Proton NMR Study of Structural Perturbation in Sperm Whale Myoglobin due to Pentaammineruthenium(III) Groups Appended to Surface Histidyl Imidazoles

Hiroo Toi,[†] Gerd N. La Mar,*[†] Ruth Margalit,[‡] Chi-Ming Che,[‡] and Harry B. Gray[‡]

Contribution from the Departments of Chemistry, University of California, Davis, California 95616, and California Institute of Technology, Pasadena, California 91125.
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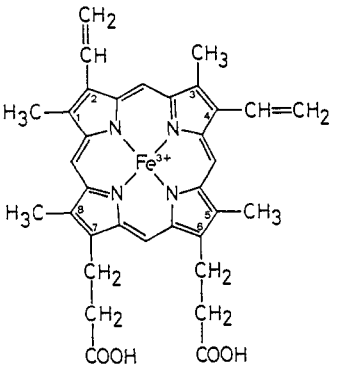
Abstract: Ruthenium-labeled sperm whale myoglobin, $[a_5Ru]_3Mb$ (a = NH_3), which exhibits novel catalytic properties, and its model compound $[a_5RuImH]Cl_3 \cdot 2H_2O$ (ImH = imidazole) have been investigated by 360-MHz ^1H NMR. The model compound in ^2H_2O exhibits three nonlabile single-proton peaks corresponding to 2′-H, 5′-H, and 4′-H of the coordinated imidazole at −31.2, −2.5, and near 4.7 ppm at 25 °C and pH 6.1, respectively. A pH titration gives a single pK 8.4 for deprotonation of the imidazole, with resulting increases of imidazole contact shifts. Ruthenium-labeled Mb and Ru-labeled apo-Mb also exhibit peaks at −36 ppm, which are assigned to the 2′-H of the imidazoles of His-12, His-81, and His-113. Integration of these 2′-H peaks relative to a heme methyl allows determination of the extent of reaction of surface histidines with pentaammineruthenium(III). Detailed comparison of the hyperfine shifted resonances for metaquo, methydroxy, metcyano, metazide, and deoxy forms of the proteins show negligible influence of the Ru chromophores on shifts, indicating protein folding essentially unaltered from that in the native protein. The difference in the high-spin/low-spin separation in the metazide form for the native and Ru-labeled proteins is estimated at only 24 cal, indicating a slightly weaker axial ligand field for the latter derivative. The decreased proton spin–lattice relaxation times and increased line widths of Ru-labeled Mb relative to native Mb complexes indicate the presence of metal–metal interactions which influence the electron-spin relaxation of the iron center.

It has recently been demonstrated[1-7] that the attachment of pentaammineruthenium(III) to surface histidines of proteins affords semisynthetic multisite metalloproteins that exhibit novel catalytic properties[7] and may serve as idealized models for studying intramolecular electron transfer across large distances. In the case of sperm whale myoglobin, the derivative contains three a_5Ru^{III} (a = NH_3) groups which ^1H NMR and tryptic digestion studies have shown to be bound to the three surface imidazoles of His-12, His-81, and His-113. The appended groups possess considerable kinetic stability with respect to dissociation and significantly alter the redox and ligand binding properties of the heme center from those of the native protein. In particular, $[a_5Ru]_3Mb$ has been found[7] to be an effective catalyst for the oxidation of a variety of organic substrates (e.g., ascorbate and durohydroquinone).

The detailed mechanistic interpretation of both the catalysis[7] and electron transfer[1-6] will be greatly simplified if it can be demonstrated that the appended a_5Ru^{III} chromophores do not alter the protein folding that determines the detailed three-dimensional geometric relationship of the heme center and the relevant surface histidines.[8-10]

Proton NMR of the paramagnetic heme center has been shown[11-16] to be particularly sensitive to the detailed interaction between the iron and the axial ligands as well as between a large number of amino acid side chains and the heme periphery. Since these interactions are the result of the specific protein folding, the hyperfine shifts provide a particularly suitable probe of the degree of perturbation of the overall protein tertiary structure. While not all protein perturbations may be evident in the NMR spectrum of a single paramagnetic state,[13,14] it is highly unlikely that significant perturbations would escape four such states.[11-14] Thus in high-spin ferric myoglobins[15] the heme methyl hyperfine shifts are clearly differentiated[16] for the two heme rotational orientations whose visible–UV spectra are indistinguishable.[17] Low-spin ferric systems, in addition to resolving many heme lines, also allow direct detection of various amino acid side chains in the heme pocket.[11-14,18-21] The high-spin ferrous form,[13,22-24] on the other hand, provides a sensitive probe for axial[22,23] as well as equatorial[23,24] interactions.

Previous ^1H NMR studies of sperm whale Mb in the metaquo,[15,16,25] methydroxy,[25,26] metcyano,[19-21] metazido,[27,28] and deoxy[22-24] forms have been reported with a considerable number of heme, A, resonance assignments based on a combination of



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[†] University of California.
[‡] California Institute of Technology.

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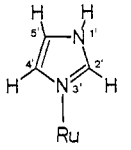
isotope labeling,[15,18,24,27] heme modification,[24,29] paramagnetic relaxation data,[19,20] and nuclear Overhauser effect measurements.[21] Since nothing is known about the NMR spectral properties of the appended paramagnetic ($S = {}^1/_2$) a_5Ru^{III}His chromophore, a ^1H NMR study of the appropriate model compound,[30] $[a_5Ru^{III}(ImH)]^{+3}$ (ImH = imidazole; see B), has also been initiated.



B

Demonstration that the ^1H NMR hyperfine shifts for $[a_5Ru]_3Mb$ derivatives are simply the sum of that of the unlabeled Mb derivatives and the Ru(III) chromophore will establish the absence of significant structural perturbations due to the Ru chromophores.

Experimental Section

Pentaammineruthenium(III) imidazole trichloride,[30] $[a_5Ru(ImH)]Cl_3 \cdot 2H_2O$, and [pentaammineruthenium(III)]$_3$ sperm whale myoglobin,[7] $[a_5Ru]_3Mb$, were prepared and characterized as described previously.

The model complex was dissolved in ^2H_2O or 90% H_2O/10% ^2H_2O (2mM). The proteins were dissolved in 99.9% ^2H_2O or 90% H_2O/10% ^2H_2O containing 0.2 M NaCl. The pH in ^2H_2O solution was altered by addition of ^2HCl or NaO^2H. The metcyano and metazido complexes were prepared by the addition of a fourfold excess of KCN or NaN_3. The reduced deoxy proteins were formed by the addition of an excess of $Na_2S_2O_4$ to a N_2-purged protein solution. Protein concentrations were generally ~1.5 mM; for the metcyano complex, the concentration was varied from 0.3 to 1.5 mM. pH readings were obtained on a Beckman Model 3550 pH meter; the readings in ^2H_2O are not corrected for the isotope effect.

Proton NMR spectra were recorded at 360 MHz on a Nicolet NT-360 quadrature FT-NMR spectrometer using a 6-μs 90° pulse over 12–40-kHz band widths with 8K data points. The strong solvent resonance was suppressed by a 500-ms (for metcyano, metazide, and deoxy form) or 50-ms (metaquo and the model compound) decoupler pulse. The peak positions are given in parts per million from internal 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), with downfield shifts taken as positive. The T_1s for the heme methyls in the metcyano complex in ^2H_2O were determined by the conventional 180°–τ–90° pulse system. Single exponential decays over at least T_1 were observed for all resolved lines. Peak line widths were determined by the Nicolet NTCCAP program.

Results

Model Compound. The ^1H NMR spectra of $[a_5Ru(ImH)]^{+3}$ in ^2H_2O at three pH values are illustrated in Figure 1. The two upfield nonexchangeable single-proton peaks y and z move further upfield with increasing pH, while a third nonlabile proton peak, x, becomes resolved from the solvent residual signal and moves

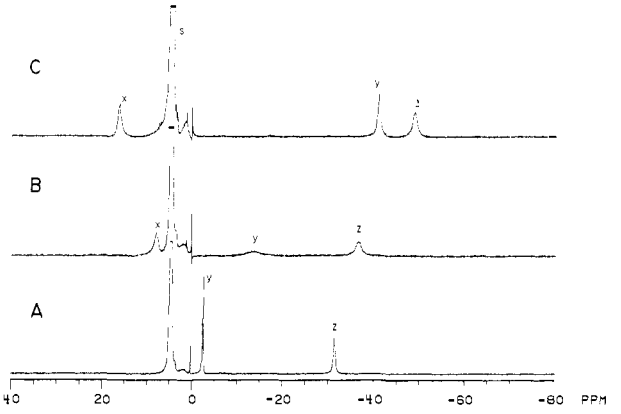


Figure 1. ^1H NMR spectra, 360 MHz, of $[a_5RuImH]^{+3}$ complex in D_2O at 25 °C: (A) pH 5.9, (B) pH 8.05, (C) pH 10.1. The chemical shifts are referenced to internal DSS. Peaks marked x, y, and z are assigned to 4′-H, 5′-H, and 2′H of coordinated imidazole (structure B), respectively (see text); s designates the residual solvent (H_2O) resonance.

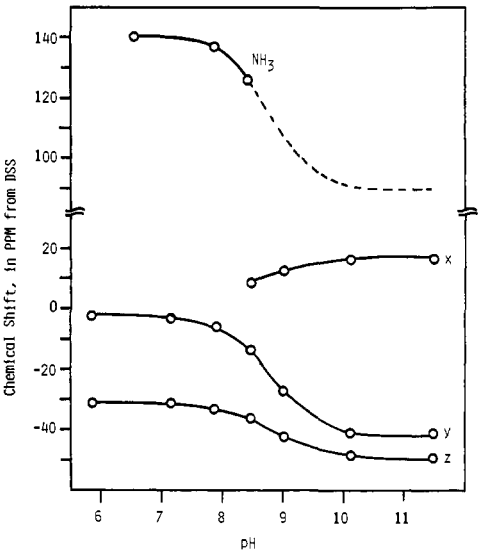


Figure 2. Plots of the chemical shifts vs. pH for ligand peaks in $[a_5RuImH]^{+3}$. The peak designations x, y, and z refer to those in Figure 1. Plots marked NH_3 indicate the pH dependence of the chemical shift of ammine resonance. Solid lines are fits to the Henderson–Hasselbalch equation for peaks y and z. The dotted line represents the predicted shift for the NH_3 peak.

downfield. These three nonlabile proton signals arise from the coordinated imidazole, B. The narrowest peak, y, can be safely assigned to the 5′-H because of its greater distance from the metal;[31-33] and x and z must originate from 2′-H and 4′-H. The single pK 8.4 observed in Figure 2 must arise from the deprotonation of the coordinated imidazole[30] to yield $[a_5Ru(Im)]^{+2}$.

In H_2O solution at acidic pH, an additional very broad signal with area consistent with 12–15 labile protons is observed at 138 ppm (not shown). This signal, which must arise from the amine ligands, moves upfield and broadens even more severely as the pH is raised. It cannot be observed at alkaline pH, but fitting the low pH data to the same pK as the nonlabile protons predicts a shift of 90 ppm for the deprotonated complex.

Protein Complexes. The ^1H NMR spectrum of $[a_5Ru]_3$apoMb is shown in A of Figure 3. Most prominent is the upfield hyperfine-shifted composite (area 1:2) at −35 to −37 ppm. Since the imidazole 5-′H is missing in histidine, this composite must

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Table I. Selected Relaxation Data for Native and Ru-Labeled Myoglobins

	metMbCN					
	native		Ru labeled		$metMbN_3$	
peak[a]	line width[b]	T_1[c]	line width[b]	T_1[c]	line width[d]	line width[d]
a	45 ± 5	90 ± 6	100 ± 20	62 ± 10	140 ± 15	300 ± 50
b					140 ± 15	250 ± 40
e	40 ± 5	126 ± 9	85 ± 15	84 ± 10	130 ± 15	290 ± 50
h	35 ± 5	138 ± 10	70 ± 10	87 ± 10		
t	90 ± 10	57 ± 7	145 ± 20	38 ± 6		

[a] Designations refer to peaks in Figure 4 for metMbCN and Figure 5 for $metMbN_3$. [b] Line width in Hz at 25 °C and pH 6.2. [c] Spin–lattice relaxation time (T_1), in ms, at 25 °C and pH 6.2. [d] Line width in Hz at 25 °C and pH 6.4.

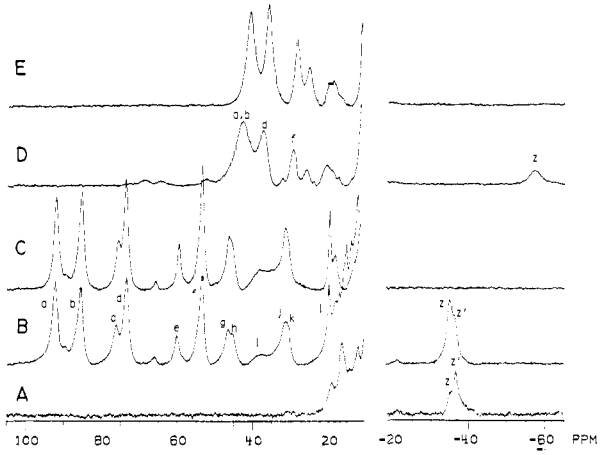

Figure 3. Hyperfine-shifted portions of the 360-MHz ^1H NMR spectra in ^2H_2O at 25 °C of (A) $[a_5Ru]_3$apoMb at pH 7.2, (B) $[a_5Ru]_3metMbH_2O$ at pH 6.3, (C) native $metMbH_2O$ at pH 6.3, (D) $[a_5Ru]_3metMbOH$ at pH 10.2, (E) native metMbOH at pH 10.1. Peaks marked by a, b, d, and f have been assigned[15] to 8-, 5-, 3-, and 1-methyl protons in the structure A, respectively.

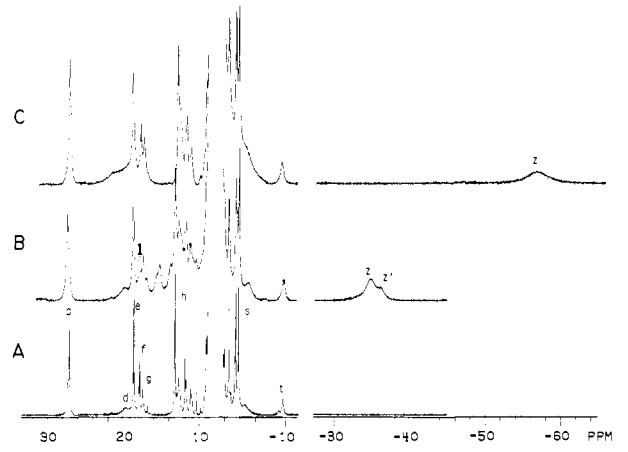

Figure 4. Hyperfine-shifted portions of the 360-MHz ^1H NMR spectra of metcyano forms of native and Ru-labeled Mb in ^2H_2O at 25 °C: (A) native-Mb at pH 6.2, (B) $[a_5Ru]_3Mb$ at pH 6.1, (C) $[a_5Ru]_3Mb$ at pH 10.1. The chemical shifts are referenced to DSS. The peak designations a, e, and h refer to the 5-, 1-, and 8-methyl proton resonances of heme,[18] repectively, and r, s, and t represent the resonances due to Ile-99.[21] Peak z,z′ arises from 2′-H of the Ru-bound His imidazoles.

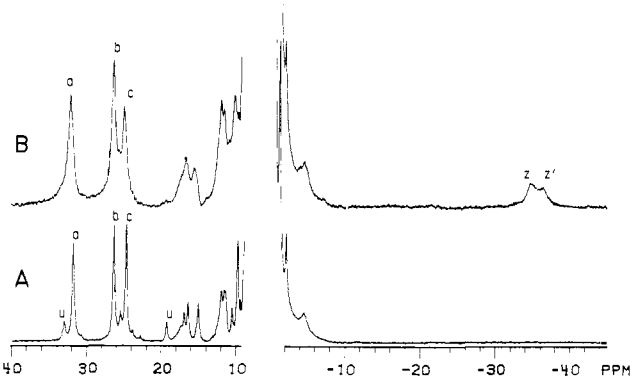

Figure 5. Hyperfine-shifted protions of the 360-MHz ^1H NMR spectra of metazide forms of native Mb (A) and $[a_5Ru]_3Mb$ (B) in ^2H_2O at 25 °C and pH 6.4. Peaks marked a, b, and c refer to 5-, 1-, and 8-methyl proton resonances of heme,[27] respectively; u is due to a minor form of Mb with the heme orientation reversed; z,z′ is due to the Ru-His 2′-H.

correspond to the same protons as peak z in the model. Multiple nonresolved resonances with smaller hyperfine shifts in the region 10–20 ppm must arise from protein side chains near the Ru chromophores.

Comparison of metaquo[15,16] (C, B in Figure 3) and methydroxy[25,26] (D, E) forms reveals resonances for native and Ru-labeled proteins with identical shifts and areas for peaks assigned to heme resonances for each protein form. In $[a_5Ru]_3metMbH_2O$, an additional composite peak is found at −37 ppm as in the apoprotein. The composite integrates to 2.9 ± 0.2 protons per molecule by comparison to the 8-CH_3 signal (a in Figure 3B). The peak moves upfield to −55 ppm in the methydroxy form, indicative of deprotonation of the RuHis, as seen in the model complex.[30] For each protein form a definite increase in line widths is observed (i.e., compare D and E in Figure 3) for the Ru-labeled vs. the native protein. While a direct determination of the pK for the acid ⇌ alkaline transition for Ru-labeled metMb is not possible by NMR due to the severe line broadening[25] at intermediate pH, an optical titration reveals that the pK is 8.4, 0.6 pH units lower than in the native protein.[34] This is consistent with the much larger positive charge in Ru_3Mb.

The effect of reacting a_5Ru with metMbCN is illustrated in A and B of Figure 4. Again the known heme as well as protein resonances[19–21] exhibit unchanged shifts upon labeling the protein, with a similar composite (2:1 area) at −36 ppm which integrates to 2.9 protons per molecule. Raising the pH to 10.1 (C in Figure 4) leads to the characteristic deprotonation of the RuHis and the upfield shift of peak z to −57 ppm. The influence of pH is completely reversible over short times (<1 h), indicating negligible hydrolysis of the Ru chromophore. The increased line broadening for the Ru-modified proteins is observed for all resolved resonances (Table I). The observed independence of linewidth over a fivefold concentration range (0.3–1.5 mM) argues strongly against aggregation. The increased net protein charges make such aggregation unlikely in aqueous solution. Both the spin–lattice (T_1) relaxation as well as the line width are increased, as seen in Table I.

In the case of $metMbN_3$ (Figure 5), the shifts are very similar for the native and Ru-labeled protein, although in this case a small, but systematic, downfield bias is observed for the heme methyls for $[a_5Ru]_3metMbN_3$ (32.01, 26.36, 24.90, 10.06, av 23.33 ppm) relative to those of native[27,28] $metMbN_3$ (31.72, 26.28, 24.64, 9.72, av 23.09 ppm). Again, the upfield composite peak z is observed, as well as the increased line widths (Table I, Figure 5).

Upon reaction with dithionite to yield $[a_5Ru]_3$deoxyMb, both the heme iron as well as the Ru chromophores are reduced, resulting in the characteristic $S = 2$ iron center and diamagnetic Ru(II). The hyperfine-shifted heme region of the ^1H NMR

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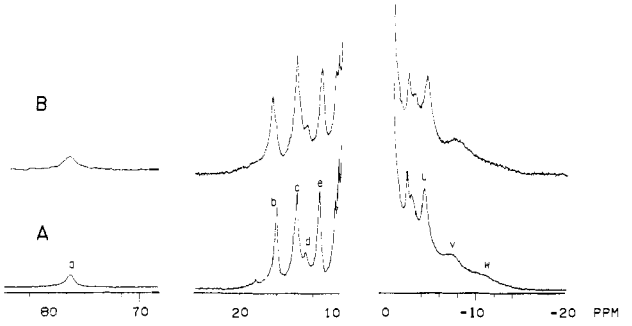

Figure 6. Hyperfine-shifted protions of the ^1H NMR spectra of deoxy forms of native and Ru-labeled Mb in ^2H_2O at 25 °C: (A) native Mb at pH 6.6, (B) $[a_5Ru]_3Mb$ at pH 6.5. The exchangeable N–H resonances, a, of the proximal histidine (F8) in H_2O are inset at left. Peaks b and e are due to heme methyls.[39]

spectrum is again unaltered[22,23] (A, B in Figure 6) except for detectable line broadening in the Ru-labeled protein. Moreover, the proximal histidyl ring NH peak[22] is also found to be independent of the presence of the ruthenium chromophore (inset to A, B in Figure 6).

Thus the hyperfine shifts for each of four paramagnetic protein forms appear to be essentially unaffected by reacting surface His-12, -81, and -113 with Ru moieties. However, in each case, even for the diamagnetic Ru(II) in deoxyMb, resonances exhibit larger line widths in the Ru-labeled protein. Determination of T_1s for native metMbCN[20,31] and $[a_5Ru]_3$metMbCN clearly reveals that the intrinsic protein spin–lattice relaxation times are shorter in the latter protein, thereby arguing against sample heterogeneity as a source of the line broadening. Line width data are given in Table I; T_1 values, when available, are also included.

Discussion

Model Complexes. The relative line widths[31,33] of the three nonlabile single-proton peaks allow direct assignment of peak y to the imidazole 5′-H (A). Assignment of z to 2-′H and x to 4′-H is concluded from the comparison of the effect of deprotonation of the imidazole on the shifts with the data reported[33] for imidazole bound to isoelectronic iron(III). The hyperfine shift patterns for the three protons, particularly in the imidazolate form, are very similar in the Ru(III) and Fe(III) systems,[33] arguing for dominant contact shifts and similar spin delocalization mechanisms. The expected dipolar shifts which characterize low-spin d^5 systems must be smaller than the contact contribution. The very large downfield NH_3 shift arises from dominant σ contact shifts.[35]

The pK 8.4 for the deprotonation of the coordinated imidazole is the same as that reported[30] for the $[a_5Ru(His)]^{+3}$ model complex and also agrees well with that observed[36] for the axial histidyl imidazole in ferricytochrome c′. Although the amine signal was not resolved at alkaline pH, the upfield bias at intermediate pH leads to an estimated 90 ppm shift in the imidazolate complex (see Figure 2), which is consistent with a decrease in ammine–Ru σ bonding,[35] as predicted by the cis effect.[37] Appended to a protein, the a_5RuHis moiety will fail to exhibit peak y since 5-′H is replaced by a methylene group, so that only the upfield 2′-H signal can be expected to be resolved at acidic or neutral pH.

Metaquo Myoglobin. The hyperfine shifts of high-spin ferric iron are predominantly contact in origin.[13-15] Since all heme resonances are resolved and the hyperfine shifts are the largest of any heme iron complex, this protein form provides the most comprehensive probe of the overall heme environment. The identical hyperfine shifts for the native and labeled protein confirm the invariant electronic structure which, in turn, requires an essentially unaltered protein conformation. The upfield peak z,z′ arises from the three His 2′-H, as confirmed by integration. While the individual His environments are barely resolved and are unlikely to yield assignments at this time, the NMR integration does provide a very quick and convenient measure of the average degree of reaction of surface histidine with a_5Ru. Additional peaks detected in $[a_5Ru]_3$metMbH_2O in the 10–20 ppm region are also present in the apoprotein and also appear unaffected upon incorporating the hemin.

Upon raising the pH, both the axial water[25,26] and the surface Ru-coordinated His[30] become deprotonated. Thus at pH 10.1 the spectrum of metMbOH[25,26] appears with shifts unaltered but a larger line width for the Ru derivative (C, D in Figure 3). The 2′-H peak (z,z′) broadens and moves upfield, as found for the model.

metMbCN. The distinctive feature of the low-spin ferric systems is large magnetic anisotropy whose resulting dipolar shifts yield large hyperfine shifts for nonbonded amino acid side chain resonances in the heme cavity.[13,14,18-21] Prominently resolved are a series of peaks (r, s, t) due to Ile-99[21] and the labile ring proton of His-E7[19,22] (not shown). Aside from the increased line widths, each resolved resonance for native metMbCN appears with unaltered shift in $[a_5Ru]_3$metMbCN, dictating that both the magnetic anisotropy and the detailed orientation of amino acid side chains in the heme pocket are essentially identical. The partially resolved composite at −36 ppm again integrates to three protons and moves to −55 ppm at alkaline pH due to deprotonating the Ru-bound histidyl imidazolate.

deoxyMb. The nature of the multiple competing and partially canceling spin delocalization mechanism in high-spin ferrous hemes[13,14,22-24,38] makes deoxyMb the most sensitive individual electronic structure for detecting either axial or equatorial structural perturbations. Beside the apparent line broadening, however, the positions of each of the heme resonances[39] (b, c, e), some of the proposed distal amino acid peaks[23] (u, v, w), and the proximal histidyl ring NH[22] are unaffected by the presence of the three appended Ru chromophores. Again, the data dictate identical axial restraints and conformational influences on the heme.

metMbN_3. Perhaps the most sensitive probe of difference in bonding is the spin equilibrium of the metazide protein.[27,28,40] The rapid thermal spin equilibrium averages the hyperfine shifts of the high-spin and low-spin ferric forms. Since the mean heme methyl hyperfine shifts differ rather substantially (55 ppm for the former and 16 ppm, for the latter spin state), very small changes in the position of the equilibrium are detectable.[28] Using the above values of the shifts for the individual spin states and the presently determined average methyl shift for native (23.09 ppm) and Ru-labeled proteins (23.33 ppm) in the equation[40]

$$\Delta G_s = -RT \ln [\alpha/(1-\alpha)] \quad (1)$$

where α is the mole fraction high spin and ΔG_s is the spin free energy, we obtain ΔG_s(metMbN_3) = 890 cal/mol and ΔG_s-($[a_5Ru]_3$metN_3) = 866 cal/mol, for a difference of only 24 cal/mol. Thus, while we are able to make a measurement that discerns a difference in bonding, namely, a very slight decrease in axial field strength in the Ru-labeled complex, the difference is extremely small and cannot be detected for a given electronic structure.

Line Broadening due to $(NH_3)_5$Ru. In each of the protein forms we find that the Ru-labeled complex exhibits the same shifts but broader resonance lines. This broadening (Table I) does not arise from sample heterogeneity since the well-resolved resonances a and t in metMbCN also exhibit a 30% decrease in T_1s. Moreover, since the excess broadening does not significantly alter the relative line width of various peaks, it is considered unlikely that the excess broadening arises from direct relaxation by the Ru(III) spins.[31,41]

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This can be verified by the observation that the components of the 2-′H signal (peak z) exhibit a line width of ~500 Hz. Since dipolar relaxation[31,41] varies as r^{-6}, the nearest heme resonances at ~16 Å can be broadened at maximum 0.1 Hz by a Ru(III) compared to the 500 Hz for 2′-H (at 4 Å).

Since the primarily iron-centered dipolar relaxation is modulated[31,42] by the iron electron spin relaxation time, T_{1e}, the broader 2-′H lines and shorter proton T_1s dictate that T_{1e} must be longer in the presence of the a_5Ru chromophores. Apparently there is some minor interaction between the iron and ruthenium centers. The effect seems to depend on the spin state of the iron (i.e., less for high spin than low spin) as well as the oxidation state, suggesting that some form of electron exchange between centers may be involved for which the rate naturally depends on the detailed redox properties of the two types of metal centers.

Conclusions

Detailed comparisons of the hyperfine-shifted region of the ^{1}H NMR spectra of metaquo, methydroxy, metcyano, metazide, and deoxy myoglobin reveal that the protein conformation near the heme cavity in $[a_5Ru]_3Mb$ is essentially unchanged from that of the native protein. For the most sensitive probe, the high-spin/low-spin equilibrium, we estimate a *difference* of only 24 cal between $metMbN_3$ and $[a_5Ru]_3metMbN_3$ in the low-spin, high-spin separation; the Ru-labeled protein is suggested to exhibit a very slightly weaker axial ligand field.

The 2′-H signal for the Ru(III)-coordinated histidyl imidazole is readily detected and integrated and provides a very rapid and quantitative index of the average degree of reaction between protein and the a_5Ru^{III} chromophore. Such ^{1}H NMR integration should prove useful in a variety of proteins. The only consistently detectable influence of the appended Ru chromophores is in the form of increased line widths (and decreased T_1s) which must reflect interaction between the metals rather than direct interaction between heme protons and the a_5Ru chromophore.

Proton NMR studies of the influence of these a_5Ru chromophores on dynamic properties of these proteins are in progress.

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Application of Multielectron Charge Relays in Chemical and Photochemical Debromination Processes. The Role of Induced Disproportionation of *N,N′*-Dioctyl-4,4′-bipyridinium Radical Cation in Two-Phase Systems[1]

Ruben Maidan,[2a] Zafrir Goren,[2a] James Y. Becker,[2b] and Itamar Willner*[2a]

Contribution from the Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, and the Department of Chemistry, Ben Gurion University of the Negev, Beer Sheva 84120, Israel. *Received February 27, 1984*

Abstract: Photochemical and chemical reduction of *N,N′*-dioctyl-4,4′-bipyridinium (C_8V^{2+}, **1**) to the corresponding radical cation $C_8V^{+\cdot}$ (**2**) leads to an induced disproportionation of $C_8V^{+\cdot}$ in a water–organic two-phase system. This process yields the two-electron reduction product *N,N′*-dioctyl-4,4′(1*H*,1*H*′)-bipyridylidene (C_8V, **3**). The induced disproportionation reaction is a result of opposite solubility properties of the disproportionation products in the two phases. The two-electron reduction product C_8V (**3**) mediates the debromination of 1,2- and 1,1-dibromo substrates.

A variety of reducing fixation processes in nature involve multielectron transfer reactions, i.e., N_2 or CO_2 fixation. Yet, the initial photoinduced electron-transfer reactions are single-electron-transfer processes. Therefore, the mode of conversion of one-electron-transfer product to multielectron charge relays seems to be of basic interest. In the past few years substantial efforts have been directed toward the design of artificial photosensitized electron-transfer cycles capable of mimicking photosynthesis.[3-5] In particular, the photoinduced decomposition of water to hydrogen and oxygen has been the subject to many reports.[6-9] The photosensitized reduction of protons to hydrogen has been accomplished in the presence of colloidal platinum using 4,4′- or 2,2′-bipyridinium radicals[6,7] (paraquats or diquats) or $Rh(bpy)_2^{2+}$ as mediators[8] for H_2 evolution. In these systems the Pt colloid provides an interface for the utilization of the single-electron-transfer products in the generation of two hydrogen radicals that combine at the solid interface. Further developments of photosensitized electron-transfer reactions in the reduction of abundant materials such as N_2 or CO_2 might involve multielectron relays formed via single-electron-transfer reactions.

One possible mode for the transformation of a single-electron-transfer product to the corresponding doubly reduced species is its disproportionation process (eq 1). This disproportionation

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