yields. The applications of this simple reaction for the preparation of spirocyclic and bridged ketosilanes, and for hirsutane synthesis, are in progress.[16]

Registry No. 1a, 113893-24-6; **1b**, 113893-25-7; **1c**, 113893-26-8; **1d**, 113893-27-9; **1e**, 113893-28-0; **2a**, 113893-29-1; **3a**, 40730-45-8; **4a**, 113893-30-4; **6c**, 113893-40-6; **9b**, 113893-31-5; *cis*-**9c**, 113893-32-6; *trans*-**9c**, 113893-33-7; *cis*-**9d**, 113893-34-8; *trans*-**9d**, 113893-35-9; **9e**, 113893-36-0; **10**, 113893-37-1; 2-methyl-2-cyclohexen-1-one, 1121-18-2; 2-methyl-2-cyclohepten-1-one, 65371-57-5; 2-methyl-2-cyclopenten-1-one, 1120-73-6; ω-trimethylsilylbutynylmagnesium chloride, 113893-38-2; 2-cyclohexen-1-one, 930-68-7; ω-trimethylsilylpentynylmagnesium chloride, 113893-39-3.

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Properties of the Fe(II)-Fe(III) Derivative of Red Kidney Bean Purple Phosphatase. Evidence for a Binuclear Zn-Fe Center in the Native Enzyme

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Purple phosphatases from pig allantoic fluid[1] and beef spleen represent a class of metallohydrolases which contain a binuclear iron center.[2-5] These enzymes exist in two redox states, a pink, catalytically active Fe(II)-Fe(III) form and a purple catalytically inactive Fe(III)-Fe(III) form. EPR spectroscopy and magnetic susceptibility measurements[4,6] have shown that the iron atoms are antiferromagnetically coupled. The oxidized Fe(III)-Fe(III) forms of these enzymes are EPR silent, whereas the reduced forms have a characteristic low-temperature EPR spectrum with a rhombic g tensor, $g = (1.94, 1.73, 1.57)$, corresponding to one unpaired electron per binuclear center.[4,5,7] Recently, we reported the purification and characterization of a purple phosphatase from red kidney bean which contains one atom of iron and one of zinc per subunit of ~60 kDa.[8] Further, we have shown that it is possible to prepare a catalytically active Fe(II)-Fe(III) form of the red kidney bean enzyme.[9]

In this report, we compare the low-temperature EPR spectra of the native Zn-Fe form **1** and the Fe(II)-Fe(III) derivative **2** of red kidney bean purple phosphatase with that of the Fe(II)-Fe(III) form of pig allantoic fluid acid phosphatase (**3**). The spectra of **2** and **3** are very similar, showing that **2** and, therefore by inference, the native Zn-Fe red kidney bean purple phosphatase (**1**) contain a binuclear complex.

A sample of **2** was prepared by treatment of a solution of the native enzyme in 0.1 M acetate buffer, pH 4.9, 0.5 M in NaCl, with ferrous ammonium sulfate and 2-mercaptoethanol ($[E]_0$ = 69 μM; $[Fe^{2+}]$ = 85.1 mM; [2-mercaptoethanol] = 136 mM) for 40 h at 25 °C. The enzyme was then separated from the other reagents by passage through a column of Sephadex G-25 equilibrated in the same buffer and concentrated by ultrafiltration. The enzyme sample so prepared (23.4 mg/mL; 0.39 mM in 60-kDa subunits) had a specific activity of 515 $U \cdot ml^{-1} \cdot A_{280}^{-1}$ in the standard assay at pH 4.9 with *p*-nitrophenyl phosphate as substrate and contained 1.92 Fe atoms and 0.24 Zn atoms per subunit. Treatment of an aliquot of this sample with 11 mM H_2O_2 for 2 min at 25 °C resulted in loss of 90% of the activity, showing that ≤10% of the activity was due to residual native (Zn-Fe) enzyme which is not inactivated by H_2O_2.[9,10]

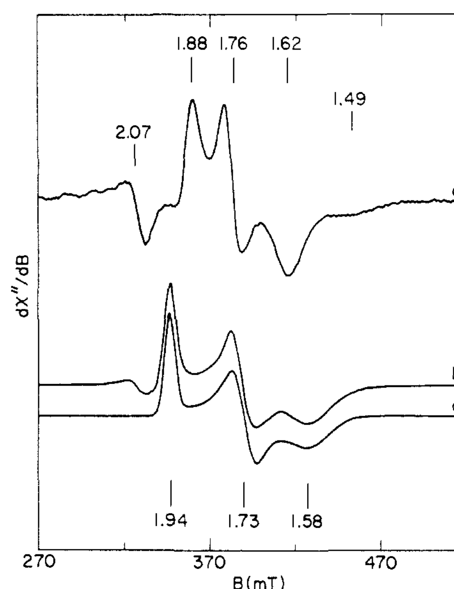

Figure 1. EPR spectra of the Fe(II)-Fe(III) derivative of (a) red kidney bean purple phosphatase (**2**) and (b) the reduced form of pig allantoic fluid acid phosphatase (**3**) at 4 K. The simulation (c) is based on an effective spin $S = 1/2$ Hamiltonian with $\tilde{g} = (1.94, 1.73, 1.58)$ and an anisotropic, Gaussian line width model with $\tilde{\sigma} = (7, 13, 27)$ mT. Spectral conditions: microwaves 9.447 GHz at 0.2 mW; 100 kHz modulation at 2 mT_{pp}; $dB/dt = 0.6$ mT/s.

Figure 1a shows the EPR spectrum at 4 K of the sample of **2** described above, together with the spectrum of a sample of the reduced form of pig allantoic fluid acid phosphatase (**3**; 0.53 mM) run under the same conditions (Figure 1b). The spectra shown in Figure 1 are clearly very similar, indicating that they arise from closely related metal ion complexes. Apart from a Cu(II) signal with $g_\perp \sim 2.06$ the main features of the spectra have g values $g < 2$ and are thus characteristic of an antiferromagnetically coupled pair of high-spin ferrous and ferric ions with zero-field splittings comparable to the exchange interaction.[11] The differences between the EPR signals of **2** and **3** are significant, however, and indicate differences in the structure of the complex. While we can simulate the spectrum of the pig enzyme with the effective g tensor and anisotropic Gaussian line width specified in the figure caption, spectrum 1a cannot be simulated by the same model. We conclude that the Fe(II)-Fe(III) signal of the bean enzyme is a superposition of at least two components, a view that is corroborated by the extra broad dip near $g = 1.49$. In this context, Averill et al.[12] have shown that the analogous EPR signal of the beef spleen enzyme is sensitive to changes in pH.

Figure 2 shows the EPR spectrum of a sample of native (Zn-Fe) red kidney bean phosphatase (**1**; 0.44 mM; specific activity 334 $U \cdot mL^{-1} \cdot A_{280}^{-1}$) at 4 K. The native Zn-Fe red kidney bean

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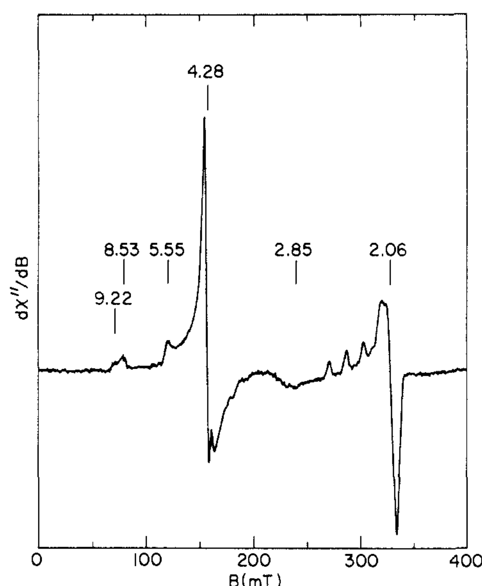

Figure 2. EPR spectrum of the native (Zn-Fe) form of red kidney bean purple phosphatase (1) at 4 K. Spectral conditions: microwaves 9.447 GHz at 20 μW; 100 kHz modulation at 2 mT_{pp}; dB/dt = 2 mT/s; 10 scans in 2000 s.

enzyme has no EPR spectrum in the $g' = 1.74$ region but shows the $g = 4.3$ signal characteristic of isolated high spin Fe(III) with large rhombicity. From measurements at different temperatures and attempts to simulate the Fe(III) signals of Figure 2 it again appears that at least two different species are present: one with rhombicity $E/D \sim 0.25$ and $D/k \sim 1.5$ K giving rise to the $g \sim 9.22$ and $g \sim 4.28$ features and another one with $E/D \sim 0.14$, $D/k \sim 0.4$ K giving rise to the features at $g \sim 8.53$, 5.55, and 2.85. Since the sample contained 1.14 Fe atom and 0.76 Zn atom per subunit, not all the Fe(III) can be paired with a diamagnetic Zn(II). One therefore expects to observe at least two types of EPR signals, but it is not clear which one to assign to the native Fe(III)-Zn(II) pair. Owing to difficulties with the integration of the Cu(II) signal overlapping the highly rhombic iron signals, attempts to quantify the number of spins proved to be unreliable. Small $g = 4.3$ signals are present in the spectra of **2** and **3** (not shown) but account for only a very small percentage of the iron in the enzyme samples. Signals at $g = 4.3$ corresponding to trace amounts of high spin Fe(III) have been observed in other samples of pig allantoic fluid and beef spleen enzymes.[4,5,7] The spectrum of Figure 2 shows a sizeable and well-resolved Cu(II) signal; weaker signals have been noted in the spectra of Figure 1. Small amounts of copper have been found in some red kidney bean preparations, frequently ≤0.1 Cu per subunit, but in one preparation (of 16), as high as 0.56 Cu/subunit. The sample used in the current preparation was not analyzed for copper. Moreover, the absolute purity of the starting enzyme in no way alters the significance of the observations.

The EPR data on the Fe(II)-Fe(III) derivative of red kidney bean phosphatase therefore show that it contains an antiferromagnetically coupled binuclear iron complex. There is good evidence that the Zn(II) atom in the native enzyme occupies the same site as the Fe(II) atom in the Fe(II)-Fe(III) derivative: (i) binding studies have shown that there is only one strong binding site for divalent metal ions per subunit,[8] and (ii) the catalytic activity of the Fe(II)-Fe(III) derivative is similar to that of the Zn(II)-Fe(III) enzyme.[8,9] Therefore, the present evidence supports the existence of a binuclear zinc-iron complex in red kidney bean purple phosphatase, the first such complex to be reported.

The catalytic subunit of calcineurin, a phosphoprotein phosphatase from bovine brain, contains stoichiometric amounts of zinc and iron,[13] and it is possible that it may have an active-site structure similar to that of the red kidney bean enzyme. Several other purple phosphatases, including enzymes from sweet potato[14] and soybean,[15] have been purified from plants, and it will be of interest to determine their relationship to the red kidney bean enzyme. The only other enzyme known to contain zinc in close proximity to a transition metal ion is the Cu-Zn superoxide dismutase in which both metal ions are linked to the same imidazole group.[16]

The significance of a binuclear center in catalysis by phosphatases remains to be established, although roles for both metal ions of a model binuclear complex in hydrolysis of a phosphate ester have been established.[17] The reason why the animal purple phosphatases have an Fe-Fe center, whereas the plant enzyme has a Zn-Fe center, is also unknown. The catalytic efficiencies of the two systems are comparable, but they differ in their susceptibility to oxidation. One could speculate that the animal enzymes in contrast to the plant enzymes may be subject to reversible redox control of their phosphatase activity.

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Registry No. Phosphatase, 9013-05-2; acid phosphatase, 9001-77-8.

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Silylation-Mediated Oxidation of 4-Aza-3-ketosteroids with DDQ Proceeds via DDQ-Substrate Adducts

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In connection with continuing interest from these laboratories in azasteroids[1] we wish to report an efficient, silylation-mediated DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) oxidation of 4-aza-3-ketosteroids to the corresponding Δ^1-lactams which proceeds via unprecedented DDQ-substrate adducts. The existing technology[2] for effecting such transformations involves several cumbersome steps or is often complicated by poor yields, unwanted byproducts, and use of selenium reagents. The new oxidation (**1** → **5**) provides a unique entry into a diverse spectrum of 17β-substituted Δ^1-4-aza-5α-androsten-3-ones (**5**) which are currently undergoing clinical evaluation for benign prostatic hypertrophy.[3] For example, treatment of **1a** with 1 mol of DDQ and 4 mol of BSTFA [bis(trimethylsilyl)trifluoroacetamide][4] in dioxane under

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