CHE 83-00590 and its predecessors. We also thank the National Science Foundation for the purchase of the Bruker FTNMR spectrometer under Grant CHE 77-05926.

Registry No. I, 53687-46-0; I-*3-d*, 82808-04-6; I-d_9, 89462-06-6; II, 53754-45-3; III, 76287-18-8; IV, 89437-71-8; A, 18321-36-3; A-*2-d*, 89278-57-9; A-*1,1,2-d*$_3$, 89437-72-9; B, 592-41-6; hydratropaldehyde, 93-53-8; 2-methyl-2-phenylpropanal, 3805-10-5; methyltriphenylphosphonium bromide, 1779-49-3; 1,3-propanedithiol, 109-80-8; 2-(1-methyl-1-phenylethyl)-1,3-dithiane, 89278-54-6; 2-(1-methyl-1-phenylethyl)-1,3-dithiane-*2-d*, 89278-55-7; 1-methyl-2-phenylpropanal-*1-d*, 89278-56-8; *cis*-2-hexene, 7688-21-3; *trans*-2-hexene, 4050-45-7.

Communications to the Editor

Influence of Heme Orientation on Oxygen Affinity in Native Sperm Whale Myoglobin

David J. Livingston,[1a] Nicki L. Davis,[1b] Gerd N. La Mar,*[1b] and W. Duane Brown[1a,c]

Institute of Marine Resources and Department of Chemistry, University of California Davis, California 95616

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Myoglobin (Mb) has long been considered one of the best understood proteins on the basis of numerous high-resolution X-ray studies,[2] a simple physiological function of muscle oxygen transport,[3] and measurements of various binding processes involved in its function.[4] It is often the 'simple" model to which more complex O_2-binding hemeproeins are compared. It was then unexpected when ^{1}H NMR studies clearly demonstrated[5] that the native protein exists not only with the structure in the heme cavity as characterized by X-ray studies, but is present as ~10% with the heme rotated by 180° about the α-γ-meso axis[6] (A and B in Figure 1). The two components exhibit indistinguishable optical spectra. However, recent NMR studies on the mechanisms of the reaction between heme- and apoMb demonstrated[7] that the resulting initial product is an equimolar mixture of the species with the heme orientations depicted in A and B of the figure. Characterization of the pH influence on the heme orientational equilibration rate has afforded the unusual ability to prepare Mb in states varying by simple relative ratios of the two heme orientations.[7,8] A wide range of O_2 affinities has been reported for Mb reconstituted with modified hemes.[9] There are few opportunities, however, when one can measure the influence on O_2 affinity of simple permutations of the same heme peripheral contacts with the protein side chains.

We report here on the O_2 affinity of sperm whale Mb as a function of the controlled and measured ratio of heme orientation, which demonstrates that the permutation of the heme methyl and vinyl peripheral contacts exerts a significant effect on the O_2-binding properties of the protein.

Oxygen affinity measurements were performed[10] at 25 °C with an original spectrophotometric–polarographic device that has been routinely used in other experiments[11] to measure O_2 affinities of several Mbs with $P_{1/2}$s ranging from 0.07 to 2.5 mmHg. Similar devices have been previously described[12] and give consistent, reproducible binding curves in good agreement with those obtained by tonometric measurements. Oxygen off rates (k_{off}) were determined at 25 °C using a Gibson–Durrum stopped-flow spectrophotometer. The procedures used for preparation of apoMb, reconstitution with hemin, and equilibration to different mixtures of major and minor component have been reported.[5,7,8] The ratio of heme orientations was monitored by the ^{1}H NMR spectrum of the metMbCN form, for which the heme orientation disorder was first observed. The ^{1}H NMR spectra for the 55:45, 65:35, and reequilibrated (10:1) ratio of native/reversed heme orientation on which O_2 binding studies were performed have been reported

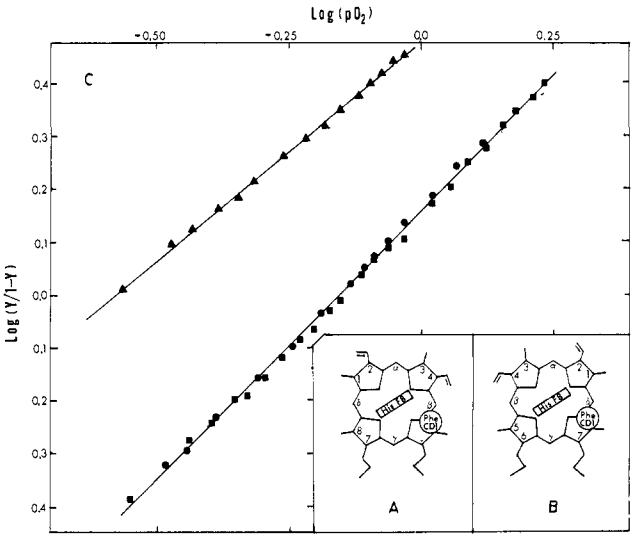

Figure 1. (A) Heme orientation as found[2] in the crystal structure. (B) Heme rotated by 180° about the α-γ-meso axis; the proximal histidyl π plane is indicated by the rectangle. (C) Hill plots (Y = fraction MbO_2) of oxygenation curves for sperm whale Mb at 25 °C. Shown are representative plots determined for a native sample (■), a 55:45 mixture of major/minor components (▲), and the previous disordered sample after reequilibrating to the native (10:1) ratio (●). For clarity, fewer points per curve are presented than were analyzed by linear regression.

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(10) Disordered metMbCN was prepared as described in ref 6 and 7, the disorder ratio was determined by ^{1}H NMR,[5] and the solutions were diluted to 70 μM protein with 50 mM sodium phosphate buffer, pH 7.4. The solution (1.9 mL) was placed in the measuring cuvette of the apparatus, and a 20-fold exess of $Na_2S_2O_4$ (in 0.1 mL) was added. The deoxy Mb sample was then swept with H_2O-saturated air and the oxygenation curve recorded at 25 °C. The presence of the cyanide had a neglible influence on O_2 binding, as determined for the native protein. Redetermination of the ^{1}H NMR spectrum of a disordered sample treated in the identical fashion as for O_2 affinity measurements indicated less than 5% change in the ratio of heme disorder components during the elapsed time (the sample was run as metMbCN by adding ferricyanide and concentrating by ultrafiltration).

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Table I. Oxygen Affinity and k_{off} Rate Data for Sperm Whale Mb

Mb sample[a]	$P_{1/2}(O_2)$ at 25 °C,[b] mmHg	n[c]	k_{off} at 25 °C,[b] s^{-1}
native	0.71 ± 0.01 (4)	0.99	22.7 ± 0.7 (3)
native[d]	0.72 ± 0.04 (2)	1.00	
reequilibrated[e]	0.69 ± 0.02 (2)	1.00	23.4 ± 0.9 (3)
55:45 mixture	0.29 ± 0.03 (4)	0.85	24.7 ± 1.4 (3)
65:35 mixture	0.37 ± 0.03 (2)	0.90	
75:25 mixture	0.47 ± 0.02 (3)	0.92	

[a] Ratio of heme orientations as depicted in A and B of Figure 1; native and reequilibrated Mb has ~10:1 ratio. [b] Number of determinations are given in parentheses. [c] Hill coefficient as determined from slope in C of Figure 1. [d] Sample was held at pH 5.1 for 2 h to simulate conditions for the disordered reconstituted samples. [e] Sample was reequilibrated to the native (~10:1) ratio from a 55:45 disordered mixture.

earlier.[5]

The measured[10] O_2 affinities are illustrated in the usual Hill plots in C of Figure 1. For the 55:45 ratio, the slope (Hill coefficient, n) is less than unity (0.85), as expected for protein components with different O_2 affinities. The slopes increase as the heme disorder equilibrates and reach unity for the native and reequilibrated reconstituted protein. The data points for the latter two samples are essentially indistinguishable, and the $P_{1/2}$s agree with published values.[13] The O_2 affinities, as reflected in the half-saturation pressure, $P_{1/2}(O_2)$, for each sample, are listed in Table I. The increase in $P_{1/2}(O_2)$ upon equilibration dictates that the "wrong" heme orientation has a significantly higher affinity. Regression analysis to the Hill equation of the $P_{1/2}$s as a function of the composition yields $P_{1/2}$(major) = 0.83 and $P_{1/2}$(minor) = 0.07 mmHg. Thus the apparent $P_{1/2}$ measured for an equilibrium mixture will overestimate the affinity of the X-ray characterized heme orientation by ca. 15%.

Alterations in $P_{1/2}$ must result from a different k_{off} or k_{on} for O_2 binding, or both. The measured k_{off}s for the native and 65:35 mixtures were identical, indicating that at least one component of the latter has normal O_2 dissociation characteristics. Long-term detector instability of our stopped-flow spectrophotometer prevented us from detecting slow off rates ($t_{1/2} > 250$ ms).

The present characterization of a significant effect of heme orientation on O_2 affinity in Mb coupled with our earlier demonstration of such disorder in several native hemoproteins[5,14,15] suggest a careful reinvestigation of the physicochemical properties for hemoproteins reconstituted with modified hemin[12,16,17] for signs of variable heme disorder. The substantial effect of heme disorder on O_2 affinity has importance not only in illustrating the correct function for a unique protein prosthetic group conformation but may have implications for the physiological role of Mb. The rate of the interconversion of the two species formed in the heme-apoMb reaction, contrary to earlier reports,[18] is extremely slow at physiological pH.[7,8] In the absence of an enzyme-mediated reconstitution dictating a unique heme orientation, heme disorder may be expected to influence the in situ Mb O_2 affinity. Calculations of the contribution of Mb to the in vivo facilitated O_2 flux[3] must consider the ratios of the two Mb conformations and the resulting effective $P_{1/2}$.

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Registry No. Heme, 14875-96-8; oxygen, 7782-44-7.

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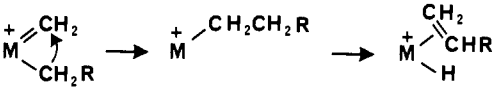
Migratory Insertion within a Cationic Alkylidene–Aryl Complex of Tungsten

Peter Jernakoff and N. John Cooper*†

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Received February 1, 1984

We have recently shown that alkylidene ligands in cationic tungsten complexes insert into cis tungsten–alkyl linkages, and we have described the reaction as a migration of the alkyl group, with its bonding electron pair, on to an unsaturated carbon which has been rendered electrophilic by the positive charge (eq 1, M



(1)

= $W(\eta\text{-}C_5H_5)_2$, R = H or CH_3).[1,2] The facility of such reactions is supported by reports of insertion within other cationic alkylidene–alkyl complexes, including both iridium[3] and, more recently, ruthenium[4] phosphine complexes, and by earlier reports of facile migratory insertion in niobium carbene complexes rendered electrophilic by the electron-withdrawing nature of the zirconoxy substituents.[5]

The transitory alkylidene–alkyl complexes were generated by hydrogen atom abstraction from paramagnetic species of the type $[W(\eta\text{-}C_5H_5)_2(CH_3)R]^{+\cdot}$, and, since such substrates are potentially available with a wide range of substituents R, we have recently been exploring the generality of the insertion reaction by examining the reactions of other paramagnetic tungstenocene alkyls with the trityl radical.[6] We now wish to report that this approach has led to the first example of insertion of an alkylidene or carbene ligand into a transition-metal–aryl bond.

A 17-electron precursor for a cationic alkylidene–aryl complex was prepared from $[W(\eta\text{-}C_5H_5)_2(CH_3)I]$[7] as shown in eq 2.

$$[W(\eta\text{-}C_5H_5)_2(CH_3)I] \xrightarrow{PhMgBr} [W(\eta\text{-}C_5H_5)_2(CH_3)Ph] \xrightarrow{[Fe(\eta\text{-}C_5H_5)_2]PF_6} [W(\eta\text{-}C_5H_5)_2(CH_3)Ph]PF_6 \quad (2)$$

Treatment of a solution of the iodide (2.14 g, 4.70 mmol) in 25 mL of diethyl ether with 4.9 equiv of 1.15 M PhMgBr in diethyl ether resulted in slow (19 h) discharge of the intense green color and formation of a red solution. Hydrolysis followed by extraction with toluene gave a red-orange material which was partially purified by chromatography on deactivated Al_2O_3 using pentane eluant. Removal of the solvent and of the volatile biphenyl contaminant under vacuum gave spectroscopically pure $[W(\eta\text{-}C_5H_5)_2(CH_3)Ph]$[8] as an orange powder (74%) which could be recrystallized from pentane at −60 °C as orange-red plates.

The neutral methyl–phenyl complex is thermally stable but can be readily oxidized to the corresponding radical cation. This was accomplished synthetically by using 1 equiv of ferrocinium hexafluorophosphate added at −78 °C as a 0.014 M solution in CH_2Cl_2 to a −78 °C solution of $[W(\eta\text{-}C_5H_5)_2(CH_3)Ph]$ (0.20 g, 0.50 mmol) in CH_2Cl_2 (10 mL). After 2 h at −78 °C the stirred mixture was warmed to −45 °C and the solvent removed. Fer-

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