exhibited the same binding volume to P-450_{LM2} as the complete b_5 macromolecule.[14]

The formation of stable protein–protein complexes of the correct structural conformation is an important component of the observed specificity of electron-transfer events in physiological systems. Various authors have suggested the involvement of charge-pair or dipolar interactions in determining the final specificity and orientation of the constituent proteins in a productive aggregate.[6-8] In this model, the close juxtaposition of opposite charges contributes to the orientation energy necessary for correct positioning of the proteins and can aid in supplying the energy necessary for equal potential electron transfer between reactant- and product-activated complexes.[10] Separation of the aggregate would then result in the exposure of this surface contact domain to solvent. The effect of pressure on an ionization equilibrium can be qualitatively understood by considering this solvation process. The electrostriction of water around a bare charge results in a smaller system volume, and hence high pressure will favor the state with the greatest net charge exposed to solvent. Hence, the ionization of acetic acid, which represents a change in total solvated charge of 2 (COOH being uncharged and COO^- + H^+ representing two charges), is accompanied by a large decrease in volume (−11 mL/mol), whereas the ionization of a primary amine or an imidazole nitrogen (which results in no net change in system charge) is less affected by pressure (−1 mL/mol).[11,12] The understanding of relatively simple chemical ionizations has been extended to encompass the more difficult case of macromolecular dissociation by Weber and co-workers.[4] In the work presented in this paper, we extend measurements of multimeric protein dissociation to the case of a heterologous dimer of physiological redox transfer proteins. If complementary charge pairing occurs between cytochrome b_5 and cytochrome P-450_{LM2} at the protein–protein interface, one might expect a large volume change for the system on dissociation of the complex due to the solvation of exposed charges. To the extent that the separation of charge in the case of heterologous dimer dissociation is analogous to the ionization of acetic acid,[11] and assuming that solvent is completely excluded in the dimer interface,[7,8] our observed volume change for the cytochrome b_5–P-450_{LM2} couple of −23.0 mL/mol is consistent with the involvement of roughly two ion pairs in the association of these two proteins.[15] Such a conclusion is consistent with that reached from protein chemical modification studies[2] and illustrates a potentially powerful application of high-pressure spectroscopy for study of electron donor–acceptor complexes.

(14) Work is in progress which will characterize the interactions with a number of heme proteins with various mutant forms of soluble rat cytochrome b_5 that have been constructed by site-specific mutagenesis techniques.

(15) Typical volume changes previously observed for spin-state transitions in heme proteins are on the order of 5 to 15 mL/mol.[16-18] However, the pressure-induced dissociation of camphor and camphor analogues from cytochrome P-450_{cam} exhibits very similar volume changes regardless of the original ferric spin state distribution.[19] The dissociation volumes for substrates bound to cytochrome P-450_{cam} differ by only 2–7 mL/mol. This suggests that the conformational change which occurs during a spin-state transition in cytochrome P-450_{cam} is small. Small-angle X-ray scattering measurements have shown that the radius of gyration for the predominantly low-spin camphor-free form and predominantly high-spin camphor-bound form are nearly equal, 23.9 ± 0.2 and 23.7 ± 0.2 Å, respectively.[20] Numerous investigators have suggested that the ferric high-spin form of cytochrome P-450 exists as a five-coordinate species.[21-23] The low-spin form of the porphyrin-chelated ferric iron is six-coordinate with H_2O or OH^- providing the sixth axial ligand.[22-26] Since the active site of P-450_{LM2} is accessible to solvent[27] thus resembling cytochrome P-450_{cam}, it is reasonable to suggest that the conformational changes which occur during the ferric spin transition of P-450_{LM2} are also small compared to the cytochrome b_5–P-450_{LM2} dissociation.

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Electrochemistry of Heptylviologen in the Presence of Phosphatidylcholine Liposomes

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This paper describes novel preliminary results on the electrochemistry of 1,1′-diheptyl-4,4′-bipyridinium ion (heptylviologen, HV^{2+}) in the presence of 1-phosphatidylcholine (PDC) liposomes. Vesicles and liposomes are currently attracting considerable interest due to the structural similarity of their bilayer walls to biological membranes.[1] To the best of this author's knowledge this is the first report on electron transfer to or from a dimensionally stable electrode and vesicle-solubilized redox substrates.

The electrochemical behavior of heptylviologen dibromide in 50 mM phosphate buffer (pH 7) is not controlled by diffusion due to the water-insoluble nature of its two reduced forms ($HV^{\bullet +}$ and HV). Cyclic voltammetry of this solution yields two close reduction peaks and three oxidation spikes upon scan reversal. The reductive behavior is explained by two consecutive one-electron reduction steps to yield the cation radical and the neutral compound.[2] The sharp peaks on the oxidative scan result from the precipitation of the $HV^{\bullet +}$ and HV forms onto the electrode surface.[3]

The addition of a cationic surfactant, such as cetyltrimethylammonium chloride (CTAC), changes this electrochemical behavior (see Figure 1A). The cation radical, $HV^{\bullet +}$, is solubilized by the cationic micelles probably via coaggregation. Conversely, the solubilizing capacity of the CTAC micelles is overwhelmed by the fully reduced HV as can be concluded from the sharp spike on the oxidative scan. In the presence of PDC liposomes[4] (see

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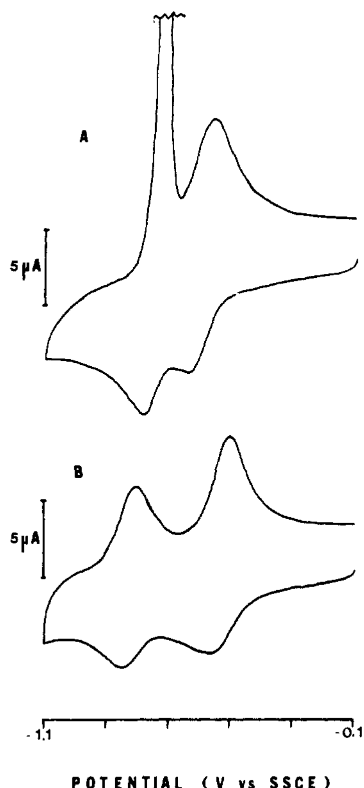

Figure 1. Cyclic voltammograms at a glassy carbon electrode (projected area = 0.08 cm^2) of 0.5 mM heptylviologen in 50 mM phosphate buffer (pH 7) also containing (A) 12 mM CTAC and (B) 12 mM PDC. The latter solution was sonicated and centrifuged before electrochemical analysis. Scan rate 50 mV/s.

Figure 1B), the electrochemistry of HV^{2+} displays two reduction and two corresponding oxidation peaks whose shape approximates that for purely diffusional behavior and is free from the redissolution spikes observed either in the absence or in the presence of CTAC micelles. This indicates that the liposomes solubilize the $HV^{\bullet+}$ and HV forms, preventing their precipitation on the electrode surface.

The peak current for the first reduction of HV^{2+} in the presence of either liposomes or micelles is linearly related to the square root of the scan rate in the range from 10 to 200 mV/s. Thus, currents are controlled by diffusion. The apparent diffusion coefficient (based on the first reduction wave and a concentration of heptylviologen of 0.5 mM) in the presence of micelles was found to be 2.3 times higher than that for the liposome solutions. This can be rationalized by the interaction of HV^{2+} with both types of aggregates. A 2-nm red shift was observed in the UV absorption of the dication in the presence of PDC liposomes, while CTAC micelles did not alter the absorption characteristics of the dication. Nonetheless, some degree of HV^{2+} coaggregation with the cationic micelles seems likely owing to the mild surfactant nature of this ion.[2] The slower overall diffusion in the presence of liposomes is essentially a consequence of the large size of these aggregates (the diameter of sonicated liposomes is about 250 Å, 5–10 times larger than those of micelles) although this seems to be partially offset by the high number of viologens associated with each liposome.[5] It must be pointed out that the diffusional problem in these solutions is far from simple and, perhaps as a reflection of this complexity, the ratios of anodic/cathodic peak currents are larger than one. The reasons are not clear yet. Deviations from the theoretical ratios for multistep electron-transfer processes have been noted previously in micellar solutions.[6] Control experiments have shown that the presence of PDC liposomes does not modify the kinetics of heterogeneous electron transfer of water-soluble couples like methylviologen and its cation radical or ferri-/ferrocyanide. No blocking of the electrode surface by lipid molecules was detected in these experiments.

Table I. Peak and Half-Wave Potentials[a] for the Reduction of HV^{2+} in Several Media

medium	reduction potentials	oxidation potentials	half-wave potentials
(A) 50 mM phosphate buffer (pH 7)	−0.70	−0.56	*b*
	−0.76	−0.65	*b*
		−0.71	
(B) (A) + 12 mM CTAC	−0.63	−0.56	−0.60
	−0.79	−0.73	*b*
(C) (A) + 12 mM PDC[c]	−0.56	−0.50	−0.53
	−0.85	−0.80	−0.83
(D) 0.1 M $TBABF_4$ in MeCN	−0.44	−0.38	−0.41
	−0.87	−0.81	−0.84

[a] All potentials (in volts) were measured against a SSCE. [b] Precipitation effects prevented the determination of reasonably accurate values. [c] Sonicated and centrifuged before electrochemical analysis.

The peak and half-wave potentials measured for the two one-electron reductions of the viologen are given in Table I. Some of these values may be affected by the partitioning of the dication between the bulk aqueous phase and the aggregates, but the observed trends are still very significant. In liposome solutions, the evident increase of the potential separation between the two reductions of HV^{2+} (both shifting toward the nonaqueous limit; see values for acetonitrile solution in Table I) clearly reflect the remarkable stabilization of the cation radical, suggesting the incorporation of this species into a fairly hydrophobic environment, such as the liposomes' membranes.[7] This is further supported by the solubilization of $HV^{\bullet+}$ and HV in liposomes (vide supra). Furthermore, preliminary ESR spectra of $HV^{\bullet+}$ show that the mobility of the cation radical is largely restrained as indicated by a broadened spectrum with some hyperfine structure superimposed, also pointing toward the incorporation of the cation radical into the lipid bilayer membrane of the liposomes.

Research efforts are under way in this laboratory to explore the effects of liposomes on the electrochemistry of hydrophobic substrates. It is hoped that these experiments could open new ways to study the electrochemistry of naturally found electron-transfer mediators in biomembranes under conditions quite similar to those encountered in biological systems.

(4) This solution was prepared according to well-established liposome formation procedures. Namely, 1 mL of a 100 mg/mL PDC ethanolic solution (Sigma) was slowly evaporated under a stream of argon. The resulting lipid film was dried overnight under vacuum at 40 °C. The dried lipid was then suspended in 10 mL of 50 mM phosphate buffer (pH 7) and sonicated under argon, in an ice-water bath, to constant turbidity with a Branson Model 185 probe sonifier. The sonicated, transparent solution was then centrifuged to remove titanium particles released by the tip of the probe. $HVBr_2$ (Aldrich), 2.8 mg, was then added to give a total concentration of 0.5 mM. A second method was tried based on cosonicating the lipid and the viologen. This produced pale blue solutions, indicative of some reduction of the viologen in solution, probably coupled to the oxidation of some PDC. Although the electrochemical results were similar, the first method was preferred.

(5) If the Poisson distribution is used to calculate the distribution of viologen ions among the liposomes (taking the aggregation number as 2400[1a]) 90% of these are found to contain from 84–116 viologen probes. On the other hand, 21% of the CTAC micelles contain one; 25%, two; 21%, three; 13%, 4; and minor percentages contain none, five, or six viologen ions.

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