same protein previously inferred by comparing the redox properties of the same cluster in different proteins of known structure [9].

Since several recent reports present modifications of the thermodynamic properties of Fe–S centers in a number of proteins and relate these modifications to more or less subtle conformational changes, the approach we introduced may be of some interest.

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O28

NMR Studies of Porcine Uteroferrin: Evidence for a Spin-coupled Binuclear Iron Cluster

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Uteroferrin is an iron-containing acid phosphatase of molecular weight near 35,000 isolated from either the allantoic fluid of pregnant sows or the uterine flushings of pseudopregnant sows [1–3]. The protein can exist in two forms – a purple (λ_{max} ~ 570 nm), enzymatically inactive, oxidized form and a pink (λ_{max} ~ 510 nm), enzymatically active, reduced form [2, 3]. The former is EPR-silent, while the latter exhibits a novel EPR signal centered near g = 1.74 [4–6], reminiscent of signals observed for the semimethemerythrins [7].

We have undertaken a ^{1}H NMR study of porcine uteroferrin focusing on paramagnetically shifted resonances in order to elucidate the active site structure and magnetic properties of the protein. Figure 1

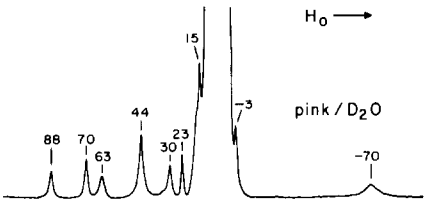

Fig. 1. 300 MHz ^{1}H NMR spectrum of pink uteroferrin.

shows the 300 MHz ^{1}H NMR spectrum of pink two-iron uteroferrin in sodium acetate buffer (D_2O), pH 4.9, at 30 °C. Well-resolved features spanning 160 ppm are observed with linewidths ranging from 300–2000 Hz. In buffered H_2O, additional resonances are observed near 89, 43, and –25 ppm.

The similarity of the EPR signal exhibited by pink uteroferrin to those of the semimethemerythrins [7] suggests the involvement of antiferromagnetically coupled Fe(III)–Fe(II) centers. Estimates for the value of the antiferromagnetic coupling constant J ($H = -2JS_1 \cdot S_2$) can be made from the temperature dependence of the isotropic shifts, since the shifts are proportional to magnetic susceptibility, assuming a temperature-invariant Fermi contact term [8]. Based on data obtained from 0–50 °C, we conclude that $-J < 20$ cm^{-1} for pink uteroferrin, in agreement with the estimate of J (-7 cm^{-1}) obtained from the temperature dependence of the intensity of the EPR signal [6].

Some of the metal ligands in pink uteroferrin can be identified by comparing the observed shifts to those of synthetic complexes. Based on our model studies, histidine is found in the coordination environments of both the ferrous and the ferric centers, while tyrosine is coordinated only to the ferric center. Other ligands including the bridging group have yet to be identified.

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