ELECTRON PARAMAGNETIC RESONANCE STUDIES ON THE FERREDOXIN FROM CLOSTRIDIUM PASTEURIANUM

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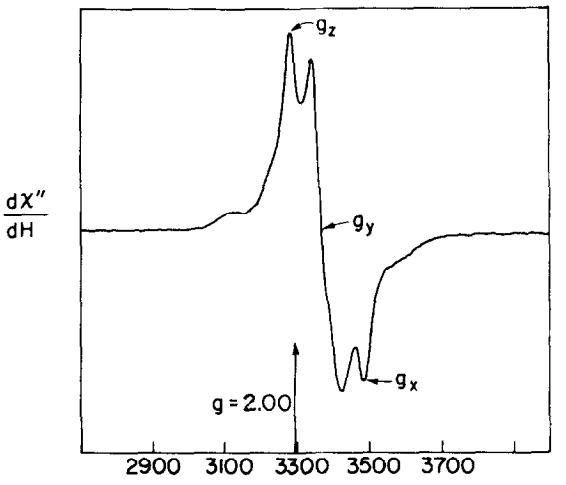
We have recently demonstrated an EPR signal in spinach ferredoxin on reduction either enzymically or with dithionite (Palmer and Sands, 1966). It is the purpose of this communication to report on the EPR spectrum found in the ferredoxin obtained from the bacterium C. pasteurianum.

The ferredoxin was purified by the method of Mortenson (1964) and had an absorbancy ratio 285 mμ; 390 mμ of 0.82; it was pure by disc electrophoresis on polyacrylamide at pH 9.0.

The oxidised enzyme exhibits a small and variable resonance at g = 2.0. This signal is typical of high spin ferric ion in a weak crystal field. On reduction with dithionite the EPR signal shown in Fig. 1 is obtained. The apparent g-values are $g_x = 1.89_2$, $g_y = 1.96_0$, $g_z = 2.00_5$ (see Fig. 1.) although the spectrum is not understood; in addition two shoulders are clearly visible one at each end of the spectrum. Inasmuch as the resonance exhibits two g-values below 2.0, it can be considered as



EPR spectrum of ferredoxin from C. pasteurianum (8.5×10^{-4} M) reduced with dithionite. The spectrum was recorded in a Varian V-4502 EPR spectrometer using 100 kc/sec. field modulation. Modulation amplitude, 3 gauss; microwave power 0.08 mwatts; microwave frequency, 9.236 Gc; temperature, 15°K.

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belonging to the g = 1.94 class of nonheme iron-proteins (Beinert, 1966). However, although the other known representatives of this class of paramagnetic iron-proteins have EPR spectra which vary from axial to rhombic symmetry with various degrees of intermediate distortion the spectrum of the bacterial ferredoxin is much more complicated than anything with which we are familiar. The possibility thus has to be considered that the sample is inhomogeneous: that is to say, we are observing overlapping spectra due to several different species either originating on the same protein molecule or from different protein molecules. An alternative possibility is that the shoulders present in the wings

of the spectrum are produced by dipole-dipole interactions between two or more paramagnetic components.

The intensity of the integrated spectrum corresponds to 0.73 μ moles of iron/ml assuming the resonance arose from a doublet. This value needs to be corrected upward by some factor due to the difference in g-values (and hence transition intensity) between the unknown and the copper - EDTA used as the reference compound (Aasa and Vanngard, 1962). The magnitude of this correction is difficult to assess but a factor of 1.2 is thought to be approximately correct which yields a corrected value of 0.88 μmoles of iron/ml. This sample of ferredoxin contained 5.0 μmoles of iron (and 4.4 μmoles of sulfide) per ml. Thus the integrated EPR intensity accounts for 17.6% of the total iron present in the protein. As shown in Table I clostridium ferredoxin contains 6 atoms of iron; consequently we observe 1.06 atoms of iron by EPR. Also detailed in Table I are experiments describing the valence state of the iron liberated from the protein by treatment with the mercurial mersalyl (sodium O-[(3-hydroxy-mercuri-2-methoxypropyl)carbamyl] - phenoxyacetate). The results show clearly that the mercurial released three of the six iron atoms in the ferric state and the remainder in the ferrous form. These results should be contrasted with the earlier observations of Blomstrom et al. (1964) who found five atoms of Fe^{II} and two atoms of Fe^{III} per mole of protein. Inasmuch as any method of chemical analysis may perturb the valence state of the metal as it originally existed in the intact protein we do not want to stress these results. Nevertheless this data,

taken with recent report of Sobel and Lovenberg (1966) that clostridial ferredoxin is a two-electron acceptor, provides encouraging support for the model we have recently proposed (Brintzinger et al. 1966) that the so-called g = 1.94 signal is due to low-spin ferric iron in essentially tetrahedral symmetry, for on reductions one would expect to have only one ferric iron present. This would account for our observed quantitation. However, in view of our lack of understanding of the anomalous line-shape of the EPR spectrum (vide supra) we must accept this correlation with reservation until more detailed studies are made.

The iron signal could also be generated by addition of TPNH, TPNH-ferredoxin reductase from spinach and neurospora DPNase. Unfortunately the presence of substantial quantities of manganous ion in the TPNH (Sigma, Type II) precluded any quantitative measurements. We are at present trying to overcome this problem.

Like its counterpart from spinach, bacterial ferredoxin exhibits an EPR spectrum substantially more temperature sensitive than that observed with the so-called g = 1.94 nonheme iron proteins: thus with the ferredoxins almost no signal is observed above liquid nitrogen temperatures and special accessories are essential to study these systems.

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TABLE I

State of Fe in Ferredoxin

	O.D. 520 mμ		moles Fe/mole Fd	
Experiment 1	Before $S_2O_4^=$	After $S_2O_4^=$	Before $S_2O_4^=$	After $S_2O_4^=$
Fd + dipyridyl + mersalyl	0.616	1.26	2.90	5.95
	O.D. 487 mμ		moles Fe/mole Fd	
Experiment 2	Before O_2	After O_2	Before O_2	After O_2
Fd + tiron. + mersalyl	0.42	0.82	3.05	6.01

In the experiment 91.2 mμ moles of ferredoxin (based on A_{390} = 20) in 3.0 ml of 0.1M tris-HCl pH 8.0 was rendered anaerobic in a two sidearm Thunberg-type cuvette and the optical spectrum obtained. Then either 2 mg. of α,α'-dipyridyl or 2 mg. of Tiron (catechol disulfonate) was added from one of the sidearms. No change in the spectrum was detected in either case; subsequently 5 mg. of Mersalyl was added from the second sidearm. The color of the metal-chelate developed immediately (independent experiments confirm that this amount of Mersalyl bleaches the protein instantly). After recording the spectrum full color development was ensured by the addition of dithionite in Experiment 1 and by stirring in air in Experiment 2. At the wave lengths employed for measurement there is a correction of 0.03 for the ferredoxin-mersalyl absorption. Under the conditions of the experiment we find extinction coefficients of 4530 for ferric-Tiron (487 mμ) and 6980 for ferrous dipyridyl (520 mμ). The iron in the sample was analyzed independently by the method of Doeg and Ziegler (1962) when a value 6.05 moles/ mole ferredoxin

was obtained. Sulfide analysis (Fogo and Popowsky, 1949) gave a value of 6.06 moles/ mole of ferredoxin.

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