ELECTRON SPIN RESONANCE SPECTRA OF SPINACH FERREDOXIN

D.O.Hall, J.F.Gibson & F.R.Whatley

Botany Department, King's College London, and

Chemistry Department, Imperial College London.

Received March 4, 1966

Spinach ferredoxin is a non-haem iron protein isolated from spinach leaves which undergoes reversible oxidation and reduction in its catalytic role as an electron carrier in photosynthesis (San Pietro and Black, 1965; Arnon, 1965). The protein contains two iron atoms per mole but only one electron is transferred per mole during oxidation-reduction (Whatley et al, 1963; Horio and San Pietro, 1964). This is in contradistinction to bacterial ferredoxin which contains seven iron atoms per mole and takes on two electrons during reduction. (Lovenberg et al, 1963; Sobel and Lovenberg, 1966). Because of its greater simplicity spinach ferredoxin was preferred for a study of the electron spin resonance (ESR) spectra of the reduced and oxidized forms. An ESR signal in the $g = 1.94$ region of the spectrum was detected in the reduced form while a $g = 4.27$ signal was found in the oxidized form. The $g = 1.94$ signal is considered characteristic of protein-bound reduced non-haem iron (Beinert and Palmer, 1965) but has not previously been noted in either spinach or bacterial ferredoxin. The $g = 4.27$ signal of the oxidized molecule may also be of significance.

Experimental. Spinach ferredoxin was prepared from Spinacea oleracea (English or French grown) and Beta vulgaris (English grown) and either used fresh or after storage at -20°C. The purification procedures described by Hill and Bendall (1960) and Tagawa and Arnon (1962) were used as well as a modification by Hill of the Tagawa and Arnon method(personal

communication); all three methods yielded spinach ferredoxin which gave ESR signals at $g = 1.94$ and 4.27.

Samples containing between 1 and 9 μmoles of spinach ferredoxin/ml (based on $E_{420} = 10.32\ cm^{-1}.mM^{-1}$) were used. 0.2 ml of a spinach ferredoxin solution was placed in a Varian ESR tube (external diam. = 4 mm). A Varian V-4502-15 ESR spectrometer with dual cavity and variable temperature accessories was used in these studies. The oxidized spectrum was recorded initially and the sample was then reduced in an atmosphere of argon with 0.1 ml of an alkaline solution containing 8 μm sodium dithionite (reduction with an excess of sodium dithionite crystals also gave a similar spectrum). The spectra were taken at temperatures over the range -80° to -190°C. Most studies were done at -180°C.

Results and Conclusions. First derivative ESR spectra of oxidized and reduced spinach ferredoxin are shown in Fig.1. A $g = 4.27$ signal is present in the oxidized molecule but this disappears and is replaced by a more intense $g = 1.94$ signal upon reduction with dithionite. The sample could be reoxidized in air and reduced again to give the same sequence of signals.

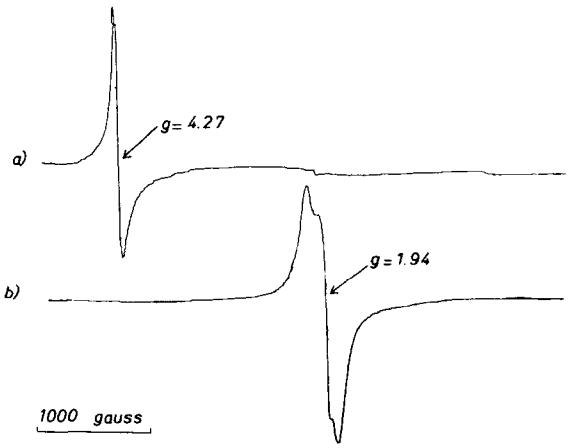

Fig.1. Spectra of oxidized and reduced spinach ferredoxin (a) oxidized (b) reduced. The conditions for ESR spectroscopy were : power 18mW; modulation amplitude 12 gauss; scanning rate 1000 gauss/min; response time 0.3 sec; temperature -180°C. Field increases from left to right.

Fig.2 shows that the $g = 1.94$ signal is remarkably temperature sensitive, broadening out rapidly with increasing temperature and disappearing above -120°C. It is also seen that decreasing the temperature from-180° to -188°C resulted in a partial splitting of the spectrum. The signifiaance of this is unknown at present but is being further investigated. The $g = 4.27$ signal of the oxidized ferredoxin was much less temperature sensitive and was still easily discernible at -80°C.

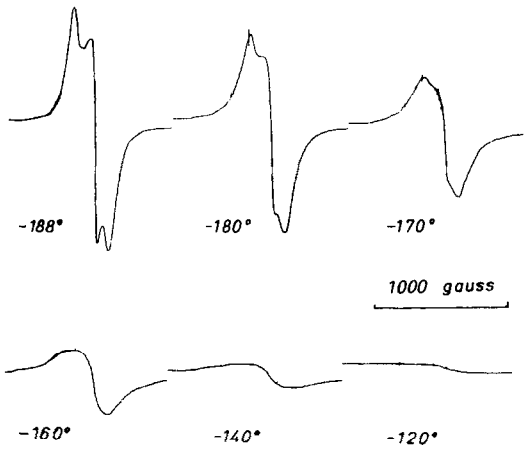

Fig.2. Variation of the $g = 1.94$ signal with temperature. ESR conditions as for Fig.1, except temperature as indicated.

In order quantitatively to estimate the amount of iron associated with each signal we numerically integrated the spectra by the method of Wyard (1965). We find that the intensities of the signals vary with different preparations and we are therefore unable to reach any definite conclusion about the relationship between the 4.27 and 1.94 signals.

Since it is well known that high-spin ferric ions in an octahedral or tetrahedral site give ESR spectra with $g = 2$, we would like to suggest that the reduced ferredoxin spectrum arises from high spin ferric ions in such an environment. Additional support comes from the observation that the non-haem iron compound rubredoxin gives a similar spectrum when oxidized but no detectable spectrum when reduced (Atherton, Mayhew and

Peel, personal communication). Significantly rubredoxin contains only one iron atom per molecule which may be presumed to be ferric when oxidized.

Acknowledgments. The authors thank Professor G. Wilkinson and Dr. R. C. Bray for providing facilities and for discussions and Mrs. S. Druery for technical assistance.

REFERENCES

Arnon, D.I. (1965), Science, 149, 1460.
Beinert, H. & Palmer, G. (1965), Adv.Enzymology, 27, 105.
Hill, R. & Bendall, F. (1960), Nature, 187, 417.
Horio, T. & San Pietro, A. (1964), Proc.Natl.Acad.Sci.U.S., 51, 1226.
Lovenberg,W., Buchanan,B.B. & Rabinowitz,J.C.(1963), J.Biol.Chem.,238,3899.
San Pietro,A. & Black,C.C. (1965), Ann.Rev.Plant Physiol., 16, 155.
Sobel,B.E. & Lovenberg,W. (1966), Biochemistry, 5, 6.
Tagawa,K. & Arnon,D.I. (1962), Nature, 195, 537.
Whatley,F.R., Tagawa,K. & Arnon,D.I.(1963), Proc.Natl.Acad.Sci.U.S.,49,266.
Wyard,S.J. (1965), J.Sci.Instr., 42, 769.