far below unity is the sulfhydryl group of a cysteine residue (0.43[8]). Presumably in the enolase-catalyzed dehydration of D-2PGA to PEP, the base is in the E—S$^-$ protonation state, whereas for the reverse hydration reaction, it is in the E—SH form.

We have been able to inactivate both yeast and muscle enolase with *p*-mercuribenzoate. Substrate protection against inactivation was observed, with Mg^{2+} not as effective as D-2PGA, which was not as effective as Mg^{2+} and D-2PGA. The inactivation was in all cases reversed by treatment with dithiothreitol.

Yeast enolase contains a single cysteine residue per monomer,[9] and it will be of interest to see if confirmation of this cysteine residue as the acid–base catalyst will come from the crystal structure of yeast enolase. Preliminary crystallographic data have been reported;[10] however, a high resolution structure has not yet been obtained.

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Mn^{2+}/Mn^{3+} and Mn^{3+}/Mn^{4+} Mixed Valence Binuclear Manganese Complexes of Biological Interest

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Binuclear and multinuclear manganese containing enzymes which occur in biology are involved in functions such as hydrogen peroxide decomposition in bacteria[1] and the oxidation of water to oxygen during photosynthesis[2], respectively. In the case of the water-oxidizing complex, the so-called S_2 oxidation state has been characterized by EPR spectroscopy to be a mixed valence manganese cluster.[3] This can be produced in two forms. The native form, which is found in active O_2-evolving samples, is considered to be either tetranuclear[4,5] or trinuclear[6] in Mn, based upon interpretation of its broad 1500 G-wide 19-line "multiline" EPR signal and unusual temperature dependence. A partially "decoupled" form is found in inactivated samples having a narrower width (1345 G), with only 16 lines and a more Curie-like temperature dependence, all features which are found in typical Mn^{3+}/Mn^{4+} binuclear centers (16-line EPR, spin $S = {}^1/_2$ ground state).[6] For the native form both different oxidation states and different numbers for the manganese ions in the cluster have been proposed in order to account for the larger spectral width due to the hyperfine interaction with ^{55}Mn. Both binuclear[7] Mn^{2+}/Mn^{3+} and tetranuclear[4] $3Mn^{3+}/Mn^{4+}$ states have been considered. Notably lacking in this analysis has been EPR data for binuclear complexes in which both types of mixed valence oxidation states, Mn^{3+}/Mn^{4+} and Mn^{2+}/Mn^{3+}, are compared within the same ligand system. The present report gives the first experimental evidence showing how ^{55}Mn hyperfine data can easily distinguish between binuclear manganese complexes having these mixed valence oxidation states.

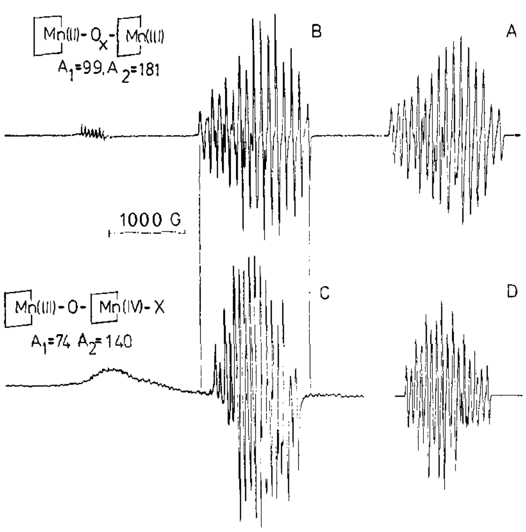

Figure 1. X-band EPR spectra at 10 K for (B) **2a** or **2b**, $[(TPP)Mn^{II}OOMn^{III}(TPP)]^-$, or $[(TPP)Mn^{II}OMn^{III}(TPP)]^-$; (C) **1**, $(TPP)Mn^{III}OMn^{IV}(TPP)X$, X = OI(Br)Ph or Br; (A) simulation of B; and (D) simulation of C using the following parameters:[12] A_1 = 99 G, A_2 = 181 G, Δ_0 = 25 G, a_1 = 3.0, a_2 = 3.0 and A_1 = 74 G, A_2 = 140 G, Δ_0 = 25 G, a_1 = −3.0, a_2 = 3.0 G with an M_I dependent Gaussian line width: $\Delta = \Delta_0 + a_1M_1 + a_2M_2$.

The tetraphenylporphyrin ligand (TPP) forms stable complexes with Mn^{2+}, Mn^{3+}, and Mn^{4+} and was employed in this study. Preparation of the Mn^{2+}/Mn^{3+} binuclear species was performed by air oxidation of $(TPP)Mn^{II}$ in chlorobenzene. $(TPP)Mn^{II}$ was prepared from (TPP)MnCl by anaerobic reduction with $NaBH_4$ in methanol and isolated by crystallization prior to use.[8] The reaction with O_2 was followed by EPR at 10 K. Prior to reaction with O_2 the only visible EPR signal was the resonance for the high spin $(TPP)Mn^{II}$ at $g = 6$.[9] Oxidation with O_2 caused this signal to decrease with concomitant appearance of a narrow free radical signal at $g = 2.0$. Subsequently, a broad underlying multiline signal appears at $g = 2$ as the free radical decays. When the reaction is complete, only the multiline signal remains and exhibits the EPR spectrum shown in Figure 1B. Here it is compared to the EPR spectrum for the mixed valence complex obtained from the room temperature decomposition of $[Mn^{IV}(TPP)X]_2O$,[10] X = $OI(Br)Ph^-$, given in Figure 1C. An identical spectrum has been assigned as a mixed valence Mn^{3+}/Mn^{4+} complex for the case of X = OCN^- and N_3^- [11] The spectrum shown in Figure 1C can therefore be assigned to the analogous mixed valence complex $(TPP)Mn^{III}OMn^{IV}(TPP)X$, **1**, where X = $OI(Br)Ph^-$ or Br^-. Both spectra arise from the ground state, as seen by a limited temperature study between 5 and 100 K (not shown).

As can be seen, both species yield 16-line "multiline spectra". Temperature dependence and hyperfine structure of this type indicate antiferromagnetic electron exchange coupling between inequivalent (valence trapped) Mn oxidation states in which the ground state has spin $S = {}^1/_2$.[12] However, the spectra differ greatly in the magnitude of their ^{55}Mn hyperfine fields; the Mn^{2+}/Mn^{3+} complex has a total width of 1410 G, while the Mn^{3+}/Mn^{4+} complex is considerably narrower at 1070 G and also exhibits somewhat larger line shape anisotropy (axial). Computer simulations of these spectra using a second-order perturbation solution to an isotropic spin hamiltonian[12] are given in Figure 1A and D. The agreement with the positions of all transitions is

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satisfactory by using hfs constants of 74 G and 140 G for **1** and 99 G and 181 G for the $\mathrm{Mn^{2+}/Mn^{3+}}$ species. The line shape of the former complex is not predicted well since the fitting utilizes isoropic line shapes, while the spectrum exhibits axial symmetry. The g factors, corrected for the second-order shift of the spectrum,[12] are 2.06 and 2.01, respectively.

Theory predicts that these dimer hfs constants (A^D) can be related to those for the individual Mn ions (A^M) by a scaling factor equal to the projection of the individual Mn ion spin onto the resultant spin of the dimer in the EPR active state, $S_i{\cdot}S/S^2$. The spin coupling formulas for these two cases predict that $|A^D/A^M| = 2$ for $\mathrm{Mn^{3+}}$ and 1 for $\mathrm{Mn^{4+}}$ and $^7/_3$ for $\mathrm{Mn^{2+}}$ and $^4/_3$ for $\mathrm{Mn^{3+}}$, in the respective dimers.[4,12] Applying these to the present data yields hfs constants A^M = 70 G and 74 G for $\mathrm{Mn^{3+}}$ and $\mathrm{Mn^{4+}}$ and 77 G and 75 G for $\mathrm{Mn^{2+}}$ and $\mathrm{Mn^{3+}}$. By comparison, the ^{55}Mn isotropic hfs constant in the mononuclear complex $\mathrm{Mn^{II}(TPP)}$ is 80 G,[9] in $\mathrm{[Mn^{IV}(TPP)Cl)]^+}$ it is 81 G,[13] and in $\mathrm{Mn^{IV}(TPP)Cl_2}$ it is estimated at 73 G.[13] Assignment of both mixed valence spectra to the same oxidation pair leads to unreasonable A^M constants for one pair. Thus the larger EPR spectral width for $\mathrm{Mn^{2+}/Mn^{3+}}$ complexes can serve as a possible means for distinguishing them from $\mathrm{Mn^{3+}/Mn^{4+}}$ complexes having ligands of comparable covalency. The basis for this discrimination is two/fold: the spin-coupling coefficients that multiply the hfs constants are larger for $\mathrm{Mn^{2+}/Mn^{3+}}$ complexes than for $\mathrm{Mn^{3+}/Mn^{4+}}$, and the individual ion hfs constants for Mn^{n+} do not vary much with n for ligands having similar covalency. Few $\mathrm{Mn^{2+}/Mn^{3+}}$ complexes have been studied by EPR to test these predictions. Confirmation of a very large multiline EPR spectral width (2000 G) in a $\mathrm{Mn^{2+}/Mn^{3+}}$ complex utilizing pyridine, amine, and acetate ligands has been observed.[14]

A 16-line EPR spectrum for a mixed valence Mn dimer has been reported for the oxidation product of $\mathrm{Mn^{II}}$ (Saldien) with $\mathrm{O_2}$.[15] The magnitude of the observed A^D hfs constants, 165 G and 83 G, was the primary basis for assigning this as a $\mathrm{Mn^{2+}/Mn^{3+}}$ species instead of $\mathrm{Mn^{3+}/Mn^{4+}}$. The predicted A^M hfs constants are then 71 G for $\mathrm{Mn^{2+}}$ and 62 G for $\mathrm{Mn^{3+}}$, considerably smaller than found here for the more covalent TPP ligand. This unexpected difference indicates either a limitation of the theory or an incorrect oxidation state assignment. An alternate formulation of this as a $\mathrm{Mn^{3+}/Mn^{4+}}$ complex leads to A^M hfs constants of 82 G and 83 G; values much closer to those found for mononuclear Mn complexes with these ligands.

Both $\mathrm{O_2}$ and $\mathrm{(TPP)Mn^{II}}$ are required for formation of the $\mathrm{Mn^{2+}/Mn^{3+}}$ complex, suggesting that oxygen forms the bridging group. Exposure of (TPP)MnCl to air does not produce a multiline signal nor does mixing equal molar quantities of $\mathrm{(TPP)Mn^{II}}$ and $\mathrm{(TPP)Mn^{III}Cl}$ in $\mathrm{CH_2Cl_2}$ under anaerobic conditions. One mechanism which is able to account for these observations is as follows.

$$\mathrm{(TPP)Mn^{II} + O_2 \rightarrow [(TPP)Mn^{III}]^+ + O_2^-} \qquad (1)$$

$$\mathrm{(TPP)Mn^{II} + O_2^- \rightarrow [(TPP)Mn^{III}O_2]^-} \qquad (2)$$

$$\mathrm{(TPP)Mn^{II} + [(TPP)MnO_2]^- \rightleftarrows [(TPP)Mn^{II}OOMn^{III}(TPP)]^-}\ (\mathbf{2a}) \qquad (3)$$

$$\mathrm{[(TPP)Mn^{II}OOMn^{III}(TPP)]^- \rightleftarrows (TPP)Mn^{IV}O + [(TPP)Mn^{III}O]^-} \qquad (4)$$

$$\mathrm{(TPP)Mn^{II} + (TPP)Mn^{IV}O \rightleftarrows (TPP)Mn^{III}OMn^{III}(TPP)} \qquad (5)$$

$$\mathrm{(TPP)Mn^{II} + [(TPP)Mn^{III}O]^- \rightleftarrows [(TPP)Mn^{II}OMn^{III}(TPP)]^-}\ (\mathbf{2b}) \qquad (6)$$

Air oxidation of $\mathrm{(TPP)Mn^{II}}$ to form $\mathrm{[(TPP)Mn^{III}]^+}$ and $\mathrm{O_2^-}$ in step (1) accounts for the loss of $g = 6$ signal and the appearance of a free radical seen by EPR. The superoxide ion reacts with a second $\mathrm{(TPP)Mn^{II}}$ to form the EPR silent peroxide, $\mathrm{[(TPP)Mn^{III}O_2]^-}$ in step (2) (loss of free radical signal). The thermally unstable complex of $\mathrm{[(TPP)Mn^{III}O_2]^-}$ has been shown to form at low temperatures by reaction (2).[16] In step (3) this anion is proposed to react further with excess $\mathrm{(TPP)Mn^{II}}$ to form the μ-peroxo complex $\mathrm{[(TPP)Mn^{II}OOMn^{III}(TPP)]^-}$ (**2a**). This mixed valence species could account for the wide multiline EPR signal in Figure 1B. It is also possible that **2a** undergoes homolytic cleavage of the peroxo bond to form the $\mathrm{[(TPP)Mn^{III}O]^-}$ and $\mathrm{(TPP)Mn^{IV}O}$ species given in step (4). Reaction of the products from step (4) with excess $\mathrm{(TPP)Mn^{II}}$ to form the EPR silent μ-oxo dimer, $\mathrm{(TPP)Mn^{III}OMn^{III}(TPP)}$ in step (5), and the EPR active mixed valence μ-oxo dimer, $\mathrm{(TPP)Mn^{II}OMn^{III}(TPP)}$ (**2b**), in step (6), could also account for the multiline signal in Figure 1B. The present EPR results do not allow us to distinguish which $\mathrm{Mn^{2+}/Mn^{3+}}$ dimer, **2a** or **2b**, is responsible for the wide EPR multiline signal.

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$\mathrm{Li^+(en)_2{\cdot}Na^-}$: A Simple Crystalline Sodide

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Since the preparation of the first crystalline sodide, $\mathrm{Na^+[cryptand(2.2.2)]{\cdot}Na^-}$, in 1974,[1,2] we have tried to prepare alkalides that have simpler and less expensive cations. Attempts to use tetraalkylammonium ions, for example, resulted in irreversible decomposition.[3] Recent success in solubilizing sodium by using solutions of lithium in methylamine and the apparent formation of solutions of $\mathrm{Li^+(CH_3NH_2)_4{\cdot}Na^-}$ in various solvents[4] prompted us to try the less volatile complexant, ethylenediamine (en). We report here the synthesis of the simple crystalline sodide, $\mathrm{Li^+(en)_2{\cdot}Na^-}$. Extension of this work to other complexants for the lithium cation and to other anions such as $\mathrm{K^-}$, $\mathrm{Rb^-}$, $\mathrm{Cs^-}$, or $\mathrm{e^-}$ could make available inexpensive, easily prepared alkalides and electrides for use as reductants in synthesis.

Vacuum-line synthesis methods, solvent purification, alkali metal handling, and analytical techniques are described elsewhere.[5-8] To prepare the title compound it is only necessary to combine lithium metal, sodium metal, and ethylenediamine in stoichiometric amounts with the aid of a suitable solvent and then remove the solvent slowly by low-temperature distillation. A

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