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Enzyme Bioelectrochemistry in Cast Biomembrane-Like Films

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Introduction

Research on the electrochemistry of enzymes is driven partly by the desire to employ reusable catalytic coatings on electrodes for biosensors and other biomedical devices.[1−3] Simultaneously, electrochemical methods are emerging as valuable tools to study protein redox chemistry.[4,5] If direct electron exchange can be achieved, electrodes can substitute for enzyme redox partners. The driving force of the reaction is then under electronic control via the applied cell potential. Diffusion is often not important in ultrathin films, data analysis is simplified, and only tiny amounts of enzyme are needed. The kinetics of enzymic reactions can be estimated from voltammetric data.[5g,6] Moreover, optical techniques coupled with electrochemistry can characterize molecular properties.[7]

Historically, several factors have plagued direct electron transfer between electrodes and proteins.[4a,b] These include (i) electroactive prosthetic groups deep within the protein structure, (ii) adsorptive denaturation of proteins onto electrodes, and (iii) unfavorable orientations at electrodes. Remarkable recent progress provides several strategies for achieving direct electron exchange between electrodes and proteins. With few exceptions,[8] special electrode preparations are required. One approach employs highly purified protein solutions and specially cleaned electrodes.[9] Another coats electrodes with promoter molecules which facilitate electron transfer by blocking adsorptive denaturation and favorably orienting the protein.[4a,b] A novel variant uses chemisorbed alkanethiol monolayers on gold, with end functional groups capable of binding proteins.[10,11] Similarly, edge plane pyrolytic graphite electrodes (PG) with carboxylate functionality can adsorb proteins.[4c] Also, redox active hydrogels can be used to deliver electrons between the electrode and enzyme.[12]

Membranes in living organisms are about half phospholipids and half protein. Many enzymes function in nature while bound to membranes.[13] Biomembrane-like structures containing proteins on electrodes were used prior to our venture into this field. For example, lipid bilayers formed on freshly cleaved Pt or Au wires were stable for 36 h and promoted electron transfer to various proteins.[14] A covalently bound surfactant bilayer on Au was used to study reactions between cytochrome *c* and cytochrome *c* oxidase.[15]

In 1991, Kunitake et al. inserted the iron heme protein myoglobin (Mb) between bilayers of dialkyl phosphate surfactants.[16] The protein was specifically oriented in these cast films. Inspired by this, Nassar and I began to explore the electrochemistry of Mb in surfactant films. Our first attempts employed liquid crystal films of didodecyldimethylammonium bromide (DDAB) on PG electrodes, which took up Mb from solution. We quickly obtained reversible electrochemistry for Mb in these films,[17] while electron transfer was not observed on bare PG electrodes in Mb solutions.

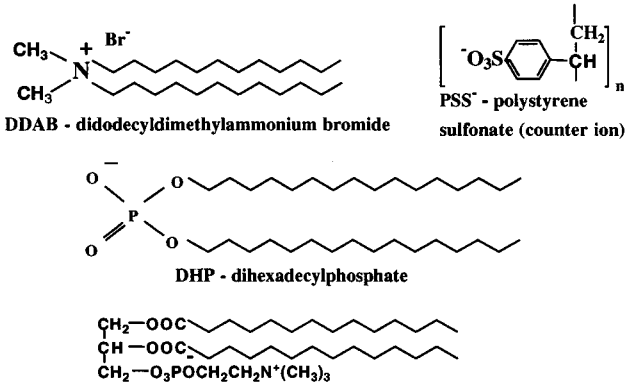
Later, we cast aqueous vesicle dispersions of Mb and insoluble surfactants onto electrodes, and found similar reversible voltammetry.[18] This method is related to vesicle spreading used to prepare supported lipid membranes.[19]

These easily prepared surfactant films enable detailed studies of protein electron transfer and catalysis in biomembrane-like environments. The sections below begin with results on various incorporated proteins. Next, structural characterization is summarized. Finally, the mechanism of electron transfer to Mb in the films and electrochemically driven enzyme-like catalysis by cyt $P450_{cam}$ and Mb are discussed.

How Are Multi-Bilayer Films Containing Proteins Made?

Surfactants are *surface active agents* with a charged or polar headgroup and a nonpolar tail.[20] The hydrophobic tails are usually long hydrocarbon chains. Stable films can be cast from surfactants that are insoluble in water and do not form micelles. Molecules fulfilling these requirements have ionic or zwitterionic headgroups and 2 or more hydrocarbon tails of 12 carbons or longer. Several surfactants used for these films are shown below.

Some insoluble surfactants used for cast films



DDAB - didodecyldimethylammonium bromide

PSS⁻ - polystyrene sulfonate (counter ion)

DHP - dihexadecylphosphate

DMPC - dimyristoylphosphatidylcholine - a phospholipid or lipid, and a component of biomembranes

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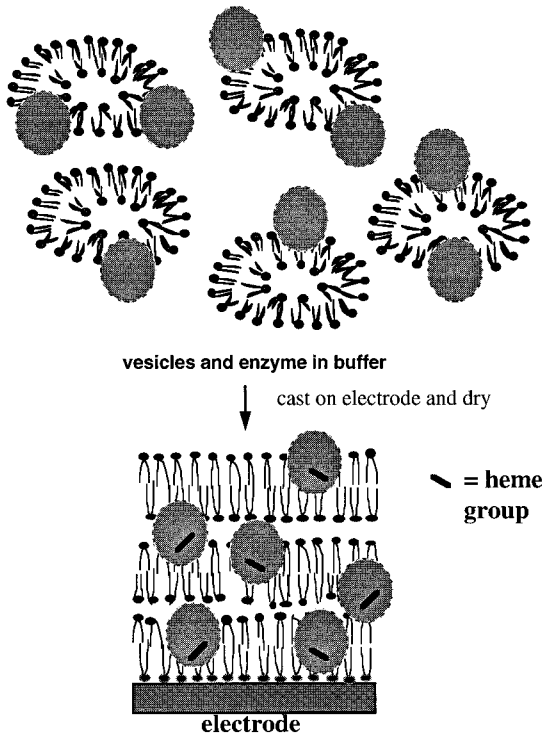


FIGURE 1. Representation of the cast vesicle method for film preparation.

The cationic surfactant (DDA^+) can have bromide or a polymer as counteranion. Phosphatidylcholines, also called lipids or phospholipids, are major components of biomembranes.

Films can be cast onto solid surfaces from solutions of surfactants in organic solvents or aqueous vesicle dispersions.[21] Evaporation of solvent leaves self-assembled multi-bilayer films, similar to stacks of biomembranes.

Cast surfactant films can exist in a solidlike gel phase, where alkyl chains are extended in *all-trans* conformations. As the temperature exceeds a critical value (T_c), these films are converted to a liquid crystal phase characterized by increased mobility of the alkyl chains.[13,20,21] The electrochemistry of enzymes is likely to be reversible only in films in the liquid crystal phase.[17]

We have used two methods to incorporate proteins into films. In the first, a solution of surfactant in chloroform is spread onto an electrode, and solvent is evaporated. The coated electrode is then placed into an electrochemical cell containing a solution of protein, which is taken up into the film. This works best with films in the liquid crystal phase. For 20 μm liquid crystal films of DDAB, incorporation of myoglobin is complete within 20 min.[17]

An alternative preparation controls the amount of protein in the film. An aqueous vesicle dispersion of surfactant is made and then mixed with a solution containing protein. A precise volume of this mixture is spread onto an electrode and dried.[18] The vesicles flatten as they dry, resulting in multiple stacks of bilayers (Figure 1).

Films made by both methods have similar electrochemical properties. Most protein−surfactant films on PG, Au, and Pt electrodes are stable for a month in buffer.[17,18] They adhere poorly to indium tin oxide (ITO) or silver, and are much less stable on such electrodes. Unlike DDAB, films of phosphatidylcholines thin significantly during their first 30 min in water, but then stabilize and give reversible voltammetry for a month thereafter.[22,23]

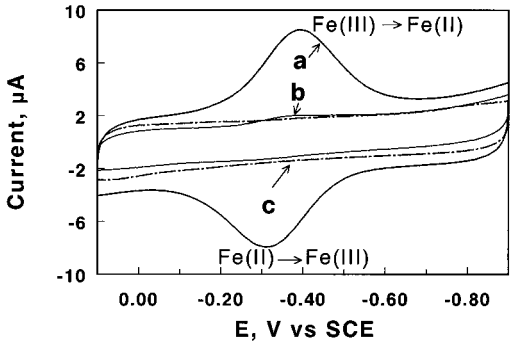


FIGURE 2. Cyclic voltammograms on basal plane PG electrodes at 100 mV s^{-1} in pH 7 buffer + 0.1 M KCl: (a) substrate-free cyt $P450_{cam}$−DMPC film in oxygen-free buffer containing no enzyme; (b) bare electrode in oxygen-free buffer containing 40 μM cyt $P450_{cam}$; (c) DMPC film in oxygen-free buffer containing no enzyme. Adapted with permission from ref 25. Copyright 1997 Royal Society of Chemistry.

Cytochrome $P450_{cam}$ in Cast Surfactant Films

Membrane-bound mammalian liver cytochromes P450 (cyt P450) metabolize drugs and lipophilic chemicals in reactions involving heme iron. They are thought to oxidatively activate pollutants for carcinogenesis.[24] Bacterial cyt $P450_{cam}$ catalyzes similar reactions as well as anaerobic dechlorination of organohalides.

The first step in these catalytic cycles involves reduction of cyt P450Fe(III) to cyt P450Fe(II). We needed to achieve this conversion on electrodes if we were to catalyze enzyme reactions. Thin films (~0.5 μm) of DMPC and DDAB containing cyt $P450_{cam}$ on PG electrodes gave reversible peaks by cyclic voltammetry (CV) in oxygen-free buffers (Figure 2a), confirming this key reaction.[25] In contrast, bare PG electrodes in cyt $P450_{cam}$ solutions gave no CV peaks (Figure 2b). The enzyme retains its native state in the films, as shown by a Soret absorption band at 447 nm after reduction and complexation with carbon monoxide (CO).

Figure 2 illustrates the power of cast surfactant films for studies of redox enzymes. Reversible cyt $P450_{cam}$ electrochemistry in solution has been observed only at low temperature in highly purified solutions, which degrade in less than a day.[26] Cast films require only routine enzyme purification and give peaks which decreased only 10% during a month of storage.[25]

Voltammetry indicated thin film electrochemistry in which all electrochemically active cyt P450Fe(III) is reduced to P450Fe(II) on the forward scan, and cyt P450Fe(II) is oxidized to P450Fe(III) on the reverse scan (Scheme 1). Integration of peaks gave average surface concentrations of electroactive cyt $P450_{cam}$ of 7×10^{-10} mol cm^{-2} in DMPC films and 30% less in DDAB films at pH 7. A rough estimate of the concentration of electroactive enzyme in

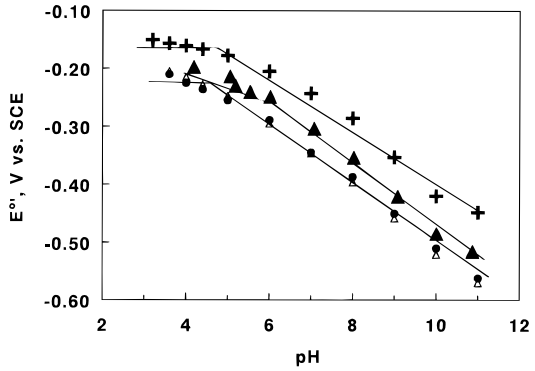


FIGURE 3. Influence of pH on average $E^{o\prime}$ from SWV of films of cyt P450−DMPC (▲), Mb−DDAB (+), Mb−DMPC (●), and Mb−dilauroylphosphatidycholine (DLPC) (△) on PG electrodes.

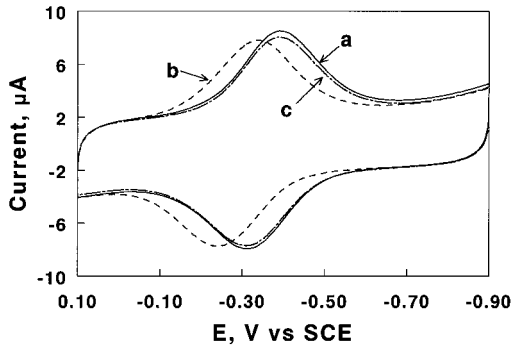
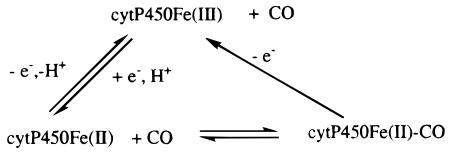


FIGURE 4. Cyclic voltammograms on basal plane PG electrodes at 100 mV s^{-1} in pH 7 buffer + 0.1 M KCl: (a) substrate-free cyt $P450_{cam}$−DMPC film in oxygen-free buffer containing no enzyme; (b) same electrode after the solution was purged with CO for 5 min; (c) same electrode after the solution was purged with N_2 for 20 min to remove CO. Adapted from with permission ref 25. Copyright 1997 Royal Society of Chemistry.

Scheme 1



the films is 2−3 mM, or about 0.03 nmol per film. Thus, only a tiny amount of enzyme is required.

Studies with myoglobin in surfactant films had shown[22,23] that the solution pH controlled electrochemistry within the films. This was true for cyt $P450_{cam}$ as well, as illustrated by the dependence of the formal (midpoint) potential ($E^{o\prime}$) on pH (Figure 3). At pH > 6, $E^{o\prime}$ shifted negatively. Similar shifts for myoglobin occur at pH > 4.6. The slopes of these lines are close to −59 mV/pH unit, suggesting[27] proton-coupled electron transfer.

Surfactant films can also be used to investigate ligation of metals in prosthetic groups. This is illustrated by the CV of cyt P450−DMPC after carbon monoxide (CO) is added. The midpoint potential (E_m) shifted +61 mV (Figure 4b) compared to the voltammogram without CO. After removal of CO, the voltammogram (Figure 4c) was nearly identical to the original (Figure 4a). Since rabbit liver P450Fe(II)−CO was shown to have a midpoint potential significantly positive of cyt P450Fe(II),[28] the shift in E_m in the presence of CO reflects formation of cyt $P450_{cam}$Fe(II)−CO.

Table 1. Electrochemical Parameters for cyt $P450_{cam}$ and Mb

film or solution	pH	$E^{o\prime}$, mV, vs NHE	k_s', s^{-1}	method/ electrode[a]	ref
cyt $P450_{cam}$−DMPC	7.0	−121 ± 2	25 ± 5	SWV/PG	25
cyt $P450_{cam}$−DDAB	7.0	22 ± 6	26 ± 8	SWV/PG	25
cyt $P450_{cam}$ soln	7.0	−303		titration[b]	32
high-purity cyt $P450_{cam}$ soln	7.4	−272[c]		CV/EPG	26
Mb−DDAB	7.0	1	31	SWV/PG	23
Mb−DMPC	7.0	−101	59	SWV/PG	23
Mb−DHP	7.4	−89	90 ± 7	SWV/PG	22
Mb soln	7.0	50	(0.07)[d]	CV/ITO	30
high-purity Mb soln	6.5	60	(3)[d]	CV/hydrophilic ITO	9

[a] SWV, square wave voltammetry; CV, cyclic voltammetry; PG, ordinary basal plane pyrolytic graphite; EPG, edge plane pyrolytic graphite; ITO, indium tin oxide. [b] Redox titration. [c] 4 °C. [d] From the bare electrode value in cm/s, converted for comparison to a hypothetical value for a 1 µm film.

CytP450Fe(II) reacts rapidly with CO,[28] but cytP450Fe(III) does not react. E_m shifts positively from the influence of CO complexing with P450Fe(II) following electron transfer (Scheme 1). Also, these results suggest that the initial electron transfer involves the heme iron of cyt $P450_{cam}$, not another group on the enzyme.

$E^{o\prime}$ and apparent average electron-transfer rate constants (k'_s) were obtained by nonlinear regression of square wave voltammograms (SWV) onto a model assuming thin film electrochemistry, dispersion of formal potentials, and Butler−Volmer kinetics (Table 1). SWV is a pulsed voltammetric method used here because of its excellent signal/noise ratio. Dispersion had been invoked previously, and a dispersion model using Marcus kinetics was used for CVs of cytochrome *c* adsorbed to carboxythiol−gold electrodes.[29] Our model gave much improved fits (Figure 5a) to SWV data compared to those assuming a single electroactive species.[25]

Analysis with the $E^{o\prime}$-dispersion model allows construction of distribution diagrams representing relative amounts of protein having a given $E^{o\prime}$ (Figure 5b). These diagrams suggest Gaussian distributions. While this model gave good fits to SWV data for cyt $P450_{cam}$[25] and Mb,[22,23] a molecular explanation is less clear. Nominally, the films behave as if there were several classes of proteins with slightly different $E^{o\prime}$ values.

Myoglobin and Other Proteins in Cast Films

The story with myoglobin (Mb) is much the same as for cyt $P450_{cam}$. Voltammetry with Mb in solution revealed little evidence of electron transfer on PG, Au, or Pt. Relatively slow electron transfer was achieved on ITO electrodes,[30] but was more reversible with a "hydrophilic" electrode and careful protein purification immediately before voltammetry.[9] Mb in films of DDAB, DMPC, and other insoluble surfactants on PG, Au or Pt electrodes gave direct, reversible electron-transfer involving the heme Fe(III)/Fe(II).[17,18,22,23,31] No special purification is needed. As with cyt $P450_{cam}$, electron exchange between electrodes and Mb is *switched on* in the surfactant films.

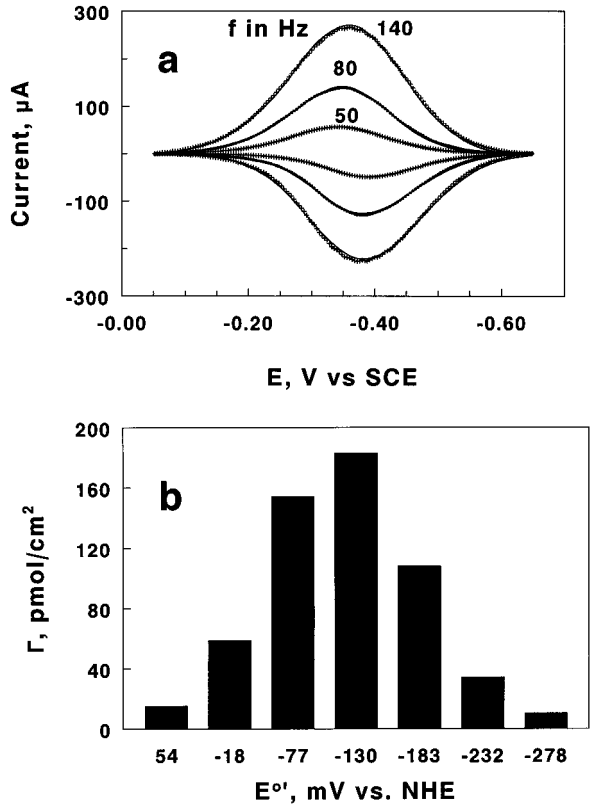


FIGURE 5. (a) Square wave voltammograms at step height 4 mV and pulse height 75 mV showing forward and reverse currents for cyt P450−DMPC films in pH 7.0 buffer. Points represent the experimental, background-subtracted data, and solid lines are the best fits by nonlinear regression onto the $E^{\circ\prime}$ dispersion thin layer model described in the text. (b) Distribution of cyt P450 in DMPC film expressed as surface concentrations (Γ, mol cm^{-2}) at discrete $E_j^{\circ\prime}$ values found from regression analysis of SWV with the model having seven $E^{\circ\prime}$ and seven Γ_j* values. Pulse 75 mV, frequency 100 Hz, step 4 mV. Adapted from with permission ref 25. Copyright 1997 Royal Society of Chemistry.

SWV of thin films of Mb in DDAB, DMPC, and DHP were fit well with the $E^{\circ\prime}$ dispersion model.[22,23] We prefer this method to obtain the average $E^{\circ\prime}$ and k'_s (Table 1). The meaning of k'_s is somewhat ambiguous for films. Its value is subject to the limitations of the model, which neglects counterion entry and exit, electron self-exchange, and molecular interactions. We interpret k'_s as a relative measure of the efficiency of the global electron-transfer process in a given film.

Subject to these qualifications, k'_s suggests much more efficient electron transfer in films than in solution and does not depend much on surfactant type (Table 1). However, surfactant type has a large effect on $E^{\circ\prime}$. For cyt P450$_{cam}$, $E^{\circ\prime}$ values in films are 180−320 mV positive of the solution value. For Mb, values in the films are 50−100 mV negative of that in solution. For both proteins, values in DDAB films are about 100 mV positive of those in DMPC films.

$E^{\circ\prime}$ values of proteins in films also depend strongly on the electrode material (Figure 6), but electron-transfer rates do not.[18] At pH 5.5, $E^{\circ\prime}$ in Mb−DDAB films ranged from −50 to +120 mV vs NHE in the order ITO < Au < PG < Pt.

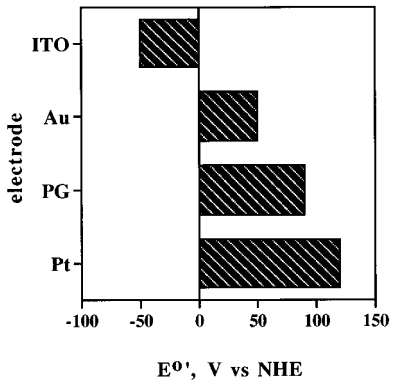


FIGURE 6. Influence of electrode material on the formal potential ($E^{\circ\prime}$) of Mb−DDAB films in pH 5.5 buffer.

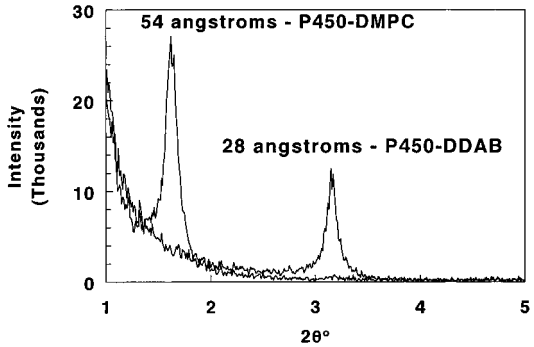


FIGURE 7. X-ray diffraction patterns for enzyme−surfactant films.

The influence of surfactant type and electrode material on $E^{\circ\prime}$ are consistent with an electrical double-layer effect at the electrode−film interface on the potential felt by the protein. Surfactant−protein interactions are also likely to be involved. Clearly, proteins in films do not give the same $E^{\circ\prime}$-values as in solution.

Reversible voltammetry of several other proteins has been achieved in surfactant films. Hemoglobin (Hb) in liquid crystal DDAB[33] had a much larger electron-transfer rate than on bare PG. Bianco et al. found that cytochromes *c*, c_3, and c_{553} in phosphatidylcholine−cholesterol−lauric acid films gave reversible voltammograms.[34a−c] The iron−sulfur protein ferredoxin from spinach gave reversible CVs in phosphatidylcholine−cholesterol films doped with dodecylamine or dioctadecyldimethylammonium bromide.[34d] Chlorella ferredoxin gave reversible voltammograms in films of synthetic surfactants.[35] We recently obtained reversible voltammetry in DDAB films for iron−sulfur protein putidaredoxin, the natural redox partner of cyt P450$_{cam}$.

Supramolecular Film Organization

X-ray diffraction at low angles suggests a stacked bilayer structure of the films. X-ray patterns of hydrated cyt P450$_{cam}$−DMPC films showed peaks corresponding to d = 54 Å (Figure 7), similar to 56 Å reported for DMPC,[36] suggesting a chain tilt angle of about 10°. For DDAB containing cyt P450$_{cam}$ or Mb, d was 28 Å, consistent with the shorter chain length and smaller headgroup. Using a fully extended DDAB length[37] of 21 Å, we estimate a tilt

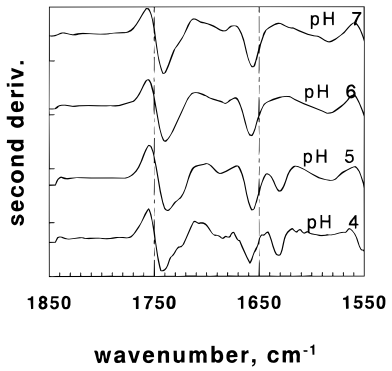


FIGURE 8. Second derivative reflectance infrared spectra of thin Mb−DMPC films at a source incidence angle of 60° prepared using buffers from pH 4 to pH 7 and cast onto vapor-deposited aluminum. The native Mb spectrum is similar to that at pH 7. Adapted from ref 38. Copyright 1995 American Chemical Society.

angle of roughly 40°, similar to the 29−34 Å found from reflectance FT-IR spectra.[17]

Further, films containing Mb and other proteins had gel-to-liquid crystal phase transition temperatures (T_c) within a few degrees Celsius of T_c for bilayer vesicle dispersions of the same surfactant.[17,22,31] This also suggests that the surfactants are arranged in bilayers. A stacked bilayer structure (cf. Figure 1) is achieved by vesicles flattening as they dry.

In general, UV−vis, ESR, and reflectance FT-IR spectroscopy showed that at buffer pH where native protein exists in solution, secondary structures of Mb and cyt $P450_{cam}$ in films are similar to native conformations.[17,23,31] ESR showed that the Mb heme iron is high spin at neutral pH.[38] This suggests water as an axial ligand of iron in Mb in the films.

Amide I infrared bands (1700−1600 cm^{-1}) reflect polypeptide backbone C═O stretching and were used to detect conformational changes in Mb within the films. Confirmation was controlled by pH. At pH 7, the amide I spectrum of Mb in DDAB or DMPC films was similar to that of pure Mb. Second derivative reflectance spectra of Mb−lipid films show that, as pH decreased from 7 to 4, the α-helix band at 1657−1659 cm^{-1} decreased (Figure 8).[23] A new band at 1630 cm^{-1} at pH ≤ 5 is assigned to disordered regions of Mb, suggesting partial unfolding of helices. Unfolding in this pH range was confirmed by visible absorbance spectra. Partial unfolding to a stable, *molten globule* state of Mb in solution at pH<5 is well documented.[39]

Visible linear dichroism and ESR anisotropy showed that Mb was specifically oriented in static films.[17,22,38] The orientation of the heme plane of Mb averaged 60° with respect to the normal to the film plane, and distributions were rather broad.[38] Heme orientation did not depend on the charge of the surfactant headgroup, suggesting that Mb may be partly imbedded in hydrophobic bilayer regions. NMR showed that anions such as Cl^- and acetate bind to Mb within the films, possibly neutralizing surface charge.

Figure 1 shows a model of Mb−surfactant films. Surfactants are arranged in stacked bilayers, with Mb imbedded in them. A major unknown is the degree of Mb penetration into the bilayers.

Why Is Electron Exchange with Electrodes Facilitated in Films?

Most protein solutions contain macromolecular impurities. Adsorption of proteins and other macromolecules onto bare electrodes, often with denaturation, can create an insulating layer which inhibits passage of electrons.[4b] Thus, reversible electrochemistry of Mb was obtained only in highly purified solutions on "hydrophilic" ITO electrodes,[9] and slow electron transfer was found on PG electrodes. Exposure of these ITO or PG electrodes to partly purified Mb solutions blocked electron transfer in the highly purified Mb solutions.[18]

We detected proteinaceous adsorbates by FT-IR and X-ray photoelectron spectroscopy on PG and Pt electrodes exposed to partly purified Mb solutions.[18] These adsorbates blocked electron transfer to solution species. Solutions of cationic surfactants removed the adsorbates from these electrodes, and allowed voltammetry of Mb to be observed. We concluded that strong adsorption of *surfactants* at the electrode−film interface helps facilitate electron transfer. Adsorbed surfactant inhibits adsorption of macromolecules which would otherwise block electron transfer between Mb and the electrode.[18]

The dependence of $E^{\circ\prime}$, k'_s, and the amount of electroactive Mb on pH revealed proton-coupled electron transfer to Mb in liquid crystal films of DDAB, DMPC, and dilauroylphosphatidylcholine (DLPC).[23] $E^{\circ\prime}$ vs pH plots for Mb (Figure 3) show an intersection point at about pH 4.6, and a slope close to −59 mV pH^{-1} at pH > 5. Discontinuities near pH 4.6 were also found in plots of k'_s, the amount of electroactive Mb, and Soret band absorbance vs pH. From pH 3.2 to pH 8, all these parameters gave good fits to equilibrium models based on eq 1. Equation 1 represents known protonations of

$$\mathrm{MbFe(III)} + m\mathrm{H}^+ \rightleftharpoons \mathrm{MbFe(III)H}_m \qquad (1)$$

$$\mathrm{MbFe(III)H}_m + \mathrm{e}^- \rightleftharpoons \mathrm{MbFe(II)H}_m \qquad (2)$$

Mb below pH 5.[39] An average pK_a of 4.6 obtained from analyses of all these data is likely to be associated with these protonations, involving histidine residues in hydrophobic pockets of native Mb. This may involve the proximal histidine bound to iron and/or the distal histidine in the heme pocket, which are likely to influence electrochemistry.

A partly unfolded form of Mb similar to the *molten globule* predominates in the films at 4 < pH < 4.6 and is reduced directly.[23] Its existence is suggested by the Soret band and FT-IR spectra discussed earlier (cf. Figure 8).

Soret and FT-IR spectra[23] also show that conformations between pH 5.5 and pH 8 are similar to native metmyoglobin [MbFe(III)]. In this pH range, protonation of MbFe(III) occurs (eq 1) prior to electron transfer (eq 2),[23] and a protonated form accepts the electron. This electron acceptor is not a molten globule. The refolding of molten

globule apomyoglobin to native apoprotein is first order with a half-time of 1 s.[40] Invoking microscopic reversibility, conversion of native to molten globule Mb would take many seconds. Full conversion from native to molten globule Mb is not possible on the millisecond time scale of voltammetry. The electron acceptor in eq 2 may be a kinetic conformer on the unfolding pathway.

The Mb orientation represents an unknown influence on the electron-transfer rate. Mb orientations were measured on static films,[38] but voltammetry is dynamic. We really need to know the Mb orientation at the electrode−film interface at the time of electron transfer. Since Mb is mobile within these films,[17] static orientation may or may not be relevant to electron-transfer kinetics.

Electrochemical Enzyme Catalysis with Surfactant Films

Mb and cyt $P450_{cam}$ can serve as oxidation or reduction catalysts. Reductions catalyzed by these proteins are easiest to achieve electrochemically, so we began with reductive dehalogenation of organohalides.[41]

Dechlorination of trichloroacetic acid (eqs 3 and 4) demonstrated the ability of heme protein−surfactant films to support electrochemically driven enzyme-like catalysis. Both Mb and cyt $P450_{cam}$ in films catalyzed this reduction.[25,41]

$$PFe(III) + e^- \rightleftharpoons PFe(II) \text{ (at the electrode, P = protein)} \quad (3)$$

$$2PFe(II) + Cl_3CCOOH + H^+ \rightarrow Cl_2CHCOOH + 2PFe(III) + Cl^- \quad (4)$$

Electrochemical catalysis was explored in most detail for reductions of ethylene dibromide and trichloroacetic acid using Mb−DDAB films. Organic reactants enter the films by diffusion from solution. Ethylene dibromide and trichloroacetic acid were dehalogenated by electrochemically generated MbFe(II). The potentials required were >1 V positive of the direct reduction potentials of the organohalides, representing a large decrease in activation energy.[41]

Conditional rate constants k'_1 for the bimolecular rate-determining reaction of MbFe(II) with organohalide were estimated by voltammetry.[41] This is an *apparent* k_1 uncorrected for organohalide concentration, unknown in the films. Thus, k'_1 reflects a comparative reaction rate rather than an absolute rate constant. The value of k'_1 for trichloroacetic acid was 8000 M^{-1} s^{-1} in Mb−DDAB films, compared to 0.2 M^{-1} s^{-1} for MbFe(II) in solution. A smaller rate enhancement was found for ethylene dibromide. Rates are enhanced by partition of the organohalides into the films, increasing the reactant concentration at the reaction site.[41]

Outlook for the Future

This Account shows that cast liquid crystal surfactant films can facilitate electron exchange between redox enzymes or proteins and electrodes. The films are easily prepared and stable for a month or more, as long as the enzyme is stable. They enable detailed studies of electron transfer, conformation, and catalysis. Various heme proteins and ferredoxins have shown enhanced electron-transfer rates in these films, and the method appears to be somewhat general. However, it is unlikely that all redox proteins will give reversible voltammetry in these films. For example, we did not obtain direct electron transfer for glucose oxidase. Systematic insight into protein requirements for successful voltammetry in these films is presently lacking.

Major advantages of the films for biochemical studies include stability, enhanced electrochemical reversibility, the ability to apply a battery of electrochemical and optical methods, and the tiny amounts of enzyme required. The films are similar to biomembranes, so it should be possible to incorporate membrane-bound enzymes. Diverse biochemical redox processes should be amenable to detailed in vitro studies in surfactant films. For example, we are currently investigating electrochemically driven enzyme-catalyzed toxic activation of pollutants using cyt P450−lipid films.

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