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PROPERTIES OF THE LOW-TEMPERATURE PHOTOSYSTEM I PRIMARY REACTION IN THE *P*-700-CHLOROPHYLL *a*-PROTEIN

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Summary

The Photosystem I primary reaction, as measured by electron paramagnetic resonance changes of *P*-700 and a bound iron-sulfur center, has been studied at 15°K in *P*-700-chlorophyll *a*-protein complexes isolated from a blue-green alga. One complex, prepared with sodium dodecyl sulfate shows *P*-700 photooxidation only at 300°K, whereas a second complex, prepared with Triton X-100, is photochemically active at 15°K as well as at 300°K. Analysis of these two preparations shows that the absence of low-temperature photoactivity in the sodium dodecyl sulfate complex reflects a lack of bound iron-sulfur centers in this preparation and supports the assignment of an iron-sulfur center as the primary electron acceptor of Photosystem I.

Fundamental to the understanding of the primary photochemical event in photosynthesis is the isolation and characterization of photosynthetic membrane components that are highly enriched in the primary reactants. Several such Photosystem I preparations are now available [1—4], allowing detailed examination of the primary reaction of this photosystem (for reviews, see refs. 5 and 6).

The functional heart of Photosystem I has been termed the *P*-700-chlorophyll *a*-protein complex [6]. A complex that is photochemically active at 300°K can be isolated after treatment of membrane fragments from some procaryotic blue-green algae with either sodium dodecyl sulfate or Triton X-100 [2,7]. These preparations both contain one *P*-700 per 40 chlorophyll *a* molecules, but they show differences in efficiency of *P*-700 photooxidation. Specifically, the kinetics are slower and the quantum requirement is higher in the sodium dodecyl sulfate-complex than in the Triton complex (unpublished observations; however, see ref. 2).

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In eucaryotic organisms it has been shown that treatment of chloroplast membranes with Triton X-100 followed by chromatography yields a *P*-700-chlorophyll *a*-protein complex that contains one *P*-700 per 40 chlorophyll *a* molecules, is photochemically active at 300°K [2,7], and shows the same quantum requirement for *P*-700 photooxidation as does the procaryotic Triton X-100 complex. However, sodium dodecyl sulfate treatment destroys the *P*-700 activity at 300°K of the eucaryotic complex [2].

The purpose of the present study was to investigate the causes of these observed differences in photochemical properties of the blue-green algal *P*-700-chlorophyll *a*-protein complexes. In particular, we questioned whether these differences relate in any way to alterations in the primary reactants of Photosystem I. These studies were carried out by examination of reactions at 15°K of *P*-700 and a bound iron-sulfur center, the latter having been proposed as the primary electron acceptor of this photosystem [5,8,9]. Our results indicate that the difference observed in photochemical efficiency of the sodium dodecyl sulfate-treated and Triton X-100 treated complexes can be ascribed to the absence of a bound iron-sulfur protein in the primary electron acceptor complex of the sodium dodecyl sulfate preparation.

The *P*-700-chlorophyll *a*-protein complex was isolated from *Phoridium luridium* var. olivaceae by two different procedures: Lamellae were solubilized with sodium dodecyl sulfate and the complex (hereafter called the sodium dodecyl sulfate complex) was isolated by the procedure of Dietrich and Thornber [1], or the lamellae were solubilized in Triton X-100 and the complex (hereafter called the Triton complex) was isolated by the method of Shiozawa et al. [2].

After the addition of sodium ascorbate and methyl viologen, the room temperature reversible light-induced absorbance changes of *P*-700 were measured in both preparations [2]. Electron paramagnetic resonance studies were carried out at 15°K in a modified JEOL X-band spectrometer equipped with an Air Products LTD-3-100 cryostat [8,10]. Samples were illuminated in the EPR cavity with wide-band red light (Corning 2-64 filter). Pertinent data were stored in an online PDP-8 computer, as previously described [8,10].

The sodium dodecyl sulfate and Triton complexes of *Phormidium* showed identical spectral properties (cf. ref. 1). The presence of *P*-700 in both preparations was demonstrated spectrophotometrically by light-induced absorbance changes at 300°K. Both preparations contain one *P*-700 per 40 chlorophyll *a* molecules.

Photooxidation of *P*-700 at 15°K in the Triton complex was evidenced by the production of an EPR free-radical signal with a *g* value of 2.0026 Fig. 1a). Photoreduction of a bound iron-sulfur center with *g* values of 2.05, 1.94, and 1.86 (Fig. 1b) accompanies this photooxidation. These light-induced EPR signals are identical to those observed in Photosystem I preparations from higher plants [4,8,9,11,12]; however, the linewidth of the $g = 1.94$ signal is broader in the blue-green algal Triton complex than in other preparations (see, for example, ref. 8). It is suggested that this broadening may reflect an alteration in the environment of the iron-sulfur center in this complex relative to its environment in less purified materials.

In contrast to observations with the Triton complex, no *P*-700 photo-

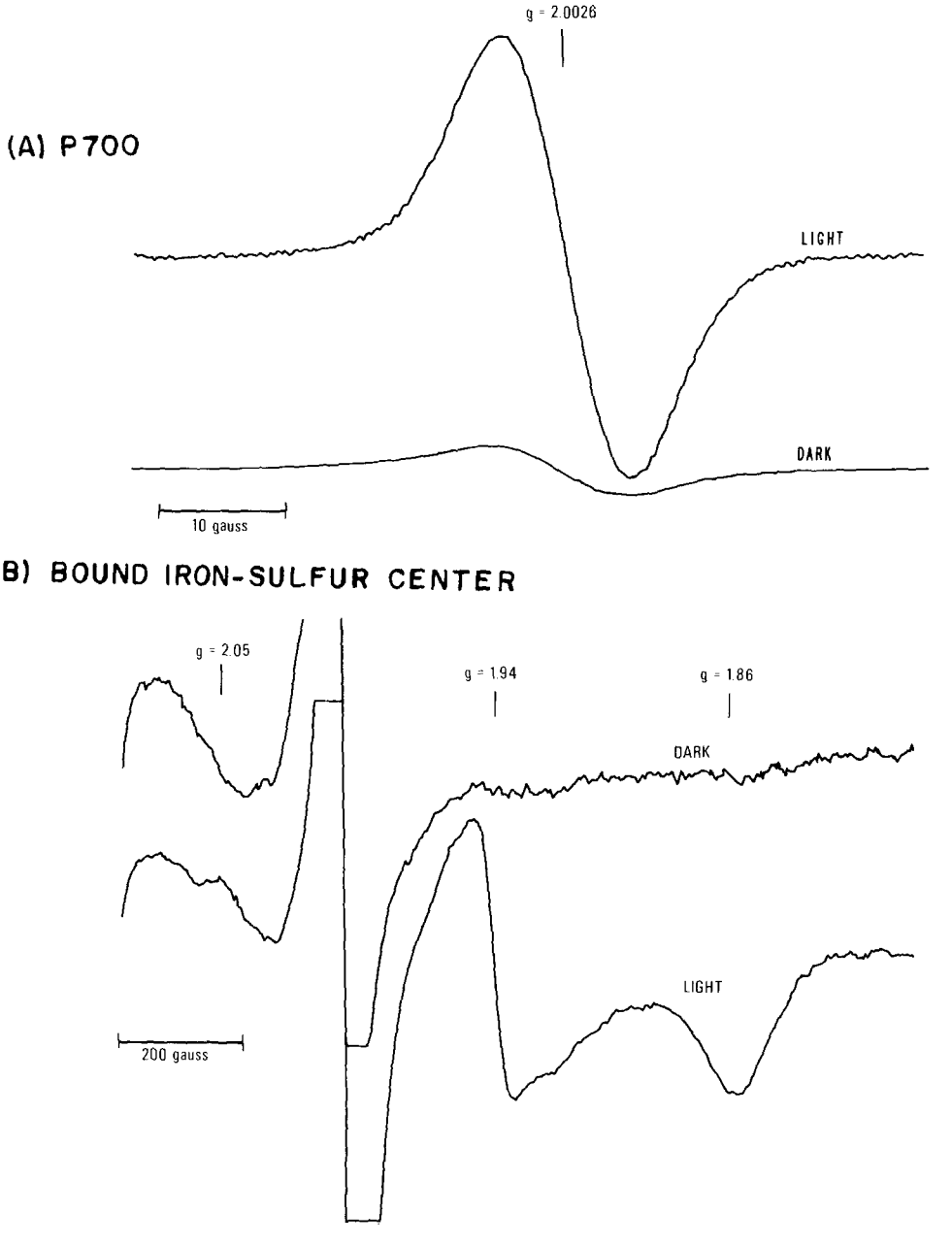

Fig.1. Photochemical reactions at 15°K in the Triton X-100 *P*-700-chlorophyll *a*-protein complex. The reaction mixture contained the chlorophyll-protein complex (chlorophyll, 0.25 mg/ml), sodium ascorbate (10 mM), and phenazine methosulfate (10 μM). Spectra were recorded in the dark and then the sample was illuminated at 15°K for 30 s. In (A) the EPR conditions were: Frequency, 9.22 GHz; microwave power, 1.0 mW; modulation amplitude, 2.3 G; amplifier gain, 40. In (B), the EPR conditions were: Microwave power, 10 mW; modulation amplitude, 10 G; amplifier gain, 400.

oxidation was observed in the sodium dodecyl sulfate complex at 15°K (Fig. 2a). Furthermore, no photoreduction of a bound iron-sulfur center was observed at 15°K in this preparation (Fig. 2b). A similar result was obtained when the EPR signal was monitored while the sample was being illuminated at 15°K.

An additional difference between the sodium dodecyl sulfate and Triton complexes was observed when the iron-sulfur centers were examined after chemical reduction. As shown in Fig. 3, the addition of sodium dithionite (in the presence of methyl viologen) resulted in the reduction of two iron-sulfur centers in the Triton complex (*g* values of 2.05, 1.94, and 1.86 and

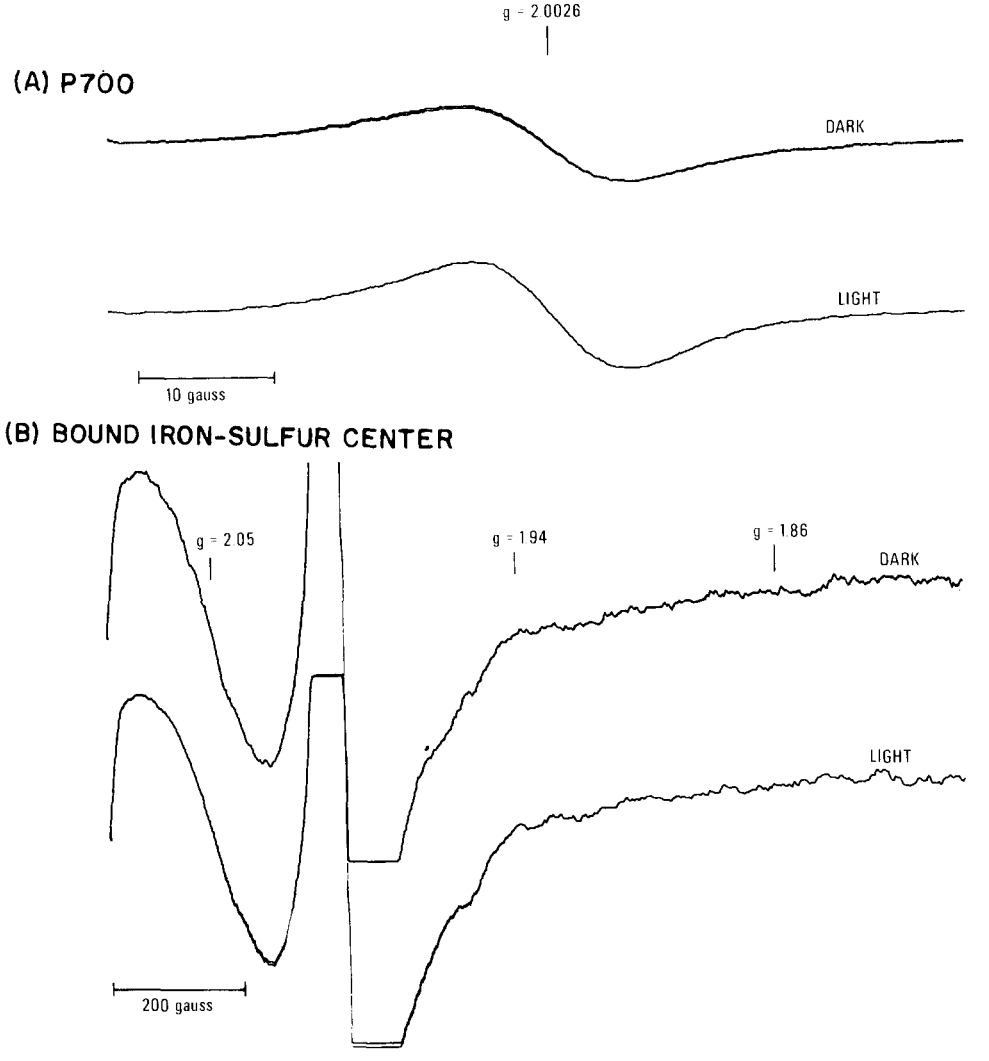

Fig.2. Photochemical reactions at 15°K in the sodium dodecyl sulfate *P*-700-chlorophyll *a*-protein complex from a blue-green alga. The reaction mixture was as in Fig. 1 except that the sodium dodecyl sulfate complex was present. EPR conditions for (A) and (B) were as in Fig. 1.

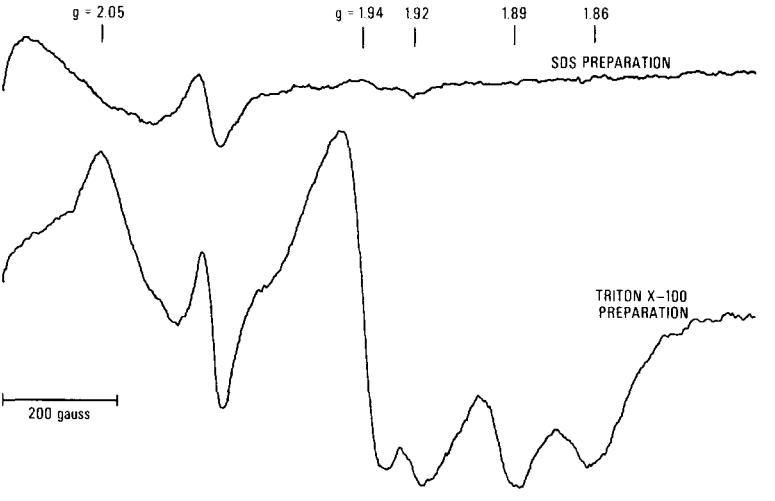

Fig.3. Chemical reduction of bound iron-sulfur centers in the sodium dodecyl sulfate (SDS) and Triton *P*-700-chlorophyll *a*-protein complexes. The reaction mixture contained either complex at a chlorophyll concentration of 0.25 mg/ml, 100 mM glycine buffer (pH 9.5), methyl viologen (0.1 mM), and sodium dithionite (2 mM). Dithionite was added under anaerobic conditions under a stream of nitrogen gas. EPR conditions were as in Figs. 1B and 2B except that an amplifier gain of 320 was used.

2.05, 1.92, and 1.89 (see refs. 13,14)) but no iron-sulfur center(s) was reduced in the sodium dodecyl sulfate complex under similar conditions.

The photochemical properties of the two preparations in this study differ dramatically at liquid-helium temperature (15°K). Only the Triton complex is able at 15°K to photooxidize *P*-700 and concomitantly to photo reduce a bound iron-sulfur center, although both the sodium dodecyl sulfate and the Triton complex are able to photooxidize *P*-700 at 300°K.

Previous evidence from several laboratories [8,9,12,15] has led to the proposal that an iron-sulfur center functions as the primary electron acceptor of Photosystem I. According to this view, the removal or alteration of this iron-sulfur center would eliminate the primary reaction of Photosystem I at 15°K. We believe that the absence of this bound iron-sulfur center in the sodium dodecyl sulfate complex explains not only the loss of photochemical activity at liquid-helium temperature but also explains the lower quantum efficiency of the photochemical event at room temperature. The activity of the sodium dodecyl sulfate complex at room temperature almost certainly arises from diffusion to the electron acceptor site of an alternate, non-physiological electron acceptor, such as oxygen or methyl viologen, which would make possible the photooxidation of *P*-700. This diffusion process would not be expected to occur at 15°K. The results of these experiments show that the low-temperature photoreaction of Photosystem I in these detergent treated complexes is a more sensitive indicator of the integrity of the total organization of the complex than is the reaction of the components at room temperature.

It was not possible to test the involvement in the Photosystem I reaction of a newly observed EPR component (g = 1.76) which has also been proposed as the primary electron acceptor of Photosystem I [16,17] because of the

low concentrations of chlorophyll available in our study.

Studies similar to those reported here cannot be performed unequivocally on preparations from eucaryotic organisms because it has been shown [2] that the addition of sodium dodecyl sulfate to chloroplast lamellae or to the Triton complex from higher plants destroys *P*-700. The recent studies of Nelson et al. [18] indicated that the bound iron-sulfur centers are destroyed in such sodium dodecyl sulfate-treated eucaryotic material as the Photosystem I preparation (chlorophyll:*P*-700 = 100:1) of Bengis and Nelson [3].

We believe that the sodium dodecyl sulfate and Triton complexes from blue-green algae used in our study, as well as the Triton complex from higher plants, are well suited for further characterization of the Photosystem I primary photochemical event because they are easily prepared, well-defined, and highly enriched in the reaction-center components. Furthermore, because the two blue-green algal preparations show critical differences in photochemical activities, further comparison of the properties of these two complexes, particularly of their polypeptide composition, should lead to a more detailed characterization of the reaction center of Photosystem I.

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