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Photosystem I charge separation in the absence of centers A and B. I. Optical characterization of center 'A_2' and evidence for its association with a 64-kDa peptide

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The flash-induced absorption transient at 698 nm in a Photosystem I subchloroplast particle showed the following characteristics after addition of 0.25–2.0% lithium dodecyl sulfate (LDS). (i) The 30-ms transient corresponding to the P-700$^+$ P-430$^-$ backreaction was replaced by a 1.2-ms transient. (ii) The amplitude of the transient did not change immediately after LDS addition, but decayed with a half-life of 10 min at pH 8.5. (iii) Methyl viologen had no effect on the magnitude or kinetics of the transient, indicating that it cannot accept an electron from this component. (iv) The difference spectrum of the transient from 400 nm to 500 nm was characteristic of an iron-sulfur protein. (v) The transient followed first-order Arrhenius behavior between 298 K and 225 K with an activation energy of 13.3 kJ/mol; between 225 K and 77 K, the 85-ms half-time remained temperature-invariant. These properties suggest that the LDS-induced absorption transient corresponds to the P-700$^+$ A_2^- change recombination seen in the absence of a reduced electron-acceptor system. In the presence of LDS, the reaction-center complex was dissociated, allowing removal of the smaller peptides from the 64-kDa P-700-containing protein. With prolonged incubation, the iron-sulfur clusters were destroyed through conversion of the labile sulfide to zero-valence sulfur. About 35% of the zero-valence sulfur was found associated with the 64-kDa protein under conditions that allowed separation of the small peptides. We interpret the long lifetime of the P-700$^+$ A_2^- transient after LDS addition and the association of zero-valence sulfur with a 64-kDa protein to indicate that A_2 is closely associated with, and perhaps integral with, the P-700-containing protein.

Introduction

The Photosystem I reaction center complex has been isolated in a number of different laboratories [1–5], but a rigorous determination of the polypeptide composition only became possible upon the purification of a Photosystem I complex free of cytochrome and coupling factor [6–10]. These complexes contain one or two large polypeptides of approx. 70-kDa (range 54–100 kDa), two moderate molecular weight peptides of 20 kDa (range 18.3–20 kDa) and 18 kDa (range 15–18 kDa), and usually two or more smaller peptides in the 10–13-kDa range.

The 64-kDa peptide has been identified as the P-700-containing protein [11] although there is still uncertainty as to the number of peptides observed in this region [11–13] and the number of copies of 64-kDa protein per P-700 [14]. The peptides corresponding to the bound iron-sulfur proteins, how-

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Abbreviations: LDS, lithium dodecyl sulfate; DCIP, 2,6-dichlorophenolindophenol; DMPD, *N,N'*-dimethyl-*p*-phenylenediamine; Temed, *N,N,N',N'*-tetramethylethylenediamine; P-700, Photosystem I reaction center chlorophyll.

ever, are as yet unassigned. A few attempts have been made at their identification; all are indirect and therefore subject to interpretation. Møller and colleagues [8] found, through incorporation experiments with radioactive iron, that radiolabel was associated with the 15.2-kDa and 18.3-kDa bands in a Photosystem I particle isolated from barley. Malkin and colleagues [17], using chaotropic agents and ESR spectroscopy, obtained evidence for the presence of centers A and B on the 19-kDa peptide. Guikema and Sherman [15], on the other hand, found radiolabel only in the low molecular weight bands (10 and 11.5 kDa) in a similar particle isolated from *Anacystis nidulans*. Lagoutte et al. [16] attempted to locate a bound iron-sulfur protein by in vivo ^{35}S labeling and carboxymethylation with [^{14}C]iodoactate. Even though the band was barely visible by staining with Coomassie brilliant blue, an apparent 8-kDa peptide became heavily labeled in a Photosystem I particle.

In this paper, we investigated the properties of P-700 and its reaction partner after treatment of a Photosystem I particle with 1% LDS. We provide spectroscopic and biochemical evidence to assign bound iron-sulfur center A_2 to the 64-kDa protein in Photosystem I.

Materials and Methods

Tris, Triton X-100, ascorbic acid, DCIP, dithiothreitol, DMPD, and zinc acetate were purchased from Sigma. LDS, acrylamide and methylbisacrylamide were obtained from British Drug Houses. SDS, Temed, glycine and ammonium persulfate were purchased from BioRad, and molecular-weight standards were from Pharmacia. All other chemicals were purchased from Fisher. Fresh spinach was obtained from a local distributor.

Photosystem I particles were isolated according to a modification of the method of Golbeck [6]. In this procedure, spinach thylakoid fragments were centrifuged for 1 h at $30000 \times g$ following overnight treatment with 1% Triton X-100. The resulting supernatant was dialyzed overnight against Tris buffer (0.025 M, pH 8.3) and concentrated 4-fold on an Amicon YM-100 membrane. The concentrated supernatant was loaded onto a 0.1–1.0 M sucrose gradient containing Tris buffer (0.025 M, pH 8.3) in the absence of detergent and centrifuged at $55000 \times g$ for 15 h. Following centrifugation, the gradient was examined by ultraviolet light and the lower non-fluorescent band was removed and dialyzed overnight against Tris buffer (0.025 M, pH 8.3). The dialyzate was concentrated 8-fold and loaded onto a second 0.1–1.0 M sucrose gradient and centrifuged for 12 h at $82500 \times g$. The lower non-fluorescent band was removed and dialyzed overnight against Tris buffer (0.025 M, pH 8.5).

The 64-kDa polypeptide was isolated according to the following procedure. The Photosystem I particles were brought to a chlorophyll concentration of 200 μg/ml, solid LDS was added to a final concentration of 1%, and the solution was stirred for 10 min at 0°C. The sample was loaded onto a 0.1–1 M sucrose gradient containing Tris buffer (0.025 M, pH 8.3) in the absence of detergent and centrifuged for 18 h at $82500 \times g$. The lower, non-fluorescent band was removed and dialyzed overnight against Tris buffer (0.025 M, pH 8.5).

Following dialysis, the particles were analyzed for chlorophyll [18], P-700 content [19], sulfide and zero-valance sulfur [16,20] and protein composition by SDS polyacrylamide gel electrophoresis [21]. Gels were silver stained using the method of Wray et al. [22]. Absorption spectra were measured with a Cary 14 spectrophotometer.

Flash-induced absorption changes were measured with a single beam spectrophotometer constructed in-house. The measuring beam was isolated from a 250 W tungsten-halogen lamp with a monochromator (Jarrell Ash) and detected with a PIN-10D photodiode (United Detector Technology). A 2-64 (Corning) blocking filter was introduced into the measuring beam in the blue region to filter second harmonic radiation. The signal was amplified with a Model 113 preamplifier (EG&G PARC) and the gain was adjusted to provide a constant voltage (typically 5.00 V) at each wavelength. After passing through a sample-and-hold circuit (designed to null the DC voltage level electrically), the signal, which now consists of only the flash-induced absorption transient, was amplified by a second Model 113 preamplifier and captured by a Model 8100 transient recorder (Biomation). A monochromator (Jarrell Ash) was placed after the sample cuvette to filter stray light and the flash

artifact. A shutter was placed immediately before the sample cuvette to prevent the measuring light from affecting the sample before measurement. Actinic flashes ($\lambda_{max} = 645$ nm) were provided by a flashlamp-pumped dye laser (Phase-R DL 1200) containing sulforhodamine 101 dye (Eastman Chemicals, Laser Grade). The flash pulse width was 0.35 μs (FWHM) at a pulse energy of 0.15 J. A microcomputer (SYM-1) controlled the sequence of events during the measurement, transferred to memory the contents of the transient recorder, and averaged the signals on completion of the appropriate number of flashes.

Flash photolysis was carried out on a 3-ml fluorescence cuvette containing photosystem I particles at 2 or 20 μg/ml chlorophyll in the buffer specified in the text. Ascorbic acid (1.7 mM) and DCIP (33 μM) were added to the sample 2 min prior to measurement; the concentrations were chosen to permit complete rereduction of P-700$^+$ in less than 10 s. Methyl viologen (33 μM) was added where specified. LDS was added from a 10% stock solution within 10 s of measurement. Unless otherwise noted, each trace is the result of a single flash. Low temperature experiments were performed in a specially constructed dewar with boiling liquid nitrogen as refrigerant. Temperature was monitored with a chrome-alumel thermocouple and an Omega Engineering 0° reference junction. The absorption transient at each temperature was the average of four flashes, spaced 10 s apart; the same sample was used for 15 determinations at various temperatures without noticeable loss of activity. The experiment was performed four times on separate, but otherwise identical samples to ensure reproducibility.

Results

Effect of LDS on the 698 nm absorption transient

In the absence of an external electron acceptor such as methyl viologen, the rereduction of P-700$^+$ in a Photosystem I particle is a competition between the backreaction from P-430$^-$ and the forward reaction from external electron donors. If the concentration of donor is kept low, the absorption change at 698 nm will show the bleaching of P-700 followed by a 30-ms transient which represents the P-700$^+$ P-430$^-$ backreaction (Fig. 1a). If methyl viologen is added to the sample to accept the electron from P-430$^-$, the transient should reflect only the reduction of P-700$^+$ from the external electron donor. In certain samples, however, we have noticed a short-lived (≈ 1 ms) absorption transient of relatively low amplitude superimposed on the slow P-700$^+$ reduction transient (see Ref. 23). We recently found that 1.0% LDS is very effective at producing the short-lived transient; as soon as the surfactant is added, the 30-ms tran-

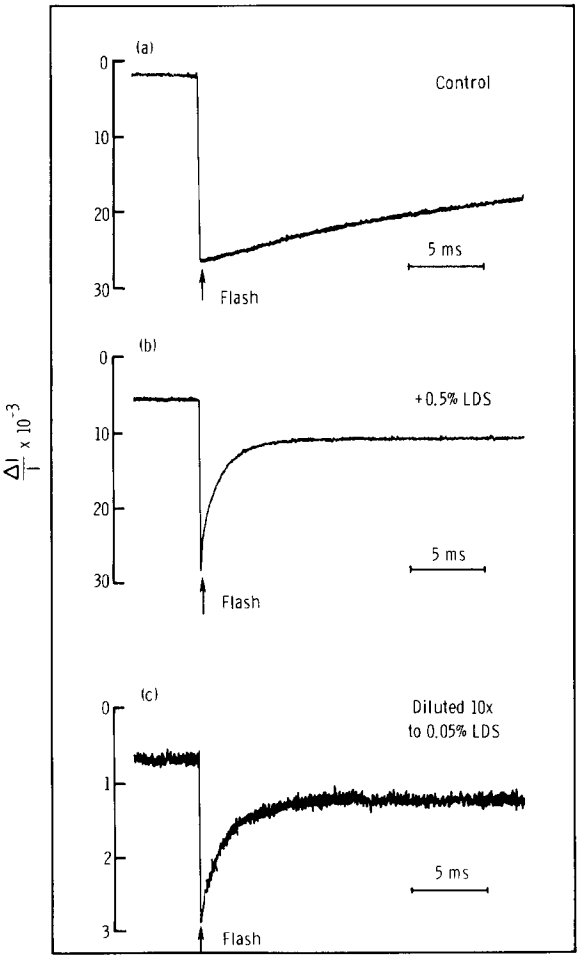

Fig. 1. Kinetic properties of the 1.2-ms transient after addition of 0.5% LDS and 10-fold dilution. (a) Control particle in Tris buffer (0.05 M, pH 8.5) containing 1.7 mM ascorbic acid and 0.033 mM DCIP; (b) same as (a), except after 30-s incubation with 0.05% LDS; and (c) same as (b), except after 30-s incubation with 0.5% LDS and 10-fold dilution to 0.05% LDS with Tris buffer (0.05 M, pH 8.5). The chlorophyll concentration was 20 μg/ml.

sient is replaced by a 1.2-ms transient (Fig. 1b). The effect occurs at chlorophyll concentrations up to 800 μg/ml, indicating that the detergent-to-chlorophyll ratio is not critical. However, the detergent concentration must be kept above a minimum level; at 20 μg/ml chlorophyll and at pH 8.3, the lowest effective concentration of LDS that produces the effect is 0.25%. A room-temperature transient of this lifetime has not been previously reported in Photosystem I; its half time does not correspond to P-430 or any known electron acceptor. The next available acceptor, A_2, becomes evident only when P-430 is reduced with dithionite at high pH values. Under these conditions, the absorption change at 698 nm has the same magnitude as the control followed by a 250-μs transient which represents the $P\text{-}700^+ A_2^-$ backreaction (not shown).

The absorption change in the LDS-treated sample shows two recovery phases: a fast phase that typically accounts for between 60 and 80% of the absorption change and a slow phase that accounts for the remainder (Fig. 1b). When the logarithm of the fast phase of the absorption change is plotted against time, the decay is found to be monotonic with a half-time of 1.2 ms (not shown). Identical kinetics are seen at 820 nm, 430 nm and 698 nm. Methyl viologen affects neither the amplitude nor kinetics of the fast phase, indicating that it cannot accept an electron from this component. The kinetics of the fast phase are completely insensitive to donor (DCIP) concentration. The kinetics of the slow phase are much slower than the $P\text{-}700^+$ $P\text{-}430^-$ backreaction kinetics (Fig. 1a and b), implying that this phase does not correspond to a population of native reaction centers that remain resistant to LDS. The kinetics of the slow phase are insensitive to methyl viologen, but are sensitive to donor (DCIP) concentration. The ratio of the fast-to-slow phase is independent of the intensity of measuring beam and laser flash as well as flash number (not shown).

If the flash photolysis is performed immediately after the addition of LDS, the transient retains the same amplitude as the control (Fig. 1a and b). There are, however, small differences in the light saturation characteristics of the two samples; the control particle saturates between 65 and 75% of the intensity needed to saturate the LDS-treated sample. We interpret this difference to be due to the solubilization of a small number of antenna chlorophyll molecules associated with the reaction center. Since the amplitude of the 1.2-ms transient is the same in the control and LDS-treated samples at high intensity flashes, the reaction center chlorophyll, P-700, is not destroyed by LDS treatment.

Stability of the LDS-induced absorption transient

The short-lived, 1.2-ms transient is unstable and begins to decay almost immediately, showing a half-time of about 10 min at pH 8.5 and 15 min at pH 7.5 (Fig. 2a). Fig. 2b shows the amplitude of the 1.2-ms transient 2.5 min after the addition of LDS at various pH values. The region of greatest stability lies between pH 7.0 and pH 9.0; above and below these values, the lifetime correspondingly declines. Although not shown, the fast phase decays at a slightly faster rate than the slow phase. At pH 7.5, and after a 5-min incubation period, the fast phase accounts for 75% of the absorbance change; at 45 min, where 79% of the transient has decayed, the fast phase accounts for 58% of the absorbance change. There are no noticeable differences in the rates of decay of either phase at concentrations of 0.5% LDS and 2% LDS.

As indicated earlier, there appears to be a

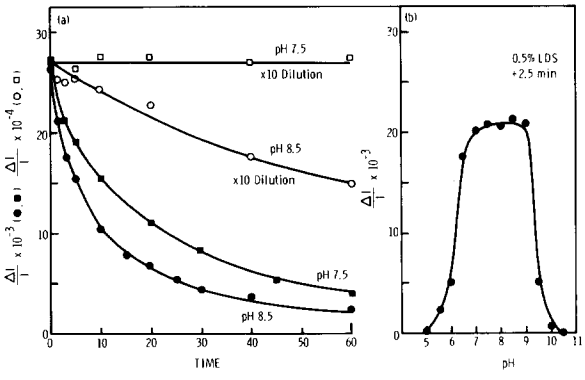

Fig. 2. Amplitude of 1.2-ms transient as a function of time of incubation. (a) Time course at pH values of 7.5 and 8.5 (0.05 M Tris buffer) in 0.5% LDS (closed points) and after 10-s incubation of pH 7.5 and 8.5 followed by dilution in Tris buffer (0.05 M) to 0.05% LDS, and (b) magnitude of the 1.2-ms transient after 2.5 min incubation with 0.5% LDS at specified pH values. The samples were incubated in buffer at the specified pH value and introduced into the sample cuvette or into the dilution buffer containing 1.7 mM ascorbic acid and 0.033 mM DCIP.

threshold value of LDS below which the replacement of the 30-ms transient by the 1.2-ms transient does not take place. Since the transition is not reversible on dilution (Fig. 1c), the decay should slow or stop if the particles are treated initially with 0.5% LDS and diluted below 0.12% LDS. As shown in Fig. 2a, the 1.2-ms component is stable for at least 60 min in a particle treated in this manner at pH 7.5. However, at pH 8.5, the decay shows a half-time of about 90 min. The retention of the 1.2-ms transient after dilution indicates that the generation of the component is completely independent of its decay. Although the latter can be partially controlled by diluting the surfactant below a minimum effective concentration, we have not been able to stop the decay completely. Passage of a sample treated for 1 min with 0.5% LDS over a size exclusion column (P-4) resulted in the loss of 40–50% of the 1.2-ms signal after 12 h storage at 4°C (not shown).

Spectral characteristics of the LDS-induced component

The relative stability of the 1.2-ms transient at pH 7.5 and its insensitivity to methyl viologen allowed an accurate point-by-point difference spectrum to be obtained. The experimental protocol, chosen to minimize error due to sample handling, involved flash photolysis of (i) a sample containing only ascorbate and DCIP as electron donors, (ii) the sample containing 30 μM methyl viologen, and (iii) the same sample containing both methyl viologen and 0.5% LDS. The difference between the first two sets of data corresponds to the spectrum of P-430; that between the second two sets of data, to the spectrum of the 1.2-ms transient. The P-430 spectrum, taken to check experimental conditions appeared as in Ref. 24. Fig. 3 shows the difference spectrum of the fast phase of the 1.2-ms transient as well as the difference spectrum of P-700. The precision of the measurement was somewhat wavelength-dependent, as shown by the error bars in Fig. 3. The broad spectral bands in the difference spectrum at 430 and 465 nm are characteristic of an iron-sulfur protein.

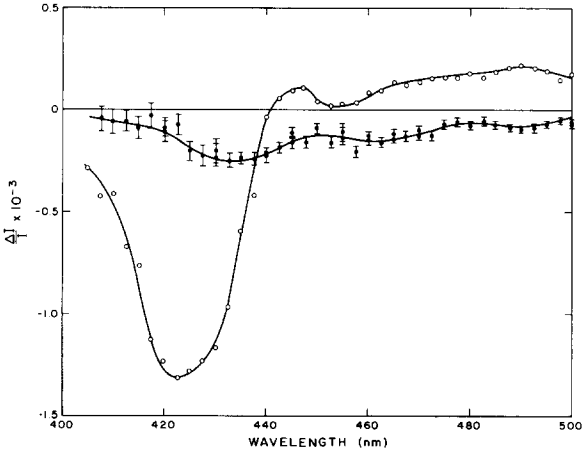

Fig. 3.Difference spectrum of P-700 and the 1.2-ms absorption transient. The former (open circles) obtained directly from a sample containing 1.7 mM ascorbate, 0.033 mM DCIP and 0.033 mM methyl viologen, and the latter (closed circles) obtained by subtracting the P-700 difference spectrum from a sample containing the above cofactors plus 0.5% LDS. The P-700 data were obtained from the average of four flashes, and the 1.2-ms transient data from an average of two flashes, each spaced 10 s apart. A fresh sample was used at each wavelength.

Temperature dependence on the lifetime of the LDS-induced transient

The effect of temperature on the fast phase of the flash-induced absorption transient is illustrated in Fig. 4. In the presence of 65% glycerol and after the addition of 1% LDS, the decay of the fast kinetic phase showed a half-time of about 2.2 ms at 298 K. Even though glycerol appeared to slow the decay of the absorption transient, it had no effect on the relative distribution of the slow and fast kinetic phases. Between 298 K and 225 K, the decay of the fast phase slowed on cooling from 2.2 ms to about 85 ms, following first-order Arrhenius behavior with an apparent activation energy of 13.3 kJ/mol. Between 225 K and 77 K, the decay kinetics of the fast phase was temperature independent with an invariant half-time of about 85 ms $\pm$ 15 ms.

Changes in polypeptide composition after LDS treatment

The photosystem I particle isolated in a sucrose gradient shows one major band at 64 kDa, which corresponds to the P-700-containing peptide [11], and about 4–6 major bands in the 11–20 kDa region (Fig. 5A). While other Photosystem I particles sometimes show two closely spaced bands in

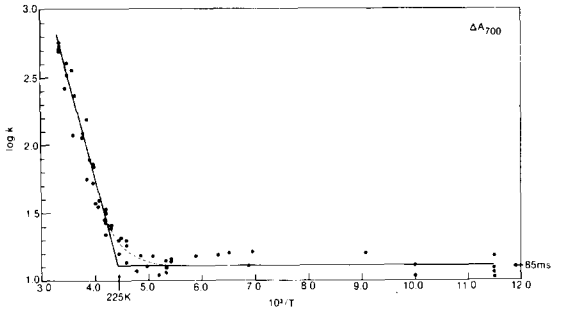

Fig. 4. Arrhenius plot for the LDS–induced absorption transient at 698 nm. The sample was treated at 300 μg/ml chlorophyll with 1% LDS for 1 min, quenched 10-fold with Tris buffer (0.025 M, pH 7.5) containing ascorbate (1.7 mM) and DCIP (33 μM), and diluted to 65% glycerol. The half-time (τ) of the fast exponential phase of the absorption transient was determined by graphical methods and the rate constant ($k = \tau^{-1}$) calculated at each temperature.

the 64-kDa region, we have not observed a doublet in the Photosystem I particle isolated here. The low molecular-weight bands occur at 18.4 kDa,

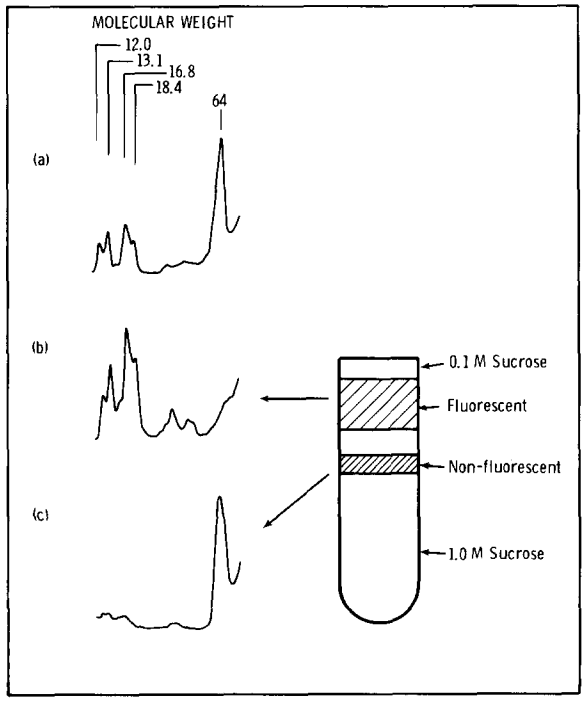

Fig. 5. Polypeptide composition of Photosystem I particle after LDS treatment and sucrose density centrifugation. Inset: chlorophyll zones after 18-fr centrifugation at 30000 × g. The lower, non-fluorescent band contain 90% of the chlorophyll. Main figure: densitometric tracing of electrophoretically separated peptides in the native particle (a), top band (b), and bottom band (c) of the separated zones.

TABLE I

P-700 WAS DETERMINED BY CHEMICAL DIFFERENCE SPECTROSCOPY. THE ABSENCE OF LDS AFTER SUCROSE DENSITY CENTRIFUGATION ALLOWED LABILE SULFIDE (S^{2-}) AND ZERO-VALENCE SULFUR (S^0) TO BE MEASURED AS DESCRIBED IN REF. 20. AVERAGE OF TWO DETERMINATIONS.

Preparation	P-700 (μM)	S^{2-} (μM)	S^0 (μM)	S^0/P-700
Nature Photosystem I	8.62	109.4	–	12.69
Lower 64-kDa zone	8.46	8.0	32.0	3.78

16.8 kDa, 13.1 kDa and 12.0 kDa with minor bands at 30–37 kDa and 8–10 kDa (the latter are not resolved in this gel). When the particle is treated briefly with 1.0% LDS at 0°C and subjected to sucrose density centrifugation, the peptides can be separated into two chlorophyll-containing zones. As shown in Fig. 5b and c, the dark green, non-fluorescent band contains the 64-kDa peptide with only traces of the lower molecular-weight peptides, whereas the light green, fluorescent band contains very little 64-kDa protein, but all of the lower molecular-weight peptides, including the 10- to 12-kDa peptides. Thus, one of the major actions of LDS is to dissociate the reaction center into at least two fractions, one of which contains only the P-700-containing protein and the majority of chlorophyll.

Association of zero-valence sulfur with a 64-kDa peptide

The photosystem I particle isolated by sucrose density centrifugation shows the normal distribution [16] of 12 atoms of labile sulfide per P-700 (Table I). Sakurai et al. [25] have shown that SDS denatures the bound iron-sulfur centers in photosystem I through conversion of labile sulfide (S^{2-}) to zero-valance sulfur (S^0). Zero-valence sulfur is relatively stable in the absence of strong nucleophiles and can serve as a highly specific label for the bound iron-sulfur apoprotein(s). As shown in Table I, the lower centrifugal zone containing the 64-kDa protein shows 0.95 mol of S^{2-} per mol of P-700; after conversion of S^0 to S^{2-}, the same particle shows 3.78 mol of sulfide per mole of P-700. Since the contamination of lower molecular-weight peptides in the lower centrifugal

zone is less than 10%, we conclude that a 64-kDa peptide contains at least one iron-sulfur cluster in the native state.

Discussion

We propose that the following events occur in the Photosystem I reaction center complex immediately after addition of LDS.

(1) the small polypeptides containing P-430 (centers A and B) dissociate from the large 64-kDa peptide(s) which contain P-700 and an iron-sulfur cluster. The experimental separation of these peptides at 0°C in a non-detergent sucrose gradient supports this contention, although strict kinetic correlation is lacking. The identification of zero-valence sulfur on a 64-kDa peptide after sucrose density centrifugation indicates that the P-700-containing protein may be an iron-sulfur protein. A similar conclusion was reached by Sakurai and San Pietro [26] after zero-valence sulfur was found on the high molecular-weight protein in Photosystem I isolated by polyacrylamide gel electrophoresis.

(2) The photochemical charge separation now occurs between P-700$^+$ and the next available acceptor, A_2^-. The P-700$^+$ A_2^- backreaction is normally seen under conditions in which centers A and B (P-430) are reduced prior to illumination. Even though the recombination time is 5-times longer, the 1.2-ms transient seen in the presence of LDS most probably represents the P-700$^+$ A_2^- backreaction obtained in the absence of nearby reduced acceptors. We suggest that the 250-μs backreaction between P-700$^+$ and A_2^- is due to an acceleration in the rate of recombination with P-700$^+$ when the electron on A_2^- is exposed to electrostatic repulsion of A^- and B^-. McLean and Sauer [27] reported that the rate constant for forward electron transfer from A_1^- to X is similar to the recombination rate of the A_1^- P-700$^+$ pair. This suggests that the forward electron transfer from A_1 to X is slower when centers A and B are in the reduced state than when they are oxidized. The deceleration in the P-700$^+$ A_2^- backreaction rate from 250 μs to 1.2 ms may reflect a similar influence from A^- and B^-. We have further identified the 1.2-ms kinetic component as the P-700$^+$ A_2^- charge separation based on (i) the expectation that the 250-μs half-time should slow in the absence of A^- and B^-, (ii) the insensitivity of the component to methyl viologen, (iii) the spectral characterization of the transient as an iron-sulfur protein and (iv) the low-temperature characteristics of the P-700$^+$ charge recombination. A_2 has been previously identified as an iron-sulfur protein by its biochemical [28] as well as spectral [29] properties. The 1.2-ms transient, therefore, does not appear to represent a new iron-sulfur protein in Photosystem I, but rather represents a known acceptor operating under a different kinetic domain.

(3) Further incubation results in the unfolding of the 64-kDa peptide(s) and denaturation of the bound-iron sulfur cluster corresponding to A_2. During this time, the 1.2-ms transient attributed to the P-700$^+$ A_2^- backreaction is lost with a half-time of about 10 min. Experimental findings indicate that bound iron-sulfur clusters in photosystem I decay in this time period after unfolding with SDS [25]. The conversion of S^{2-} to S^0 occurs this time period although it is evident from Table I that a small percentage of labile sulfide remains in the native state. The existence of at least four moles of S^0 per P-700, however, indicates that a 64-kDa peptide in Photosystem I is most likely an iron-sulfur apoprotein.

The observation of a temperature-independent decay of signal A_2 below 225 K is adequately explained by a quantum mechanical tunneling model observed in other biological electron transfer processes [30,31]. The P-700$^+$ A^- pair detected as Signal I in chloroplasts shows first order Arrhenius behavior between 270 K and 150 K with an apparent activation energy of 23.0 kJ/mol [32]. A fast optical signal interpreted at the time as the P-700$^+$ A_1^- pair (but now known to represent the P-700 triplet state; see Ref. 33) follows first-order Arrhenius behavior between 294 K and 100 K with an apparent activation energy of about 4.2 kJ/mol [34,35]. The half-time of 85-ms between 225 K and 77 K observed after LDS-treatment is in agreement with the previously measured 130-ms lifetime for the P-700$^+$ A_2^- pair determined under conditions where centers A and B are reduced prior to illumination [35]. The near-identical lifetimes for the P-700$^+$ A_2^- pair under non-reducing (Fig. 4) and reducing conditions (Ref. 34) suggests

and A^- and B^- exert only a minor influence on the P-700$^+$ A_2^- backreaction time at low temperature. This suggests that there may be temperature-dependent changes in the conformational arrangement of A_2^-, A^- and B^- which might lead to changes in magnetic coupling between A_2^-, A^- and B^-.

The conclusion that the 64-kDa peptide in Photosystem I is an iron-sulfur protein requires further elaboration in light of the possibility that two non-identical peptides may exist in the 64-kDa region. On denaturing SDS polyacrylamide gels, this region shows a rather broad band that occasionally splits into a doublet. Okamura et al. [14] believe that two copies of the 64-kDa protein exist, only one of which contains P-700. Vierling and Alberte, however, report that two proteins seen in their preparations are conformational variants of a single polypeptide [36]. The issue is further complicated by the finding that the chloroplast genome contains two non-identical, but closely related, genes for the P-700 apoprotein [37].

By analogy with other Fe-S proteins, we assume the iron ligands of center A_2 to be cysteinyl residues; however, the nucleotide sequence of the ps I-A1 and ps I-A2 genes in maize shows that the polypeptides PS I-A1 and PS I-A2 contain only four and two cysteine residues, respectively [37]. According to the hydropathy plot, the cysteines at positions 160 and 444 of PS I-A1 are located in two of the 11 membrane-buried helices. The cysteines at positions 575 and 584 are separated by eight amino acids, one of which is a helix-disrupting proline (a proline also occupies amino acid 574) and are located in the extramembrane region. In PS I-A2 the extramembrane cysteines are conserved at positions 560 and 569, but the membrane-buried cysteines are replaced by threonine and serine. The amino acids between the two conserved cysteines are also conserved in PS I-A1 and PS I-A2 polypeptides as are two flanking amino acids prior to the first cysteine. Since 2Fe-2S and 4Fe-4S clusters require four cysteines as ligands to the iron atoms, either (1) the polypeptide PS I-A1 contains the entire cluster, or (2) a single iron-sulfur cluster may span two polypeptides. The interpolypeptide cluster is attractive because of the extramembrane location of the two conserved cysteine regions and because PS I preparations have been judged to contain two polypeptides of molecular weight 58 000 to 70 000. An analogous situation exists in the nitrogenase Fe-protein (Av2) from *Azotobacter vinelandii*, where a single iron-sulfur cluster acts as a bridge between two apparently identical subunits [38,39]. The PS I reaction center core may consist of either one or two PS I-A1 peptides or one peptide each of PS I-A1 and PS I-A2, both of which provide two cysteines for an iron-sulfur complex that bridges the two peptides.

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