rhombic 2.10 signal reached a maximum intensity corresponding to ~0.5 spin/molecule, while the axial 2.06 signal was reduced to a minimum value of 0.3 spin/molecule. Since the light beam was focused onto only a part of the sample, complete conversion of the axial 2.06 signal to the rhombic 2.10 signal was not expected. Most interestingly, this photoinduced process was found to be reversible by warming up the sample and letting it stand for 10 min at 150 K. An EPR spectrum recorded at 9 K after warming is shown in Figure 1F. The rhombic 2.10 signal disappeared, and the axial 2.06 signal recovered approximately 80% of its original intensity. Storing the sample in liquid nitrogen for 24 h restored the full intensity of the axial 2.06 signal. For a given sample the irradiation-warming cycle could be repeated several times with consistently reproducible results.

The rhombic 2.10 signal could also be induced by partially oxidizing the reduced hydrogenase under an argon atmosphere.[12] In order to show that CO is involved in the above mentioned photoconversion process, we produced a partially reoxidized hydrogenase sample under argon exhibiting the 2.10 signal and subjected it to the higher temperature (150 K). It was found that the argon-induced rhombic 2.10 signal retained its full intensity and did not convert into the axial 2.06 signal.

Both the axial 2.06 and the rhombic 2.10 signals have been observed previously in the [Fe] hydrogenases isolated from *D. vulgaris*,[6,12] *Clostridium(C.) pasteurianum*,[13,14] and *Megasphaera elsdenii*.[15] In the case of *C. pasteurianum* hydrogenase I the axial 2.06 signal was induced by reacting CO with preparations which exhibited the rhombic 2.10 signal.[13] The axial 2.06 signal was shown to represent a CO-bound cluster by the observation of ^{13}C resonances in an ENDOR study of the ^{13}CO-treated enzyme.[16] In the case of the [Fe] hydrogenase from *D. vulgaris*, the relationship between these two signals has not been extensively studied. It should be noted in this regard that the axial 2.06 signal can also be induced by chemical oxidants.[6] Furthermore, the physiological significance of the axial 2.06 signal has long been a controversy. It has been proposed that the axial 2.06 signal observed in *D. vulgaris* hydrogenase was caused by the unintentional exposure of the enzyme to oxygen and represented irreversibly inactivated hydrogenase.[12] However, our recent investigation has shown that *D. vulgaris* hydrogenase with the fully developed axial 2.06 signal (induced by CO) could be reversibly activated.[17]

The present study suggests that the CO-induced 2.06 and the rhombic 2.10 signals observed in *D. vulgaris* hydrogenase originate from the same iron-sulfur cluster with the axial 2.06 signal representing the putative CO-bound cluster and the rhombic 2.10 signal, the unligated cluster. The effect of irradiation is to flash off the bound CO. The facts that the rhombic 2.10 signal retains its intensity at low temperature and that it can be converted back to the axial 2.06 signal by raising the temperature suggest that the flashed-off CO remains in the protein matrix but is separated from the cluster by an energy barrier(s). At higher temperatures, the CO is capable of crossing the barrier and recombining with the cluster, a plausible mechanism which bears similarity with that described for the CO binding of myoglobin where certain energy barriers were postulated.[18] Since CO is a competitive inhibitor of *D. vulgaris* hydrogenase, it is apparent that both the axial 2.06 and the rhombic 2.10 signals are of physiological significance. In general, metal centers in proteins can be grouped into catalytic substrate-binding sites as well as electron-transfer centers. The observed photoreaction of the 2.06 signal demonstrates that in the [Fe] hydrogenase from *D. vulgaris* a specific iron-sulfur cluster is involved in ligand binding. The fact that the 2.06 and the 2.10 signals are commonly observed in the [Fe] hydrogenases suggests further that the [Fe] hydrogenases must share this unique active center.

(11) In addition to the rhombic 2.10 signal, a weak EPR signal with g values at 2.21 and 2.15 was also detected upon irradiation. However, its intensity did not increase with irradiation time. A control experiment on buffer solution indicated that this weak signal was intrinsic to the protein sample.

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A General Treatment of Hydrogen Bond Complexation Constants in Tetrachloromethane

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We wish to report a generalized treatment applicable to 1:1 hydrogen bond complexation constants (as log K values) for a large number of acid:base pairs in solvent tetrachloromethane. Recently, we have shown[1] that when log K values for a series of acids (hydrogen bond donors) against a given reference base are plotted versus log K values for the acid series against any other reference base, there results a set of lines that intersect at a point where log $K = -1.1$, when equilibrium constants are expressed in molar concentration units. Because the order of solute hydrogen bond acidity is independent of the reference base (for exceptions see

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Table I. A Selection of Some α_2^H and β_2^H Values[1,2]

solute	α_2^H	β_2^H
water	0.353	*a*
methanol	0.367	*a*
2,2,2-tribromoethanol (TBE)	0.478	*a*
2,2,2-trichloroethanol (TCE)	0.500	*a*
2,2,2-trifluoroethanol (TFE)	0.567	*a*
hexafluoro-2-propanol	0.771	*a*
phenol	0.596	*a*
4-chlorophenol	0.670	*a*
4-nitrophenol	0.824	*a*
1-naphthol	0.608	*a*
2-naphthol	0.612	*a*
carbazole	0.469	
indole	0.436	
pyrrole	0.408	
hexamethylphosphortriamide	0	1.000
Ph_3PO	0	0.919
DMSO	0	0.775
DMF	0	0.663
$(MeO)_3PO$	0	0.762
$(PhO)_3PO$	0	0.624
acetone	0.04	0.497
diethyl ether	0	0.450
ethyl acetate	0	0.446
triethylamine	0	0.669
pyridine	0	0.625

[a] For these solutes, reliable β_2^H values are not yet available. No log *K* values involving these solutes as bases were used to obtain eq 3.

later) it was possible to obtain an "average" hydrogen bond acidity for solutes in CCl_4 that we denoted as log K_A^H. These were then transformed[1] into a solute hydrogen bond acidity scale, α_2^H, simply via eq 1.

$$\alpha_2^H = (\log K_A^H + 1.1)/4.636 \quad (1)$$

Similarly, it can be shown[2] that when log *K* values for a series of bases (hydrogen bond acceptors) against a given reference acid are plotted versus log *K* values for the base series against any other reference acid, a set of lines is obtained that also intersect at the point where log $K = -1.1$, as above. It is then possible to obtain an "average" hydrogen bond basicity for solutes in CCl_4, denoted as log K_B^H, and then to transform them into a basicity scale via eq 2, where the factor 4.636 is chosen so that $\beta_2^H = 1.0$ for the base hexamethylphosphortriamide.[2] This factor has no physical significance but serves merely to yield a convenient range of α_2^H and β_2^H values.

$$\beta_2^H = (\log K_B^H + 1.1)/4.636 \quad (2)$$

We now show that these α_2^H and β_2^H values, that refer specifically to solute hydrogen bond complexation at 298 K in CCl_4, can be combined in a general equation that can be used to predict a vast number of hitherto unknown log *K* values. We have available a matrix of 89 primary α_2^H and 215 primary β_2^H values that contains 1312 experimental log *K* values for complexation in CCl_4. On plotting these log *K* values versus the product $\alpha_2^H \cdot \beta_2^H$ for the relevant acid–base combinations, there results an excellent line given by eq 3, where the number of data points is 1312, the

$$\log K = (7.354 \pm 0.019)\alpha_2^H \cdot \beta_2^H - (1.094 \pm 0.007) \quad (3)$$

correlation constant is 0.9956, and the standard deviation is 0.093 log units. Within experimental error, the intercept corresponds to the already observed magic point of log $K = -1.1$, as required. Since the complete matrix contains $89 \times 215 = 19\,135$ possible log *K* values, we are now in a position to predict the remaining 18 000 log *K* values with an estimated precision of around 0.1 log units. We have available[1,2] a total of some 150 α_2^H and 500 β_2^H values (including primary and secondary values), so that estimates of log *K* can be made for a further 57 000 acid–base combinations. These include CH, SH, NH, OH aliphatic and aromatic acids and nearly all the basic functional groups encountered in organic chemistry. In Table I are given a selection of the α_2^H and β_2^H values we have used.[1,2] It should be noted that eq 1–3 are not completely general, in that some particular acid–base combinations are excluded, specifically weak acids such as pyrrole, indole, 5-fluoroindole, Ph_2NH, $CHCl_3$, etc. with bases such as pyridine, amines, and ethers.[3] But note that combinations of these acids with other bases are included.

Table II. Observed and Calculated log *K* Values for Combinations Not Used in the Data Base[a]

	base		
acid	Ph_3PO	$(MeO)_3PO$	$(PhO)_3PO$
2-naphthol	3.06	2.35	1.76
	(3.04)	(2.34)	(1.71)
1-naphthol	2.97	2.32	1.72
	(3.01)	(2.31)	(1.70)
phenol	2.82	2.20	1.62
	(2.93)	(2.25)	(1.64)
TFE	2.77	2.17	1.60
	(2.74)	(2.08)	(1.51)
TCE	2.30	1.69	1.21
	(2.29)	(1.71)	(1.20)
TBE	2.16	1.54	1.09
	(2.14)	(1.58)	(1.10)
carbazole	1.94	1.37	1.10
	(2.08)	(1.53)	(1.06)
indole	1.75	1.23	0.98
	(1.85)	(1.35)	(0.91)
pyrrole	1.48	1.03	0.78
	(1.66)	(1.19)	(0.78)

[a] Observed values in CCl_4 at 298 K from ref 11; calculated values from eq 3 shown in parentheses.

We believe that eq 3 can be generalized to eq 4, where *m* and *c* may depend on the solvent (and also on the standard state) but are independent of acid and base, judging from preliminary work we have carried out by using cyclohexane and 1,1,1-trichloroethane as solvents.

$$\log K = m\alpha_2 \cdot \beta_2 + c \quad (4)$$

An early report,[4] containing important data on hydrogen bonding of bases toward pyridine-*N*-oxide in cyclohexane, suggested that the standard free energy of hydrogen bonding (as $\Delta G°$ or log *K*) could be represented by an additive sum, rather than by a product term. Terent'ev[5] later claimed that a product term was not applicable to $\Delta G°$ values (although it was to $\Delta H°$). More recently[6] there has been a specific proposal that an $\alpha\beta$ product is necessary in the interpretation of processes in which solute acids hydrogen bond to solvent bases ($\alpha_2\beta_1$) and solute bases hydrogen bond to solvent acids ($\alpha_1\beta_2$). This $\alpha\beta$ formalism has already been used in the discussion of a number of physicochemical and biochemical (toxicological) properties of solutes.[7-9] The present report, however, provides the first direct confirmation of an $\alpha_2\beta_2$ relationship, and we feel lends considerable support to the notions

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of $\alpha_2\beta_1$ and $\alpha_1\beta_2$ relationships. We note that product terms are also involved in the E/C equation of Drago.[10] We have not attempted to relate our parameters to Drago's E and C values, partly because many of the acids listed by Drago are Lewis acids and are not hydrogen bond acids, but also because the E/C equation is specifically designed to correlate enthalpies of complexation, whereas our eq 3 is couched in terms of Gibbs energies of complexation (as log K).

In order to illustrate the power of the simple eq 3, we have calculated log K values for some acid-base combinations recently studied by Ruostesuo et al.[11] and published *after* our data base, leading to eq 3, had been set up. Observed and calculated log K values are given in Table II. The average deviation between observed and calculated values is 0.02 log units, and the standard deviation only 0.05 log units, the latter well within our value of 0.09 log units for 1312 complexation constants, eq 3.

Note that throughout this paper, all acidities refer to solute hydrogen bond acidity. In forthcoming publications we shall point out the large differences that exist between these acidities and acidities that refer to full proton transfer.

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Direct Electrochemistry of the Undecapeptide from Cytochrome c (Microperoxidase) at a Glassy Carbon Electrode

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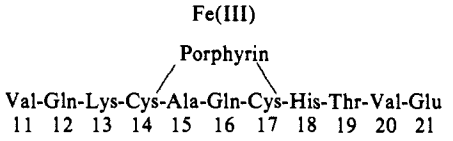
Application of cyclic voltammetry to the study of electron transfer of various metalloproteins has clarified several interesting aspects of the redox reaction of cytochrome c,[1,2] ferredoxins,[3,4] and plastocyanins.[5] In particular, direct electrochemistry of the redox process of these systems has been achieved in the presence of appropriate promoters, and some aspects of the mechanism of electron transfer between buried redox sites of the proteins and the electrode surface have been clarified.

We report here an electrochemical investigation of the undecapeptide obtained by hydrolysis of horse heart cytochrome c (called microperoxidase),[6,7] which contains iron protoporphyrin IX covalently bound by thioether bridges to Cys 14 and 17 (where the numbers refer to the amino acidic sequence of native horse heart cytochrome c).



This heme-peptide represents a good system in order to gain further information on (a) the factors controlling rapid electron transfer between a biopolymer and the electrode surface including the role played by electrostatic interactions and (b) the electrochemical behavior of the heme group when relatively unshielded by the protein but still covalently bound to cysteines and in a soluble state.[8]

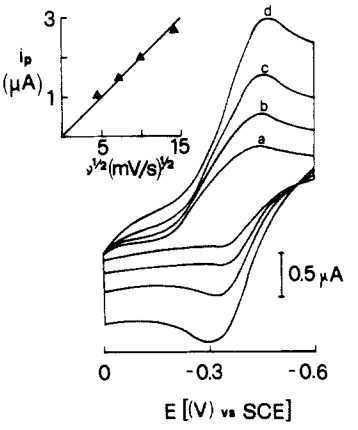


Figure 1. Dc cyclic voltammetry of microperoxidase (0.5 mg/mL) in $NaClO_4$ (0.1 M) and phosphate buffer (0.02 M) at pH 7.0, in the potential range 0 to -0.60 V vs SCE; sweep rate: (a) 20; (b) 50; (c) 100; (d) 200 mV/s. Temperature: 25 °C. Inset shows a plot of i_p vs (scan rate)$^{1/2}$.

The results reported below clearly indicate that the heme peptide undergoes rapid and reversible electron transfer at a glassy carbon electrode, with $E_{1/2}$ approximately -160 mV vs NHE (at pH 7.0 and 25 °C). The promotion of electron transfer by Mg^{2+} ions has also been investigated and found to be significant but not crucial.

Experiments were carried out in 20 mM phosphate buffer, pH 7.0, with either 100 mM sodium perchlorate or 25 mM magnesium perchlorate as supporting electrolyte. Microperoxidase was obtained from SIGMA (U.S.A.) or prepared in our laboratory following the method of Harbury and Loach.[7] An Amel 473 multipolarograph equipped with an Amel 863 recorder was used for voltammetric measurements.

Figure 1 shows the dc cyclic voltammograms of microperoxidase (0.5 mg/mL) in the presence of 100 mM Na^+. A well-defined electrochemistry is observed; the cathodic and anodic peaks are similar in shape and magnitude, with a i_{pa}/i_{pc} ratio close to unity. For a fully reversible one-electron-transfer reaction, a peak separation ΔE_p = 57 mV (at 25 °C), independent of the scan rate, is expected.[9] In agreement with previous studies,[3,5] we found that ΔE_p increases with scan rate, the smallest value of about 90 mV being obtained at a scan rate of 20 mV s^{-1}. The calculated redox potential, $E_{1/2} = -160 \pm 8$ mV vs NHE, lies within the range of the potentiometric value reported by Harbury and Loach under similar conditions ($E_{1/2} \simeq -190$ mV vs NHE, at pH 7.0 and 25 °C).[8] As shown in the inset to Figure 1, i_{pc} is proportional to the square root of the scan rate; thus, the redox process of microperoxidase at the electrode surface is diffusion-controlled.[9] From the slope of the curve, a value of $D_0 = 2 \times 10^{-6}$ cm^2 s^{-1} for the diffusion coefficient was calculated.[10] Thus, the dc cyclic voltammetry shows that microperoxidase takes part in a rapid one-electron reaction at the glassy carbon electrode in the absence of mediators. In accordance with the procedure of Nicholson,[11] the rate constant for heterogeneous electron transfer was calculated to be $k_s = 3 \pm 1 \times 10^{-3}$ cm s^{-1}, based on $n = 1$, $\alpha = 0.5$ and $T = 25$ °C.

Cyclic voltammograms performed at different concentrations of microperoxidase (0.1-1 mg/mL) gave results similar to those shown in Figure 1. Therefore, no indication of gross effects related

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