which we were able to measure them with precision for each of the new complexes. The upper limit is determined by the decomposition reaction, which is evidenced by the onset of nonisosbestic behavior in sets of spectral scans. It should be noted that the thermodynamic parameters ΔH and ΔS remain constant when R^3 changes from CH_3 to phenyl, whereas both parameters change when benzyl is substituted for the methyl group at R^2. Studies on the cobalt(II) lacunar complexes[13] commonly give values of ΔS in the region -62 ± 3 eu, essentially the entropy of dioxygen.[14] The benzyl-phenyl derivative appears to involve substantial reorganization within the complex in addition to removing the degrees of freedom of dioxygen. The alteration in ΔH suggests that the reorganization enlarges the void so as to enhance the O_2 binding.

The new complexes open many new possibilities. The structural modifications we have used are trivial compared to those that are possible, and future studies will almost certainly address such subjects as the use of simple iron coordination compounds in the energy-efficient reduction of dioxygen and the selective oxygenation of organic substrates.[15]

(14) Jones, R. D.; Summerville,, D. A.; Basolo, F. *Chem. Rev.* **1979**, *79*, 139.

(15) Support by the National Institutes of Health and the National Science Foundation is gratefully acknowledged. The aid of the staff and use of the facilities of the Cooperative Institute for Research in Environmental Sciences of the University of Colorado in the preparation of this paper is deeply appreciated.

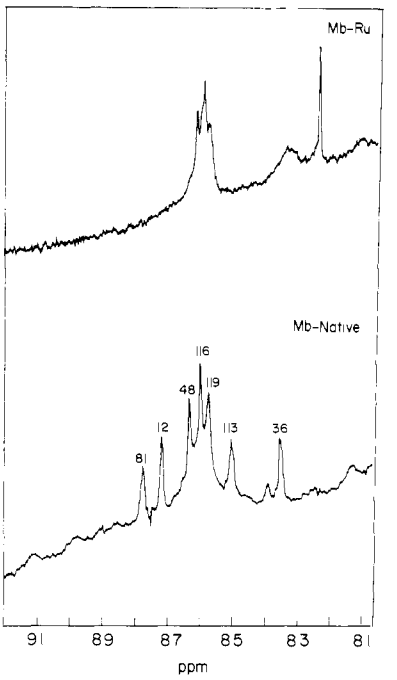

Figure 1. Proton NMR spectra (Bruker WH500) of Mb and Ru_3Mb in the region of imidazole ^1H(C-2) resonances (D_2O/DCl, pH 5.5; internal DSS). Prominent peaks attributable to the C-2 proton resonances of His-12, His-81, and His-113 in the spectrum[11] of native Mb are absent in the Ru_3Mb spectrum.

Oxidation-Reduction Catalytic Activity of a Pentaammineruthenium(III) Derivative of Sperm Whale Myoglobin

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Received September 2, 1982

The extent to which the oxidation-reduction properties of a metalloprotein can be manipulated by the attachment of redox-active inorganic groups to polypeptide chain ligands is a matter of current interest in our laboratory. Our initial studies in this area have centered around pentaammineruthenium(III) (a_5Ru^{3+}) and pentacyanoferrate(II) derivatives of horse heart cytochrome *c*.[2-6] In accord with Matthews (who has studied the a_5Ru^{3+} derivatives of several proteins),[7-10] we have found that a_5Ru^{3+} has

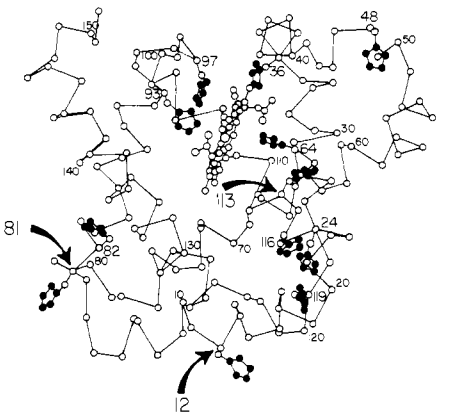

Figure 2. Drawing of the Mb structure that highlights the three a_5Ru^{3+} binding sites. The shortest His-113 imidazole edge to heme edge distance is roughly 12 Å. The His-12 and His-81 imidazole edge-heme contacts are all over 15 Å.

Table I. Reactivity Parameters for Oxidations Catalyzed by Ru_3Mb and Related Species at 25 °C

substrate	pH	E°, mV[a]	catalyst	K_m, M	k_{cat}, s^{-1}
ascorbate	5.5	55	Ru_3Mb	1.5×10^{-5}	0.60
			Ru_3apoMb	1.9×10^{-5}	0.0063
			a_5RuIm^{3+}	1.3×10^{-5}	0.0035
durohydroquinone	7	65	Ru_3Mb	2.8×10^{-5}	0.30
			Ru_3apoMb	1.1×10^{-4}	0.058
hydroquinone	7[b]	120[b]	Ru_3Mb	1.1×10^{-4}	0.0042

[a] Versus NHE. [b] At pH 5 (E° = 360 mV) catalytic turnover is not observed.

a strong preference for surface-accessible histidine residues; furthermore, we have been able to establish that a_5Ru-His(protein) complexes possess considerable kinetic stability (with respect to dissociation) in *both* Ru^{II} and Ru^{III} states. In recent experiments we have found that three a_5Ru^{3+} groups can be attached to sperm whale myoglobin and that the ligand-binding and oxidation-reduction properties of $(a_5Ru^{3+})_3Mb$ or Ru_3Mb are dramatically

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(2) Yocom, K. M.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. E.; Worosila, G.; Isied, S. S.; Bordignon, E.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7052-7055.

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(5) Winkler, J. R.; Nocera, D. G.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798-5800.

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(10) Recchia, J.; Matthews, C. R.; Rhee, M.-J.; Horrocks, W. D., Jr. *Biochim. Biophys. Acta* **1982**, *702*, 105-111.

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different from those of the native protein.

The reaction of $a_5RuH_2O^{2+}$ with sperm whale myoglobin was allowed to proceed for 24 h under anaerobic conditions at pH 7, and the Ru-labeled protein was then oxidized and purified by standard procedures, to give Ru_3Mb.[11] Absences of the imidazole C-2 proton resonances of His-12, His-81, and His-113 in the high-field NMR spectrum of Ru_3Mb pinpoint those residues as the sites of attachment of the a_5Ru^{3+} groups (Figure 1).[12,13]

The three attached a_5Ru^{3+} groups do not appear strongly to perturb the Mb conformation, as judged by comparative measurements of electronic and vibrational spectra. The ability of the heme in Ru_3Mb to bind anions, however, is enhanced greatly over that of the native protein. Cyanide, for example, binds strongly both to the Fe^{III} *and* Fe^{II} forms of Ru_3Mb.[14] The electrostatic influence of the three a_5Ru^{3+} groups is likely responsible for this impressive change in anion affinity, as it is for the very high pI value (~9.2) for Ru_3Mb.

The reduction of O_2 by a variety of organic substrates is catalyzed quite effectively by Ru_3Mb. Good substrates for this "synthetic oxidation-reduction enzyme" include ascorbate and durohydroquinone. Comparisons of reactivity parameters (K_m, k_{cat}) for several substrates with a_5RuIm^{3+}, Ru_3apoMb, and Ru_3Mb suggest that the presence of a dioxygen binding site in Ru_3Mb greatly enhances the base line turnover rate of an a_5RuIm^{3+}-type catalytic system (Table I). In view of the proximity of His-113 to the heme (Figure 2), it is likely that $a_5Ru(His\text{-}113)^{2+}$ transfers an electron rapidly to the heme-dioxygen complex,[15] thereby producing some form of heme-bound peroxide intermediate (whose dissociation may prove to be the rate-limiting step). Clearly, our preliminary work has established that Ru_3Mb is an interesting multisite catalytic system that deserves detailed mechanistic examination.

Acknowledgment. We thank Gary Campbell and Walther Ellis for assistance with certain spectroscopic measurements, and we are indebted to Joan Shelton, Roger Shelton, and Walter Schroeder for performing the tryptic hydrolyses. This research was supported by National Science Foundation Grant CHE80-24863. Fellowship support (R.M.) from Martin Marietta Corp. is acknowledged. NMR experiments were performed at the Southern California Regional NMR Facility supported by National Science Foundation Grant CHE79-16324.

Registry No. Cyanide, 57-12-5; oxygen, 7782-44-7; ascorbic acid, 50-81-7; durohydroquinone, 527-18-4; hydroquinone, 123-31-9; $[Ru(NH_3)_5H_2O][PF_6]_2$, 34843-18-0.

(11) The reaction between $[Ru(NH_3)_5H_2O](PF_6)_2$ (40-fold excess) and sperm whale myoglobin (Type II, Sigma) at room temperature (pH 7.3; 0.05 M Tris·HCl) was terminated by applying the solution to a Sephadex G-25 column. The Ru_3Mb sample was oxidized by $Co(phen)_3(ClO_4)_3$ and then separated from unreacted Mb by using a CM-52 column (linear gradient of NaCl in 50 mM Tris·HCl, pH 7.3). Ru_3Mb was characterized by isoelectric focusing on LKB ampholine PAG plates (pH range 3.5-9.5; native Mb, pH ~7; Ru_3Mb, pH ~9.2); the Ru:Mb ratio was determined to be 3:1 (±3%) by employing $[^{106}Ru(NH_3)_5Cl]Cl_2$ to prepare $^{106}Ru(NH_3)_5H_2O^{2+}$ and analyzing ^{106}Ru in the protein derivative.

(12) The imidazole C-2 proton resonances in native sperm whale Mb have been assigned (Botelho, L. H.; Friend, S. H.; Matthew, J. B.; Lehman, L. D.; Hanania, G. I. H.; Gurd, F. R. N. *Biochemistry* **1978**, *17*, 5197-5205).

(13) Isolation of the His-81- and His-113-containing tryptic peptides of Ru_3Mb has confirmed those two a_5Ru^{3+} attachment sites, but the His-12-containing peptide has eluded characterization, owing in part to its poor solubility properties (Shelton, J. B.; Shelton, J. R.; Schroeder, W. E., unpublished results). However, the presence of an $a_5Ru(His\text{-}12)^{3+}$ unit may be inferred from our observation that the fluorescence of nearby tryptophans (Trp-7, Trp-14) in apoMb is strongly quenched in Ru_3apoMb.

(14) Very concentrated cyanide solutions ($[CN^-] \sim 1$ M; $[Mb] \sim 40\ \mu M$) are required for >95% formation of the $Mb(Fe^{2+})\cdot(CN^-)$ complex (Keilin, D.; Hartree, E. F. *Biochem. J.* **1955**, *61*, 153-171). In contrast, >95% formation of $Ru_3Mb(Fe^{2+})\cdot(CN^-)$ occurs at $[CN^-] \sim 0.1$ mM for $[Ru_3Mb\text{-}(Fe^{2+})] \sim 10\ \mu M$.

(15) Dioxygen reduction by ruthenium(II) ammine complexes apparently involves formation of superoxide anion as a reactive intermediate (Stanbury, D. M.; Haas, O.; Taube, H. *Inorg. Chem.* **1980**, *19*, 518-524). It would be highly surprising if reduced Ru_3Mb with its multiple redox centers did not choose a more felicitous route to peroxide. Indeed, our observation that reduced Ru_3Mb reacts much more rapidly than a_5RuL^{2+} with dioxygen indicates that it does.

Structure and Reactivity of Sterically Hindered Lithium Amides and Their Diethyl Etherates: Crystal and Molecular Structures of $[Li\{N(SiMe_3)_2\}(OEt_2)]_2$ and $[Li(\overline{NCMe_2CH_2CH_2CH_2CMe_2})]_4$

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Received July 12, 1982

Bulky amides of the alkali metals are extensively employed as reagents in organic chemistry by virtue of the combination of their strong Brønsted basicity and their low nucleophilicity, especially with respect to electrophilic carbon centers;[1] lithium derivatives of secondary amines have a particularly pivotal role. An objective of the present communication is to provide X-ray structural data for two of the key compounds, bis(trimethylsilyl)amido-[2] and (2,2,6,6-tetramethylpiperidinato)lithium in order to place the steric arguments on a firm basis. A further purpose is to note that these compounds are not only bases but also Lewis acids,[3] and hence the choice of donor solvent may be significant.[4] The formation of a lithium amide solvate is expected to affect both the state of molecular aggregation of the amide[6,8] and its hydrocarbon solubility.[10] These properties are of considerable significance in making an appropriate choice of lithium amide reagent as an amido transfer reagent for the synthesis of an amide of another metal. In our own work, we have a strong preference for a *crystalline* lithium amide monoetherate as reagent, because of the following features: (i) confidence with regard to reagent purity and concentration, (ii) availability of a nonpolar solvent as the reaction medium, and (iii) ease of manipulation.[12]

(1) Cf.: Lappert, M. F.; Power, P. P.; Sanger, A. R.; Srivastava, R. C. "Metal and Metalloid Amides"; Ellis Horwood-John Wiley: Chichester, 1980; pp 689-691.

(2) The X-ray structure of $\{Li[N(SiMe_3)_2]\}_3$ has been determined: Mootz, D.; Zinnius, A.; Böttcher, B. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 378. Rogers, R. D.; Atwood, J. L.; Grüning, R. *J. Organomet. Chem.* **1978**, *157*, 229.

(3) The formation of a 1:1 OEt_2 adduct of $Li[N(SiMe_3)_2]$ has been noted: Wannagat, U.; Niederprüm, H. *Chem. Ber.* **1961**, *94*, 1540.

(4) We believe that for metal amides this has not previously been explicitly stated; although for lithium alkyls the role of different solvents such as OEt_2, THF, TMEDA, or PMDETA (pentamethyldiethylenetriamine), upon their structure and reactivity is beginning to be documented.[5]

(5) Lappert, M. F.; Raston, C. L.; Skelton, B. W.; White, A. H. *J. Chem. Soc., Chem. Commun.* **1982**, 14.

(6) Variable-temperature ^1H and ^7Li NMR spectra of $Li[N(SiMe_3)_2]$ in several donor solvents were examined and the results interpreted in terms of a monomer ⇄ dimer equilibrium in solution.[7]

(7) Kimura, B. Y.; Brown, T. L. *J. Organomet. Chem.* **1971**, *26*, 57.

(8) We suggest that the lower the state of molecular aggregation, the greater the amide reactivity and selectivity; the extreme case would be for a monomeric solvated lithium amide, and we predict that for example $Li[N(SiMe_3)_2](PMDETA)$ will prove to be a monomer in the crystal and will be outstandingly reactive and selective as a proton abstractor. As circumstantial evidence we cite the monomeric $Li[CH(SiMe_3)\text{-}o\text{-}MeC_6H_4](PMDETA)$.[9]

(9) X-ray data: Lappert, M. F.; Raston, C. L.; Skelton, B. W.; White, A. H., unpublished work.

(10) We find that although $\{Li[N(SiMe_3)_2]\}_3$ and $\{Li[\overline{NCMe_2(CH_2)_3CMe_2}]\}_4$ have some *n*-pentane solubility, this is significantly increased for $\{Li[N(SiMe_3)_2](OEt_2)_2\}_2$ or $\{Li[\overline{NCMe_2(CH_2)_3CMe_2}](OEt_2)\}_n$ (the structure of this compound, and hence the value of *n*, is not yet known). We had previously reported[11] that $[Li(OAr)(OEt_2)]_2$ (Ar = C_6H_3-4-Me-2,6-*t*-Bu_2) is soluble in *n*-C_6H_{14}, whereas $[Li(OAr)]_n$ (of unknown structure) is insoluble.

(11) Cetinkaya, B.; Gümrükcü, I.; Lappert, M. F.; Atwood, J. L.; Shakir, R. *J. Am. Chem. Soc.* **1980**, *102*, 2086.

(12) Our most recent papers on metal amides using $\{Li[N(SiMe_3)_2]\text{-}(OEt_2)\}_2$ concerns $\{ErCl[N(SiMe_3)_2](\mu\text{-}Cl)_2Li(THF)_2\}$,[13] and using $[Li(N\text{-}t\text{-}Bu_2)(OEt_2)]_x$ or $\{Li[\overline{NCMe_2(CH_2)_3CMe_2}](OEt_2)\}_n$ concerns $M(N\text{-}t\text{-}Bu_2)_2$ or $M[\overline{NCMe_2(CH_2)_3CMe_2}]_2$ (M = Ge or Sn).[14]

(13) Lappert, M. F.; Singh, A.; Atwood, J. L.; Hunter, W. E. *J. Chem. Soc., Chem. Commun.* **1981**, 1191.

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