acid-labile Mn in the sample.[13]

Although Mn catalase was originally identified on the basis of its insensitivity to azide and cyanide,[1,14] it has since been shown[2,3,15] that all three Mn catalases are weakly inhibited by these anions. We observe no change in the EPR spectrum following addition of 25-fold excess of either NaCN or NaN_3. However, addition of 250-fold excess of NaN_3 results in loss of the 16-line signal and formation of the Mn(II) 6-line EPR spectrum. The enzyme shows no activity following treatment with 250-fold excess NaN_3, and activity is not recovered following dialysis.

There is substantial interest in mixed-valence Mn complexes because of their potential relevance to the photosynthetic oxygen-evolving complex. Many mixed valence Mn(II)/Mn(III)[16] and Mn(III)/Mn(IV)[11,17] dimers have been prepared in recent years. These complexes characteristically show a 16-line EPR signal similar to that observed for Mn catalase, with Mn(III)/Mn(IV) dimers showing the closest similarity.[18] On the basis of similarities in the optical spectra, it has been proposed[17c,f] that the Mn catalase contains a μ-oxo, di-μ-carboxylato bridged Mn(III) dinuclear core. One-electron oxidation of a model system having this core[17c] gives a species having an EPR spectrum similar to that for Mn catalase.

In conclusion, we have presented the first direct evidence for a dimeric Mn active site in the *L. plantarum* Mn catalase. This site is a mixed valence Mn cluster having spectroscopic properties similar to one of the forms of the *T. thermophilus* catalase. A low-resolution crystal structure of the latter enzyme[19] is consistent with a dinuclear Mn site; however, the resolution is not sufficient to provide additional structural details. The simplicity of the *L. plantarum* EPR spectra suggest that this enzyme will be amenable to more detailed spectroscopic investigation. Experiments along these lines are in progress.[20]

(13) Samples were denatured by acidifying to pH 0 with concentrated HCl and heating at 50 °C for 15 min. The concentration of Mn(II) released, as determined by comparison with standard Mn(II) solutions, was similar to that determined by neutron activation analysis, demonstrating that these conditions are efficient in converting protein bound Mn to the $Mn^{II}(H_2O)_6$ complex. Quantitation was estimated by double integration of the 16-line and the 6-line spectra measured at 6 K. Integrals were calculated over 2722–4722 G. The dominant feature in the EPR spectrum of the denatured protein is the 6-line signal arising from the $m_s = -1/2$ to $1/2$ transition, with some additional contribution from the other allowed transitions. The experimental ratio of double integrated intensities was 10:1 (6-line:16-line). The expected ratio ranges from 6:1 (assuming only $m_s = -1/2$ to $1/2$ transitions contribute to 6-line spectrum) to 23:1 (assuming all allowed transitions contribute to 6-line spectrum), thus at least 60% of the acid-labile protein-bound manganese is present in the $S = 1/2$ dinuclear cluster.

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π-Localization in Aromatic Ligands: Formation of Mixed-Metal η^2:η^2-μ-Arene Complexes of Ruthenium(II) and Osmium(II) Ammines

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Recently we reported the synthesis of a novel class of pentaammineosmium(II) compounds in which an arene is coordinated η^2 to the metal center.[1] Relative to others reported,[2] these complexes offer unusual kinetic stability, allowing their convenient manipulation and study at room temperature. Both theoretical calculations[3] and crystallographic data[4] indicate that the η^2 mode of ligation disrupts the aromaticity of the arene. Bearing this in mind, it does not seem surprising that $[Os(NH_3)_5(\eta^2\text{-}C_6H_6)](OTf)_2$, (**1**), is inherently unstable with respect to the liberation of benzene and formation of $[\{Os(NH_3)_5\}_2(\eta^2{:}\eta^2\text{-}\mu\text{-}C_6H_6)](OTf)_4$.[1] This shows that the affinity of pentaammineosmium(II) for an η^2-coordinated arene is greater than for the unperturbed ligand and suggests that the loss in stabilization by π electron localization is greater in the first stage of metal binding than in the second. These considerations led us to investigate the reaction of **1** with other metal centers in order to evaluate the ability of pentaammineosmium(II) to activate arenes through the localization of their π electrons.

Though our attempts to form an arene complex of $\{[Ru(NH_3)_5]^{2+}\}$ with either benzene or naphthalene have been unsuccessful,[5] we find that when a MeOH solution of $[Ru(NH_3)_5(CH_3OH)]^{2+}$ is treated with 1 equiv of **1** the formation of the mixed-metal μ-arene complex **2** is observed.[6]

$$[Ru(NH_3)_5]^{2+} + [Os(NH_3)_5(\eta^2\text{-}C_6H_6)]^{2+} = [Os(NH_3)_5Ru(NH_3)_5(\eta^2{:}\eta^2\text{-}\mu\text{-}C_6H_6)]^{4+}$$
2

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(5) The affinity of olefins for $\{[Ru(NH_3)_5]^{2+}\}$ and other π acids has been widely demonstrated. See: Ford, P. C. *Coord Chem. Rev.* **1970**, 75. Also see ref 14. Reducing $Ru(NH_3)_5(OTf)_3$ in the presence of excess arene in either acetone or MeOH resulted solely in the corresponding (solvent)pentaammine complex.

(6) All reactions were carried out under argon. $Ru(NH_3)_5(OTf)_3$ (ref 18) (50 mg) and Zn/Hg (250 mg) were combined in MeOH (4.0 mL), and the solution was stirred 0.5 h. The clear yellow solution was pipetted away from reducing agent and added to 43 mg of $[Os(NH_3)_5(\eta^2\text{-}C_6H_6)](OTf)_2$ (see ref 1). After an additional 0.5 h, the reaction mixture was added slowly to Et_2O (20 mL) upon which an oily solid forms. The oil was worked into a solid from acetone and Et_2O and dried under vacuum. Alternatively, **2** can be isolated as a chloride salt by the addition of excess bis(triphenylphosphoranylidene)-ammonium chloride to the original reaction mixture.

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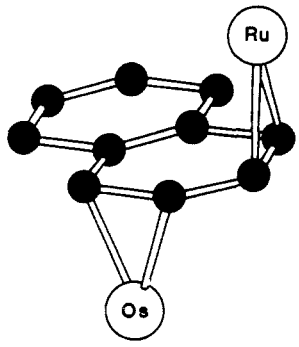

Figure 1. Proposed stereochemistry of the ion $[Os(NH_3)_5Ru(NH_3)_5(\eta^2:\eta^2\text{-}\mu\text{-}C_{10}H_8)]^{4+}$.

Owing to its lability in solution, our attempts to prepare a pure salt of **2** have been unsuccessful. However, the identity of **2** was confirmed by ^1H NMR;[7] chemical shifts in acetone-d_6 for both the arene and osmium–ammine protons in **2** are within 0.5 ppm of those found for its binuclear Os–Os analogue.[1] The ruthenium ammine protons are shifted about 1 ppm upfield relative to those for osmium, an observation made with other pentaammine complexes of these metals.[8] Both proton NMR and cyclic voltammetric experiments suggest that **2** in several weakly coordinating solvents is in labile equilibrium with **1** and the $[Ru(NH_3)_5(S)]^{2+}$ complex (where S = H_2O, MeOH, acetone, DME).[9] In contrast, the π-acid acetonitrile reacts rapidly ($t_{1/2}$ = 15 min) and irreversibly with **2** to generate equivalent amounts of **1** and the solvent complex:

$$[Os(NH_3)_5Ru(NH_3)_5(\eta^2:\eta^2\text{-}\mu\text{-}C_6H_6)]^{4+} + CH_3CN = [Os(NH_3)_5(\eta^2\text{-}C_6H_6)]^{2+} + [Ru(NH_3)_5(CH_3CN)]^{2+}$$

The naphthalene complex $[Os(NH_3)_5(\eta^2\text{-}C_{10}H_8)]^{2+}$ (**3**) was found to be significantly more stable than its benzene analogue.[10] In accord with expectation based on this finding, the reaction of this material with $\{[Ru(NH_3)_5]^{2+}\}$ in methanol affords a binuclear product, less labile than the benzene analogue, which can be conveniently isolated as a pure solid, **4**.[11] ^1H NMR, microanalytical, and electrochemical data[12] for this material are consistent with the formation of the species $[Os(NH_3)_5Ru(NH_3)_5(\eta^2:\eta^2\text{-}\mu\text{-}C_{10}H_8)]^{4+}$. On the basis of homonuclear-decoupled NMR data for **4** and the similarity of these chemical shifts to that for the binuclear osmium analogue,[13] we assign the metals to the 1,2- and 3,4-η^2 positions as indicated in Figure 1. Congruent with this are theoretical calculations[3] which show this configuration to be more stable than one in which both rings are ligated, as there is a minimum disruption of aromaticity for this former arrangement.

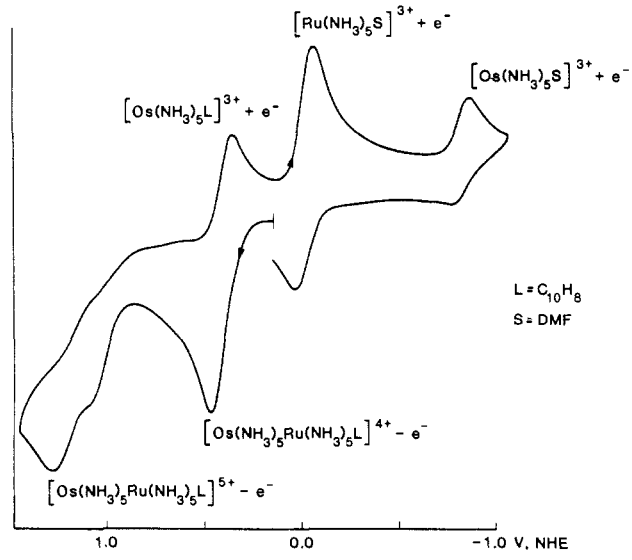

Figure 2. Cyclic voltammogram of the compound $[Os(NH_3)_5Ru(NH_3)_5(\eta^2:\eta^2\text{-}\mu\text{-}C_{10}H_8)](OTf)_4$ in DMF.

Electrochemistry of $[Os(NH_3)_5Ru(NH_3)_5(\eta^2:\eta^2\text{-}\mu\text{-}C_{10}H_8)](OTf)_4$. The cyclic voltammogram of **4** (Figure 2) in DMF reveals a reversible couple at $E_{1/2}$ = 0.47 V, NHE, indicating that this osmium-based oxidation is not affected significantly by ruthenium coordination. ($E_{1/2}$ for the 3+/2+ couple of **3** is 0.41 V).[11] That the mixed-valence species is stable for several seconds suggests that even Os(III) is effective at localizing the arene ligand. The second or ruthenium-based oxidation gives rise to a broad irreversible wave with $E_{p,a}$ = 1.28 V, NHE, about 0.4 V positive to that for typical (olefin)pentaammineruthenium complexes.[14] Even after consideration of solvent differences and the inductive effect of Os(III), the remarkable stability that the mixed valence form shows toward oxidation is noteworthy. The return scan shows that comparable amounts of $[Os(NH_3)_5(\eta^2\text{-}C_{10}H_8)]^{3+}$ and $[Ru(NH_3)_5(DMF)]^{3+}$ are formed upon full oxidation.[15]

The $\eta^2:\eta^2\text{-}\mu$-bonding mode has rarely been observed for arene ligands.[16] To our knowledge, this is the first report of such a complex with mixed metals. Coordination of pentaammineosmium(II) to arenes clearly activates these ligands toward olefinic chemistry. Currently, our efforts are focused on hydrogenation,[17] cycloadditions, and other typical olefinic reactions with complexes such as **1**.

Acknowledgment. We gratefully acknowledge Mikiya Sekine

(7) ^1H NMR of **2**: (acetone-d_6; ppm relative to TMS) 6.76 (dd, 1 H), 6.65 (dd, 1 H), 5.18 (dd, 1 H), 4.83 (dd, 1 H), 4.60 (dd, 1 H), 4.55 (dd, 1 H), 4.93 (br, 3 H), 4.14 (br, 3 H), 3.68 (br, 12 H), 2.41 (br, 12 H); (CD_3CN) 6.54 (dd, 1 H), 6.42 (dd, 1 H), 4.68 (dd, 1 H), 4.33 (dd, 1 H), 4.2 (m, 2 H), 4.23 (br, 3 H), 3.48 (br, 3 H), 3.1 (br, 12 H), 1.88 (br, 12 H).

(8) E.g., for the complex $[[M(NH_3)_6](OTf)_2$ (^1H NMR in acetone-d_6) M = Ru, 2.52 ppm; M = Os, 3.44 ppm.

(9) With time, **2** apparently suffers the loss of ammonia producing a complex whose NMR is consistent with the formation of $[Os(NH_3)_5Ru(NH_3)_3(\eta^2:\eta^6\text{-}\mu\text{-}C_6H_6)]^{4+}$. This reaction is currently under investigation.

(10) In acetone **3** has a half-life greater than 1 week. The synthesis of **3** follows that described for its benzene analogue. See ref 1. Characterization of **3**: Anal. Calcd for $C_{12}H_{23}OsS_2F_6O_6N_5$: C, 20.54; H, 3.30; N, 9.98. Found: C, 20.82; H, 3.30; N, 9.72. ^1H NMR (acetone-d_6) 7.70 (d, 1 H), 7.48 (d, 1 H), 7.3 (m, 3 H), 7.02 (d, 1 H), 5.42 (d, 1 H), 5.23 (dd, 1 H), cyclic voltammetry (acetone; NaOTf; 100 mV/s); $E_{1/2}$ = 0.41 V (NHE). This value is expected to differ by as much as 100 mV from that obtained in DMF.

(11) Synthesis of **4**. $Ru(NH_3)_5(OTf)_3$ (ref 18) (140 mg) was reduced in MeOH (1.5 mL) with Zn/Hg (200 mg) for 20 min. The reduced solution was then added to 142 mg of **3**, and the reaction mixture was stirred 1 h. Addition to 75 mL of Et_2O resulted in a yellow precipitate which was washed and dried under vacuum. Yield: 75%. Attempts to recrystallize this material in CH_3CN resulted in **3** and $[Ru(NH_3)_5(CH_3CN)]^{2+}$ after 4 days.

(12) Characterization of **4**. Anal. Calcd for $C_{18}H_{38}OsRuS_4F_{12}O_{12}N_{10}$: C, 14.18; H, 3.23; N, 11.81. Found: C, 14.00; H, 3.23; N, 11.68. ^1H NMR (acetone-d_6; ppm vs TMS) (a) 4.81 (d, 1 H), (b) 4.86 (d, 1 H), (c) 5.18 (dd, 1 H), (d) 5.45 (dd, 1 H), (e) 7.46 (d, 1 H), (f) 7.57 (d, 1 H), (g) 7.27 (m, 2 H), (h) 3.63 (br, 12 H), (i) 2.46 (br, 12 H), (j) 4.98 (br, 3 H), (k) 4.21 (br, 3 H). Homonuclear decoupling for resonances a–d does not affect the splitting pattern of the aromatic protons (e–g) and indicates the following relationship: H_a–H_d–H_c–H_b. As with other η^2-arene complexes, the site of metal coordination is marked by a large upfield shift in the proton resonance relative to the free ligand value. Cyclic voltammetry (CH_3CN; TBAH; 100 mV/s) $E_{1/2}$ = 0.70 V; $E_{p,a}$ = 1.40 V (NHE).

(13) The complex $[\{Os(NH_3)_5\}_2(\eta^2:\eta^2\text{-}\mu\text{-}C_{10}H_8)](OTf)_4$ can be generated from **3** by procedures outlined in ref 19. ^1H NMR (acetone-d_6; ppm vs TMS) 5.08 (d, 2 H), 4.70 (d, 2 H), 7.38 (m, 2 H), 7.16 (m, 2 H), 3.65 (br, 24 H), 4.96 (br, 6 H). A 1,2-η^2:3,4-η^2 stereochemical assignment is uniquely consistent with both the number of resonances and the observation of two high field doublets.

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Measurement of ^{13}C Relaxation Times in Proteins by Two-Dimensional Heteronuclear ^{1}H-^{13}C Correlation Spectroscopy

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We have developed a heteronuclear 2D NMR experiment for measurements of ^{13}C T_1 values in proteins. This experiment was applied to determine spin-lattice relaxation times for the α-carbons of basic pancreatic trypsin inhibitor (BPTI). No isotope enrichment was used. The experiment consists of a 2D double DEPT sequence (Figure 1) that is related to 1D sequences proposed recently.[1,2] In a first step, polarization is transferred from protons to carbons by a DEPT sequence. This is followed by a carbon 90° pulse which converts S_x to $-S_z$. During the relaxation delay, unwanted transverse proton and carbon magnetization are destroyed by a long proton pulse and a homospoil pulse.[1] The initial DEPT sequence has the effect of saturating the magnetization of ^{13}C bound protons. This saturation is maintained by applying a train of proton 90° pulses for the remainder of the delay τ_2. This also eliminates magnetization of protons that are not coupled to ^{13}C. After the relaxation delay, the carbon z-magnetization is turned into the x-y plane, frequency labeled during t_1, and finally converted into proton magnetization for detection. The experiment was conducted on a 20 mM solution of BPTI in D_2O at 36 °C, pH 4.55. Measurements were performed on a General Electric GN-500 spectrometer. Sixty-four scans were collected for each of the 350 t_1 increments. The delay between scans was 3 s, corresponding to about twice the proton T_1's. Ten data sets were collected for τ_2 values of 8 ms, 53 ms, 93 ms, 128 ms, 178 ms, 228 ms, 278 ms, 353 ms, 2 s, and 5 s, respectively. The spectrum with τ_2 = 8 ms is shown in Figure 2. The assignments given in the figure have been obtained previously on a Bruker AM-360 spectrometer.[3] The remaining ambiguities of the α-carbon assignments were resolved due to the better resolution at the higher field strength. If the DEPT transfer were perfect, cross-peak intensities would relax from $-4 \times M_0$ at $\tau_2 = 0$ to $+1 \times M_0$ at $\tau_2 = \infty$, where M_0 corresponds to the carbon equilibrium polarization. In practice, the DEPT transfer reached only values of $-2.5 \times M_0$. Relaxation times were determined by measuring the cross-peak intensities, $I(\tau_2)$, as a function of the relaxation delay, and a linear least-squares fit of $\ln I = \ln [(I(\tau_2) - I(\infty))/(I(0) - I(\infty))]$ versus τ_2 was performed. The slope is equal to the inverse of the relaxation time T_1. To test the accuracy of the T_1 measurements obtained by this method, we used a sample of 20 mM α-^{13}C enriched alanine. The value of T_1 obtained by using a direct detection inversion recovery experiment was 4.4 s while the proposed pulse sequence gave a value of 3.8 s. For the β-carbon, the values were 2.3 and 2.1 s, respectively. The sequence of Figure 1 thus seems to yield values for T_1 that are consistently 10% to 15% shorter than those measured with direct detection methods. Being aware of this we have measured the T_1 values in BPTI for the α-carbons of all residues except for the glycines where the cross peaks are suppressed due to the DEPT sequence, and for a few other residues where the signals were not resolved or were broadened due to internal mobility (C14, Y23).[4] The whole relaxation time series took 10 days of instrument time. Figure 3 shows the experimental data points and the fitted curves for the residues Cys 51 and Ala 58. Figure 4 shows the distribution of T_1 values for the various residues in the protein. The average T_1 of the α-carbons for the whole protein is 358 ms with the longest T_1 being 477 ms for Ala 58 and the shortest being 305 ms for Arg 17. This may be compared with measurements at lower field strength (90.5 MHz) where an average value of 230 ms was estimated from the envelope of 1D spectra in a conventional inversion recovery experiment.[5] In this previous study it has been estimated that the overall rotational correlation time for BPTI is 4×10^{-9} s. This is consistent with our data which also confirm that we are in the regime of slow molecular motion ($\omega\tau_c > 1$). The variation in the T_1 values is smaller than the variation in the

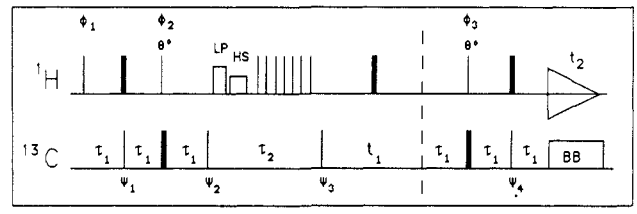

Figure 1. Double DEPT pulse sequence for measurements of ^{13}C relaxation times in 2D heteronuclear ^{13}C-^{1}H correlated spectra. The delays τ_1 are tuned to $1/2J_{CH}$. The length of the long pulse and the homospoil pulse were 1 ms each. The pulses in the train of 90° pulses were spaced 5 ms apart. We have used the following phase cycles: $\phi_1 = x, -x$; $\phi_2 = y, y, -y, -y$; $\phi_3 = x, x, x, x, -x, -x, -x, -x$; $\psi_1 = x$; $\psi_2 = y, -y, -y, y$; $\psi_3 = y, y, y, y, y, y, y, y, -y, -y, -y, -y, -y, -y, -y, -y$; $\psi_4 = x, x, -x, -x$; receiver = $x, x, -x, -x, -x, -x, x, x, -x, -x, x, x, x, x, -x, -x$. The phases of the 180° pulses were cycled independently. Quadrature detection in ω_1 was achieved using time-proportional phase incrementation of ψ_3. Decoupling of the ^{13}C spins during acquisition was done using the Waltz-16 decoupling sequence.

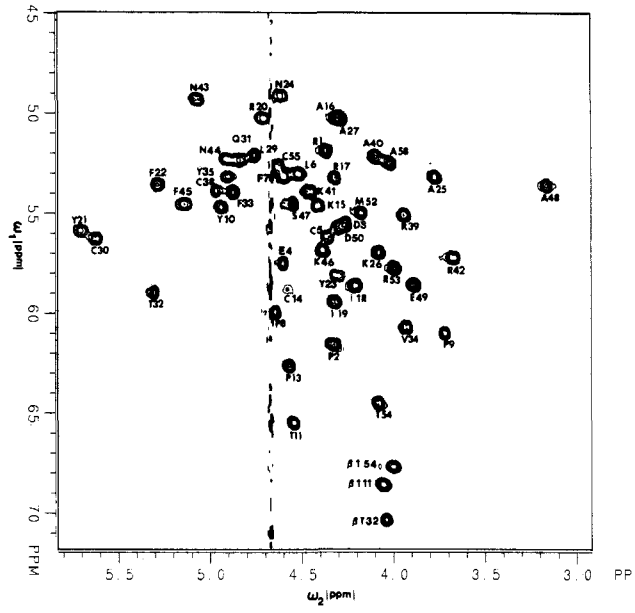

Figure 2. Region of the C$^{\alpha}$H cross peaks of a spectrum of a 20 mM solution of BPTI in D_2O recorded with the pulse sequence of Figure 1. The flip angle of the θ pulses was set to 90° to select for CH cross peaks. The value of τ_1 was set to 3.6 ms and τ_2 was 8 ms. Each FID was recorded with 4096 points and 350 FID's were collected. The data were Fourier transformed with use of a line broadening of 3 Hz and a 45° shifted sine bell window in ω_2 while a 60° shifted sine bell window was used in ω_1. The interferograms in t_1 were zero filled to 1024 points.

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