THE EFFECT OF THE ^{57}Fe HYPERFINE INTERACTION ON THE EPR SPECTRUM OF SPINACH FERREDOXIN[1]

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Recently several models have been proposed to explain the novel EPR at g = 1.94 found in reduced non-heme iron proteins (Blumberg and Peisach, 1965; Brintzinger et al., 1966; Gibson et al., 1966; Van Voorst and Hemmerich, 1967). The two-iron model of Gibson et al. (1966) predicts that the hyperfine interaction which would occur if the natural isotope ^{56}Fe were replaced with the magnetic isotope ^{57}Fe should produce a splitting of approximately 37 gauss in the EPR spectrum (Thornley et al., 1966).

The demonstration of the g = 1.94 resonance in spinach ferredoxin reduced either chemically or enzymically (Palmer and Sands, 1966) has encouraged us to prepare ^{57}Fe spinach ferredoxin and to measure its EPR spectrum. The results of these experiments are reported here.

METHODS

Spinach ferredoxin was prepared by the method of Tagawa and Arnon (1962). The isotopic iron-57 (as Fe_2O_3, 92.8%, Union Carbide) was exchanged into the protein by a method similar to that of Lovenberg et al. (1963). The efficiency of the exchange was monitored by adding a little radioactive ^{59}Fe to the ^{57}Fe and measuring the radioactivity of the reconstituted protein. Biological activity of the protein was measured spectro-

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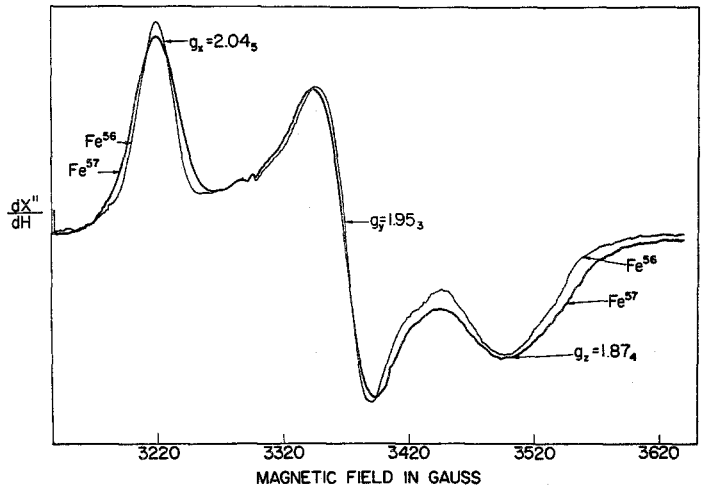
photometrically using the ferredoxin mediated reduction of cytochrome c by TPNH and ferredoxin-TPN reductase. EPR spectra were obtained with a Varian V-4500 X-band spectrometer with 100 kHz field modulation and a variable low-temperature device capable of reaching 17°K.

RESULTS

The technique of Lovenberg et al. (1963) for exchanging iron into the ferredoxin of Clostridium pasteurianum works well with spinach ferredoxin. The product so obtained, whether containing ^{56}Fe or ^{57}Fe, has 80-95% of the activity of the starting material. Furthermore the critical ratio A_{420}/A_{275} is invariably as good as the starting material and usually higher. A value of 0.45 has been obtained with a range of 0.40-0.45 (five experiments with ^{56}Fe; three experiments, ^{57}Fe). The optical spectra of the oxidised and reduced product (^{56}Fe and ^{57}Fe) are indistinguishable from native ferredoxin in the visible region, as are the circular dichroism spectra (^{56}Fe only; insufficient ^{57}Fe protein available). The oxidised protein shows no EPR in the range 0-5 kilogauss; the chemically reduced protein shows a g = 1.94 EPR signal (see below). The extent of exchange is found to be almost 100% as judged by the radioactivity of the isolated product. The iron content determined by the optical density at 420 mμ (ϵ = 4700), by radioactivity measurement, and by total iron determinations with o-phenanthroline (Massey, 1957) are in good agreement; values of 0.19, 0.175 and 0.185 μatoms Fe/mg. protein, respectively, are typical.

The EPR spectrum of native and ^{57}Fe substituted spinach ferredoxin reduced with dithionite is shown in Fig. 1. It is clear that there is an electron-nuclear hyperfine interaction as judged by the decrease in intensity and broadening at g_x (low field peak) and g_y (center slope). However, it is also clear that the effect is small. The apparent gain in intensity at g_z (high field trough) is real and is observed in all samples of the ^{57}Fe protein and in the reconstituted ^{56}Fe protein. The latter is indistinguishable from the native protein at g_x and g_y. This is the only dif-



Legend to Fig. 1.

EPR spectra of ^{56}Fe and ^{57}Fe spinach ferredoxin reduced with dithionite. Protein concentration 1.12 x 10^{-4}M for both samples and spectra were run in quartz capillaries of identical internal diameter. Field Modulation: frequency, 100 kHz; amplitude, 6 gauss. Klystron Frequency, 9.221 GHz. Temp., 32°K.

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ference we have observed so far between the native and reconstituted proteins. The EPR spectra have identical shapes at 40°K and 20°K.

The magnitude of the hyperfine interaction has been estimated by simulation of the ^{57}Fe EPR spectrum on the IBM 7090. With both one-iron and two-iron models for the protein a value of ca 14 gauss is obtained for the hyperfine coupling constant and thus no distinction between these two possibilities is afforded by these data. The observed hyperfine coupling is somewhat smaller than the 22 gauss found with an iron protein from Azotobacter (Shethna, et al., 1964) and substantially less than the value of 37 gauss calculated by Thornley et al. (1966) on the basis of the two-iron model of Gibson et al (1966). Thus if this latter model is indeed correct, the results reported here imply that the hyperfine interaction

with the individual iron atoms is about 4 gauss rather than the 10 gauss assumed for the calculations.

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