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Accelerated Publications

Characterization of a Photosystem I Core Containing P700 and Intermediate Electron Acceptor A_1[†]

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ABSTRACT: A new photosystem I core has been isolated that is devoid of the bound iron–sulfur clusters but preserves electron flow from P700 to the intermediate electron acceptor A_1. The particle is prepared by incubation of a *Synechococcus* sp. PCC 6301 photosystem I core protein (which contains electron acceptors A_0, A_1, and F_X) with 3 M urea and 5 mM $K_3Fe(CN)_6$ to oxidatively denature the F_X iron–sulfur cluster to the level of zero-valence sulfur. In this apo-F_X preparation, over 90% of the flash-induced absorption change at 820 nm decays with a 10-μs half-time characteristic of the decay of the P700 triplet state formed from the backreaction of $P700^+$ with an acceptor earlier than F_X. Chemical reduction at high pH values with aminoiminomethanesulfinic acid results in kinetics identical with those seen in the P700 chlorophyll *a* protein prepared with sodium dodecyl sulfate (SDS-CP1, which contains only electron acceptor A_0); the flash-induced absorption change decays primarily with a 25-ns half-time characteristic of the backreaction between $P700^+$ and A_0^-, and the magnitude of the total absorption change is larger than can be accounted for by the P700 content alone. Addition of oxygen results in a reversion to the 10-μs kinetic decay component attributed to the decay of the P700 triplet state. At 77 K, the optical transient in the apo-F_X preparation decays with a 200-μs half-time characteristic of the backreaction between $P700^+$ and A_1^-. In contrast, an SDS-CP1 particle shows an absorption change of equal magnitude but is followed by a 1.3-ms transient characteristic of the decay of the P700 triplet state formed from the $P700^+$ A_0^- backreaction. The high efficiency of P700 triplet formation at room temperature, the ability to be chemically reduced, and the 200-μs backreaction at 77 K indicate that charge separation in the apo-F_X preparation occurs between P700 and intermediate electron acceptor A_1.

The photosystem I reaction center in plants and cyanobacteria consists of a primary electron donor, P700, and a series of five electron acceptors: A_0, a chlorophyll *a* monomer; A_1, probably a quinone (phylloquinone or 5′-monohydroxyphylloquinone); and F_X, F_B, and F_A, all [4Fe-4S] clusters. The function of these acceptors is to prevent rapid charge recombination by delocalizing the electron from the initial site of charge separation between $P700^+$ and A_0^-. The polypeptide locations of these components are known: P700, A_0, A_1, and F_X reside on the photosystem I core protein containing the *psaA* and *psaB* gene products, while F_A and F_B are both located on the *psaC* gene product. The photosystem I reaction center also contains several additional low molecular mass polypeptides, labeled *psaD* through *psaK*. The *psaD* and *psaF* gene products are considered to be ferredoxin and plastocyanin docking proteins, but the roles of the other low molecular mass polypeptides remain undefined [for review, see Lagoutte and Mathis (1990) and Golbeck and Bryant (1990)].

In a recent series of papers, Golbeck et al. (1988a) and Parrett et al. (1989) showed that the addition of chaotropic agents to a spinach or cyanobacterial photosystem I complex results in the removal of the F_A/F_B polypeptide, as well as

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other peripheral low molecular mass polypeptides, from the photosystem I reaction center. In this *psaA*- and *psaB*-containing preparation, termed the photosystem I core protein, charge separation occurs between P700 and F_X at both room and cryogenic temperatures. Significantly, electron transfer from P700 to the F_A/F_B clusters can be restored by combining the photosystem I core protein with either a freshly isolated spinach F_A/F_B holoprotein (Golbeck et al., 1988b) or a *Synechococcus* sp. PCC 6301 F_A/F_B apoprotein concomitant with rebuilding the iron–sulfur clusters (Parrett et al., 1990). In both cases, the successful restoration of electron transfer from P700 to F_A/F_B indicates that the *Synechococcus* sp. PCC 6301 photosystem I core protein had been isolated intact. We also showed that the F_X iron–sulfur cluster could be oxidatively denatured to the level of zero-valence sulfur with 3 M urea and 5 mM $K_3Fe(CN_6)$ and rebuilt by the addition of $FeCl_3$, Na_2S, and β-mercaptoethanol (Parrett et al., 1990). Flash kinetic spectroscopy and low-temperature ESR[1] spectroscopy indicated ~80–85% restoration of electron flow from P700 to the renatured F_X iron–sulfur cluster.

One consequence of the ability to restore electron flow to F_X is that the preceding electron acceptor, A_1, must remain undamaged throughout these manipulations. A preliminary optical study of the apo-F_X preparation at room temperature (Parrett et al., 1990) showed a flash-induced absorption change that decayed in the microsecond time range. This transient, attributed to the decay of the triplet state of P700, accounted for >95% of the total absorption change. This contribution was recognized to be significantly higher than the relatively modest yield of P700 triplet observed in SDS-CP1 preparations where the majority of the backreaction is the decay of the $P700^+ A_0^-$ radical pair directly to the ground state (Sêtif et al., 1985). Sêtif and Bottin (1989) recently provided evidence that $P700^+ A_1^-$ backreaction decays directly to the ground state at cryogenic temperatures but occurs through the P700 triplet state—at an efficiency greater than 90%—at room temperature. Given these criteria, careful examination of the optical kinetic properties should allow us to determine the identity of the terminal electron acceptor in the apo-F_X preparation.

MATERIALS AND METHODS

The photosystem I complex containing the full array of electron acceptors (A_0, A_1, F_X, F_A, F_B) was isolated from *Synechococcus* sp. PCC 6301 (*Anacystis nidulans* TX-20) membrane fragments with 1% Triton X-100 followed by sucrose density gradient ultracentrifugation (Golbeck et al., 1988a). The photosystem I complex contains 110 chlorophyll molecules, 12 non-heme irons, and 12 acid-labile sulfides per P700. The photosystem I core protein was isolated from the photosystem I complex by treatment with 6.8 M urea, followed by ultrafiltration over a YM-100 membrane (Parrett et al., 1989). The photosystem I core protein contains 110 chlorophyll molecules, 4 non-heme irons, and 4 acid-labile sulfides per P700. SDS-CP1 particles were isolated from the photosystem I complex by incubation with 1% SDS for 12 h at room temperature followed by washing over a YM-100 ultrafiltration membrane. The SDS-CP1 particle contains 110 chlorophyll molecules and <0.3 acid-labile sulfides per P700. All particles were further purified by ultracentrifugation for 18 h at 113000*g* (SW-27 rotor) in a 0.1–1.0 M sucrose density gradient containing 50 mM Tris buffer, pH 8.3, and 0.04% Triton X-100. The apo-F_X particle, which contains P700, A_0, and A_1 (see text), was isolated by incubating the photosystem I core protein for 3 h with 3 M urea and 5 mM $K_3Fe(CN)_6$ in 50 mM Tris, pH 8.3. The urea and $K_3Fe(CN)_6$ were removed by dialysis in 50 mM Tris, pH 8.3, followed by dialysis in 50 mM Tris, pH 8.3, and 5 mM Tiron to remove the excess iron. The residual Tiron and the Tiron chelate were removed by dialysis for 12 h in 50 mM Tris, pH 8.3, and 0.04% Triton X-100. All photosystem I preparations were concentrated to 1000 μg of Chl/mL by ultrafiltration over a YM-100 membrane and stored in 20% glycerol at −80 °C. Chlorophyll was determined in 80% acetone.

Flash-induced optical absorption changes were determined in the microsecond time range at 820 nm with a single-beam spectrometer consisting of a 40-mW collimated laser diode measuring beam (Spindler & Hoyer Model DC 25F) and a planar diffused photodiode detector (United Detector Technology PIN-10D) reverse biased to 15 V and dropped across a 50-Ω resistor. The signal was amplified with a 70-MHz bandwidth voltage preamplifier (PAR Model 115), digitized with a Nicolet Model 4094A digital oscilloscope, and ported to a Macintosh Plus computer for data manipulation and storage. Absorption changes were determined in the nanosecond time range with a Schottky barrier photodiode detector (PIN-8LC) and a 400-MHz bandwidth transimpedance amplifier (UDT Model 700) reverse biased to 30 V. The signal was further amplified with a 600-MHz bandwidth voltage amplifier (Tektronix 11A52), digitized at a rate of 4 gigasamples/s with a Tektronix DSA 601 oscilloscope, and ported to a Macintosh IIci computer for data manipulation and storage. Curve fitting and data analysis were performed with a commercial software package (IGOR, WaveMetrics). Excitation was provided by a 2.3-MW nitrogen laser (PTI Model PL2300) with a 600-ps pulse width at 337.1 nm. Low-temperature optical studies were performed at 700 nm with a single-beam spectrophotometer described previously (Parrett et al., 1989). The sample was placed in a 1-cm path-length polystyrene cuvette at 5 μg of Chl/mL containing 0.033 mM DCPIP and 1.7 mM ascorbate in 60% glycerol and 0.10 M Tris, pH 8.3.

ESR studies were performed on a Varian E-109 spectrometer equipped with an Air Products LTD liquid helium transfer cryostat. The spectrometer was interfaced to a Macintosh IIci computer via a Keithley digital voltmeter (Model 195A) and an IEEE-488 bus controller (I/O Tech Mac 488A) for signal averaging and base-line subtraction. Sample temperatures were monitored with a thermistor situated directly below the sample tube. Light-minus-dark difference spectra were obtained by illuminating the sample with a 150-W xenon lamp.

RESULTS

Flash-Induced Absorption Changes Due to the P700 Triplet State. The flash-induced absorption changes at 820 nm in four different photosystem I preparations are shown in Figure 1. The corresponding low-temperature ESR spectra are depicted in Figure 2. The optical transient in the photosystem I complex (Figure 1A), which contains P700 and electron acceptors A_0, A_1, F_X, F_B, and F_A, decays with a 30-ms back-

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[1] Abbreviations: PSI, photosystem I; Chl, chlorophyll; ^{3}P700, P700 triplet state; ESR, electron spin resonance; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; DCPIP, 2,6-dichlorophenolindophenol; AIMS, aminoiminomethanesulfinic acid (also known as formamidinesulfinic acid or thiourea dioxide); IR, infrared; photosystem I complex, multiprotein reaction center isolated with Triton X-100, containing P700 and acceptors A_0, A_1, F_X, F_B, and F_A; photosystem I core protein, reaction center isolated from the photosystem I complex with chaotropes, containing P700 and acceptors A_0, A_1, and F_X; apo-F_X, reaction center isolated from the photosystem I core protein with urea and ferricyanide, containing P700 and acceptors A_0 and A_1; SDS-CP1, reaction center isolated from the photosystem I complex with SDS, containing P700 and acceptor A_0.

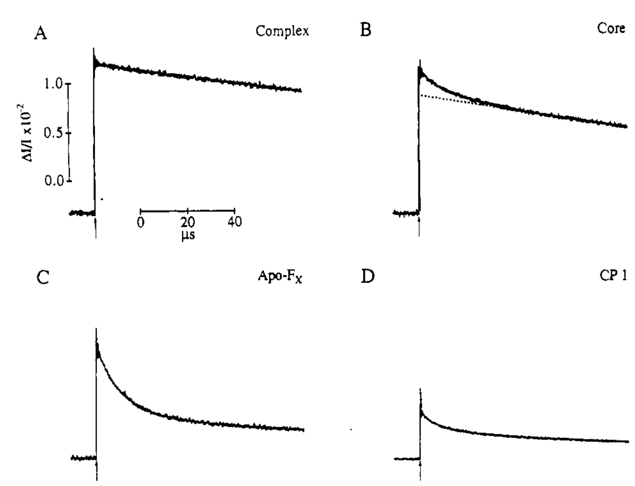

FIGURE 1: Flash-induced absorption changes at 820 nm in (A) photosystem I complex, (B) photosystem I core, (C) photosystem I apo-F$_X$ preparation, and (D) SDS-CP1 particle. All measurements were performed at 100 μg/mL Chl in 50 mM Tris buffer, pH 8.3, containing 1.7 mM ascorbate and 0.033 mM DCPIP. Each trace represents the average of 32 flashes provided at a frequency of 1 Hz.

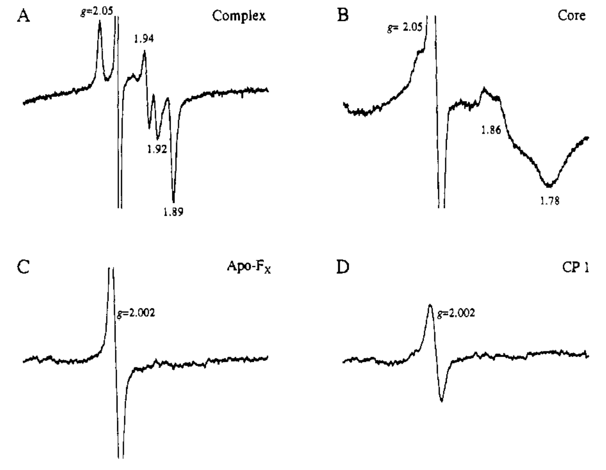

FIGURE 2: Electron spin resonance spectra of (A) photosystem I complex, (B) photosystem I core, (C) photosystem I apo-F$_X$ preparation, and (D) SDS-CP1 particle. The samples were frozen during illumination with dithionite in 50 mM glycine, pH 11.25, at a chlorophyll concentration of 500 μg/mL. The spectra shown are the average of four scans, each of which represent the difference between the light-on and the light-off (before light-on) spectrum. The conditions for (A) were gain, 5×10^3; microwave power, 10 mW; amplitude modulation, 10 G, field position, 3400 G; scan width, 900 G; temperature, 19 K; and microwave frequency, 9.292 GHz. Conditions for (B, C, and D) were gain, 5×10^3; microwave power, 40 mW; amplitude modulation, 40 G; field position, 3400 G; scan width, 900 G; and temperature, 4.5 K.

reaction (the decay kinetics in Figure 1A,B appear faster than 30 ms due to the low-frequency roll-off of the AC-coupled amplifier). The charge separation occurs between P700 and the terminal iron–sulfur centers F$_A$/F$_B$, shown by the ESR resonances at g = 2.05, 1.94, 1.92, and 1.89 (Figure 2A). When the photosystem I complex is treated with a chaotropic agent such as 6.8 M urea, the magnitude of the charge separation remains unaffected (Figure 1B), but the 30-ms backreaction is largely replaced with a 1.2-ms backreaction (not shown). In this preparation, charge separation occurs between P700 and iron–sulfur center F$_X$, shown by the ESR resonances at g = 2.05, 1.86, and 1.78 (Figure 2B). Chaotropes have been found to be very effective at removing the peripheral low molecular mass polypeptides, including the F$_A$/F$_B$ protein, from the photosystem I core protein without destroying F$_X$ (Parrett et al., 1989). A fast-decaying component with a ~10-μs half-time can be seen in Figure 1B, which represents the destruction of about 18% of F$_X$. The addition of 3 M urea and 5 mM $K_3Fe(CN)_6$ to the photosystem I core protein for 3 h results in the oxidative denaturation of the remaining acid-labile sulfides to zero-valence sulfur (Parrett et al., 1990) and culminates in the loss of the characteristic ESR resonances of F$_X$ (Figure 2C). The room temperature, flash-induced absorption change in this apo-F$_X$ preparation (Figure 1C) shows a biphasic decay. The fast phase has a half-time of 10 μs, which is similar to the 4.5-μs half-time found in *Synechocystis* PCC 6803 for the decay of the P700 triplet state. The slow phase remains uncharacterized. The SDS-CP1 preparation (Figure 2D) also lacks the characteristic ESR resonances of F$_X$, F$_B$, and F$_A$. Similarly, the flash-induced optical transient (Figure 1D) shows a biphasic decay. The fast phase has a 10-μs half-time and is known to be due to the decay of the P700 triplet state formed from the backreaction between P700$^+$ and A$_0^-$ (Sétif et al., 1981; Ikegami et al., 1987). In the near-IR, the P700 triplet has a flat positive band between 730 and 820 nm and an extinction coefficient between 6000 and 7000 M^{-1} cm^{-1} (Sêtif et al., 1981, 1985). By use of a nominal value of 6500 M^{-1} cm^{-1} at 820 nm for both the ^{3}P700 – P700 and P700$^+$ – P700 differential absorption changes, the Chl/P700 ratio in the apo-F$_X$ preparation (Figure 1C) is 138, which is greater than the Chl/P700 ratio of 106 found in the P700- and F$_X$-containing photosystem I complex (Figure 1A) and photosystem I core protein (Figure 1B). This discrepancy indicates that, in spite of the loss of iron–sulfur center F$_X$, ~80% of P700 is still active on a microsecond time scale. The Chl/P700 ratio in the SDS-CP1 preparation is 285, which indicates that less than 40% of the available P700 is active on a microsecond time scale. The Chl/P700 ratio of all four photosystem I preparations, determined by chemical difference spectroscopy, is 110.

Flash-Induced Absorption Changes Due to the P700$^+$ A$_0^-$ Recombination. The flash-induced absorption changes on a nanosecond time scale in both the apo-F$_X$ and SDS-CP1 preparations are depicted in Figure 3. The total magnitude of the optical transient in the apo-F$_X$ preparation (Figure 3A) was found to be nearly equal to that of both the photosystem I complex and the photosystem I core protein (not shown), indicating little loss of photoactive P700. The decay of the optical transient is biphasic: there exists a 10-μs decay component attributed to the decay of the P700 triplet, and a faster decay component which only accounts for a small fraction of the total absorption change (the decay kinetics of the slow phase in Figure 3 appear faster than 10 μs due the 500-kHz low-frequency roll-off of the transimpedance amplifier). Although some of the fast component is lost in the 15-ns response time of the photodiode detector, the magnitude of the absorption change at the onset of the flash can be calculated by fitting to a double exponential and extrapolating to time zero. As shown by the fitted line and inset, about 13% of the absorption change decays with a half-time of ~25 ns. The total absorption change corresponds to 100 Chl/P700, which is slightly more P700 than can be accounted for by the chemical difference spectrum. In an SDS-CP1 preparation, the majority of the absorption change decays on the nanosecond time scale (Figure 3B) and explains the loss of absorption seen on the microsecond time scale in Figure 1D. The fitted curve, shown by the solid line and inset, indicates that the fast phase has a half-time of 25 ns, and the total absorption change corresponds to 62 Chl/P700. This is significantly more P700 than can be accounted for by the chemical difference spectrum. However, previous studies have shown that the 25-ns component is due to the decay of the P700$^+$ A$_0^-$ radical pair

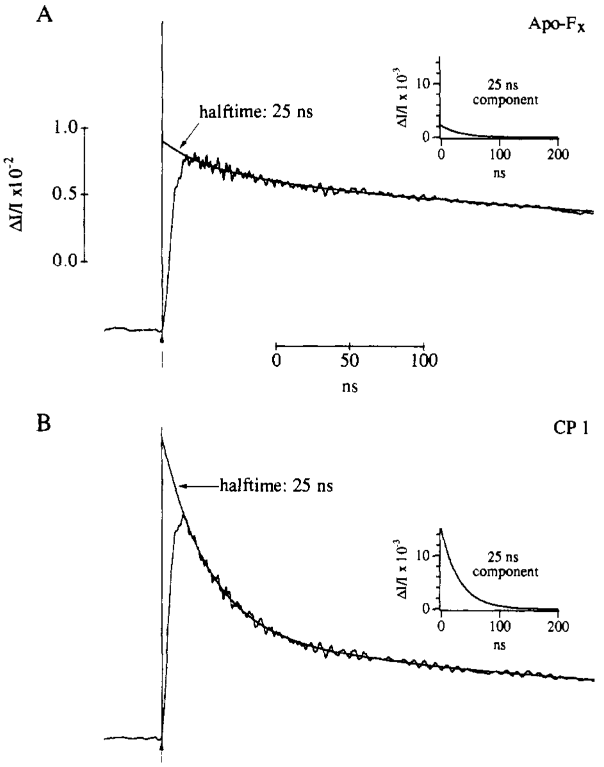

FIGURE 3: Room temperature flash-induced absorption changes at 820 nm on the nanosecond time scale of (A) apo-F$_X$ preparation and (B) SDS-CP1 particle. All measurements were performed at 100 μg/mL Chl in 50 mM Tris buffer, pH 8.3, containing 1.7 mM ascorbate and 0.033 mM DCPIP. The predicted nanosecond kinetic component, determined from biexponential regression, is depicted in the inset. Each trace represents the average of 32 flashes provided at a frequency of 1 Hz.

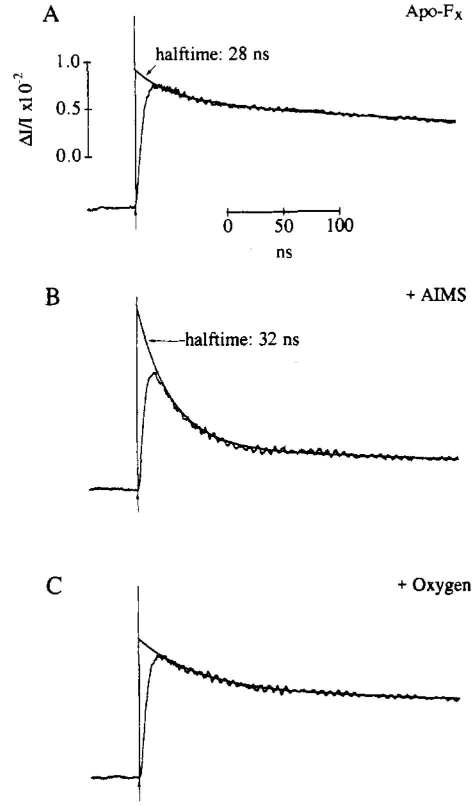

FIGURE 4: Kinetics of absorption changes measured at 820 nm of photosystem I apo-F$_X$ preparation. (A) Measurement performed at 100 μg/mL Chl in 50 mM glycine buffer, pH 11.25, containing 1.7 mM ascorbate and 0.033 mM DCPIP. (B) Addition of excess aminoiminomethanesulfinic acid, 0.33 μM each of methyl viologen, 1,1′-trimethylene-2,2′-bipyridilium dibromide, and 4,4′-dimethyl-1,1′-trimethylene-2,2′-bipyridiliium dibromide and incubation for 40 min. (C) Addition of oxygen by purging the cuvette for 2 min with air. Each trace represents the average of 32 flashes provided at a frequency of 1 Hz.

directly to the ground state (Sêtif & Bottin, 1989; Ikegami et al., 1987), and hence, the combined chlorophyll anion and chlorophyll cation charge-separated pair will contribute to the total absorption change at 820 nm. The A$_0^-$ – A$_0$ difference spectrum published by Ikegami et al. (1987) shows a rather intense band in the near-infrared, with a peak around 760 nm that is somewhat larger than expected. If we use an extinction coefficient of 15 mM^{-1} cm^{-1} at 820 nm [based on Figure 4 from Ikegami et al. (1987)] and correct for the contribution of the A$_0^-$ – A$_0$ differential absorption change, the Chl/P700 ratio becomes 200, a value at variance with the Chl/P700 ratio of 110 expected from the chemical difference spectrum. Some error will be introduced by the extrapolation procedure to obtain ΔI (probably no more than 10%) and by the uncertainty in the A$_0^-$ – A$_0$ differential extinction coefficient (perhaps as much as 100%), but neither will alter the fundamental conclusion that the total absorption change at 820 nm in Figure 3 is larger than can be accounted for by P700$^+$ and ^{3}P700 alone. Also, the slow phase derived from the decay of the P700 triplet contributes less to the total amplitude of the absorption change in SDS-CP1 than in the apo-F$_X$ preparation. The 25-ns component in apo-F$_X$ (Figure 3A) can therefore be attributed to the charge separation between P700$^+$ and A$_0^-$ followed by the backreaction to the ground state and explains the slight increase in apparent photochemical P700 in this preparation. Most likely, some A$_1$ becomes damaged or removed during the treatment protocol to denature F$_X$, and this may explain the less-than-quantitative yield of F$_X$ during the reconstitution protocol (Parrett et al., 1990).

Chemical Reduction of Electron Acceptor A_1. The difference in the amount of room temperature P700 triplet formed between the apo-F$_X$ preparation and SDS-CP1 is significant and indicates that different terminal electron acceptors may be functioning in these two particles. According to a recent study (Sêtif & Bottin, 1989), when F$_X$ is reduced by weak illumination, the backreaction between oxidized P700$^+$ and the reduced secondary acceptor A$_1^-$ decays through the P700 triplet state with an efficiency greater than 90%. With strong background illumination, the majority of the backreaction occurs with a 25–30-ns half-time characteristic of the decay of the P700$^+$ A$_0^-$ radical pair directly to the ground state. Under conditions of strong illumination, a double reduction of A$_1$ is proposed, with the result that electron transfer is blocked at the level of A$_0$. Iron–sulfur center F$_X$ remains oxidized under these conditions, which would imply that the redox potential of doubly reduced A$_1$ is greater than –710 mV. Since it is possible to generate redox potentials in excess of –650 mV in aqueous solution at high pH values, we reasoned that if the 10-μs backreaction were due to A$_1^-$ backreacting with P700$^+$ through the triplet state, strong chemical reduction (leading, presumably, to the doubly reduced species) should lead to a transition in the kinetics and amplitude of the optical transient to those of the P700$^+$ A$_0^-$ backreaction. Alternately, if the 10-μs backreaction were due to A$_0^-$ backreacting with P700$^+$, a chlorophyll molecule would not be chemically reducible, and we would expect to find no effect on the backreaction kinetics. As shown in Figure 4A, the apo-F$_X$ preparation is stable at the high pH value of 11.25 and displays the normal kinetic behavior with the major, 10-μs decay component. After addition of aminoiminomethanesulfonic acid and 40-min incubation, the magnitude of the absorption change

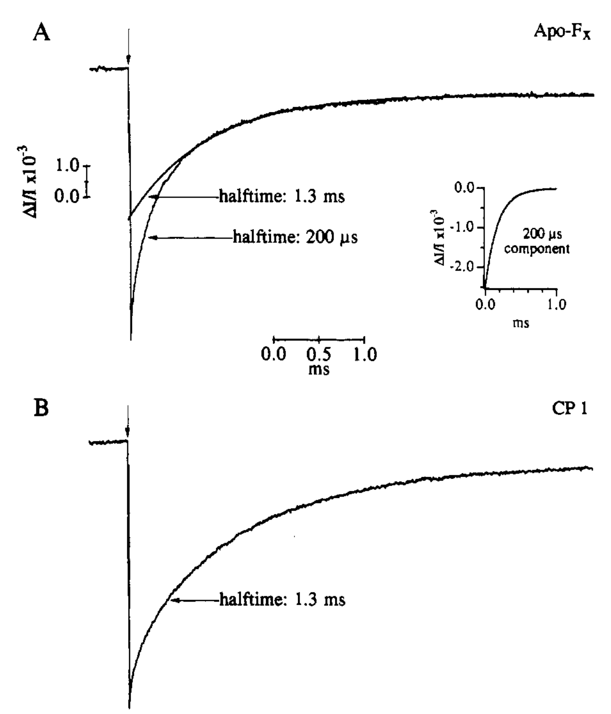

FIGURE 5: Flash-induced absorption changes at 77 K measured at 700 nm of (A) apo-F_X preparation and (B) SDS-CP1 particle. All measurements were performed at 5 µg/mL Chl in 60% glycerol and 50 mM Tris, pH 8.3, containing 1.7 mM ascorbate and 0.033 mM DCPIP. The traces represent the average of 64 flashes provided at a frequency of 1 Hz. The predicted microsecond kinetic component, determined from biexponential regression, is depicted in the inset.

increases, and the decay kinetics change to show a major, 25-ns component and a minor, 10-µs component (Figure 4B). These are the same kinetics seen in SDS-CP1 particles (Figure 3B) and in photosystem I reaction centers where the quinone has been solvent extracted (Biggins & Mathis, 1988). The increase in absorption, over and above that expected on the basis of the P700 content alone, is good evidence for the presence of a charge-separated pair between P700$^+$ and A_0^-. Furthermore, the fraction of the absorption change derived from the decay of the P700 triplet state (Figure 4B, slow phase) has declined significantly and is similar to that found in SDS-CP1 (Figure 3B). When oxygen is bubbled in the cuvette, the 10-µs half-time, P700 triplet is fully restored (Figure 4C), and when the residual 25-ns component is taken into account, the magnitude of the absorption change decreases to that expected on the basis of the P700 content. The fraction of the absorption change derived from the P700 triplet (Figure 4C, slow phase) has also returned to near-control levels. The apo-F_X preparation can be cycled between (B) and (C) at least one additional time without loss of signal, indicating that the acceptor retains its integrity throughout these manipulations.

Low-Temperature Optical Studies. The low-temperature optical behavior at 700 nm of the apo-F_X preparation and SDS-CP1 is shown in Figure 5. Because the P700$^+$ A_1^- backreaction decays directly to the ground state at 10 K whereas the P700$^+$ A_0^- backreaction occurs through the triplet state of P700 (Sêtif & Bottin, 1989), this difference can provide a test of the hypothesis that the terminal acceptor functioning in the apo-F_X preparation is A_1. At 77 K, the flash-induced absorption transient in the apo-F_X preparation (Figure 5A) decays with biphasic kinetics; ~50% decays with a half-time of 200 µs (inset), and the remainder decays with a half-time of 1.3 ms. The former is nearly identical with the 220-µs half-time reported for the low-temperature backreaction between P700$^+$ and A_1^- in the cyanobacterium *Synechocystis* PCC 6803; the latter has the half-time characteristic of the decay of the P700 triplet (Sêtif et al., 1981). We reported earlier (Parrett et al., 1990) a half-time of 600 µs in apo-F_X for the flash-induced absorption change at 77 K which we credited to P700 triplet decay. However, after reanalyzing the data, we found a poor fit to a single exponential and a much better fit to the 200-µs and 1.3-ms kinetic components shown here. The presence of the 1.3-ms component might be due to the partial removal or destruction of the A_1 acceptor, where a certain fraction of the reaction centers decay through the triplet state of P700. Alternately, in *Synechococcus* sp. PCC 6301, a certain fraction of the backreaction from A_1^- to P700$^+$ may proceed through the triplet state of P700 at 77K, and we are currently investigating this possibility. In contrast, the flash-induced absorption transient in the SDS-CP1 preparation (Figure 5B) shows little or no contribution on the microsecond time scale (inset) but decays primarily with the 1.3-ms half-time due to the decay of the P700 triplet.

DISCUSSION

We report the characterization of a new photosystem I core preparation containing P700 and the intermediate electron acceptor, A_1. We had speculated earlier (Parrett et al., 1990) that the ability to restore electron flow to F_X after oxidative denaturation and rebuilding of the bound iron-sulfur cluster presupposes that the intermediate electron acceptor A_1 must remain functional and active throughout these manipulations. A_1, however, is difficult to measure directly; we were disinclined to use photoaccumulation techniques because the origins of the optical and ESR-based signals are unclear [see Golbeck and Bryant (1990)]. The evidence for A_1 as the terminal electron acceptor is therefore indirect: (1) The flash-induced kinetics of the apo-F_X photosystem I reaction center shows a 10-µs, room temperature optical transient (we have observed variable half-times, ranging from 3 to 10 µs, in these preparations [see Parrett et al. (1990)]), and the decay is due almost entirely to the P700 triplet. This efficiency of triplet formation is significantly higher than in SDS-CP1, where the majority of the backreaction between P700$^+$ and A_0^- decays directly to the ground state. In contrast, the P700$^+$ A_1^- backreaction has been proposed to decay primarily through the triplet state of P700 (Sêtif & Bottin, 1989). (2) The change in kinetics from a P700 triplet to a direct backreaction to the ground state after addition of aminoiminomethanesulfinic acid at pH 11.25 indicates the reduction of an electron acceptor other than a chlorophyll molecule. A possible candidate is a quinone, such as phylloquinone or 5′-monohydroxyphylloquinone, which are known to be present in photosystem I at a ratio of two molecules per P700. These quinones are capable of undergoing a double reduction at the redox potential of −660 mV and the pH of 11.25 used in this study. The quinone present in *Synechococcus* sp. PCC 6301 is probably not phylloquinone because only 0.22 ± 0.09 molecule per P700 can be extracted from purified photosystem I complexes (data not shown). In contrast, a spinach photosystem I complex contains 1.97 ± 0.05 phylloquinone per P700 when assayed under the same conditions (extraction with wet ether followed by HPLC analysis on a reverse-phase, C-8 column with methanol as eluant). It has recently been shown that *Anacystis nidulans* (probably identical with the *Synechococcus* sp. PCC 6301 used in this study) contains 5′-monohydroxyphylloquinone (Ziegler et al., 1989), and we are currently checking the possibility that the photosystem I reaction center used here contains this prenylquinone. (3) The low-temperature behavior of the apo-F_X photosystem I core can be interpreted as the backreaction between A_1^- and P700$^+$ directly to the ground state. The backreaction kinetics of photosystem I acceptors are quite variable, depending on the identity of the organism, the type of preparation, and the presence of reduced electron acceptors in close proximity. In spinach, for example, a 120-μs decay phase has been proposed to be due to the recombination reaction between P700$^+$ and A_1^- when the iron–sulfur centers are in the state $[F_XF_B^-F_A^-]$, and a 20-μs phase from the same reaction has been proposed when the iron–sulfur centers are in the state $[F_X^-F_B^-F_A^-]$. In *Synechocystis* PCC 6803, the P700$^+$ A_1^- backreaction occurs with a half-time of 220 μs when the iron–sulfur centers are in the state $[F_XF_B^-F_A^-]$ and with a half-time of 23 μs when the iron–sulfur centers are in the state $[F_X^-F_B^-F_A^-]$. Our data indicate that in *Synechococcus* sp. PCC 6301 the 200-μs kinetic phase is due to recombination between P700$^+$ and A_1^- when F_X, F_B, and F_A are physically absent.

The conclusion that A_1 remains active in the apo-F_X preparation explains the finding that electron transfer can be restored to F_X after addition of $FeCl_3$, Na_2S, and β-mercaptoethanol, whereas it cannot be restored under identical conditions in SDS-CP1. The SDS-CP1 reaction center has been extensively studied and is considered the most fundamental photosystem I preparation. It is composed of the *psaA* and *psaB* reaction center polypeptides and contains only the primary electron-transfer components P700 and A_0. Phylloquinone has been found in a spinach SDS-CP1 particle (Schoeder & Lockau, 1986), but the photoreduction of A_1 [assuming the optical and ESR spectroscopic signatures are due to phylloquinone; see Golbeck (1987)] has not been observed. We have shown elsewhere (Parrett et al., 1990) that prior exposure to 1% SDS precludes reconstitution of the F_X iron–sulfur cluster in the photosystem I core protein. On the basis of evidence presented here, we suggest that the inability to restore electron flow to F_X in SDS-CP1 is due to the lack of functional A_1. The most likely explanation is that SDS has displaced A_1 from its binding site, thereby precluding forward electron transfer from A_0^- to the F_X iron–sulfur cluster. The oxidative denaturation of F_X with urea–ferricyanide, on the other hand, leaves the electron acceptor, A_1, functional and intact. The particle described is therefore a subset of the photosystem I core protein and a superset of the SDS-CP1 preparation: the components are associated with the reaction center heterodimer composed of the *psaA* and *psaB* polypeptides, and the particle is efficient in electron transfer between P700, A_0, and A_1.

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