× 50 mL); the combined organic layers are washed with water (93 × 100 mL) and dried over $MgSO_4$. The crude 3-(6-hydroxyhexyl)cyclooctanone obtained after removal of the solvent is used without further purification in the following oxidation.

3-(6-Oxohexyl)cyclooctanone (4).[18] To a solution of 1.9 mL (22 mmol) of oxalyl chloride in 50 mL of dichloromethane is added at −60 °C a solution of 3.1 mL (44 mmol) of DMSO in 10 mL of dichloromethane. After stirring for 10 min at −60 °C, a solution of the crude 3-(6-hydroxyhexyl)cyclooctanone in 20 mL of dichloromethane is added dropwise. The mixture is stirred for another 15 min at −60 °C, followed by addition of 13.9 mL (0.1 mol) of triethylamine. The mixture is warmed up to room temperature, and 100 mL of water is added; the organic layer is separated, and the aqueous layer is extracted with 100 mL of dichloromethane. The combined organic layers are dried over $MgSO_4$, and the solvent is removed in vacuo. To the crude product is added 50 mL of diethyl ether, and the precipitate formed is filtered off; removal of the solvent from the filtrate is followed by Kugelrohr distillation (130–140 °C/0.1 Torr), yielding 2.55 g (57%) of **4** as a colorless liquid: IR (neat) ν = 2935 (vs), 2857 (vs), 2718 (m), 1724 (vs), 1700 (vs), 1466 (s), 1446 (s), 1103 (s) cm^{-1} 250 MHz ^1H NMR δ = 9.67 (t, 1 H, J = 1.8 Hz), 2.4–2.2 (m, 6 H), 2.0–1.2 (m, 17 H); ^{13}C NMR δ = 216.9 (s), 202.4 (d), 47.0 (t), 43.6 (t), 42.8 (t), 37.8 (d), 36.8 (t), 33.2 (t), 29.0 (t), 27.6 (t), 26.7 (t), 24.5 (t), 23.6 (t), 21.8 (t); MS, m/z (%) = 224 (5, M^+), 206 (10), 196 (10), 188 (10), 181 (15), 173 (7), 163 (15), 153 (14), 148 (100), 135 (15), 133 (15), 125 (84), 121 (14), 111 (25), 107 (30), 97 (59), 95 (26), 93 (29), 83 (43), 81 (49), 79 (43), 69 (29), 67 (40), 55 (78); high-resolution mass spectrum (CI, M + H^+) calc. 225.1855, found 225.1848.

Bicyclo[6.5.1]tetradec-1(2)-ene (5).[6] The McMurry reagent is prepared by heating a mixture of 4.62 g (30 mmol) of titanium trichloride and 5.88 g (90 mmol) of a 3% zinc–copper couple in 300 mL of DME to reflux for 4 h. To the refluxing mixture a solution of 750 mg (3.3 mmol) of **4** in 150 mL of DME is added over a period of 24 h; after the addition, reflux is continued for another 2 h. The mixture is cooled to room temperature and filtered through Celite; to the filtrate is added 1 L of water. The aqueous layer is extracted with pentane (4 × 100 mL), and the combined organic layers are dried over $MgSO_4$. Removal of the solvent is followed by Kugelrohr distillation (80–90 °C/0.1 Torr), furnishing 243 mg (38%) of **5** as a colorless liquid. The in- and out-isomers can be separated by column chromatography on $AgNO_3$-impregnated silica gel with ethyl acetate/pentane = 1:100 as eluent: IR (neat) ν = 3033 (w), 2921 (vs), 2857 (s), 1481 (m), 1456 (m) cm^{-1}; 500 MHz ^1H NMR out-**5**, δ = 5.41 (dt, 1 H, J = 0.8/8.1 Hz), 2.61 (dd, 1 H, J = 3.6/14.0 Hz), 2.26–2.14 (m, 5 H), 1.96 (m, 1 H), 1.75–1.23 (m, 16 H); in-**5**, δ = 5.02 (t, 1 H, J = 8.0 Hz), 2.54–2.48 (m, 2 H), 2.35 (dd, 1 H, J = 8.2/14.0 Hz), 2.17 (t, 1 H, J = 13.3 Hz), 2.02–1.30 (m, 18 H), 1.22 (m, 1 H); ^{13}C NMR out-**5**, δ = 138.7 (s), 127.4 (d), 39.5 (t), 37.8 (d), 31.2 (t), 31.0 (t), 30.6 (t), 29.7 (t), 26.8 (t), 26.0 (t), 25.4 (t), 25.1 (t), 24.5 (t), 23.6 (t); in-**5**: δ = 145.6 (s), 120.5 (d), 40.1 (t), 38.4 (t), 36.6 t), 35.8 (d), 30.0 (t), 29.8 (t), 28.1 (t), 27.7 (t), 26.2 (t), 23.5 (t), 20.3 (t), 19.0 (t); MS, m/z (%) = 192 (15, M^+), 149 (10), 135 (20), 121 (49), 110 (21), 107 (17), 95 (49), 93 (38), 81 (77), 79 (56), 67 (63), 65 (12), 55 (20), 53 (18), 43 (100), 41 (99); high-resolution mass spectrum calc 192.1878, found 192.1866.

***in,out*-Bicyclo[6.5.1]tetradecane (*in,out*-6).** A mixture of 43 mg (0.22 mmol) of in-**5** and 5 mg of 5% rhodium on charcoal in 10 mL of diethyl ether is hydrogenated at 50 psi for 1 day. The catalyst is filtered off, and the solvent is removed in vacuo; the crude product is purified by Kugelrohr distillation 80–90 °C/0.1 Torr), yielding 40 mg (92%) of *in,out*-**6** as a colorless liquid: IR (neat) λ = 2919 (vs), 2848 (s), 1477 (s), 1450 (m) cm^{-1}; 500 MHz ^1H NMR δ = 1.,80–1.16 (m, 24 H), 1.06 (m, 1 H), 0.90 (m, 1 H); ^{13}C NMR δ = 39.2 (t), 37.3 (t), 37.3 (d), 31.0 (t), 28.6 (t), 28.5 (t), 27.7 (t), 27.5 (d), 27.3 (t), 24.1 (t), 24.1 (t), 21.0 (t), 20.3 (t), 19.9 (t); MS, m/z (%) = 194 (10, M^+), 166 (35), 152 (8), 137 (8), 123 (28), 110 (60), 96 (100), 81 (83), 79 (16), 69 (24), 67 (51), 55 (36), 41 (27); high-resolution mass spectrum calc 194.2035, found 194.2032.

***out,out*- and *in,in*-Bicyclo[6.5.1]tetradecane (*out,out*- and *in,in*-6).** A mixture of 49 mg (0.25 mmol) of *out*-**5** and 5 mg of 5% rhodium on charcoal in 10 mL of diethyl ether is hydrogenated at 50 psi for 6 days. The catalyst is filtered off, the solvent is removed in vacuo, and the crude product is purified by Kugelrohr distillation (80–90 °C/0.1 Torr), furnishing 45 mg (91%) of a mixture of *in,out*-**6** (ca. 50%), *out,out*-**6** (ca. 40%), and *in,in*-**6** (ca. 10%) as a colorless liquid: IR and MS, see in,-out-**6**; 500 MHz ^1H NMR *out,out*-**6**, δ = 1.92 (m, 1 H), 1.80–1.20 (m, 25 H); ^{13}C NMR *out,out*-**6**, δ = 41.9 (d), 35.9 (t), 35.2 (t), 32.4 (t), 29.6 (t), 29.1 (t), 26.3 (t), 24.4 (t); *in,in*-**6** δ = 43.1 (d), 40.7 (t), 37.2 (t), 32.7 (t), 26.0 (t), 23.5 (t).

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Microporous Aluminum Oxide Films at Electrodes. 7. Mediation of the Catalytic Activity of Glucose Oxidase via Lateral Diffusion of a Ferrocene Amphiphile in a Bilayer Assembly

Christian Bourdillon*,† and Marcin Majda*

Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received August 14, 1989*

Abstract: Glucose oxidase was immobilized in the head group region of an organized bilayer self-assembled in a porous template of aluminum oxide film at a gold electrode. Immobilization of the enzyme involved interactions with the (ferrocenylmethyl)dimethyloctadecylammonium amphiphile, which forms the outer monolayer of the bilayer assembly. The same amphiphile mediates electrooxidation of glucose oxidase via lateral diffusion along the bilayer between the enzyme and the electrode surface. The enzyme immobilization does not restrict electroactivity of the ferrocene surfactant but it appears to bind ca. 60% of its population and thus restricts its participation in the electron transport process. These data lead to a postulate that the enzyme–surfactant interactions involve a much larger number of the ferrocene surfactant molecules than expected from the consideration of just electrostatic interactions.

Formation of organized molecular assemblies designed to perform a specific function is one of the emerging major goals in electrochemical research and other areas of chemistry.[1] Electrocatalytic systems are just one class of such assemblies. Their chemical composition often requires several components: catalyst, electron transfer mediator, and the structural components of a supporting matrix.[2] The structural design of such systems

† Permanent address: Laboratoire de Technologie Enzymatique, Universite de Compiegne, BP 649, 60206 Compiegne, France.

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presents several challenges. Appropriate local for all the components must be provided to comply with the dynamic demands of the electrocatalytic processes.[2c,d] The transport of reagents and products through an electrode assembly has to be efficient. The electron and ion transport related to the overall electrolysis need also be sufficiently rapid to best take advantage of the chemical characteristics of the catalyst.[2b]

In this paper, we present the design and dynamic properties of an organized electrocatalytic assembly which consists of glucose oxidase (GOd) immobilized in the head group region of an organized amphiphilic bilayer. The catalytic activity of the enzyme is coupled to the electrode surface through a cationic ferrocene amphiphile which is a major component of the bilayer. The idea that we demonstrate in this project involves a synergistic action of the ferrocene amphiphile which binds the enzyme and also serves as an electron transfer mediator via lateral diffusion along the bilayer assembly.

Redox enzymes such as GOd cannot exchange electrons directly at the electrode surface and thus their usefulness in the design of electroenzymatic processes and amperometric sensors depends on success in developing means of coupling the catalytic activity of an enzyme with the electrode surface via redox mediators. Some recent strategies in the development of integrated enzyme/mediator systems for the electrocatalytic oxidation of glucose involve various modes of direct immobilization of the enzyme and its mediators at the electrode surface,[3] entrapment of the enzyme in a conducting polymer,[4] and chemical modification of GOd by covalent attachment/insertion of redox mediators.[5] Also, Degani and Heller showed recently that the FAD/$FADH_2$ redox centers of glucose oxidase can be addressed by cationic redox polymers electrostatically or covalently bound to the enzyme.[6] This approach simulates the natural binding of the oxidoreductase enzymes with the electron transfer proteins, which act as biological electron transport relays.

The strategy presented here relies on our ability to produce organized bilayer assemblies in a microporous aluminum oxide template at a gold electrode described previously.[7] In this initial report, we show that the immobilization of GOd is stable over a period of at least 48 h and that it involves interactions with a large number of the ferrocene surfactant molecules (ca. 10^2), which probably aggregate around the enzyme rather than reside within the bilayer structure. Our results indicate that all of the ferrocene surfactant molecules remain electroactive following the binding of the enzyme. However, only the molecules not associated with the enzyme take part in the electron transport process. The steady-state catalytic electrooxidation of glucose is controlled by either the electron transport kinetics or the kinetics of the mediation reaction.

Experimental Section

Reagents. (Ferrocenylmethyl)dimethyloctadecylammonium chloride ($C_{18}Fc^+$) was synthesized according to a literature procedure.[8] Its characterization in a bilayer assembly was also described earlier.[7a] Octadecyltrichlorosilane (OTS) (Petrarch Systems Inc.) was vacuumed-distilled into glass ampules that were opened immediately prior to use. Glucose oxidase (EC 1.1.3.4) (Type VII) was obtained from Sigma Chemical Co. All other chemicals were reagent grade.

Porous Aluminum Oxide Coated Disk Electrodes. The aluminum oxide films were produced by electrooxidation of Al foil (0.01 cm thick, 99.997% purity, Johnson Matthey, Materials Technology, U.K.) in 4% phosphoric acid. All the procedures involved in their formation, detachment from the aluminum substrates, barrier layer dissolution, characterization, and the fabrication of electrodes were described in our earlier report.[9] The Al_2O_3 films were 2.5 to 3.5 μm thick with an average pore diameter of 80 nm. The assembly of the OTS monolayer preceded the gold vapor deposition and the fabrication of electrodes as described earlier.[7a] The geometric area of the disk electrodes was 0.071 cm^2. The internal surface area of the aluminum oxide films was 16 ± 15% times the geometric area of the underlying electrode surface per 1 μm of the oxide film thickness.[7a,9]

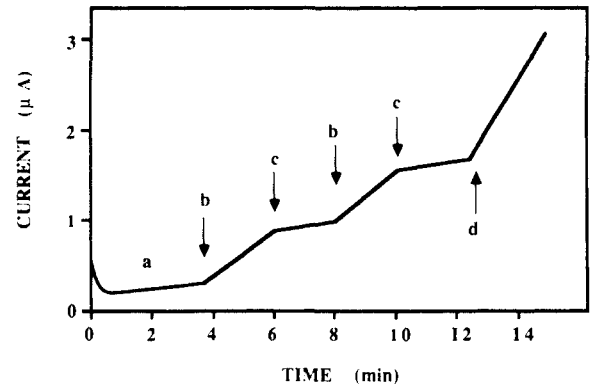

Figure 1. Typical amperometric data obtained during the electrochemical assay of glucose oxidase immobilized in a OTS/$C_{18}Fc^+$ bilayer assembly of a porous aluminum oxide coated gold electrode (0.07 cm^2 Au electrode coated with 2.5 μm thick oxide film). The current corresponds to the electrooxidation of hydroquinone produced in reaction 1 at a rotating glassy carbon disk electrode (see Experimental Section for details): (a) background level; (b) immersion of a Au/Al_2O_3/OTS/$C_{18}Fc^+$/GOd electrode into the test solution; (c) withdrawal of the Au/Al_2O_3/OTS/$C_{18}Fc^+$/GOd electrode from the test solution; (d) addition of 50 μL of standard GOd solution.

Immobilization of Glucose Oxidase. Gold electrodes coated with OTS treated porous aluminum oxide films (Au/Al_2O_3/OTS) were first rinsed with methanol, followed by 0.1 M phosphate buffer, and exposed for 15 min to a 5×10^{-4} M solution of $C_{18}Fc^+$ in 0.1 M pH 6.5 phosphate buffer. This resulted in self-assembly of a $C_{18}Fc^+$ monolayer along the oxide pores as described before.[7a] The Au/Al_2O_3/OTS/$C_{18}Fc^+$ electrode with 0.1 M KNO_3 was rinsed and then transferred to a 1 mg/mL GOd solution in the same phosphate buffer for 30 min. Following the GOd immobilization, the electrode was rinsed with a large volume of 0.1 M KNO_3, 0.01 M pH 6.5 phosphate buffer solution and stored in the same solution until needed for further experiments.

GOd Assay in the Bilayer Assembly. The assay of the immobilized enzyme was done by an electrochemical method described in the literature.[10] Briefly, the assay is a controlled potential, rotating disk electrode, amperometric measurement of the rate of hydroquinone generation in the reaction

$$\text{glucose} + \text{benzoquinone} \rightarrow \text{gluconolactone} + \text{hydroquinone} \quad (1)$$

catalyzed by glucose oxidase. A 0.2 cm in diameter glassy carbon rotating disk working electrode was rotated at 2000 rpm and potentiostated at +0.450 V vs SCE in a standard electrochemical cell. A typical current–time profile tracing various stages of the experiment is shown in Figure 1. We began the experiment by obtaining a stable current background level at the working electrode in 10.0 mL of solution containing 0.1 M, pH 6.5 buffer, 0.5 M glucose, and 5 mM benzoquinone. The solution was deaerated and then continuously bubbled with Ar. Benzoquinone solution was made daily from the freshly sublimed substrate. The small slope of the initial segment a in Figure 1 corresponds to the slow, spontaneous oxidation of glucose by benzoquinone. At point b, an Au/Al_2O_3/OTS/$C_{18}Fc^+$/GOd electrode was introduced to the cell which resulted, as expected from eq 1, in an increased rate of hydroquinone generation. At point c, the slope of the current–time curve returned to the background level as the aluminum oxide coated gold electrode containing immobilized glucose oxidase was withdrawn from the cell. An increase in the slope value of the segment b–c would indicate a desorption of GOd from the electrode film into the solution. To monitor the stability of the enzyme immobilization during these experiments, steps b and c were carried out twice in each assay, as shown in Figure 1. Finally, at point d, 50.0 μL of a standard (0.01 mg/mL)

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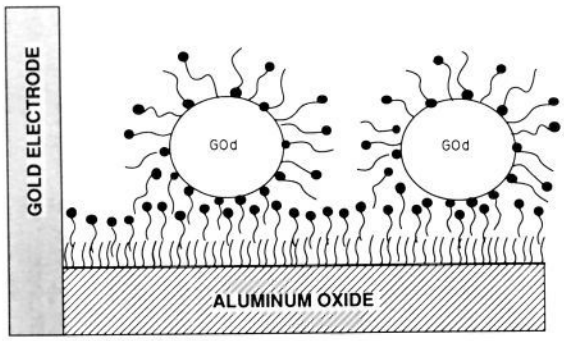

Figure 2. Simplified schematic representation of the bilayer assembly with the immobilized glucose oxidase. Attached to the Al_2O_3 surface is the monolayer of OTS. The second half of the bilayer consists of the $C_{18}Fc^+$ molecules. The analysis of the electron transport dynamics led us to postulate that $C_{18}Fc^+$ molecules also aggregate around the entire glycoprotein shell of the enzyme (for details see Dynamics of Electron Transport Processes section). The Al_2O_3 surface is a fragment of a cylindrical channel ca. 80 nm in diameter and 2.5 μm long of a porous aluminum oxide film attached to the Au electrode.[7] The orientation of the bilayer relative to the gold electrode stems from the perpendicular orientation of the pores in the Al_2O_3 films with respect to the electrode surface.

glucose oxidase solution was injected into the cell to calibrate the measurement. The sensitivity of the assay under these conditions is 5×10^{-14} mol of GOd in the solution. A typical level of the immobilized enzyme in an electrode film was ca. 10^{-12} mol. The current slopes are not sensitive to variations in the rate of bubbling or magnetic stirring of the solution. This result proves that the concentrations of glucose and benzoquinone are sufficiently high to present negligible diffusional constraint to the enzymatic reaction in the porous structure of the Al_2O_3 film.

Instrumentation. The electrochemical experiments were done with the PAR Model 173, 175, and 179 instruments and a BAS Model 100A electrochemical analyzer, all used in a routine fashion. An IBM Model EC/219 rotating disk electrode system was used in the electrocatalytic experiments.

Results and Discussion

Enzyme Immobilization and Its Stability. The scheme of glucose oxidase immobilization developed in this project relies on the interactions between the negatively charged enzyme (the isoelectric pH of GOd is ca. 4.0)[11] and the positively charged head groups of the octadecyl ferrocene amphiphile in the bilayer assembly. A simplified schematic structure of this system is shown in Figure 2. A more detailed discussion of the enzyme–ferrocene amphiphile interactions, based on the measurements of the dynamics of the electron transport process, is presented in the last section of Results and Discussion. The aluminum oxide surface shown in Figure 2 is a fragment of a microporous aluminum oxide film overcoating the electrode surface. Such Al_2O_3 films have been developed and used extensively in our laboratory.[2d,7,9] The oxide pores form a dense array of channels perpendicular to the electrode surface with an overall permeability of 40% to 65%.[9] The monolayer attached to the aluminum oxide surfaces is formed by the spontaneous assembly of octadecyltrichlorosilane (OTS) involving the formation of siloxane bridges to the oxide surface and between the OTS molecules.[12] The second half of the bilayer is formed by an exposure of the OTS treated oxide films to a solution of (ferrocenylmethyl)dimethyloctadecylammonium bromide ($C_{18}Fc^+$) as described in the Experimental Section. On the basis of a voltammetric assay carried out in the pure supporting electrolyte at 10 mV/s, this results in a stable $C_{18}Fc^+$ coverage of ca. 1.8×10^{-10} mol/cm^2, in good agreement with our previously published data.[7a]

The GOd immobilization involves a simple exposure of a Au/Al_2O_3 electrode with the immobilized $OTS/C_{18}Fc^+$ bilayer

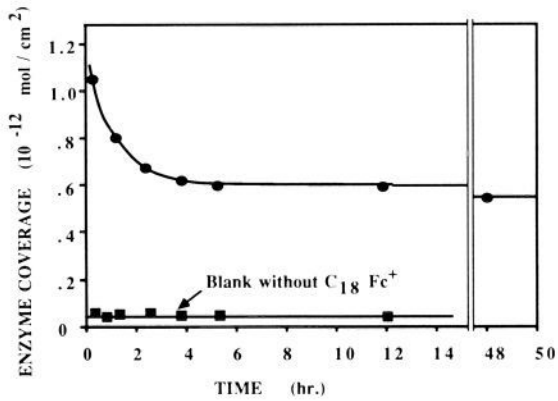

Figure 3. Test of the long-term activity of the enzyme in a $Au/Al_2O_3/OTS/C_{18}Fc^+/GOd$ assembly. The data were obtained by the amperometric assay (see Figure 1 and the Experimental Section). The gold electrode assembly was stored in the working buffer containing 0.1 M KNO_3, 0.01 M phosphate buffer at pH 6.5 between the enzyme activity measurements.

to a solution of the enzyme as described in the Experimental Section. Following the immobilization, the electrode was transferred and stored in a working buffer solution containing 0.01 M pH 6.5 phosphate buffer and 0.1 M KNO_3. The presence of nitrate in this solution was required to maintain the stability of the $C_{18}Fc^+$ and thus of the GOd in the bilayer.[7a] The quantity of the active enzyme in the electrode film was monitored periodically by an electrochemical assay with benzoquinone as a mediator (see the Experimental Section), during which the electrode carrying the enzyme was disconnected and acted only as a support for the immobilized enzyme.

Long-term stability of the enzyme in the electrode film is documented in Figure 3. The "blank" line in Figure 3 was obtained by exposing the $Au/Al_2O_3/OTS$ electrode to the GOd solution without the prior assembly of $C_{18}Fc^+$. In view of an additional finding that the contact angle of water on an OTS treated surface decreases from 112° to 0°, we believe that GOd adsorbs at the hydrophobic Al_2O_3/OTS surfaces but becomes inactive, presumably as a result of unfolding. The 40% decrease of the enzyme activity in the first 4 h in the upper trace of Figure 3 is probably related to a slow loss of the $C_{18}Fc^+$ molecules and the loss and/or deactivation of the enzyme as it penetrates the bilayer structure and adsorbs at the surface of the OTS monolayer.

Typical levels of the glucose oxidase coverage on the bilayer coated Al_2O_3 surface were in the range $6–10 \times 10^{-13}$ mol/cm^2. Assuming an average GOd diameter of 6.8 nm,[11] 20% to 30% of the bilayer surface was covered with the enzyme molecules. This may be a low estimate as the assay reports only the catalytically active enzyme.

Electrocatalytic Oxidation of Glucose. The voltammetric characteristics of the $Au/Al_2O_3/OTS/C_{18}Fc^+$ system and its electrocatalytic behavior following the GOd immobilization are illustrated in Figure 4. The voltammogram **2**, which shows the electrochemistry of the $C_{18}Fc^+$ in the bilayer, could be obtained either in the presence of glucose but prior to the GOd immobilization or in the glucose-free solution following the enzyme immobilization. At a slow scan rate of 10 mV/s, the level of electroactivity of the $C_{18}Fc^+$ and its voltammetric behavior under these two sets of conditions are indeed the same. This point will be discussed further in the next section.

The steady-state electrocatalytic behavior of the immobilized glucose oxidase is shown in Figure 4, curve 3. There are three reactions accounting for the electrocatalytic oxidation of glucose.

$$\text{GOd(ox)} + \text{glucose} \rightarrow \text{GOd(red)} + \text{gluconolactone} \quad (2)$$

$$\text{GOd(red)} + 2C_{18}Fc^{2+} \rightarrow \text{GOd(ox)} + 2C_{18}Fc^+ + 2H^+ \quad (3)$$

$$C_{18}Fc^+ - e^- \rightarrow C_{18}Fc^{2+} \quad (4)$$

Since the catalytic current of glucose oxidation is observed in the absence of the mediator in the electrolyte solution ($C_{18}Fc^+$ is

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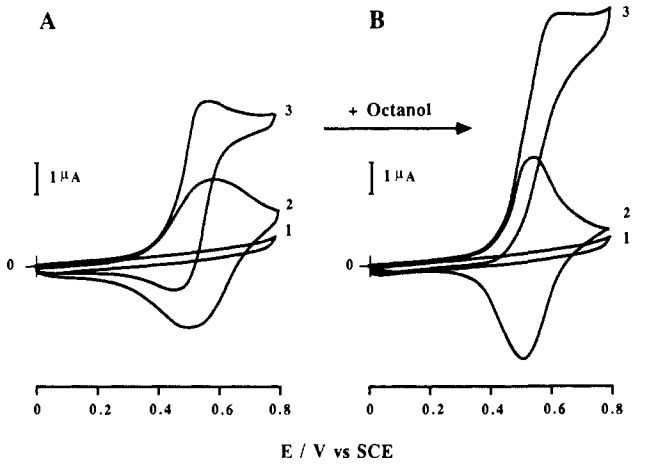

Figure 4. Cyclic voltammetric behavior of the $Au/Al_2O_3/OTS/C_{18}Fc^+/GOd$ system in 0.1 M KNO_3, 0.01 M phosphate buffer at pH 6.5 (A) without octanol and (B) in the presence of ca. 3 mM octanol; v = 10 mV/s; A = 0.07 cm^2. Trace 1: Background scan recorded with the $Au/Al_2O_3/OTS$ electrode in the presence of 0.02 M glucose. Trace 2: Recorded with the $Au/Al_2O_3/OTS/C_{18}Fc^+/GOD$ electrode in the absence of glucose. Trace 3: The same as trace 2 but in the presence of 0.02 M glucose.

confined to the bilayer assembly), the electron transport is accomplished via the lateral diffusion of the $C_{18}Fc^+$ and $C_{18}Fc^{2+}$ molecules between the electrode and the GOd sites attached throughout the assembly in the oxide film. To verify this, and to eliminate a possibility that the GOd mediation is done, at least in part, by the diffusion of the desorbed $C_{18}Fc^+$ molecules in the solution, we added deliberately $C_{18}Fc^+$ to the solution (50 μM level) and did not observe any increase of the catalytic current. Furthermore, the steady-state catalytic current in Figure 4 does not change with stirring or when the working electrode is rotated. These observations confirm our assertion that the electron transport is carried out by the lateral diffusion of the amphiphilic ferrocene molecules. It is also apparent that the magnitude of the catalytic current is independent of the mass transport of glucose to the electrode film and thus the slow step in the electrocatalysis is either the electron transport along the oxide pores or the kinetics of the GOd reoxidation reaction (equation 3). The level of the catalytic current becomes sensitive to stirring, however, when the phosphate buffer concentration is lowered to 1 mM. This is not surprising in view of the proton release in reaction 3 and known diminution of GOd activity in low-pH solutions.[13]

Figure 4B shows that the catalytic current increases when the glucose solution is made close to saturation with n-octanol (ca. 3 mM). We have demonstrated previously that the presence of octanol in the electrolyte solution leads to its intercalation into the bilayer assembly and caused more rapid lateral diffusion of the amphiphiles.[7] Thus, the current increase observed in Figure 4B strongly indicates that the rate of electrocatalysis is limited by the rate of electron transport in the bilayer assembly, which primarily involves the lateral diffusion of the ferrocene amphiphile.[7b] However, the current increase could also be related to the improved dynamics of the GOd's $FADH_2$ oxidation by the amphiphilic ferrocenium cations. This process probably involves several elements related to the bilayer fluidity such as mobility of the GOd bound ferrocene amphiphiles and rotational mobility of the enzyme. Both in the presence and in the absence of n-octanol, the steady-state catalytic current depended on the concentration of glucose in the electrolyte. These data are shown in Figure 5. Detailed elucidation of the enzymatic and the mediation kinetics awaits further research.

Dynamics of Electron Transport Processes. Since the most significant feature of the $Au/Al_2O_3/OTS/C_{18}Fc^+/GOd$ assembly is electron transfer mediation via lateral diffusion of the ferrocene amphiphile, we have investigated the effect of GOd immobilization on the dynamics of $C_{18}Fc^+$ lateral transport. On the basis of

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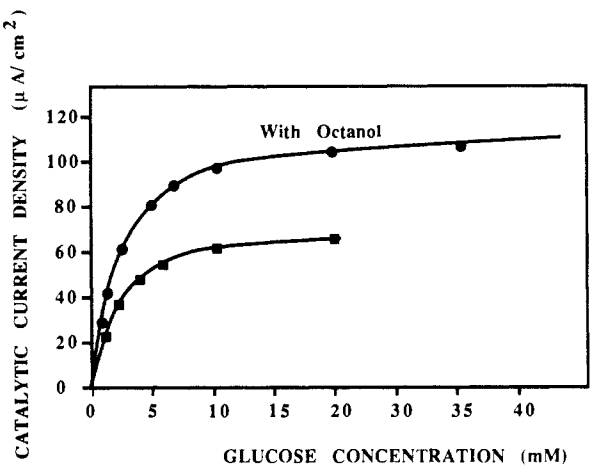

Figure 5. Dependence of the steady-state catalytic current of Figure 4 on the concentration of glucose. Experimental details are the same as those in Figure 4.

standard chronocoulometric experiments, we observe that GOd immobilization did not decrease the activity level of the $C_{18}Fc^+$ but it did result in a decrease of the diffusion coefficient of its lateral motion. For example, in a typical series of experiments carried out with a single electrode, the $C_{18}Fc^+$ was initially loaded to the level of 1.8×10^{-10} mol/cm^2. The diffusion coefficient measured in a 1.0 M KNO_3 solution nearly saturated with n-octanol was 9.4×10^{-8} cm^2/s. This electrode was then transferred to a GOd loading buffer for 30 min as described above. In the following step, it was transferred back to the 1.0 M KNO_3 solution with n-octanol. The $C_{18}Fc^+$ coverage decreased only slightly to 1.5×10^{-10} mol/cm^2 (most likely due to desorption),[7a] but its diffusion coefficient decreased by 61% to 3.7×10^{-8} cm^2/s. The extent of the decrease in D_{app} value correlated with the extent of GOd loading in the electrode film. In the example above, the GOd coverage was 3.3×10^{-13} mol/cm^2 of the Al_2O_3 internal surface area. In the case of another electrode with a GOd coverage of 1.3×10^{-13} mol/cm^2 level, the lateral diffusion coefficient of $C_{18}Fc^+$ decreased only 20% (9.3×10^{-8} to 7.4×10^{-8} cm^2/s).

These data suggest that the $C_{18}Fc^+$ molecules involved in the GOd binding are significantly less mobile. Thus, only a fraction of the amphiphilic ferrocene centers is involved effectively in the electron transport process. In view of the fact that all of the sites remain electroactive, we can treat this case as a system with two parallel diffusional pathways coupled by a fast electron exchange reaction.[2d,9] In such a case, the apparent diffusion coefficient, D_{app}, depends on the diffusion coefficients and the mole fractions of the species in each channel

$$D_{app} = x_m D_m + x_b D_b \quad (5)$$

where the subscripts m and b represent mobile and bound $C_{18}Fc^+$ centers. If we assume that D_b is negligible compared to D_m, then the fraction of the ferrocene sites participating in the mediation process $x_m = D_{app}/D_m$. Using the data given above where D_m = 9.4×10^{-8} cm^2/s and D_{app} = 3.7×10^{-8} cm^2/s, one obtains x_m = 39%. Knowing that the GOd and the total $C_{18}Fc^+$ coverages in the bilayer are 3.3×10^{-13} and 1.5×10^{-10} mol/cm^2, respectively, it appears that $C_{18}Fc^+$ binding by glucose oxidase involves a much larger number of molecules (in the order of 10^2) than expected if one considers only electrostatic interactions between $C_{18}Fc^+$ and the GOd. At pH 6.5 used here, GOd is expected to have a net charge of −6 to −10.

Considering these data, we envisage a model where the $C_{18}Fc^+$ interactions with GOd result in aggregation of the amphiphilic ferrocene molecules around the entire glycoprotein shell of the enzyme. We show this schematically in Figure 2. These interactions could involve both electrostatic and hydrophobic forces as well as some degree of $C_{18}Fc^+$ intercalation. The fact that the GOd immobilization is relatively stable, and that in the slow scan voltammetric experiments all of the ferrocene centers are electrochemically active, suggests that some of the GOd-bound $C_{18}Fc^+$

molecules still constitute a part of the bilayer assembly. Apparently, the dynamics of the entire system is sufficiently fast to produce these results. By proposing the involvement of lateral diffusion as a means of electron transport, we do not intend to exclude completely electron hopping in accounting for the full electroactivity of the system. We showed earlier for a similar system that translational diffusion is a dominant mechanism of the charge transport in bilayers.[7b] This finding may not be directly applicable to the present, more complicated case.

On the basis of this model, one might predict that the aggregation of $C_{18}Fc^+$ molecules around the immobilized GOd would leave some free space on the OTS surfaces. Consistent with this postulate, we found that the re-exposure of the $Au/Al_2O_3/OTS/C_{18}Fc^+/GOd$ electrode assembly to the original $C_{18}Fc^+$ loading solution resulted in a significant increase in the loading level of the ferrocene amphiphile. For example, in the case of the two electrodes mentioned above, the $C_{18}Fc^+$ loading increased 67% and 43% (compared to its initial value prior to the GOd immobilization) for the electrodes with GOd coverage of 3.3×10^{-13} and 1.3×10^{-13} mol/cm^2, respectively. This substantiates our aggregation model.

Conclusions

We have described here the structure and behavior of an organized multimolecular assembly designed to carry out enzymatically catalyzed electrooxidation of glucose. A key feature of this system is the immobilization of glucose oxidase in the head group region of an organized bilayer assembly. The electron transfer mediation of GOd is accomplished by the lateral diffusion of the octadecylferrocene amphiphile, one of the components of the bilayer. The overall electroenzymatic reaction is controlled by the lateral electron transport rate, or the kinetics of the mediation reaction. A simple analysis of the effect of GOd immobilization on the dynamics of the lateral diffusion suggests that the enzyme molecules aggregate around them a large number of the ferrocene surfactant molecules which remain electroactive but most likely do not participate in the lateral charge transport process. A number of important questions have emerged from this research. The issues concerning the kinetic aspects of this system as well as the questions addressing the nature of the $GOd/C_{18}Fc^+$ interactions and the structure of their aggregates are the subjects of our current investigations.

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Synthesis, Properties, and Molecular Structure of Highly Distorted Hexakis(trimethylsilyl)benzene[1]

Hideki Sakurai,* Keisuke Ebata, Chizuko Kabuto, and Akira Sekiguchi

Contribution from the Department of Chemistry and Organosilicon Research Laboratory, Faculty of Science, Tohoku University, Aoba-ku, Sendai 980, Japan. *Received April 10, 1989*

Abstract: Hexakis(trimethylsilyl)benzene (**2**), the most distorted benzene to a chair form, has been prepared as yellow crystals by methylation of hexakis(bromodimethylsilyl)benzene. The structure of **2** has been determined by X-ray crystallography. Crystals of **2** are orthorhombic, space group $P2_12_12_1$, a = 14.579 (3) Å, b = 18.927 (4) Å, c = 12.069 (9) Å, V = 3330.3 (10) Å^3, D_c = 1.02 g/cm^3, Z = 4. The benzene ring is remarkably deformed to a chair form with torsion angles of 9.8° (average) for C_{ar}–C_{ar}–C_{ar}–C_{ar}, and the six Si atoms are located up and down alternately from the benzene ring with torsion angles of 60.5° (average) for Si–C_{ar}–C_{ar}–Si. In solution, **2** exists as an equilibrium mixture of chair and boat forms. The quite unique chemical features of **2** are also demonstrated, for example, by photolysis with a light ($\lambda > 300$ nm) to afford hexakis(trimethylsilyl)bicyclo[2.2.0]hexa-2,5-diene (Dewar benzene), whereas thermolysis led to the formation of 1,1,3,4,6,6-hexakis(trimethylsilyl)-1,2,4,5-hexatetraene.

The molecular structures of overcrowded compounds are of interest in order to determine how the molecules relieve their inherent repulsive nonbonded interactions.[2] Hexa-*tert*-butylbenzene (**1**)[3] (Chart I) and hexakis(trimethylsilyl)benzene (**2**)[3,4] have not been synthesized yet, and hence, these sterically crowded molecules are one of the interesting synthetic targets. Hexakis(trimethylgermyl)benzene (**3**) has recently been prepared by Mislow and co-workers, and its unique molecular structure has been demonstrated.[3] The benzene ring of **3** distorts to a chair form in the ground state due to the nonbonded steric repulsion.[3,5] In contrast, the benzene ring of 1,3,5-tris(*N*,*N*-dialkylamino)-2,4,6-trinitrobenzene (**4**) highly distorts to a boat form by both steric and electronic reasons.[6]

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