Electrochemical Studies of Cyanometmyoglobin and Metmyoglobin: Implications for Long-Range Electron Transfer in Proteins

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Abstract: The electron-transfer reactions of high-spin metmyoglobin and low-spin cyanometmyoglobin have been studied electrochemically. The striking differences seen for the reactions of these complexes are apparent when the solutions studied contain both metmyoglobin and cyanometmyoglobin. The redox potential for cyanometmyoglobin has shifted negatively over 400 mV, from +0.046 to −0.385 (±0.015) V vs NHE, and the rate of electron transfer has increased by more than 1 order of magnitude compared with that for metmyoglobin, from 7×10^{-6} to 5.4 (±0.9) $\times 10^{-4}$ cm s^{-1}. Only subtle effects are observed when the high-spin complexing ligand is fluoride. The reorganizational energy accompanying spin-state change of the heme iron is believed to be the primary factor controlling the rates of electron-transfer reactions described here. As expected, results from cyclic voltammetric experiments show that when cyanometmyoglobin undergoes electron transfer, $Mb(III)CN^-$ and $Mb(II)CN^-$ are the oxidized and reduced forms involved. Surprisingly, when metmyoglobin undergoes electron transfer, it also appears that it is the six-coordinate $Mb(III)H_2O$ and $Mb(II)H_2O$ that undergo electron-transfer reactions and that dissociation of water from $Mb(II)H_2O$ is unexpectedly slow, k = 1.0 (±0.5) s^{-1}, and produces an electroinactive five-coordinate Mb(II) under these experimental conditions. Support for the conclusions given above comes from the comparison of experimental and calculated cyclic voltammograms.

Introduction

Myoglobin, the small heme protein (MW 17 800[1]) found primarily in cardiac and red skeletal muscles, functions physiologically in its reduced state to store dioxygen and facilitate its transport to the mitochondria.[2-4] Since the determination of its structure by X-ray crystallography and the sequencing of its single polypeptide chain,[5-7] myoglobin has been used as a model compound to study the dioxygen-binding properties of the larger, more complex hemoglobin. Deoxymyoglobin binds dioxygen reversibly according to the equation

$$Mb(II) + O_2 \rightleftarrows Mb(II)O_2 \qquad (1)$$

Here in its +2 state, the heme iron is either five-coordinate high-spin or six-coordinate low-spin with dioxygen as the sixth ligand. The heme iron of metmyoglobin, $Mb(III)H_2O$, is +3 and six-coordinate high-spin with water as the sixth ligand.

The electron-transfer reactions of myoglobin and cytochrome *c*, a low-spin electron-transfer protein, are often compared. Although functionally different, both myoglobin and cytochrome *c* contain a single heme iron with the same fifth ligand, i.e., an imidazole nitrogen of a histidine residue. In myoglobin, the distal histidine does not bind and the sixth position is free to bind exogenous ligands, thus allowing the study of the effects of ligand binding on electron-transfer properties without mutagenesis. The sixth ligand in cytochrome *c* is the sulfur atom of a methionine residue. One of many examples of mutagenesis is the recent work of Raphael and Gray[8] in which a large negative shift in potential was observed when cysteine was substituted for methionine-80 in cytochrome *c*. In myoglobin, the distal histidine is important because it can hydrogen bond to and stabilize other ligands, as considered recently by a number of authors.[9-11] In particular, stopped-flow time-resolved spectrophotometry was used to investigate the kinetics of decay of the intermediate formed after dithionite reduction of cyanometmyoglobins that differed at the distal position: native myoglobins containing His-E7, mutants substituted at this position, and native myoglobins that contain other amino acid residues in this position such as *Aplysia limacina* myoglobin.[9]

The reduction of aquometmyoglobin occurs between two high-spin states according to the equilibrium equation

$$Mb(III)H_2O + e^- \rightleftarrows Mb(II) + H_2O \qquad (2)$$

A mechanistic question to be answered is whether the water molecule dissociates before or after the reductive electron transfer. Tsukahara[12] used cyanogen bromide to bind the distal histidine of metmyoglobin, which prevented the binding of water at the sixth position of the heme iron. Dithionite reduction of the resulting five-coordinate high-spin iron(III) to five-coordinate high-spin iron(II) was shown to occur more rapidly than the reduction of native metmyoglobin. The unique binding properties resulting from this treatment were also described by Shiro and Morishima.[13] Their ^1H NMR studies show that the complexation of cyanogen bromide to the distal histidine prevents the binding to the heme iron of cyanide, methylamine, and dioxygen while allowing the binding of azide and carbon monoxide. Although the treatment with cyanogen bromide simplifies the reaction in eq 2, the question posed earlier remains unanswered.

The reduction rates of five myoglobin derivatives by dithionite were studied by Cox and Hollaway.[14] The experimentally determined order, imidazole $\gg CN^- > SCN^- \gg N_3^- \gg F^-$, follows both the spectrochemical series and the nephelauxetic series.[15] The tendency of the ligand to cause ligand field splitting as well as to allow "cloud" expansion of the metal's d orbitals is important. Their rapid-wavelength-scanning stopped-flow spectrophotometric

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studies clearly show that reduction occurs with cyanide bound to the iron but only imply that imidazole remains bound during reduction. The primary mechanism for fluorometmyoglobin, on the other hand, is for ligand dissociation to occur before reduction. They suggest two possible paths for "outer-sphere" reduction, either through the porphyrin ring or through the empty $p\pi$ orbitals of the ligand, which fluoride lacks.

The time frame for the spectra obtained by Cox and Hollaway,[14] showing the formation of unstable cyanomyoglobin after dithionite reduction, suggested that the electron-transfer reactions of the two low-spin cyanomyoglobin derivatives could be studied by cyclic voltammetry.[16] The mechanism for this reaction is given by the following equations:

$$Mb(III)CN^- + e^- \rightleftarrows Mb(II)CN^- \quad (3)$$

$$Mb(II)CN^- \rightleftarrows Mb(II) + CN^- \quad (4)$$

A negative shift in the formal potential of more than 400 mV for these electron-transfer reactions at pH 9 was reported in the presence of a large excess of cyanide. The large negative shift in the formal potential is expected because of the greater affinity of metmyoglobin for cyanide compared to that of the reduced species according to eq 5.[17,18] Literature values for the equilibrium constant are approximately 2×10^5 M^{-1}.[19,20]

$$Mb(III)H_2O + CN^- \rightleftarrows Mb(III)CN^- + H_2O \quad (5)$$

Much earlier it had been assumed that both hydrogen cyanide and the cyanide ion would react with the heme iron although the only product is $Mb(III)CN^-$.[21-23] However, Awad et al.[24] conclude that the binding of hydrogen cyanide to metmyoglobin is not important.

Using both electrochemical and spectroelectrochemical methods, we showed earlier that under anaerobic conditions myoglobin will undergo direct electron transfer at optically transparent tin-doped indium oxide electrodes.[25] In that work, the electron-transfer reaction was found to be quasi-reversible to irreversible. A formal potential, $E^{\circ\prime}$, also determined in that work agreed with the value of 0.046 V vs NHE at pH 6.95 that had been reported much earlier by Taylor and Morgan.[26] Following the determination of the formal potential for the reaction shown in eq 3 at pH 9.0,[16] a more detailed study of the electron-transfer reactions of $Mb(III)CN^-$ and $Mb(III)H_2O$ was performed using direct cyclic voltammetry, and the results are reported here. The mechanisms for the electron-transfer and ligand-binding reactions of cyanometmyoglobin and aquometmyoglobin are described together with the relevant rate constants and equilibrium constants. These results include the answer to the question as to when water dissociates during reduction of $Mb(III)H_2O$: the reduction of $Mb(III)H_2O$ to give the final product, five-coordinate Mb(II), is not a concerted process, a result with important consequences for the interpretation of electron-transfer kinetics.

Experimental Section

Prior to use, lyophilized myoglobin from horse skeletal muscle (Sigma) was dissolved in one of two buffers, ionic strength 0.2 M: Tris/cacodylic acid,[27] pH 7.0, or Tris/Tris·HCl, pH 9.0. The myoglobin solution was then filtered through a YM30 filter (Amicon) to remove high molecular weight species, shown to be present by gel electrophoresis,[28,29] which interfered with electron transfer. The cacodylic acid (hydroxydimethylarsine oxide, 98% pure from Sigma) was twice recrystallized from 2-propanol. All other chemicals were ACS reagent grade and used as received. These included tris(hydroxymethyl)aminomethane (Trizma Base, reagent grade, Sigma), tris(hydroxymethyl)aminomethane hydrochloride (Aldrich), sodium cyanide (Aldrich), and sodium fluoride (Fisher). All water used was purified with a Milli RO-4/Milli-Q system (Millipore Corp.) and on delivery exhibited a resistivity of 18 MΩ.

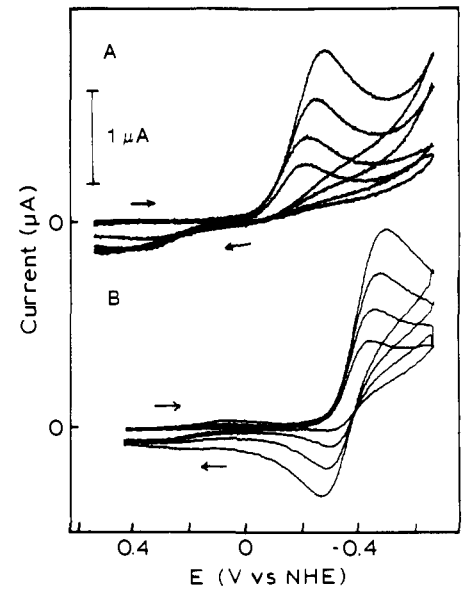

Figure 1. Background-subtracted cyclic voltammetry of metmyoglobin: (A) 86 μM metmyoglobin, pH 7.0; (B) 67 μM metmyoglobin, pH 7.0, with excess cyanide to give initial concentration ratios, [Mb(III)H_2O]:[CN^-], of 1:300. Scan rates: 20, 50, 100, 200 mV/s.

A Lucite electrochemical cell of conventional design[30] was used for all experiments with provision for nitrogen bubbling through a solution volume of approximately 5 mL. The working electrode was a tin-doped indium oxide film deposited on glass (Donnelly Corp.).[31] A new electrode was used for each set of determinations. Pretreatment and cleaning of the working electrode have been described elsewhere.[32] The area of the working electrode was approximately 0.3 cm^2 in all experiments. A Ag/AgCl reference electrode in 1 M KCl and a platinum auxiliary electrode completed the cell. The reference electrode was calibrated against saturated quinhydrone[33] in the appropriate buffer at the completion of each experiment. Electrochemical methods and procedures as well as concentration determinations have been described elsewhere.[25]

All determinations were carried out at ambient temperature, 20 (±1) °C, under nitrogen (Grade 4.5 from Airco, Inc.) which had been passed over hot copper turnings in a Sargent-Welch furnace to scavenge dioxygen and then bubbled through water to both water-saturate and cool the nitrogen. The buffer and sample solutions were purged with nitrogen for at least 30 min prior to beginning a series of determinations. Myoglobin solutions must be nitrogen purged longer than pure buffers to remove dioxygen.[34] Standard addition experiments were used for experiments involving low ligand concentrations by adding small volumes of buffer containing the desired moles of cyanide to the myoglobin solution in the electrochemical cell to minimize dilution effects. A weighed amount of the sodium salt (cyanide or fluoride) was added to the solution in the cell for a ligand concentration greatly in excess of the myoglobin

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Table I. Formal Potentials and Heterogeneous Rate Constants for Electron Transfer

	$E^{\circ\prime}$ (mV vs NHE)	$k^{\circ\prime}$ (cm s^{-1} × 10^5)	pH
Mb(III)CN	−385 (±15)	54 (±9)[a]	7.0
	−408 (±9)	70 (±9)[b]	9.0
Mb(III)H_2O	+46[c]	0.7	7.0

[a] Based on five sets of background-subtracted cyclic voltammograms. [b] Based on four sets of background-subtracted cyclic voltammograms. [c] Taylor and Morgan.[26]

concentration to avoid sample dilution.

Results

Remarkable differences in the electron-transfer reactions for Mb(III)H_2O and Mb(III)CN^- were observed in this work using direct cyclic voltammetry. Figure 1 shows cyclic voltammetric curves for these two forms of myoglobin. For Mb(III)H_2O (Figure 1A), the large difference between the reductive peak potential (ca. −0.28 V) and the oxidative peak potential (ca. +0.40 V) shows that the rate of this electron-transfer reaction is slow, i.e., quasi-reversible to irreversible,[35] and we have reported on this reaction earlier.[25,34]

Figure 1B shows a much smaller separation between reductive and oxidative peak potentials in the cyclic voltammetric results obtained for Mb(III)CN^-, and at slower scan rates the anodic peak disappears. This is an example of the EC reaction mechanism in which a heterogeneous electron-transfer reaction occurring at an electrode (E) is followed by a chemical reaction in solution (C) that consumes the electroactive reduced species[35,36] and is given by eqs 3 and 4 shown earlier. At the fastest potential scan rate shown, the effect of the reaction involving dissociation of cyanide from the reduced cyanomyoglobin is minimal (eq 4). The difference in peak potential, ΔE, of this cyclic voltammogram can be used to estimate the formal potential, $E^{\circ\prime}$, for the electrode reaction given by eq 3. As discussed in the Introduction, the formal potential has been shifted over 400 mV negatively (see Table I) compared with the formal potential for metmyoglobin. The slower potential scan cyclic voltammograms in Figure 1B show sensitivity to the reaction given in eq 4; essentially no oxidative current is observed for Mb(II)CN^-.

The ΔE values from cyclic voltammograms such as those shown in Figure 1B allowed the calculation of the formal heterogeneous electron-transfer rate constants, $k^{\circ\prime}$, for cyanometmyoglobin at pH 7 and 9 which are given in Table I.[37] These rate constants for the electrode reactions given by eq 3 are quasi-reversible and are over an order of magnitude larger than the rate constants for the reactions depicted in Figure 1A (i.e., eq 2). The reason for the difference in these rates was not immediately obvious and led to the experiments described below.

The large molar excess in cyanide ligand concentration relative to the metmyoglobin concentration was of concern. Cyanide might have been functioning as an electron-transfer promoter. This led to experiments on solutions in which the metmyoglobin to cyanide molar ratio was closer to unity. Figure 2 shows the cyclic voltammograms of solutions containing molar concentration ratios, [Mb(III)H_2O]:[CN^-], of 1:1, 1:2, and 1:5. (Note: dashed lines are simulated results to be discussed below.) In Figure 2A, approximately 50% complexation of the metmyoglobin by cyanide is expected on the basis of an equilibrium formation constant of 5×10^6 M^{-1} and a K_a for HCN of 6.2×10^{-10} M. In these cyclic voltammograms, there is clear evidence of reductive electrode reactions for both metmyoglobin and cyanometmyoglobin that occur at the expected potentials, as shown in Figure 1. In Figure 2B, where cyanide complexation is 75%, the cathodic peaks for the reduction of metmyoglobin have further decreased and the cathodic peaks for the reduction of cyanometmyoglobin have

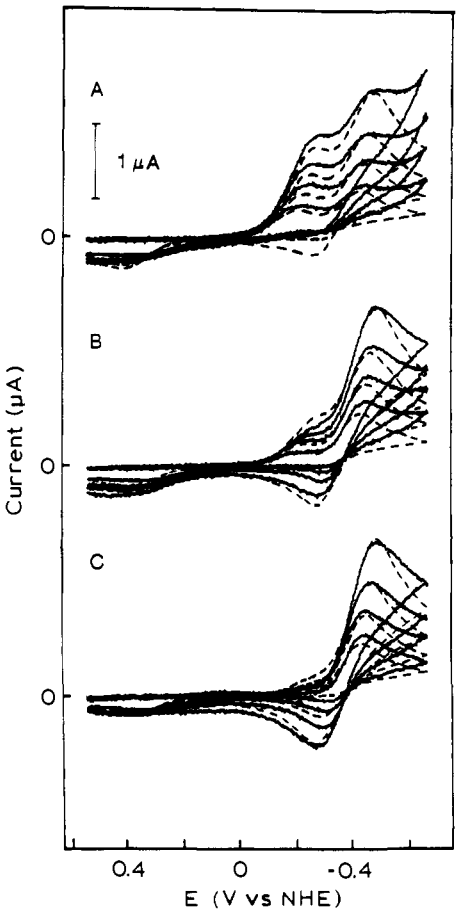

Figure 2. Background-subtracted cyclic voltammetry of metmyoglobin after standard additions of cyanide. Scan rates: 20, 50, 100, 200 mV/s. Dashed lines indicate calculated data using the rate constants, k_f and k_b, for water dissociation from and complexation to reduced myoglobin, and k_f and k_b, for cyanide dissociation from and complexation to reduced cyanomyoglobin. Total metmyoglobin concentration and percent complexation with cyanide: (A) 83 μM, 53%; (B) 80 μM, 75%; (C) 72 μM, 90%.

increased. No anodic peaks for the reoxidation of cyanomyoglobin are seen at the slower scan rates due to the dissociation of cyanide. In Figure 2C, where metmyoglobin complexation to cyanide is 90%, the cathodic peaks for the reduction of metmyoglobin are almost absent while the peaks for the electron-transfer reactions of cyanomyoglobin have both increased.

The cyclic voltammetric results shown in Figures 1 and 2 qualitatively support the reaction mechanisms as described thus far, and an attempt to more quantitatively model these experiments was undertaken. Using finite-difference digital simulation procedures,[35,38,39] the cyclic voltammetry[40-42] of solutions containing only metmyoglobin was examined. Using experimentally determined values for all simulation parameters (i.e., diffusion coefficient, electrode area, concentration, formal potential, and transfer coefficient[25]), except for the formal heterogeneous electron-transfer rate constant which was allowed to vary, the results shown in Figure 3A were obtained. This simple simulation cannot reproduce the qualitative shape of the voltammogram in the region of the oxidative wave.

As discussed in the Introduction, metmyoglobin is six-coordinate with water occupying the sixth position. The final reduction

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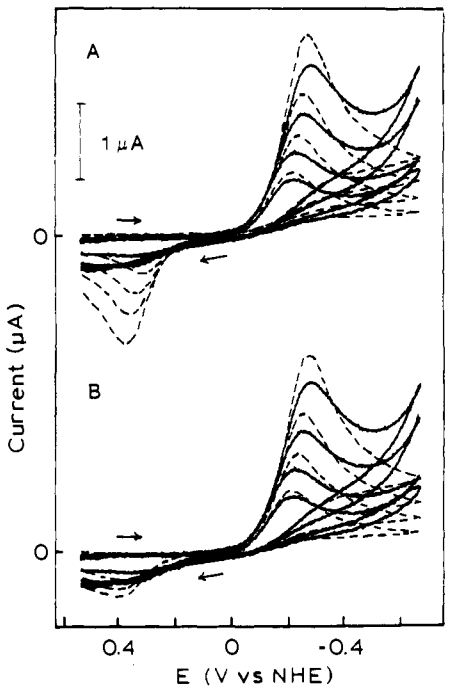

Figure 3. Cyclic voltammetry of metmyoglobin. Scan rates: 20, 50, 100, 200 mV/s. Dashed lines indicate calculated data. (A) Calculations do not include rate constants, k_f and k_b, for water dissociation from and complexation to reduced myoglobin (conditions given in the caption of Figure 1A). (B) Calculations do include these rate constants.

product is five-coordinate deoxymyoglobin (eq 2). The failure of the simulations, which treat the reduction as though it were a concerted process described by eq 2, suggests that the cyclic voltammograms instead reflect the occurrence of an EC reaction mechanism:

$$Mb(III)H_2O + e^- \rightleftarrows Mb(II)H_2O \quad (6)$$

$$Mb(II)H_2O \rightleftarrows Mb(II) + H_2O \quad (7)$$

In our earlier work, the effect of such an extended mechanism on the voltammetry was not considered,[25] and errors associated with background subtraction were believed to limit the quality of agreement between experiment and simulation. In this work, the mechanism of eqs 6 and 7 was included in the simulation program with reasonable results, as shown in Figure 3B. The calculations include the rate constants, k_f and k_b, for the dissociation of water from reduced myoglobin after reduction (eq 7), and the best fit values were found to be $k_f = 1.0\ (\pm 0.5)\ s^{-1}$ and $k_b = 0.5\ (\pm 0.2)\ s^{-1}$. Error estimates have been qualitatively set from inspection of simulations of kinetic parameters covering these ranges. To illustrate the sensitivity of these simulations to these kinetic parameters, the cyclic voltammogram at 50 mV/s from Figure 3B is shown in Figure 4 with simulations using three sets of values for k_f and k_b within the error ranges given above.

Returning to Figure 2, simulations for solutions with three different percentages of cyanide complexation using appropriate concentrations and a single set of simulation parameters show good fit between experiment and simulation. Comparing numerous standard addition experimental results with simulated results, such as the one shown in Figure 2, produced an equilibrium formation constant for the complexation of metmyoglobin and cyanide of $5 \times 10^6\ M^{-1}$. This value is a factor of 10 larger than literature values.[19,20] However, the values in these earlier reports were based on kinetic experiments in which the rate constant for the dissociation was described as being only an estimate. The magnitude of this equilibrium constant is also consistent with the shift in the formal potential for the cyanometmyoglobin complex compared with metmyoglobin.[16] These simulations also used the rate constants given previously for the dissociation of water as well as for

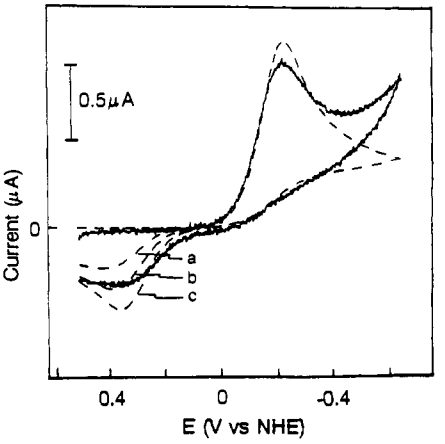

Figure 4. Cyclic voltammogram at 50 mV/s from Figure 3B. Rate constants for water dissociation from, k_f, and complexation to, k_b, reduced myoglobin respectively: (a) $1.5\ s^{-1}$, $0.3\ s^{-1}$; (b) $1.0\ s^{-1}$, $0.5\ s^{-1}$; (c) $0.5\ s^{-1}$, $0.7\ s^{-1}$.

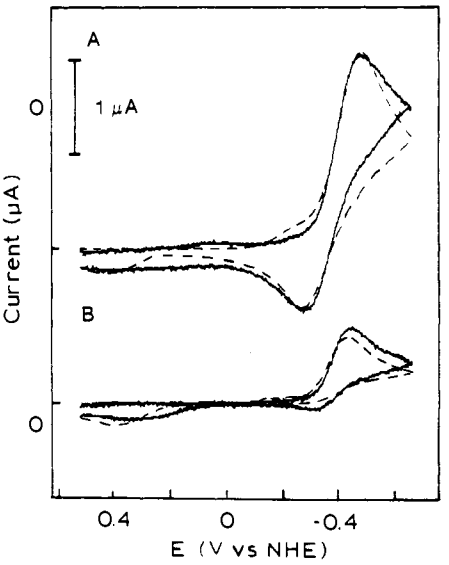

Figure 5. Cyclic voltammograms of cyanometmyoglobin (conditions given in the caption of Figure 2C). Scan rates: (A) 200 mV/s; (B) 20 mV/s.

the dissociation of cyanide from the two reduced species. For eq 4, a value of $k_f = 0.3\ s^{-1}$, rather than the literature value of 0.09 s^{-1},[14] together with the value from this reference for $k_b = 0.23\ M^{-1}\ s^{-1}$ produced better agreement between simulations and experiment.

Several points regarding the quality of the agreement between simulation and experiment need to be made. The agreement is not good in the vicinity of the switching potential (−0.6 V) in most of these cyclic voltammograms. This is due to the difficulty in obtaining reliable background-subtracted cyclic voltammograms at this negative potential limit. Other effects observed during the oxidative potential scan that need to be discussed are not clear in Figure 2 because of the superposition of so many traces. Thus, for clarity Figure 5 reproduces the experimental and simulated cyclic voltammograms from Figure 2C for only the scan rates of 20 and 200 mV/s. At the faster scan rate, the reoxidation of reduced cyanomyoglobin dominates. There is not adequate time for the dissociation of the cyanide ligand (eq 4). At the slower scan rate, no oxidation of reduced cyanomyoglobin is observed while the oxidation of reduced aquomyoglobin is evident at ca. +0.4 V. Clearly, cyanide has dissociated from the heme iron during this slow potential scan rate. The agreement between simulation and experiment for the important reaction mechanism features during the oxidative potential scans described above is good.

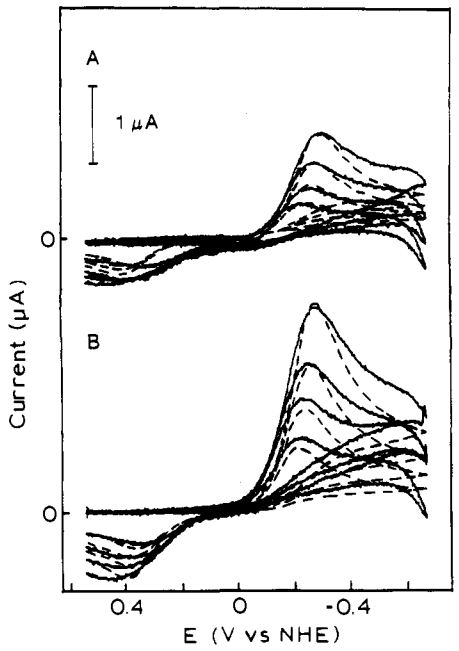

Figure 6. Background-subtracted cyclic voltammograms of metmyoglobin with and without fluoride present: (A) 82 μM metmyoglobin, pH 7, containing excess fluoride (concentration ratios, [metMb]:[F^-], of 1:250 resulting in 50% complexation); and (B) the solution containing no fluoride. Scan rates: 20, 50, 100, 200 mV/s.

Studies were also conducted using the fluorometmyoglobin complex which forms slowly with a color change according to eq 8. This complex provides a six-coordinate high-spin heme iron

$$Mb(III)H_2O + F^- \rightleftarrows Mb(III)F^- + H_2O \qquad (8)$$

complex with an anionic ligand for comparison with the cyanide and water complexes. Solutions containing an initial molar concentration ratio of $[Mb(III)H_2O]:[F^-] = 1:250$ were prepared. Approximately 50% of the total myoglobin concentration at pH 7.0 is complexed with fluoride, on the basis of an equilibrium formation constant for the fluorometmyoglobin complex of 41.4 M^{-1} [43] and a K_a for HF of 6.8×10^{-4}. Figure 6 compares the cyclic voltammetric results upon the addition of fluoride to metmyoglobin (Figure 6A) to those for metmyoglobin without fluoride (Figure 6B). The cyclic voltammetric peaks are reduced by approximately 50% without change in shape following the formation of the fluorometmyoglobin complex. These data show that fluorometmyoglobin is electroinactive, and on the time scale of these experiments, this complex does not dissociate as metmyoglobin is reduced. Simulations for this reaction mechanism that include the fluoride-binding equilibrium (eq 8) with rate constants for complex formation and dissociation of $k_f = 2.4\ M^{-1}\ s^{-1}$ and $k_b = 0.058\ s^{-1}$ are in good agreement with experiment as shown in Figure 6A. To further demonstrate that fluorometmyoglobin dissociation has no effect on these cyclic voltammetric results, the association and dissociation rate constants were not included in a simulation. No difference was seen. Others have proposed that fluoride dissociation is required before reduction for this high-spin complex of metmyoglobin on the basis of reductive chemical reactions.[14,44,45]

Conclusions

The results presented here include three main observations. First, for cyclic voltammetry of solutions of Mb(III)L, L = H_2O and CN^-, it is only the six-coordinate Mb(III)L and Mb(II)L that undergo electron transfer. This is expected from early studies with L = CN^- but is quite unexpected for L = H_2O. Second, they show that the rate of heterogeneous electron transfer is markedly greater for the cyano derivatives of myoglobin, $Mb(III)CN^-$ and $Mb(II)CN^-$, compared to that for metmyoglobin, $Mb(III)H_2O$, and aquomyoglobin, $Mb(II)H_2O$. Third, we confirm the suggestion that $Mb(III)F^-$ is electroinactive and fluoride must first dissociate to form the electroactive $Mb(III)H_2O$.

The conclusion that the Mb(III)L complexes are electron transfer active and that the dissociation of L from the resulting Mb(II)L is relatively slow for *both* L = H_2O and CN^- arose because of the sensitivity of cyclic voltammetry to ligand dissociation following reduction. The small and scan-rate-dependent anodic peaks pointed to the operation of an EC mechanism in both cases with ligand release following reduction.[36] Figure 5 shows there is good agreement between experiment and simulation for a mechanism where reduction of $Mb(III)H_2O$ is followed by water dissociation from the electroactive $Mb(II)H_2O$ to form electroinactive Mb(II). Six-coordinate $Mb(III)H_2O$ is high-spin and five-coordinate Mb(II) is high-spin, but it is likely that six-coordinate $Mb(II)H_2O$ is low-spin. Heme proteins with a strong-field axial ligand, such as imidazole in the case of myoglobin, lead to six-coordinate reduced states that are low-spin.[46] Previous work suggests that metmyoglobin reduction must occur with water bound because the reduction rate for metmyoglobin correlates with the rates of reduction for other six-coordinate metmyoglobin complexes.[44] Evidence for a transient six-coordinate $Mb(II)H_2O$ in this work finds support in the low-temperature spectral studies of Gasyna.[45] One application of these observations is in the study of long-range electron transfer in mixed-metal hemoglobin hybrids. The photoinitiated cycle of electron transfer between a Zn porphyrin (or Mg porphyrin) and an Fe^{III}·L porphyrin within the hybrid is now shown to involve retention of ligand L in all states, most specifically the electron-transfer intermediate with Zn·porphyrin$^+$ and Fe^{II}·L porphyrin.[16] More generally, *any* discussion of electron-transfer measurements that involve the reduction of $Mb(III)H_2O$ must include a recognition that the initial product is $Mb(II)H_2O$ and not the thermodynamic product, Mb(II).[53,54]

Calculations show the ligand dissociation rates of $Mb(II)H_2O$ following reduction to be similar to those of Mb(II)CN. While it was suggested that this EC reaction mechanism would be impossible to study spectroscopically because the ligand is also the solvent,[14] this mechanism has been probed by cyclic voltammetry in this work. The values determined here for the dissociation of water from and complexation of water with reduced myoglobin are $k_f = 1.0\ (\pm 0.5)\ s^{-1}$ and $k_b = 0.5\ (\pm 0.2)\ s^{-1}$. Error estimates have been qualitatively set from inspection of simulations of kinetic parameters covering these ranges. Simulations of cyclic voltammetry using a reversible rate of heterogeneous electron transfer for metmyoglobin show no evidence for water dissociation.

Substantial work by many groups has focused on determining the parameters that control protein electron-transfer rates.[12,47,48] Important parameters that have been considered include distance of electron transfer, electron path, extent of heme exposure, orientation of the heme, solvent effects, and reorganizational energy accompanying spin-state changes. These studies have found some agreement between experiment and Marcus theory.[49] It has been established that electron transfer can occur through protein interiors over distances greater than 20 Å. Hoffman and coworkers have reported electron transfer over a distance of 25 Å in a mixed-metal hybrid of hemoglobin in which zinc or mag-

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nesium has been substituted for two of the four iron atoms.[16,50-52] Gray and co-workers have complexed pentaammineruthenium to the surface histidines of both myoglobin and cytochrome c to include a second electron-transfer site at a known distance.[47,48,53,54] A comparison of the electron-transfer reactions of these Ru-modified myoglobins shows the rate to decrease exponentially as distance increases. The electron-transfer reactions in the present work occur over long distances, distances comparable to those just described. However, we suggest that the differences in the rates of electron transfer for the myoglobin complexes studied in this work, 7×10^{-6} cm s^{-1} for myoglobin and 5.4 ($\pm$0.9) $\times 10^{-4}$ cm s^{-1} for cyanomyoglobin, cannot be explained by distance, electron path, extent of heme exposure, orientation of the heme, or solvent effects.[55] As it is probable that any six-coordinate ferromyoglobin is low-spin due to the ligand field strength of imidazole coordinated at the fifth position,[46] then the electron-transfer reaction of $Mb(III)CN^-$ to $Mb(II)CN^-$ is low-spin to low-spin but the electron-transfer reaction of $Mb(III)H_2O$ to $Mb(II)H_2O$ is high-spin to low-spin.

The rates for electron transfer for heme iron redox couples generally follow the order low-spin/low-spin > high-spin/high-spin $\gg$ high-spin/low-spin or low-spin/high-spin due to the reorganizational energy accompanying changes in spin state.[56,57] It is known that the iron in the heme of low-spin cyanometmyoglobin lies in the heme plane and that the porphyrin ring is flat.[58,59] Reduction to low-spin cyanomyoglobin results only in a slight increase in the iron radius. High-spin metmyoglobin with water as the sixth ligand has the iron out of the heme plane due to longer bonds between the iron and the nitrogens of the porphyrin ring. The ring is described as domed or puckered. (In any of the four combinations of spin state and oxidation number, the porphyrin ring "nitrogen-to-center" distance remains approximately the same or close to 2.01 Å.[59]) Reduction from high-spin metmyoglobin to low-spin aquomyoglobin should result in the shortening of the Fe–N bonds as the iron is pulled into the porphyrin plane. The reorganizational energy change for these electron-transfer reactions should exceed that for the analogous electron-transfer reactions between low-spin states. This energy difference appears to cause the decrease in the electron-transfer rate that is observed here. These results also agree with those of Tsukahara for electron transfer between two high-spin five-coordinate species.[12]

Further work will involve examining the effects described above for myoglobin complexes that incorporate ligand-binding features such as size, charge, and the spin state of the heme iron in the complexed state.

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A Magnetic Resonance Study of the Inclusion Compounds of Sodium in Zeolites: Beyond the Metal Particles Model

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Abstract: The reaction between sodium vapor and three different zeolites—Na-X, Na-Y, and Na-A—has been studied quantitatively, as a function of the concentration of metal. Its efficacy in introducing sodium into the zeolite pores, selectively and on a large scale, is demonstrated through ^{23}Na solid-state NMR measurements. The ESR singlet lines, previously thought to originate from metallic clusters within the zeolite pores, have been closely examined to test in some detail the properties of sodium in zeolites against those expected of the smallest metal particles. Although they exhibit g values characteristic of sodium metal, it is clear that these ESR spectra are not consistent with a simple metal particles model, and that talk of quantum size effects in such systems is premature. An alternative conceptual framework for the study of this class of compounds is developed, which focuses on the interaction of (ionized) sodium valence electrons with the zeolite cations, and with each other. The model described is consistent with many aspects of the experimental observations and suggests that such compounds may contribute to our understanding of the metal–nonmetal transition.

Introduction

The well-defined class of crystalline aluminosilicates known as zeolites, many of which are naturally occurring minerals, are composed of corner sharing SiO_4 and AlO_4 tetrahedra, arranged into three-dimensional frameworks in such a manner that they contain regular channels and cavities of molecular dimensions (see Figure 1). The presence of aluminum (formally Al^{3+}) in such a framework, in place of silicon (Si^{4+}), produces a net negative charge, which is balanced by cations resident in the cavities. These cations are usually coordinated to water molecules and often have a high degree of mobility, readily exchanging with others in aqueous solution. The water molecules can be removed by heating, leaving the zeolite cations imperfectly coordinated to the anionic framework, a situation which can result in the generation of considerable electric fields within the zeolite;[1] other molecules of suitable size can then be absorbed by the dehydrated zeolite.

Perhaps the first to anticipate that, through the filling of their pore space with other solid materials, zeolites might be used as templates for a new kind of solid-state chemistry was Barrer,[2] who spoke of forming "structures heterogeneous on the molecular scale with oxide threads and clusters having the pattern of the channel

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