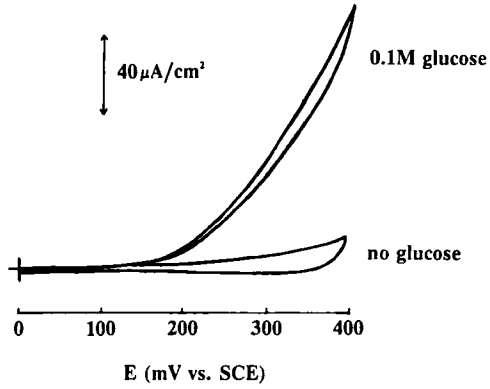

FIGURE 2 Cyclic voltammograms for the ferrocene-modified polysiloxane/glucose oxidase/carbon paste electrodes (scan rate: 5mV/s) in pH 7.0 phosphate buffer (with 0.1M KCl) solution with no glucose present and in the presence of 0.1M glucose. The electrode contained the 1:2 copolymer as the electron relay system (see Figure 1).

voltammetry changes dramatically, with a large increase in the oxidation current and no increase in the reduction current. The fact that the reduction current does not increase along with the oxidation current is indicative of the enzyme-dependent catalytic reduction of the ferricinium ion produced at oxidizing potential values. Upon comparison of the voltammograms with and without glucose present, it is apparent that the ferrocene-containing siloxane polymer can act as an efficient electron transfer relay system between the $FAD/FADH_2$ centers of glucose oxidase and a carbon paste electrode. Voltammograms made with carbon paste electrodes containing only glucose oxidase with no polymeric relay system do not display this catalytic behavior.

Constant potential measurements. A typical glucose response trace (current vs. time) is shown in Figure 3 for a carbon paste electrode containing glucose oxidase and the ferrocene-modified siloxane 1:2 copolymer as mediator. This trace clearly shows the rapid response and good sensitivity of the sensor to clinically relevant glucose concentrations. The time response of sensors containing the ferrocene-modified siloxane homopolymer or the other copolymers is similar to that shown in Figure 3, although the magnitude of the response does vary. This variation in response is apparent from the glucose calibration curves shown in Figure 4 for sensors containing each of the ferrocene-siloxane polymers in Figure 1. As described above, the molar amount of the ferrocene moiety is the same for each sensor.

As mentioned above, siloxane polymers are known to be extremely flexible.[19] This flexibility will, of course, be sensitive to the amount of side-chain substitution present along the polymer backbone. For instance, in the homopolymer used in these studies, the presence of a ferrocenylethyl moiety bound to each silicon subunit should provide a degree of steric hindrance, and thus a barrier to rotation about the siloxane backbone. The importance of this feature can be studied by comparing the mediating ability of this polymer with that of the various copolymers, as in Figure 4. By, in effect, systematically replacing a fraction of the ferrocenylethyl

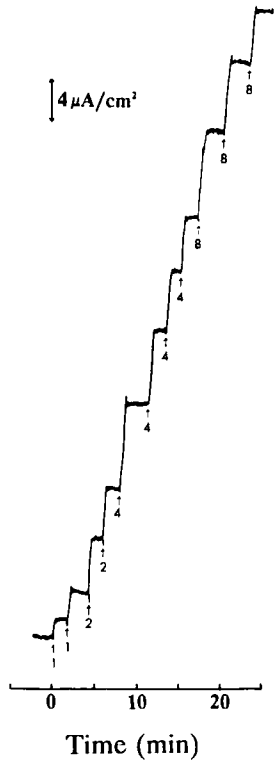

FIGURE 3 Typical response of the ferrocene-modified polysiloxane/glucose oxidase/carbon paste electrode to addition of glucose at E = +300mV (vs. SCE). The electrode contained the 1:2 copolymer. Indicated are the number of 100μl aliquots of 0.1M glucose added to the test solution (initial solution volume: 10ml).

groups with methyl groups, it is possible to adjust the flexibility of the polymer as well as the average spacing between the redox sites. From the glucose calibration curves, it is apparent that these factors play a key role in the interaction between the polymeric relay system and the FAD redox centers in glucose oxidase. The results of the electrochemical measurements in Figure 4 show an enhanced sensitivity to glucose for the electrodes containing the 1:1 and 1:2 copolymers. On the other hand, when the average spacing between the electron relays becomes very large, as in the case of the 1:7.5 copolymer, the increased polymer flexibility does not result in an increase in the measured catalytic current. Since the total molar amount of the mediating species is kept constant in each electrode (the copolymers have fewer redox sites per mole, so more material is necessary), then a change in the constant potential experiments can be attributed to a change in the interaction between the enzyme and the polymeric relay system. These results demonstrate the possibility of systematically increasing this interaction in order to optimize the response of the sensor.

Conclusions. The four polymeric relay systems described in this work effectively mediate the electron transfer from reduced glucose oxidase to a conventional carbon

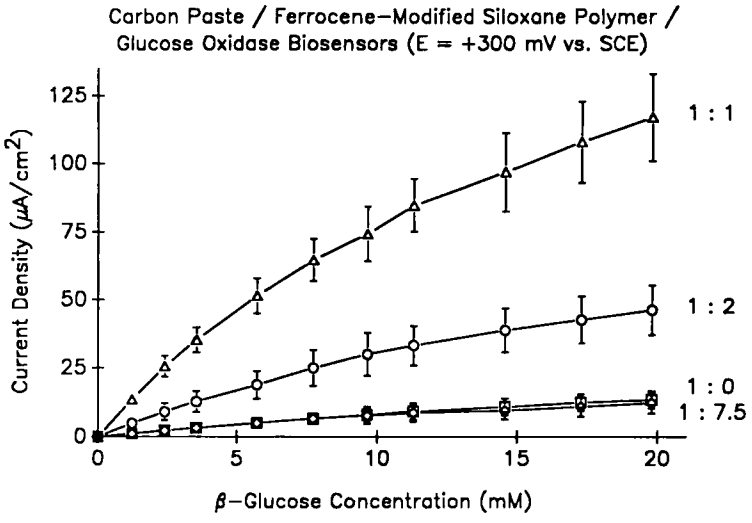

FIGURE 4 Glucose calibration curves for the ferrocene-modified polysiloxane/glucose oxidase/carbon paste electrodes at E = +300mV (vs. SCE). The m:n ratios (see Figure 1) are indicated next to each curve; 1:0 refers to the homopolymer. Each curve is the mean result for three electrodes.

paste electrode. It is clear from the glucose calibration curves that the response of the sensors can be optimized through systematic changes in the polymeric backbone. The optimal electron relay system appears to consist of a compromise between an increased polymer flexibility and a decreased spacing between the individual relay sites. These results, as well as those described previously,[14,17,18] show that the mediating ability of the polymers is quite general; work is presently underway to extend these studies to other flavoenzyme systems.

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