Table I. IR and UV/Vis Data for Photolysis of $CpMn(CO)_3$ in the Presence of Either Haloalkane or THF at 195 K. Photoacoustic Data, Recorded at 298 K, for the Substitution of CO by Haloalkane or THF According to Scheme I[a]

	ν_{CO} (cm^{-1})		λ_{max} (rel ϵ)	$\Delta H_{1,5}$	$\Delta H_{5,3}$	$k_2 \times 10^6$	ΔH_{Mn-S} ±2
	neat[b]	hexanes[b]	±5 (nm) (neat)	(kcal/mol)	(kcal/mol)	(L/mol·s)	(kcal/mol)
CH_2Cl_2	1867	1877	393(1.2)	46.7 ± 1.1	−9.0 ± 1.0	4.9 ± 2.6	−17
	1946	1953	501(1.0)				
CH_2Br_2				47.1 ± 0.9	−12.2 ± 1.2	8.1 ± 2.5	−20
n-BuCl	1867	1874	393(1.2)	46.9 ± 1.0	−10.5 ± 1.8	3.4 ± 0.8	−19
	(1932)	(1939)	493(1.0)				
n-BuBr	1870	1876	394(1.9)	46.7 ± 1.0	−13.3 ± 1.3	5.3 ± 1.2	−21
	(1932)	(1939)	508(1.0)				
n-$C_5H_{11}Br$	1869	1877	392(2.3)	46.6 ± 0.6	−13.4 ± 3.2	5.4 ± 2.6	−21
	(1932)	(1939)	494(1.0)				
THF	1845	1858	396(1.1)	46.2 ± 1.2[c]	−16.1 ± 1.4[c]	4.4 ± 0.4[c]	−24
	(1932)	(1939)	507(1.0)				
$CpMn(CO)_3$	2021	2026					
	1932	1939					

[a] Errors are 1σ. [b] Values of ν_{CO} in parentheses are estimated on the basis of overlap with the lower energy ν_{CO} band of $CpMn(CO)_3$. [c] Reference 17.

confirms that **3**-XR contains two CO ligands and that the haloalkane ligand is easily substituted. Since oxidative addition of a haloalkane to the metal center is likely to be difficult to reverse, the haloalkane appears to remain intact and is probably coordinated to the metal center through the halogen lone pairs.[15]

The energetics of coordination of the haloalkanes to the metal center were examined with photoacoustic calorimetry.[16-18] Photolyses of $CpMn(CO)_3$ in heptane solutions of RX (0.2-1.3 M) initially yield the heptane-solvated intermediate **5** with an enthalpy of reaction $\Delta H_{1,5}$ (Scheme I). These values are identical within experimental error and agree well with previously reported values.[17,18] Subsequent substitution of the heptane by haloalkane occurs with the bimolecular rate constant k_2 and enthalpy of reaction $\Delta H_{5,3}$ to give the corresponding **3**-XR (Table I).

In the $CpMn(CO)_2$ system all the haloalkanes studied are weaker ligands than is THF. The bromoalkanes are more strongly bound to the Mn center than are the corresponding chloroalkanes, and although the kinetic data is less definitive, it appears that k_2 values for the bromoalkanes are greater than k_2 values for the corresponding chloroalkanes. The dihalomethanes are less strongly bound to the metal than the corresponding monohaloalkanes, yet k_2 is greater for the dihalomethanes. The weaker bond is a result of the electron-withdrawing character of the second halogen reducing the donating ability of the coordinated halogen. However, the second halogen atom increases the number of possible binding sites for coordination to the metal center; hence k_2 is greater.

If the strength of the Mn-heptane bond ($-\Delta H_{Mn-hept}$, Scheme I) in **5** is known, the enthalpy of the haloalkane-metal bond in **3**-XR (ΔH_{Mn-S}) can be calculated using eq 1.

$$\Delta H_{Mn-S} = \Delta H_{5,3} + \Delta H_{Mn-hept} \quad (1)$$

From previous studies, $\Delta H_{Mn-hept}$ is −8 ± 1 kcal/mol.[17,18] The calculated values of ΔH_{Mn-S} range from −17 kcal/mol for CH_2Cl_2 to −21 kcal/mol for 1-bromobutane and 1-bromopentane. Thus the bromoalkanes have overall metal-ligand bond strengths that are nearly sufficient to allow isolation of stable intermolecular bromoalkane complexes.

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Electrochemical Probes of Protein Folding

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Intense fundamental and practical interest is focused on the factors that control the thermodynamics and kinetics of protein folding. Yet, most details, particularly regarding the pathways for folding, remain obscure.[1] In part, this situation reflects the difficulties of initiating folding via rapid (submillisecond) processes. To approach this and related problems, we have studied protein folding induced by rapid oxidation/reduction techniques. As a first step in such studies, we have found that, under limiting conditions, unfolding thermodynamics can be estimated using electrochemical techniques. As these conditions are modified, simple electrochemical methods (e.g., cyclic voltammetry) can also provide kinetic information on folding rates and (perhaps) intermediates. Cytochrome *c* is an ideal protein for such investigations. The thermodynamics[2,3] and kinetics[2,4-6] of cytochrome

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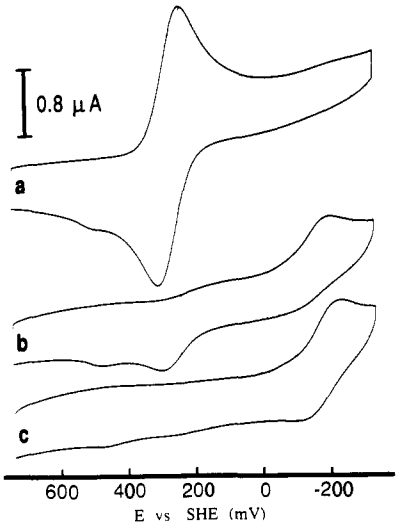
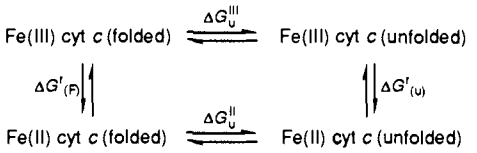


Figure 1. Cyclic voltammetry of horse heart Fe(III) cytochrome *c* at a 4,4′-dithiodipyridine-modified gold electrode: 2.0 mM N_2-purged cytochrome *c* in 0.1 M pH 6.0 Kphos at 23 °C; scan rate, 25 mV/s: (a) no guanidine, (b) 3.5 M guanidine, and (c) 7.0 M guanidine. The cathodic feature at −100 mV and anodic feature at +470 mV are electrode artifacts, due to an impurity in "ultrapure" gdn and gold (oxide) surface effects.

c folding have been extensively studied. Reduced Fe(II) cytochrome *c* appears, by a variety of criteria, to be far more stable toward unfolding than is oxidized Fe(III) cytochrome *c*.[7] However, the magnitude of this difference remains rather uncertain.[3]

As outlined below, combining electrochemical data with an appropriate thermodynamic cycle provides a quite precise measure of this differential stability. The direct electrochemistry of cytochrome *c* is well developed for the folded state,[8a,b] though no report has appeared of the direct redox potential of the unfolded state of cytochrome *c*.[8c]

Following literature protocols[8] using a 4*S*,4′*S* bipyridine modified gold electrode,[8b] cyclic voltammetry of folded oxidized horse heart cytochrome *c* yields a nearly reversible redox response with E° = +286 mV vs SHE (see Figure 1a). As guanidine·HCl (Gdn) is added, this formal potential of folded cytochrome *c* shifts to +264 mV, remaining constant at that value from 0.5–3 M Gdn. This shift reflects the stabilization of the Fe(III) potential by Cl^- binding.[9] With increasing Gdn concentration, the voltammetric peaks for native cytochrome *c* disappear, and a new cathodic peak is observed ($E^{\circ\prime}$ = −167 mV), which becomes reversible when the concentration Gdn > 6 M (Figure 1b,c). Although relatively large peak separations are observed ($\Delta E_{pp} \geq 100$ mV), peak current scales linearly with (scan rate)$^{1/2}$. Thus, under these conditions, the electrochemical response of the unfolded state is quasi-reversible and diffusion controlled. The relatively low potential for unfolded cytochrome *c* is consistent with coordination of a single histidine and a water molecule or nitrogenous base, to the heme,[10,11] which is likely to be highly solvent exposed. The peak currents observed for horse cytochrome *c* in the sequence in Figure 1 provide estimates of the concentrations of Gdn which induce unfolding of the Fe(III) cytochrome *c* ($D_{1/2} \approx 2.5$ M) and Fe(II) cytochrome *c* ($D_{1/2} \approx 5$ M). These estimates agree well with the literature.[2,3] More importantly, the measured redox potentials provide a high precision measurement of the *difference* in stability between oxidized and reduced cytochrome *c*, which is not plagued by the uncertainties attendant with long extrapolations of solution conditions. Consider the following thermodynamic cycle:



Clearly, the difference $\Delta G_u^{III} - \Delta G_u^{II} = \Delta\Delta G_u$ is simply given by $\Delta G'_{(F)} - \Delta G'_{(U)} = -0.239nF(\Delta E^\circ) = -10$ kcal/mol for horse heart cytochrome *c*.[12] This large stabilization reflects, in part, the large increase in methionine bond strength which accompanies reduction. The estimated $\Delta\Delta G_u$ value in fact agrees quite well with values obtained by (linear) extrapolation methods.[3] The electrochemical approach thus reinforces and extends conventional spectroscopic approaches to protein folding thermodynamics.

Finally, we briefly consider the kinetic implications of these initial experiments. Note that, from Figure 1, there exists a region between 3 and 5 M [Gdn] where the reduced protein is stable toward unfolding while the oxidized protein is not. Thus, in this range, when the unfolded, oxidized protein is reduced ($E_{app} \sim$ −200 mV), one observes the corresponding reoxidation peak not at the potential for unfolded protein ($E \sim$ −200 mV) but at that for the folded protein ($E \sim$ 300 mV). This refolding peak is most pronounced in Figure 1b, where the Gdn concentration is well above $D_{1/2}$ for oxidized cytochrome *c*, but well below $D_{1/2}$ for reduced cytochrome *c*. Clearly, folding has occurred during the time of the reverse sweep (ca. 2 s). A small return wave (~10% of the cathodic peak) is observed, independent of scan rate. This species is logically assigned to the known, slow-refolding component observed in kinetic studies,[5,6] which appears to arise from the (slowly equilibrating) *cis*-proline isomers of unfolded cytochrome *c*. In principle, the rate of the folding process might be monitored by varying the sweep rate. Up to a scan rate of 1 V/s, the folding process remains unresolvably fast (i.e., $\tau < 100$ ms). Faster scans are precluded by the relatively slow interfacial charge transfer of cytochrome *c*. Thus, other kinetic techniques (e.g., ring disk voltammetry) are more appropriate to obtain millisecond resolution, and such studies are in progress.

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