IDENTIFICATION OF A g = 1.90 HIGH-POTENTIAL IRON-SULFUR PROTEIN IN CHLOROPLASTS

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Summary: A new bound iron-sulfur protein has been identified in spinach chloroplasts. In the reduced form, this protein has an electron paramagnetic resonance spectrum at 20°K with g-values of 2.02 and 1.90. The midpoint oxidation-reduction potential (E_m) of the protein, which is pH-independent, is +290 mV. These properties are similar to those of the "Rieske" g = 1.90 iron-sulfur protein of mitochondrial Complex III.

The presence in chloroplasts of bound iron-sulfur proteins that can be distinguished from soluble ferredoxin was first indicated in electron paramagnetic resonance (EPR) studies by Malkin and Bearden (1). These bound iron-sulfur proteins have been extensively characterized *in situ* (2-10), and it has been proposed that one, which undergoes low-temperature photoreduction, is the primary electron acceptor of Photosystem I (1,2,6-9).

In this communication we report the presence in chloroplasts of another bound iron-sulfur protein with EPR and oxidation-reduction properties significantly different from those of the previously detected bound chloroplast iron-sulfur proteins. The properties of this protein are similar to the properties of the "Rieske" g = 1.90 protein, first identified in Complex III of the mitochondrion (11,12).

Materials and Methods

Whole chloroplasts and washed, broken chloroplast fragments were prepared from spinach as previously described (13,14) and were suspended in either 50 mM Tricine buffer (pH 8.0) + 20 mM NaCl + 1 mM EDTA (pH 8.0) or the buffers indicated in the legend to Fig. 2. Digitonin Photosystem I chloroplast fragments (D-144) were prepared by the procedure of Anderson and Boardman (15).

EPR spectra at 20°K were recorded with a modified JEOL X-band spectro-

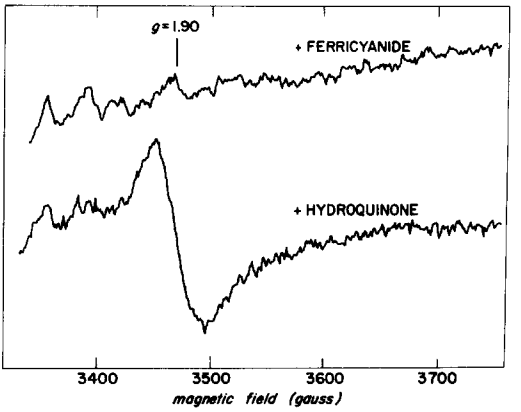

Fig. 1. Presence of a g = 1.90 iron-sulfur protein in chloroplasts. Washed, broken spinach chloroplasts (chlorophyll concentration, 2 mM) in 20 mM Tricine buffer (pH 8.0) + 20 mM NaCl + 1 mM EDTA (pH 8.0) were incubated with 0.5 mM potassium ferricyanide prior to freezing to 77°K. After the EPR spectrum was recorded at 20°K, the sample was thawed and reduced with hydroquinone (5 mM) and the spectrum was again recorded (at 20°K). EPR instrument settings: frequency, 9.2 GHz; microwave power, 10 mW; modulation amplitude, 10 G.

meter as previously described (7,14).

Results and Discussion

When washed, broken chloroplasts are treated with the oxidant potassium ferricyanide, no EPR signal is present in the g = 1.90 region (Fig. 1). However, subsequent reduction of the chloroplast sample with hydroquinone results in the appearance of an EPR signal centered at g = 1.90. With some preparations, it was also possible to observe an additional resonance line at g = 2.02 which accompanied hydroquinone reduction but, in general, the low-field line was difficult to detect because of an interfering Cu^{+2} signal in this spectral region. The EPR spectrum of the chloroplast protein (g-values of 2.02 and 1.90 on reduction) is similar to that of the "Rieske" g = 1.90 iron-sulfur protein in mitochondrial Complex III (11,12,16,17) and, on this basis, we would suggest that chloroplasts contain a similar iron-sulfur protein.

The g = 1.90 iron-sulfur protein is firmly bound to the chloroplast lamellae, as evidenced by the observation that repeated washing of the fragments had no effect on the intensity of the g = 1.90 signal. The g = 1.90

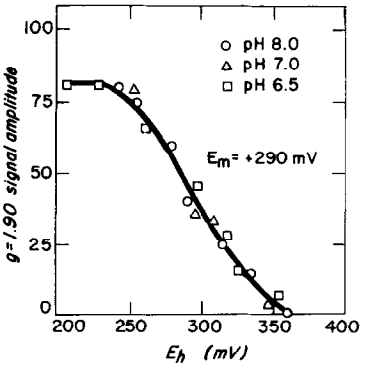

Fig. 2. Oxidation-reduction potential of the g = 1.90 iron-sulfur protein *in situ*. Washed, broken chloroplasts (chlorophyll concentration, 2 mM) were suspended in either 50 mM Tricine buffer (pH 8.0), 50 mM potassium phosphate buffer (pH 7.0), or 50 mM Hepes buffer (pH 6.5) + 20 mM NaCl + 1 mM EDTA (pH 8.0). The oxidation-reduction potential was monitored in a cell similar to that used by Dutton (22). After the oxidation-reduction potential was adjusted with ferricyanide to +400 mV it was lowered by the addition of aliquots of a hydroquinone solution and samples were taken at the desired potentials. Samples were frozen to 77°K prior to the recording of the EPR spectra at 20°K. EPR settings as in Fig. 1.

signal was also found in Photosystem I chloroplast fragments prepared by digitonin treatment. This latter finding suggests a possible role for the iron-sulfur protein in cyclic electron-transfer reactions associated with Photosystem I.

The optimum temperature for detection of the g = 1.90 signal was between 15°K and 25°K. At higher temperature (40°K), the signal was considerably broadened, as it is from other iron-sulfur proteins (18).

The oxidation-reduction potential of the g = 1.90 iron-sulfur protein in untreated chloroplasts is +290 mV (n = 1) (Fig. 2). This midpoint potential is pH-independent in the pH range from 6.5 to 8.0. The midpoint oxidation-reduction potential of the chloroplast g = 1.90 iron-sulfur protein is similar to that reported for the "Rieske" g = 1.90 protein in Complex III of the mitochondrion, the latter having a reported value of +280 mV (17,19). In addition, the EPR signal of an iron-sulfur protein with similar oxidation-reduction properties has recently been observed in *Chromatium* chromatophores (20,21) and

in chromatophores of several purple iron-sulfur bacteria (17; R. C. Prince and P. L. Dutton, personal communication).

The present findings, taken in conjunction with these previous reports, suggest that the *in situ* properties of the $g = 1.90$ iron-sulfur protein are similar in a large number of diverse membrane-bound energy-transducing systems.

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