defined by the remaining five ring atoms. In **2** the corresponding displacement is 0.62 Å. This type of distortion is attributed to the presence of the transannular ferrocenyl unit.[11]

The coordination of the lithium atom to N(3) in the solid state is consistent with a significant amount of the negative charge in **3** residing on the skeletal nitrogen atoms adjacent to P(3). Interestingly, the NMR spectra for **3** (see above) show that N(3) and N(2) are equivalent, which indicates that the lithium ion either dissociates from N(3) or fluctuates rapidly between N(3) and N(2) in solution at room temperature. These possibilities, together with the reactivity and mechanism of formation of **3**, are under investigation.

Acknowledgment. We thank the U.S. Army Research Office for financial support.

Supplementary Material Available: An ORTEP drawing of **3** and tables of positional and displacement parameters and bond distances and angles (13 pages). Ordering information is given on any current masthead page.

A New Class of Amperometric Biosensor Incorporating a Polymeric Electron-Transfer Mediator

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Typical amperometric glucose electrodes based on glucose oxidase undergo several chemical or electrochemical steps which produce a measurable current that is linearly 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 of facilitating this electron transfer is to introduce oxygen into the system since it is the natural 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. Alternatively, one may use a nonphysiological redox couple to shuttle electrons between the $FADH_2$ and the electrode. 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 clinical applications, however, sensors based on electron-shuttling redox couples suffer from an inherent drawback: the soluble mediating species can diffuse away from the electrode surface into the bulk solution, which would preclude their use as implantable probes.

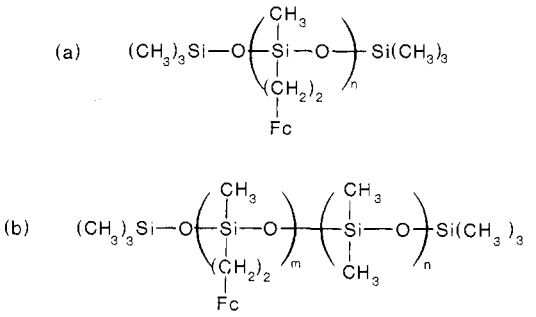

Figure 1. Schematic diagrams of the polymers used in the redox polymer/glucose oxidase/carbon paste electrodes: (a) methyl(ferrocenylethyl)siloxane homopolymer; (b) methyl(ferrocenylethyl)dimethylsiloxane copolymer. Fc refers to $(\eta^5\text{-}C_5H_4)Fe(\eta^5\text{-}C_5H_5)$ or ferrocenyl; n is approximately 35 in the homopolymer; in the copolymer, the m:n ratio is approximately 1:2, with the subunits being randomly distributed (i.e., a random block copolymer).

With this in mind, we have investigated systems where the mediating species is chemically bound to a flexible polymer backbone 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. The resulting electron-transfer relay system acts in a manner similar to that described by Degani and Heller,[10,11] who chemically attached the electron relays to the enzyme itself. In the present work, however, the necessary electrical communication between the $FAD/FADH_2$ centers and the electrode has been achieved without modifying the enzyme, which can cause a measurable decrease in enzyme activity. The polymers used in this study are shown in Figure 1. The synthesis and characterization of these materials are described elsewhere;[12] the molecular weight of these polymeric materials is approximately 4000. 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.

Figure 2 shows typical cyclic voltammetric results for carbon paste electrodes which were modified with each of the polymeric relay systems in Figure 1 and glucose oxidase. Carbon paste has previously been used for amperometric enzyme electrodes with freely diffusing redox mediators.[13,14] With no glucose present in solution, the voltammetry shows a ferrocene oxidation at approximately 0.3 V (vs the saturated calomel electrode, SCE) for a scan rate of 5 mV/s, while the corresponding reduction of the ferricinium ion occurs at 0.2 V; these values are typical for ferrocene derivatives.[2] Upon addition of glucose, the voltammetry changes dramatically, with a large increase in the oxidation current and a slight lowering of 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 polymer-bound ferrocene/ferricinium moieties can act as an efficient electron-transfer relay system between the $FAD/FADH_2$ centers of glucose oxidase and the carbon paste electrode.

In order to test the utility of the redox polymer/glucose oxidase/carbon paste electrodes as analytical probes, the catalytic current was measured at a constant applied potential for a large

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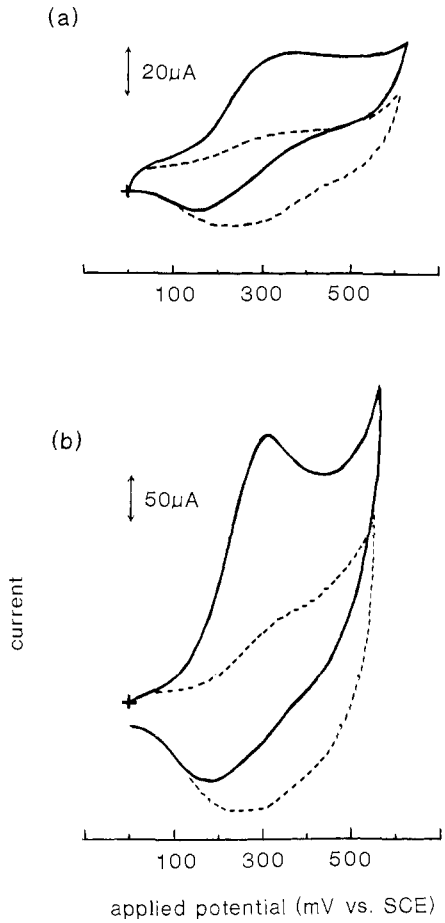

Figure 2. Cyclic voltammograms for the ferrocene-modified polysiloxane/glucose oxidase/carbon paste electrode (scan rate: 5 mV/s) in pH 7.0 phosphate buffer (with 0.1 M KCl) solution with no glucose present (dashed line) and in the presence of 100 mM β-D-glucose (solid line). The electrode in (a) contained the methyl(ferrocenylethyl)siloxane homopolymer shown in Figure 1a, while that in (b) contained the methyl(ferrocenylethyl)-dimethyl (1:2) siloxane copolymer shown in Figure 1b. The electrodes were constructed by thoroughly mixing 100 mg of graphite powder with a measured amount of the ferrocene-containing polymer (the latter was first dissolved in chloroform); the molar amount of the ferrocene moiety was the same for both electrodes. After evaporation of the solvent, 10 mg of glucose oxidase (129 000 units/g) and 20 μL of paraffin oil were added, and the resulting mixture was blended into a paste. The paste was packed into a 2 mm deep well at the base of a glass electrode housing (6 mm inner diameter). The reference electrode was a saturated calomel electrode (SCE), and the auxiliary electrode consisted of a platinum wire. The solutions were deoxygenated with nitrogen prior to each experiment. All measurements were made at 23 (±2) °C. Note the different current scales in (a) and (b).

range of glucose concentrations. Typical values for the variation of the steady-state current density (at +400 mV vs SCE) with glucose concentration are shown in Figure 3. The electrodes are clearly sensitive to small changes in glucose concentration over a clinically useful range of concentrations. In addition, because of the excellent stability of the siloxane polymers and the fact that the ferrocene/ferricinium moieties are not free to diffuse away from the electrode, these sensors display an excellent response over long periods of time: for the sensor containing the homopolymer relay system, the measured response to 10 mM glucose after 2 months is approximately half of the initial value. In contrast, previous glucose sensors based on ferrocene-modified pyrrole polymers[15] failed to respond to glucose after only 2 days of use because of the instability of the polymer films. In the present case, it is also important to note that the decrease in sensor response can be partly attributed to the fact that, as described in Figure

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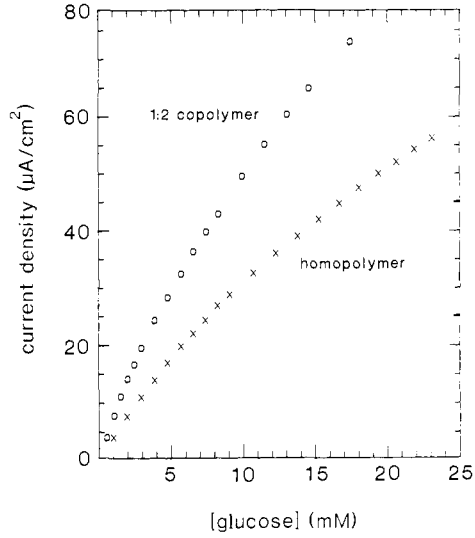

Figure 3. Typical results for the variation of the steady-state current with glucose concentration at +400 mV (vs the SCE) measured with the ferrocene-modified polysiloxane/glucose oxidase/carbon paste electrodes. Results are shown for electrodes containing methyl(ferrocenylethyl)siloxane homopolymer (×) and methyl(ferrocenylethyl)-dimethyl (1:2) siloxane copolymer (○). After application of the potential, the background current was allowed to decay to a constant value before samples of a stock glucose solution were added to the gently stirred buffer solution. Steady-state current values were attained approximately 2 min after each addition. All measurements were made at 23 (±2) °C.

2, the enzyme is physically adsorbed to the electrode material and is free to diffuse out of the sensor. The use of a membrane covering for the electrode or an enzyme immobilization procedure should therefore increase the sensor's lifetime even further.

This work demonstrates that electrical communication between the $FAD/FADH_2$ redox centers of glucose oxidase and a conventional electrode can be facilitated by a network of donor–acceptor relays covalently bound to a siloxane polymer. The unique flexibility of the polysiloxane backbone, which has a very small energy barrier to rotation,[16] allows these relay moieties to interact intimately with the enzyme molecule and achieve a close contact with the $FAD/FADH_2$ centers. This appears to be a vital consideration since, as has been demonstrated in our laboratory and elsewhere,[17] redox polymers such as poly(vinylferrocene) cannot achieve this close contact with the enzyme's redox centers and thus cannot serve as effective electron-transfer relay systems. 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. In the case of the copolymer, the redox species are bound to only one-third of the silicon subunits, resulting in a more flexible electron-transfer relay system. As shown in Figures 2 and 3, for an equimolar amount of the ferrocene species, the copolymer system is more effective at mediating the electron transfer between reduced glucose oxidase and the carbon paste electrode. This demonstrates that the flexibility of the polymeric relay system may indeed be important in enhancing the interaction between the mediating species and the redox centers of the enzyme. This point is presently under investigation in our laboratory using a variety of ferrocene-modified siloxane copolymers as electron-transfer relay systems. While the ferrocene/ferricinium couple is clearly a very effective relay, we are also investigating other redox species within the polysiloxane framework in order to lower the necessary applied potential. Finally, preliminary results indicate that these polymeric relay systems can be used in con-

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junction with other oxidases to further develop this new class of amperometric biosensor.

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1,1-Difluoroalkyl Glucosides: A New Class of Enzyme-Activated Irreversible Inhibitors of α-Glucosidases

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There is presently an upsurge of interest in glycosidase inhibitors. Such compounds have been shown to be important tools in mechanistic studies on glycohydrolases,[1] as well as having promising therapeutic applications.[2] However, only a few examples of enzyme-activated irreversible inhibitors of glycosidases have been described. They include conduritol epoxides,[3] glycosylmethyltriazenes,[4] and the aziridine triol derivatives of piperidine.[5] These compounds are activated in the first protonation step of the enzyme-assisted glycoside hydrolysis. Due to their high intrinsic chemical reactivity, insufficient specificity could limit their use as drugs. Inhibitors activated only after or during enzymic cleavage of the glycosidic bond should be more selective. The first examples of this new class are 2-deoxy-2-fluoroglycosides.[6] These inhibitors are believed to be cleaved before inactivating the enzyme by the formation of a covalent glycosyl–enzyme complex. We now wish to report a novel enzyme-activated irreversible inhibitor of yeast α-glucosidase based on the activation, following hydrolytic cleavage, of the aglycon moiety of the molecule.

α-Glucosidases catalyze the hydrolysis reaction shown in Scheme I where R can be either a glycosidic residue or an aglycon leaving group. Our approach toward irreversible inhibition of α-glucosidase was to design R in such a way that the glucosidase **1** would be a stable substrate, while the alcohol **2** would be spontaneously and rapidly converted into a reactive alkylating agent.

1,1-Difluoroalkyl glucosides **3** (Scheme II) were chosen to illustrate this concept. The α,α-difluoro alcohols **4** (products of the α-glucosidase-catalyzed hydrolysis of **3**) are known[7] to rapidly lose HF and to be transformed into acid fluorides **5**. These acylating agents are then expected to form a covalent adduct **6** with a nucleophilic residue of the enzyme-active site.[8]

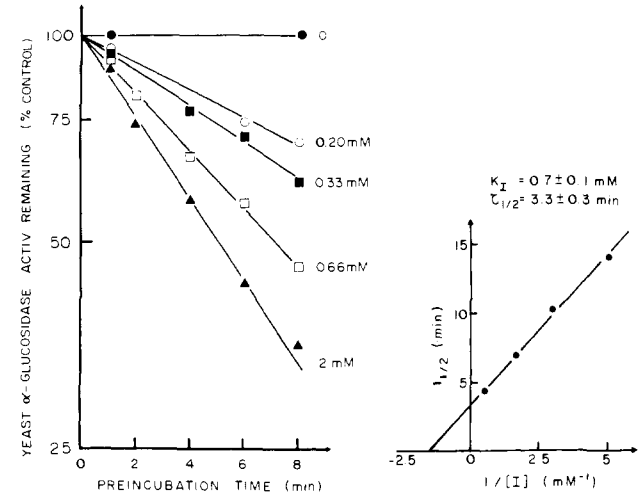
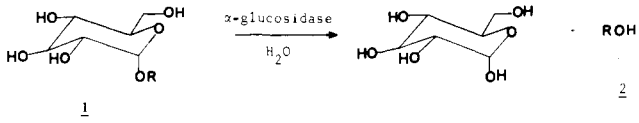
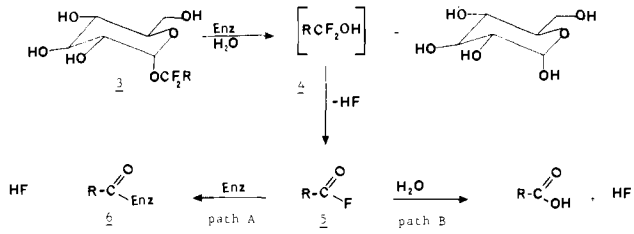


Figure 1. Time- and concentration-dependent inhibition of yeast α-glucosidase by **7**. The enzyme was incubated at 37 °C with 0.2 M phosphate buffer (pH 6.9) and various concentrations of inhibitor. At given time intervals, aliquots were withdrawn and assayed for the remaining activity according to ref 14. In the right-hand part of the figure, the times of half-inactivation ($t_{1/2}$) are plotted against the reciprocal of the **7** concentrations, according to Kitz and Wilson.[15]

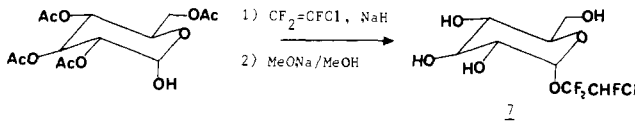
Scheme I



Scheme II



Scheme III



In order to evaluate this concept, we prepared 2-chloro-1,1,2-trifluoroethyl α-D-glucopyranoside **7** (Scheme III) in two steps from 2,3,4,6-tetraacetyl-α-D-glucopyranose.[11] The latter is first regioselectively condensed with trifluorochloroethylene[12] (DMF, −20 °C, 4 h) in the presence of a catalytic amount of NaH; then the acetyl groups are removed (MeONa/MeOH) to give 2-chloro-1,1,2-trifluoroethyl α-D-glucopyranoside as a 3/1 mixture of α/β anomers in 65% overall yield. The two anomers were separated by flash chromatography on silica gel, and compound **7** was isolated in pure (>95%) α-anomeric form as a mixture of 2 epimers.[13]

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