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Resolution and reconstitution of the cyanobacterial Photosystem I complex

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We had shown previously that addition of urea to a *Synechococcus* 6301 Photosystem I complex leads to dissociation of the 8.9 kDa, F_A/F_B polypeptide, from the P-700- and F_X-containing Photosystem I core protein (Golbeck et al. (1988) FEBS Lett. 240, 9–14). In the presence of chaotropes, the iron-sulfur clusters in the 8.9 kDa, F_A/F_B polypeptide are unstable, and degrade to the level of zero-valence sulfur (Parrett et al. (1989) Biochim. Biophys. Acta 973, 324–332). We now report that addition of $FeCl_3$, Na_2S, and β-mercaptoethanol to a mixture of the low molecular mass polypeptides and the purified Photosystem I core protein results in complete restoration of light-induced charge separation between P-700 and F_A/F_B, including (i) the 30 ms room temperature charge recombination between $P\text{-}700^+$ and $[F_A/F_B]^-$ and (ii) the characteristic light-induced ESR spectrum of F_A and F_B with g values of 2.05, 1.94, 1.92 and 1.89. Analysis by SDS-PAGE shows that the reconstituted 8.9 kDa, F_A/F_B polypeptide has rebound to the Photosystem I core protein. The purified Photosystem I core protein was treated with 3 M urea and 5 mM potassium ferricyanide to oxidatively denature F_X to the level of zero-valence sulfur; light-induced charge separation in the apo-F_X core protein results in a 3 μs optical transient due to the relaxation of the P-700 triplet state. Addition of $FeCl_3$, Na_2S and β-mercaptoethanol results in restoration of light-induced charge separation between P-700 and F_X, including (i) the 1.2 ms room temperature charge recombination between $P\text{-}700^+$ and F_X^- and (ii) the characteristic light-induced ESR resonances of F_X with g values of 2.05, 1.86 and 1.78. Addition of $FeCl_3$, Na_2S and β-mercaptoethanol to a mixture of the F_X and F_A/F_B apoproteins results in reconstitution of electron flow from P-700 to F_A/F_B, indicating quantitative reinsertion of the F_X as well as the F_A/F_B iron-sulfur clusters and quantitative rebinding of the 8.9 kDa polypeptide to the Photosystem I core protein. This reconstitution technique makes possible novel studies of Photosystem I, including chemical or genetic modification of the F_X or F_A/F_B apoproteins followed by reinsertion of the iron-sulfur clusters and rebinding of the low molecular mass polypeptides to produce a functional Photosystem I complex.

Introduction

The Photosystem I reaction center is a membrane-bound, multiprotein complex which participates in the light-induced transfer of an electron from plastocyanin to ferredoxin (see Refs. 1, 2 for review). Photoexcitation of one of approx. 100 antenna chlorophyll molecules leads to charge separation between the primary electron donor, P-700, and the primary electron acceptor, A_0. The electron then passes through the intermediate acceptors A_1 and F_X to the terminal acceptors F_B and F_A. The identity of these electron transport components is now known: P-700 is probably a chlorophyll *a* dimer,

Abbreviations: PS I, Photosystem I; Chl, chlorophyll; ESR, electron spin resonance; DCPIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulfate; Tiron, 4,5-dihydroxy-1,3-benzenedisulfonic acid; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; NHI, non-heme iron.

Definitions: Photosystem I complex: multiprotein reaction center isolated with Triton X-100, containing P-700 and acceptors A_0, A_1, F_X, F_B and F_A; Photosystem I core protein: reaction center heterodimer of the psaA and psaB proteins isolated from the Photosystem I complex with chaotropes, containing P-700 and acceptors A_0, A_1 and F_X; F_A/F_B polypeptide: 8.9 kDa polypeptide of psaC containing iron-sulfur clusters F_A and F_B.

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A_0 is a chlorophyll *a* monomer, A_1 is most likely phylloquinone (vitamin K_1), F_X is considered to be an interpolypeptide [4Fe-4S] cluster, and F_A and F_B are both [4Fe-4S] clusters.

The Photosystem I core protein responsible for binding P-700, A_0 [3], A_1 [4–6], and F_X [7–10] consists of one copy each of the 82.4 and 83.0 kDa reaction center polypeptides [11,12]. The Photosystem I complex contains at least eight or nine additional low-molecular-mass polypeptides (excluding LHCI in higher plants and algae) ranging from 4.0 to 17.9 kDa. With the exception of the 8.9 kDa polypeptide, which binds the F_A/F_B iron-sulfur clusters [13–17], the low-molecular-mass polypeptides are poorly characterized. Chemical cross-linking studies, however, have provided evidence that the 17.9 and 17.3 kDa polypeptides in spinach serve as 'docking' sites for ferredoxin [18,19] and plastocyanin [20], respectively.

The *psaA, psaB* and *psaC* genes code for the 83.0 and 82.4 kDa reaction center polypeptides [21,22] and the 8.9 kDa, F_A/F_B polypeptide [23–25], respectively. The deduced amino acid sequences of the *psaA* and *psaB* gene products from maize have approx. 45% homology and contain 81 histidines, which may bind the majority of the core chlorophyll molecules in Photosystem I. Also present are three conserved cysteine residues on the *psaA* gene product and two conserved cysteine residues on the *psaB* gene product in all organisms sequenced to date. Since recent EXAFS [26] and Mössbauer [27] studies of the Photosystem I core protein have shown that F_X is a [4Fe-4S] cluster, and since all known low potential iron-sulfur clusters require four cysteine residues as ligands, F_X is most likely an interpolypeptide [4Fe-4S] cluster with two cysteine ligands provided by each of the high molecular mass proteins in the reaction center heterodimer [2,7].

We reported earlier that the Photosystem I core protein could be isolated from a *Synechococcus* Photosystem I complex by treatment with chaotropes followed by ultrafiltration over a YM-100 membrane [10,28]. The *Synechococcus* F_A/F_B apoprotein could be recovered in the YM-100 filtrate, but the iron-sulfur clusters were found to be in the sulfur-zero state. We later reported that the intact spinach F_A/F_B polypeptide, isolated from thylakoid fragments by methanol and acetone precipitation, could be rebound to the *Synechococcus* Photosystem I core protein, leading to complete restoration of electron flow from P-700 to F_A and F_B [17]. Since the ability to rebind the intact spinach F_A/F_B polypeptide indicated that the *Synechococcus* Photosystem I core protein remained undamaged by chaotrope treatment, we questioned whether the *Synechococcus* F_A/F_B apoprotein, recovered in the YM-100 filtrate, could be reconstituted with synthetic iron-sulfur clusters formed from inorganic iron and sulfide and rebound to the Photosystem I core protein. Although reconstitution of water-soluble iron-sulfur proteins such as spinach and Clostridial ferredoxins has been accomplished [29–32], previous attempts to reconstitute the ferredoxin-like iron-sulfur clusters of F_A and F_B in an oxidatively denatured Photosystem I complex have been unsuccessful [33,34].

In this paper, we report that the iron-sulfur clusters in the *Synechococcus* F_A/F_B apoprotein can be rebuilt with $FeCl_3$ and Na_2S in the presence of β-mercaptoethanol, and rebound to the *Synechococcus* Photosystem I core protein. The reconstituted Photosystem I complex is fully functional in electron transfer between P-700 and F_A/F_B. We also report that oxidatively denatured F_X in the *Synechococcus* Photosystem I core protein can be rebuilt with $FeCl_3$ and Na_2S in the presence of β-mercaptoethanol. Optical and ESR analyses indicate that reconstituted-F_X is fully functional in accepting electrons from P-700.

Materials and Methods

Isolation of the Synechococcus Photosystem I complex and the F_A/F_B polypeptide

The Photosystem I complex was isolated from *Synechococcus* 6301 membrane fragments with 1% Triton X-100 followed by sucrose-density ultracentrifugation [10]. The complex contains approx. 115 chlorophyll molecules, P-700, and the electron acceptors A_0 A_1 F_X F_B and F_A [2]. The native-F_A/F_B polypeptide was isolated from lyophylized *Synechococcus* membrane fragments by butanol extraction similar to that described in Ref. 16, except that excess dithionite was present during the purification procedure. SDS-CP1 particles were isolated from the *Synechococcus* Photosystem I complex by incubation for 12 h with 1% SDS at room temperature. The SDS-treated reaction center was purified by ultracentrifugation in a 0.1 to 1 M sucrose gradient containing 50 mM Tris buffer (pH 8.3) and 0.1% Triton. After 40 h centrifugation at $113000 \times g$ (SW-27 rotor) the lower green band (containing SDS-CP1) and the upper band (containing the low molecular mass polypeptides) were collected and dialyzed overnight against 50 mM Tris (pH 8.3). Both fractions were concentrated by ultrafiltration and stored at $-80\,^{\circ}C$ in 20% glycerol.

In situ resolution and reconstitution of the Photosystem I complex

A solution of 9 M urea in 100 mM Tris (pH 8.3), was added dropwise to a rapidly stirring solution of the Photosystem I complex (1000 μg Chl/ml) to a final concentration of 6.8 M urea and 250 μg Chl/ml. After 10 min, a 60 μl aliquot of this solution was diluted in a 3 ml anaerobic cuvette containing N_2-purged Tris buffer (pH 8.3), 0.5% β-mercaptoethanol and 30 μM DCPIP. In this chaotrope-treated sample, the flash-induced 1.2 ms backreaction of $P\text{-}700^+$ F_X^- had totally replaced the

30 ms backreaction of P-700$^+$ $[F_A/F_B]^-$. An aliquot of anaerobic 30 mM $FeCl_3$ was then slowly added to a final concentration of 0.15 mM, and after an additional 5 min, an aliquot of anaerobic 30 mM Na_2S was slowly added to a final concentration of 0.15 mM. The progress of the reconstitution was followed by observing the replacement of the flash-induced 1.2 ms backreaction of P-700$^+$ F_X^- with the 30 ms backreaction of P-700$^+$ $[F_A/F_B]^-$.

Separation of the Photosystem I core protein and the low molecular mass polypeptides

The low-molecular-mass polypeptides were dissociated from the Photosystem I core protein by treatment with chaotropes. When the 1.2 ms backreaction had totally replaced the 30 ms backreaction (as described above), the stock solution was diluted to 100 μg Chl/ml with 50 mM Tris buffer (pH 8.3) and dialyzed against the same buffer for 12 h. The Photosystem I core protein was recovered by ultrafiltration over a YM-100 membrane and purified by ultracentrifugation in a 0.1 to 1 M sucrose gradient containing 50 mM Tris buffer (pH 8.3) and 0.1% Triton X-100. The low molecular mass polypeptides were recovered from the YM-100 filtrate and concentrated by ultrafiltration over a YM-5 membrane to the same volume as the purified Photosystem I core protein. The 8.9 kDa, F_A/F_B polypeptide (*psaC* gene product) was present in the low-molecular-mass fraction; however, the iron-sulfur clusters were found to be oxidized to the level of zero-valence sulfur [28]. Because losses are negligible, equal volumes of the Photosystem I core protein and the fraction containing the low molecular mass polypeptides contain equimolar amounts of the Photosystem I polypeptides.

Rebinding of the low molecular mass polypeptides to the Photosystem I core protein

The freshly-isolated native-F_A/F_B polypeptide from *Synechococcus* was added in more than 10-fold molar excess to the Photosystem I core protein at 100 μg Chl/ml in 50 mM Tris (pH 8.3) and 0.1% β-mercaptoethanol. After 1 h incubation at room temperature, the solution was washed twice over a YM-100 membrane with 50 mM Tris (pH 8.3), and 0.05% Triton X-100 to remove unbound F_A/F_B polypeptide. Rebinding of the F_A/F_B apoprotein to the Photosystem I core protein was attempted by adding a 10-fold molar excess of the fraction containing the low-molecular-mass polypeptides derived from the YM-100 filtrate. The reconstituted-F_A/F_B protein was rebound to the Photosystem I core protein using the following protocol: (i) a solution of 50 mM Tris (pH 8.3) was purged with oxygen-free nitrogen in a closed reaction vessel; (ii) after 2 h, β-mercaptoethanol was added through a septum to a final concentration of 0.5%; (iii) a 10-fold molar excess of the fraction containing the low molecular mass polypeptides was added to the Photosystem I core protein (10 μg Chl/ml) and the solution was purged with a low flow rate (to minimize foaming) of oxygen-free nitrogen; (iv) after 10 min, an aliquot of anaerobic 30 mM $FeCl_3$ was slowly added to a final concentration of 0.15 mM; (v) after 5 min, an aliquot of anaerobic 30 mM Na_2S was slowly added to a final concentration of 0.15 mM. This solution was allowed to incubate in the dark and at room temperature for 12 h. The reaction vessel was uncapped and the solution was transferred under a flow of oxygen-free nitrogen to an ultrafiltration cell equipped with a YM-100 membrane, concentrated to near-dryness, and washed twice with 50 mM Tris (pH 8.3), containing 0.05% Triton X-100. The washed solution was diluted to 500 μg Chl/ml, followed by dialysis for 12 h in 50 mM Tris (pH 8.3), containing 5 mM Tiron. The residual Tiron and Tiron-iron chelate were removed by dialysis for 12 h in 50 mM Tris (pH 8.3), containing 0.05% Triton X-100. The reconstituted Photosystem I complex was concentrated to 1000 μg Chl/ml and stored at −80 °C in 20% glycerol.

Oxidative denaturation of the F_X iron-sulfur cluster

Apo-F_X was prepared by treating a Photosystem I core protein (50 μg Chl/ml) for 2 h with 3 M urea and 5 mM potassium ferricyanide in 50 mM Tris (pH 8.3). The urea and potassium ferricyanide were removed by dialysis for 12 h in 50 mM Tris (pH 8.3), followed by dialysis for 12 h in 50 mM Tris (pH 8.3), and 5 mM Tiron to remove the excess iron. The residual Tiron and the Tiron-iron chelate were removed by dialysis for 12 h in 50 mM Tris (pH 8.3), and 0.05% Triton X-100. The apo-F_X Photosystem I core protein was concentrated by ultrafiltration over a YM-100 membrane to 1000 μg Chl/ml and stored at −80 °C in 20% glycerol.

Reconstitution of the F_X iron-sulfur cluster

Reconstitution of the F_X iron-sulfur cluster was accomplished using the following treatment protocol: (i) a solution of 50 mM Tris (pH 8.3) was purged with oxygen-free nitrogen in a closed reaction vessel; (ii) after 2 h, β-mercaptoethanol was added through a septum to a final concentration of 0.5%; (iii) the apo-F_X Photosystem I core protein was added to 10 μg Chl/ml and the solution purged with a low flow rate (to minimize foaming) of oxygen-free nitrogen; (iv) after 10 min, an aliquot of anaerobic 30 mM $FeCl_3$ was slowly added to a final concentration of 0.15 mM; (v) after 5 min, an aliquot of anaerobic 30 mM Na_2S was slowly added to a final concentration of 0.15 mM. This solution was then allowed to incubate in the dark and at room temperature for 12 h. The reaction vessel was uncapped and the solution transferred under a flow of oxygen-free nitrogen to an ultrafiltration cell equipped with a YM-100 membrane and concentrated with oxygen-free nitrogen. The reconstituted-F_X Photosys-

tem I core protein was washed twice by repeated dilution to 10 μg Chl/ml (50 mM Tris (pH 8.3) containing 0.05% Triton X-100) and concentrated to near dryness. The washed solution was diluted to 500 μg Chl/ml, followed by dialysis for 12 h in 50 mM Tris (pH 8.3) and 5 mM Tiron. The excess Tiron and Tiron-iron chelate were removed by dialysis for 12 h in 50 mM Tris (pH 8.3) and 0.05% Triton X-100. The reconstituted-F_X Photosystem I core protein was concentrated over a YM-100 membrane to 1000 μg Chl/ml and stored at −80 °C in 20% glycerol.

Optical spectroscopy

Flash-induced absorption transients were determined at 698 nm with a single beam spectrometer consisting of an Oriel 250 W quartz-tungsten source, two Jarrell-Ash 1/4 meter monochromators (one before and one after the sample cuvette), and a Schottky-barrier photodiode (UDT PIN 8LC). For millisecond resolution, the signal was DC-coupled, amplified with a pair of PAR Model 113 preamplifiers, digitized using a Nicolet 4094A digital oscilloscope and ported to a Macintosh Plus computer for signal averaging, data manipulation, and storage. An analog sample-and-hold circuit, located between the two amplifiers, nulled the measuring beam voltage 1-ms before the flash. For microsecond resolution, the signal was AC-coupled, amplified with a PAR Model 115 preamplifier, and digitized with a Biomation 8100 Waveform Recorder. The data were ported via an IEEE-488 bus controller (I/O Tech Mac488A) to a Macintosh Plus computer for signal averaging, data manipulation, and storage. Excitation was provided by either a Xenon flashlamp (PRA Model 6100E) or a 2.3 MW nitrogen laser (PTI Model PL2300). Low-temperature optical studies were performed in a 1 cm pathlength polystyrene cuvette containing 5 μg Chl/ml, 0.033 mM PMS and 1.7 mM ascorbate in 60% glycerol and 0.10 M Tris (pH 8.3). The cuvette was initially placed in an optically transparent Dewar 1 cm above the liquid nitrogen surface and placed in darkness. No cracks developed in the frozen glass when the cooling was performed slowly. After 10 min of precooling, excess liquid nitrogen was added until the cuvette was completely submerged. After an additional 10 min, the excess liquid nitrogen was removed and the Dewar was placed in the spectrometer for optical measurements. A chromel/alumel thermocouple (Omega Engineering) was used to monitor the temperature.

ESR spectrocopy

ESR studies were performed on a Varian E-109 spectrometer interfaced to a Macintosh Plus computer via a Keithley Model 195A digital voltmeter and an IEEE-488 bus controller [28]. The programs to control the optical and ESR spectrometers, and to analyze the data, were written in-house in FORTH.

SDS-PAGE

Electrophoresis (SDS-PAGE) was performed in a 14 cm × 16 cm × 1.5 mm slab gel (Hoefer SE 600) containing a linear 13–21% polyacrylamide gradient and a 4% stacking gel (both 30:0.8, acrylamide:bis). Protein samples were incubated in 0.0625 M Tris (pH 6.8), 2% SDS, 10% glycerol and 5% β-mercaptoethanol for 24 h at 30 °C. The samples were applied to the stacking gel containing 50 mM Tris H_2SO_4 (pH 6.1), 0.1% SDS, 1 mg/ml APS, and 1 μl/ml TEMED. Electrophoresis was carried out at 23 °C at a constant current of 15 mA for 12–14 h. Gels were stained with Coomassie Brilliant Blue and scanned with an LKB laser densitometer. Molecular masses were calculated on the basis of the R_F values of soluble proteins from 6.21 to 66.0 kDa (Sigma Nos. MW-SDS-7, MW-SDS-70 and MW-SDS-200) treated at 37 °C for 2 h and run in alternate wells.

Other methods

Chlorophyll was determined colorimetrically. Acid-labile sulfide was determined as described in Ref. 35; spinach ferredoxin at a purity ratio of 0.46 (A_{420}/A_{276}) served as an absolute standard for acid-labile sulfide. Non-heme iron was determined colorimetrically as described in Ref. 34.

Results

In situ resolution and reconstitution of the Photosystem I complex

The flash-induced absorption transient at 698 nm in an isolated Photosystem I complex results from charge separation between P-700 and the terminal iron-sulfur clusters F_A/F_B. In the absence of added donors and acceptors, the charge-separated state is relatively long-lived, and charge recombination occurs between $P\text{-}700^+$ and $[F_A/F_B]^-$ with a half-time of approx. 30 ms (Fig. 1a). If the Photosystem I complex is treated with chaotropic agents, such as 6.8 M urea or 2 M NaI, the long-lived optical transient is progressively replaced by a faster optical transient with a 1.2 ms half-time [10,28]. We have shown through spectroscopic and biochemical techniques that the 1.2 ms optical transient represents the backreaction between $P\text{-}700^+$ and F_X^- in reaction centers deficient in a functional F_A/F_B polypeptide [7–10,17,28].

Fig. 1b shows the 1.2 ms charge recombination of $P\text{-}700^+ F_X^-$ in the chaotrope-treated Photosystem I complex after the addition of 6.8 M urea followed by 50-fold dilution. The backreaction kinetics appear identical to those in the isolated Photosystem I core protein; even after 12 h, no change in the 1.2 ms charge recombination is observed. However, when $FeCl_3$, Na_2S and β-mercaptoethanol are added under anaerobic conditions and incubated for 12 h, much of the long-lived optical transient becomes restored at the expense of the

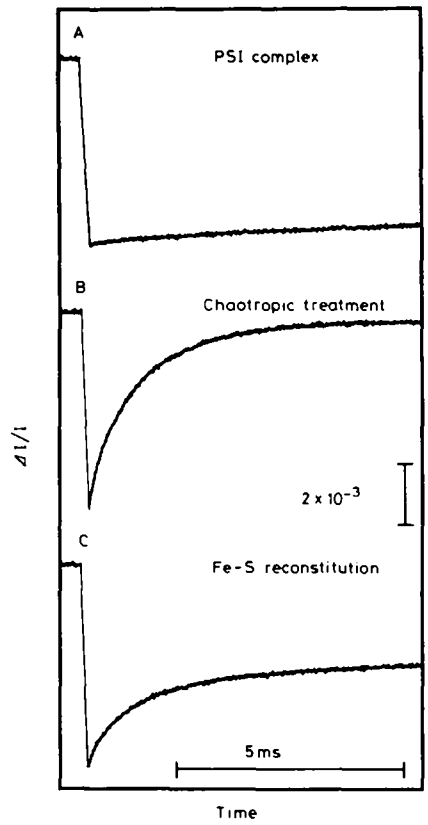

Fig. 1. Flash-induced absorption changes of P-700 before and after in situ resolution and reconstitution of the Photosystem I complex. (A) Absorption transient in the Photosystem I complex before chaotropic treatment. (B) Absorption transient 10 min after chaotropic treatment. (C) Absorption transient 12 h after the addition of inorganic iron and sulfide in the presence of β-mercaptoethanol and under anaerobic conditions. All measurements were performed at 5 μg Chl/ml in 50 mM Tris buffer (pH 8.3), containing 1.7 mM ascorbate and 0.033 mM DCPIP.

1.2 ms optical transient (Fig. 1c). The change in the backreaction kinetics would imply that the F_A/F_B clusters are replacing the F_X cluster as the terminal electron acceptor(s). Since chaotropic agents have been shown to cause denaturation of the F_A/F_B iron sulfur clusters in addition to dissociating the Photosystem I core complex [28], two discrete events would need to occur during the reconstitution protocol: first, the iron-sulfur clusters must be reinserted into the 8.9 kDa, F_A/F_B apoprotein and second, the F_A/F_B holoprotein must be rebound to the Photosystem I core protein.

Reconstitution of the Photosystem I complex from the F_A/F_B apoprotein and the Photosystem I core protein

The apparent recovery of electron flow from P-700 to F_A/F_B has several important implications. First, if the F_A/F_B holoprotein is rebinding to its site rather than simply serving as a soluble electron acceptor, the affinity of the F_A/F_B polypeptide for the Photosystem I core protein must be very high, since both proteins are inherently present in a 1 : 1 ratio and at a concentration of only approx. 50 pmol/ml. Second, the F_A/F_B holoprotein must bind tighter to the Photosystem I core protein than the F_A/F_B apoprotein, since the latter can be removed by ultrafiltration [10,28] and therefore may exist free in solution. If these suppositions hold true, the magnitude and kinetics of the P-700 backreaction after rebinding the reconstituted F_A/F_B polypeptide to the Photosystem I core protein can be used as a sensitive and accurate method for determining the efficiency and yield of F_A/F_B cluster reconstitution.

To test these assumptions, reconstitution of the Photosystem I complex was performed by combining the F_A/F_B polypeptide with the purified Photosystem I core protein followed by extensive washing over a YM-100 ultrafiltration membrane (to remove the unbound low molecular mass polypeptides and soluble components added for the reconstitution of the iron-sulfur clusters). For a control preparation, the native-F_A/F_B polypeptide, isolated from *Synechococcus* 6301 according to Ref. 16, was added to the purified Photosystem I core protein in greater than 10-fold excess (to ensure complete rebinding) and washed by ultrafiltration. As shown in Fig. 2a, rebinding results in a rapid (< 2 min) transition in the flash-induced absorption change from a 1.2 ms optical transient due to the $P\text{-}700^+ F_X^-$ backreaction to a 30 ms transient due to the $P\text{-}700^+ [F_A/F_B]^-$ backreaction. Attempted rebinding of the F_A/F_B apoprotein (present in the YM-100 filtrate) to the Photosystem I core protein results in no perceptible change in the $P\text{-}700^+$ backreaction kinetics, regardless of the molar ratios of the two proteins (Fig. 2b). However, when the F_A/F_B apoprotein (present in the YM-100 filtrate) is added to the isolated Photosystem I core protein at a 10-fold molar excess, incubated for 12 h with $FeCl_3$, Na_2S and β-mercaptoethanol, and washed by ultrafiltration, the 1.2 ms optical transient due to the $P\text{-}700^+ F_X^-$ backreaction was completely replaced by the 30 ms transient characteristic of the $P\text{-}700^+ [F_A/F_B]^-$ backreaction (Fig. 2c).

The ESR spectra of these preparations are shown in Fig. 3. When the native-F_A/F_B holoprotein is rebound to the Photosystem I core protein, washed by ultrafiltration, and illuminated during freezing, both F_A and F_B become photochemically reduced (Fig. 3a). This spectrum is characterized by interaction between F_A and F_B which gives rise to g values of 2.05, 1.94, 1.92 and 1.89. When rebinding of the F_A/F_B apoprotein to the Photosystem I core protein is attempted (Fig. 3b) there is no evidence of photochemically-reduced F_A and F_B, thus confirming the denatured state of these clusters. However, when the F_A/F_B apoprotein is reconstituted with $FeCl_3$, Na_2S and β-mercaptoethanol in the presence of the Photosystem I core protein, washed by ultrafiltration, and illuminated during freezing, the characteristic spectrum of interacting F_A and F_B is recovered (Fig. 3c).

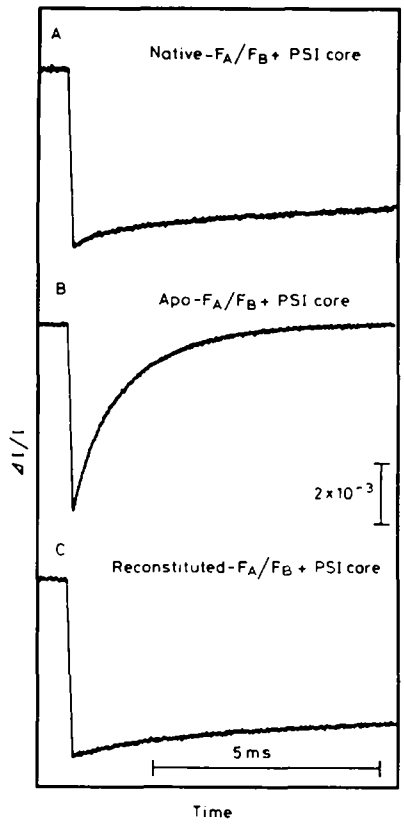

Fig. 2. Flash-induced absorption changes of P-700 after reconstitution of the F_A/F_B polypeptide in the presence of the isolated Photosystem I core protein. (A) Absorption transient in the Photosystem I core protein after rebinding of the native-F_A/F_B polypeptide. (C) Absorption transient in the Photosystem I core protein after attempted rebinding of the F_A/F_B apoprotein. (B) Absorption transient in the Photosystem I core protein after reconstitution of the F_A/F_B apoprotein with inorganic iron, sulfide and β-mercaptoethanol in the presence of the Photosystem I core protein followed by washing over a YM-100 ultrafiltration membrane. All measurements were performed as described in Fig. 1.

The kinetics of reconstitution for the F_A/F_B apoprotein in the presence of Photosystem I core protein, shown in Fig. 4, indicate that at a 10:1 molar ratio, the reconstitution follows biphasic kinetics; the reaction is approx. 60% complete in 50 min and 95% complete in 12 h. At a 20:1 molar ratio, the reaction is nearly 95% complete in 60 min (compare 10:1 and 20:1 ratios in Fig. 4). However, if the F_A/F_B apoprotein is incubated separately with inorganic iron and sulfide in the presence of β-mercaptoethanol for 12 h and the Photosystem I core protein is then added, there is a much faster (less than 2 min) rate of restoration of electron flow from P-700 to F_A/F_B (data not shown). This would indicate that the rate-limiting step is the assembly of the artificial iron-sulfur clusters and/or the insertion of the clusters into the F_A/F_B apoprotein and not the rebinding of the F_A/F_B holoprotein to the Photosystem I core protein. In the absence of Na_2S, but in the presence of $FeCl_3$ and β-mercaptoethanol, the yield of reconstitution is 27%. When an iron-sulfur protein is treated with 2 M or 4 M urea in the presence of an oxidant, the bound iron-sulfur clusters become oxidized to the level of zero-valence sulfur [31,33,34]. Although the iron is lost, the sulfur remains bound to the cysteine residues in the form of [CysS–S^0–S^0–SCys]. Addition of a nucleophile, such as dithiothreitol or β-mercaptoethanol, results in scission of the sulfur–sulfur bond and reduction of the sulfur to the level of sulfide; this is presumably the source of sulfide

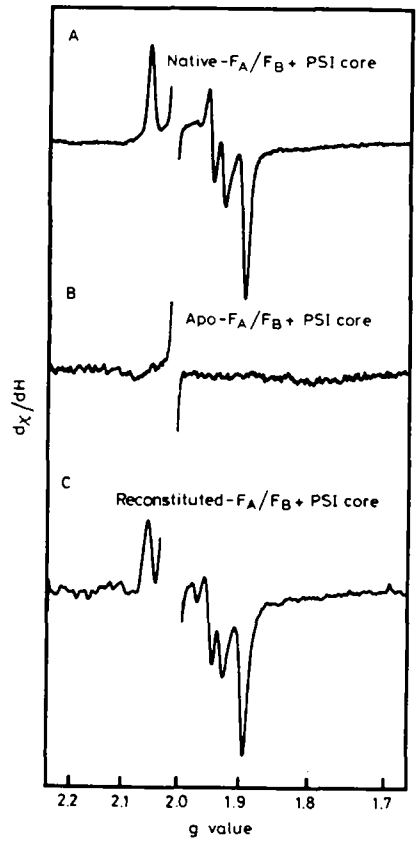

Fig. 3. ESR spectra after reconstitution of the F_A/F_B polypeptide in the presence of the isolated Photosystem I core protein. The samples were illuminated during freezing. (A) Light-minus-dark ESR spectrum of the native-F_A/F_B polypeptide after rebinding to the Photosystem I core protein. (B) Light-minus-dark ESR spectrum of the F_A/F_B apoprotein after attempted rebinding to the Photosystem I core protein. Note that F_X is not observed under these conditions of temperature, microwave power and amplitude modulation. (C) Light-minus-dark ESR spectrum of the reconstituted-F_A/F_B polypeptide after reconstitution in the presence of the Photosystem I core protein followed by washing over a YM-100 ultrafiltration membrane. The spectra were resolved by subtracting the light-off (before light-on) from the light-on spectrum and digitally smoothed with a 2.5 G window. The spectrum in A was determined at 500 μg Chl/ml and amplified 3-fold in software. The spectra in B and C were determined at 150 μg Chl/ml and amplified 10-fold in software. The $g = 2.0026$ region containing $P\text{-}700^+$ was deleted for reasons of clarity. The samples were suspended in 50 mM Tris buffer (pH 8.3), containing 1 mM sodium ascorbate and 0.3 mM DCPIP. Spectrometer conditions: temperature, 16 K; microwave power, 20 mW; microwave frequency, 9.128 GHz; receiver gain, $5.0\cdot10^3$; modulation amplitude, 10 G at 100 kHz.

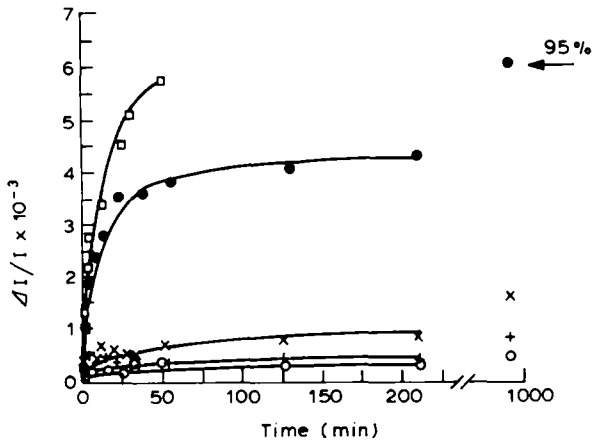

Fig. 4. Time-course of recovery of electron flow from P-700 to F_A/F_B during iron-sulfur cluster reconstitution. The 95% indicates 95% reconstitution. The reconstitution was performed by anaerobic incubation of a 10:1 ratio of the F_A/F_B apoprotein to the purified Photosystem I core protein with: 0.15 mM $FeCl_3$ and NaS_2, (●); 0.15 mM $FeCl_3$, (×); 0.15 mM Na_2S, (+); no $FeCl_3$ or Na_2S, (○); 0.15 mM $FeCl_3$ and with NaS_2 at a 20:1 ratio of the F_A/F_B apoprotein to the purified Photosystem I core protein, (□). In all cases, 0.5% β-mercaptoethanol was present. The recovery of the flash-induced, room temperature 30 ms backreaction between $P\text{-}700^+$ and $[F_A/F_B]^-$ served as the criterion of reconstitution. The analysis was conducted in the optical cuvette in the presence of the reconstitution reagents, and without further purification.

for the limited reconstitution. In the absence of $FeCl_3$, the yield of reconstitution is only 13%, and in the absence of both $FeCl_3$ and Na_2S, the yield is less than 7%. The source of iron is either contaminating iron or adventitously-bound iron derived from the chaotrope-induced denaturation of the F_A/F_B clusters. The exchange ligand β-mercaptoethanol is required for reconstitution under all conditions, and we observe no higher rates or yields when the reconstitution is performed in the presence of 2 M urea or increased iron or sulfide concentrations.

The polypeptide composition of the Photosystem I complex from *Synechococcus* 6301, determined by SDS-PAGE, is shown in Fig. 5a. The major polypeptides include the 82.4 and 83 kDa psaA and psaB proteins, which run together as a broad band centered at 55 kDa, and two low molecular mass polypeptides at 16.4 kDa and 8.9 kDa. The latter corresponds to the F_A/F_B, psaC protein, and the former corresponds to the ferredoxin-docking, psaD protein based on its molecular mass, its relative position in the gel, and on the known heavy staining with Coomassie brilliant blue R-250 (Bryant, D., personal communication). In addition, there are about three minor polypeptides at 7.5 kDa, 13.4 kDa and 17.2 kDa (the former is resolved in partially depleted complexes; see Fig. 5b and Ref. 10). The F_X-containing Photosystem I core protein, when isolated with 6.8 M urea, is composed primarily of the 82.4 and 83 kDa, psaA and psaB proteins (Fig. 5b). However, under these less stringent treatment conditions (urea vis à vis NaI; see Refs. 10, 27), two of the minor polypeptides at 13.4 and 17.2 kDa are also present. The low molecular mass polypeptides, which have been removed from the Photosystem I core protein by treatment with 6.8 M urea, are recovered in the YM-100 filtrate and can be concentrated over a YM-5 membrane (Fig. 5c). Surprisingly, this fraction contains a larger number of low molecular mass polypeptides than are resolved in the control Photosystem I complex (Fig. 5a), and we suspect that the heavily stained protein band located between the stacking and resolving gels (not shown) contains these missing polypeptides. While this problem will be addressed in a separate work, it is sufficient for the purpose of this study that the 8.9 kDa and 16.4 kDa polypeptides are totally removed by treatment with 6.8 M urea, and are clearly resolved in the

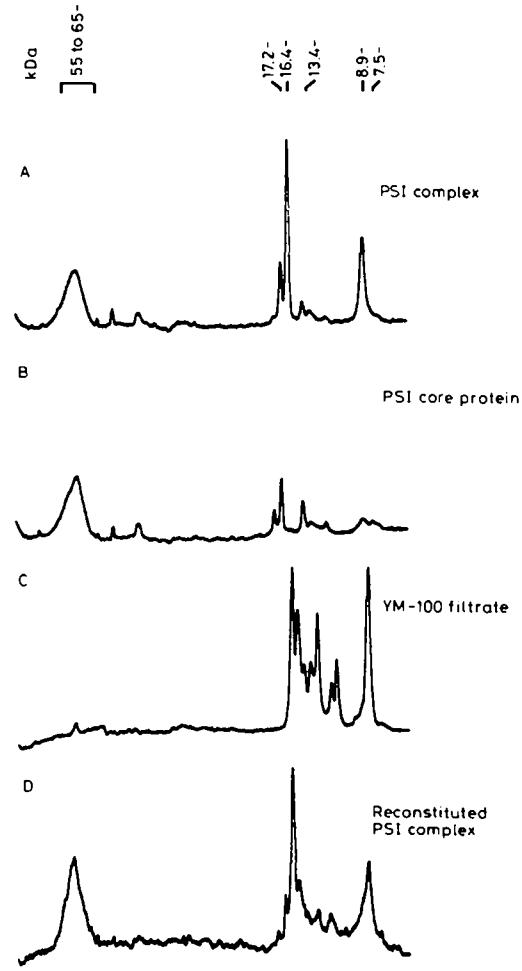

Fig. 5. Laser-densitometric tracings of SDS-PAGE after resolution and reconstitution of the Photosystem I reaction center. (A) Photosystem I complex. (B) Photosystem I core protein. (C) YM-100 filtrate obtained after chaotropic treatment of the Photosystem I complex. (D) Reconstituted Photosystem I complex produced by anaerobic incubation of the Photosystem I core protein with the YM-100 filtrate in the presence of inorganic iron, sulfide and β-mercaptoethanol, followed by washing over a YM-100 ultrafiltration membrane.

control Photosystem I complex (Fig. 5a). When the Photosystem I complex is reconstituted by adding the YM-100 filtrate to the Photosystem I core protein in the presence of Na_2S, $FeCl_3$ and β-mercaptoethanol under anaerobic conditions, the 8.9 and 16.4 kDa polypeptides rebind in approximately the same molar ratio as the control (Fig. 5d). Repeated ultrafiltration over a YM-100 membrane in the presence of 0.1% Triton X-100 does not change the pattern of rebound polypeptides. We observe no rebinding of the 8.9 kDa polypeptide when the F_A/F_B clusters are in the denatured state. Studies are now underway with isolated and purified proteins to determine if there is an order-of-addition that must be followed in the binding of the remaining low molecular mass polypeptides.

Properties of the reconstituted-F_X iron-sulfur cluster

The successful reconstitution of the F_A/F_B iron-sulfur clusters prompted us to attempt reconstitution of the F_X iron-sulfur cluster. Fig. 6 shows the 1.2 ms optical transient in the *Synechococcus* Photosystem I core protein resulting from the backreaction between $P\text{-}700^+$ and F_X^- in a reaction center deficient in the F_A/F_B polypeptide. When the F_X iron-sulfur cluster is oxidatively-denatured by treatment with 3 M urea and 5 mM potassium ferricyanide for 2 h, the 1.2 ms optical transient is largely lost on the time-scale of the measurement (Fig. 6b). However, when analyzed at a faster digitizing rate (Fig. 7a), a 3 μs optical transient was found with a magnitude nearly identical to the control (compare, Fig. 6a). Since the half-time slows to 600 μs at 77 K (Fig. 7b), we conclude that this transient is due to relaxation of the P-700 triplet formed from a backreaction of $P\text{-}700^+$ with an acceptor earlier than F_X [36,37]. Incubation of the apo-F_X photosystem I core protein with $FeCl_3$, Na_2S and β-mercaptoethanol results in a time-dependent transition from the 3 μs optical transient to the 1.2 ms optical transient characteristic of the $P\text{-}700^+ F_X^-$ backreaction (Fig. 6c).

The ESR spectra of the native-F_X, apo-F_X and reconstituted-F_X Photosystem I core proteins are shown in Fig. 8. The light-induced spectrum of native-F_X (Fig. 8a) has ESR resonances at $g = 2.05$, 1.86 and 1.78, and is similar to that described earlier [8–10,28]. Fig. 8b shows that after oxidative denaturation, less than 15%

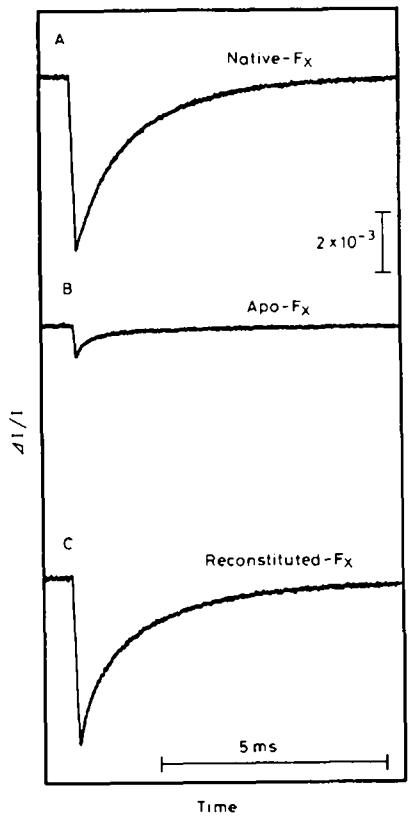

Fig. 6. Flash-induced absorption changes of P-700 in the Photosystem I core protein on a ms timescale. (A) Absorption transient in the native-F_X Photosystem I core protein. (B) Absorption transient in the apo-F_X Photosystem core protein prepared by oxidative denaturation with 3 M urea and 5 mM potassium ferricyanide, followed by washing over a YM-100 ultrafiltration membrane. Studies at a faster digitizing rate showed that the P-700 absorption transient has approximately the same magnitude as the native Photosystem I core protein but relaxes with a half-time of 3 μs (see Fig. 7a). (C) Absorption transient in the reconstituted-F_X Photosystem I core protein prepared by anaerobic incubation of apo-F_X with inorganic iron and sulfide in the presence of β-mercaptoethanol, followed by washing over a YM-100 ultrafiltration membrane.

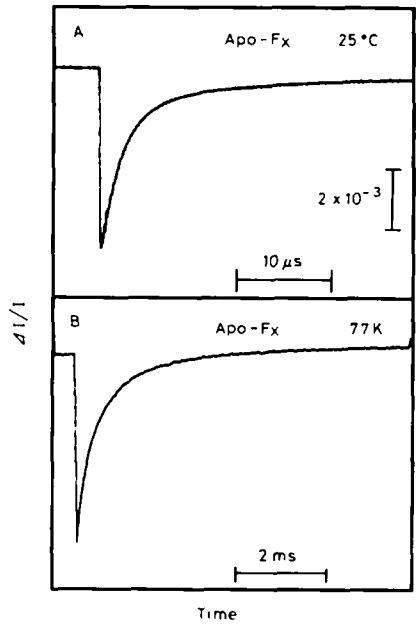

Fig. 7. Flash-induced absorption changes of P-700 in the Apo-F_X Photosystem I core protein on a microsecond timescale. (A) Absorption transient in the apo-F_X Photosystem I core protein at 25 °C (note the faster digitizing rate as compared to Fig. 6b). (B) Absorption transient in the apo-F_X Photosystem core protein at 77 K. The measurements in (A) were performed at 5 μg Chl/ml in Tris buffer (pH 8.3) containing 0.05% β-mercaptoethanol and 0.033 mM PMS. The 77 K sample contained 60% glycerol and 1.7 mM ascorbate in place of β-mercaptoethanol.

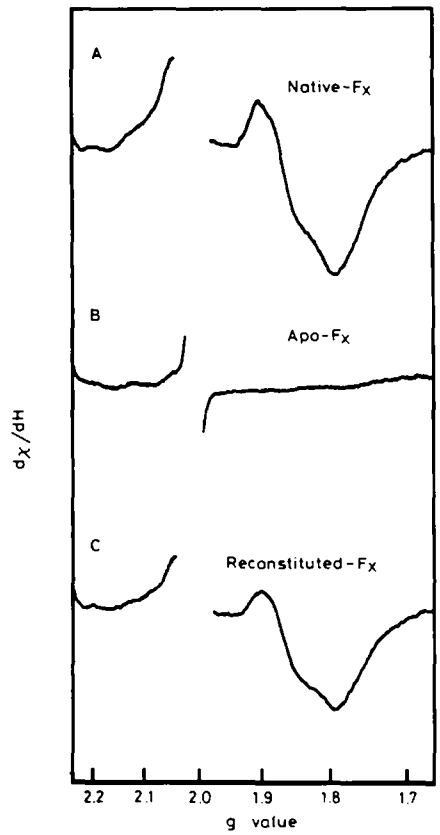

Fig. 8. ESR spectra after reconstitution of F_X in the Photosystem I core protein. The samples were illuminated during freezing. (A) Light-minus-dark ESR spectrum of native-F_X. (B) Light-minus-dark ESR spectrum of apo-F_X. (C) Light-minus-dark ESR spectrum of reconstituted-F_X. The samples were suspended in 50 mM Tris (pH 8.3), containing 1 mM ascorbate and 0.3 mM DCPIP at 500 μg Chl/ml. The spectra were resolved by subtracting the light-off (before light-on) from the light-on spectrum, amplifying 3.5-fold in software, and digitally smoothed with a 3.5 G window. The $g = 2.0026$ region containing P-700$^+$ has been deleted for reasons of clarity. Spectrometer conditions: temperature, 6 K; microwave power, 40 mW; microwave frequency, 9.101 GHz; receiver gain, $5 \cdot 10^3$; modulation amplitude, 40 G at 100 kHz.

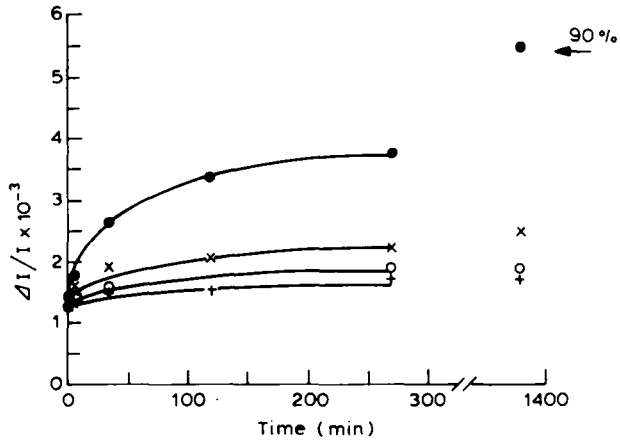

Fig. 9. Time-course of recovery of electron flow from P-700 to F_X during iron-sulfur cluster reconstitution. The 90% refers to 90% reconstitution. The reconstitution was performed by anaerobic incubation of the apo-F_X Photosystem I core protein with: 0.15 mM $FeCl_3$ and NaS_2, (●); 0.15 mM $FeCl_3$, (×); 0.15 mM Na_2S, (+); no $FeCl_3$ or Na_2S, (○). In all cases, 0.5% β-mercaptoethanol was present. The recovery of the flash-induced, room temperature 1.2 ms backreaction between P-700$^+$ and F_X^- served as the criterion of reconstitution. The analysis was conducted in the optical cuvette in the presence of the reconstitution reagents and without further purification.

of light-inducible F_X is present, which is in general agreement with the optical determination of apo-F_X shown in Fig. 6b. After reconstitution with $FeCl_3$, Na_2S and β-mercaptoethanol, more than 80% of the ESR spectrum of F_X is recovered (Fig. 8c).

In the presence of iron, sulfide and β-mercaptoethanol, F_X is reconstituted within 1200 min and with a typical yield of 70–95% (Fig. 9; note that 20% residual F_X is present in this preparation). When the reconstitution is performed in the absence of added sulfide, the protein-bound zero-valence sulfur serves as the ultimate source of sulfide, and results in a yield of 10% in addition to the residual F_X. Reconstitution in the absence of added iron results in a yield of 5% in addition to the residual F_X, in which cases the iron is probably derived from non-specifically bound iron. The exchange ligand β-mercaptoethanol is required for reconstitution under all conditions, and we observe no higher rates or yields when the reconstitution is performed in the presence of 2 M urea or increased iron or sulfide concentrations. Attempts to reconstitute the F_X iron-sulfur cluster in the SDS-CP1 preparation [38] have been unsuccessful (data not shown). We have also been unsuccessful in reconstituting the F_X iron-sulfur cluster in the apo-F_X Photosystem I core protein after SDS-treatment identical to the SDS-CP1 protocol.

Table I shows the non-heme iron and acid-labile sulfide content of the various Photosystem I preparations. The Photosystem I complex, which includes iron-sulfur clusters F_X, F_B and F_A, contains 13.6 mol of non-heme iron and 12.0 mol of acid-labile sulfide per mol of P-700, and is consistent with the [4Fe-4S] iden-

TABLE I

Non-heme iron and sulfur content of the various Photosystem I preparations

	NHI/P-700	S^{2-}/P-700	S^0/P-700	Chl/P-700
PSI complex	13.6	12.0	–	115
PSI core protein	6.0	4.8	–	115
Apo-F_X	2.2 [a]	0.3	3.2	115
Reconstituted-F_X	80 [b]	24 [b]	–	115
SDS-CP1	–	0.3 [c]	3.8 [c]	110

[a] After dialysis against Tiron.
[b] See text.
[c] From Ref. 7.

tity of the three iron-sulfur clusters. The Photosystem I core protein, which includes only iron-sulfur center F_X, contains 6.0 mol of non-heme iron and 4.8 mol of acid-labile sulfide per mol of P-700, and is consistent with its identity as a single [4Fe-4S] cluster [26,27]. Since the Photosystem I core protein is now considered to consist of only two polypeptides (the *psaA* and *psaB* gene products), the cluster identity of F_X and polypeptide stoichiometries of the *psaA* and *psaB* gene products converge to indicate that F_X consists of only one [4Fe-4S] cluster per reaction center. The apo-F_X containing Photosystem I core protein contains 0.3 mol of acid-labile sulfide and 2.2 mol of non-heme iron. This remaining approx. 2 mol of adventitious iron which is present in the Photosystem I complex and the Photosystem I core protein is difficult to remove, and may account for the partial reconstitution of the iron-sulfur cluster F_X or F_A/F_B in the absence of added iron. The addition of 1 mM dithiothreitol to the apo-F_X containing Photosystem I core protein prior to sulfide analysis shows that the ratio of zero-valence sulfur to P-700 is 3.2. This is most likely the source of sulfur for the partial reconstitution of the F_X iron-sulfur cluster in the absence of added sulfide. It is also similar to the zero-valence sulfur content of SDS-CP1. The content of non-heme iron and acid-labile sulfide after reconstitution were both significantly greater than 12 mol per mol of P-700; most likely artificial iron-sulfur clusters and/or adventitiously bound iron (and sulfide) account for the excess. The extra iron must bind very tightly, since it remains attached to the reconstituted Photosystem I complex after repeated dialysis against iron chelators. Finally, the retention of approx. 115 Chl/P-700 throughout the course of the resolution/reconstitution studies indicates that the reaction center chlorophylls remain unaffected by the treatment protocol.

Reconstitution of the Photosystem I complex from the F_A/F_B apoprotein and the apo-F_X Photosystem I core protein

As a final demonstration of reconstitution in *Synechococcus*, we combined the F_A/F_B apoprotein (present in the YM-100 filtrate) with apo-F_X (derived from the oxidative denaturation of the Photosystem I core protein) in a 10 : 1 ratio and attempted reconstitution of all three iron-sulfur clusters. We found that addition of $FeCl_3$, Na_2S and β-mercaptoethanol to a mixture of the apo-F_X Photosystem I core protein and the F_A/F_B apoprotein resulted in reconstitution of electron flow from P-700 to F_A/F_B, measured by optical flash-photolysis (data not shown). The time-course and yield of the reconstitution was similar to that of the rebuilding of F_X in the Photosystem I core protein alone. This overall reconstitution indicates that quantitative reinsertion of the F_X iron-sulfur cluster probably occurs prior to rebinding of the 8.9 kDa polypeptide, and may influence the ability of the F_A/F_B holoprotein to bind tightly to the Photosystem I core protein.

Discussion

We have shown that the Photosystem I reaction center can be resolved into its most basic components: (i) the Photosystem I core protein containing the psaA and psaB polypeptides and the electron transfer components P-700, A_0, (A_1 by inference), and F_X (or if desired apo-F_X), and (ii) the 8.9 kDa, F_A/F_B apoprotein. Most importantly, these individual components can now be reconstituted and reassembled to produce a functional and intact Photosystem I complex. Reconstitution and reassembly of the F_A and F_B iron-sulfur clusters are indicated by: (i) the in situ regeneration of the 30 ms optical transient between $P\text{-}700^+$ and $[F_A/F_B]^-$ after anaerobic incubation of the chaotrope-treated Photosystem I complex with inorganic iron and sulfide in the presence of β-mercaptoethanol, (ii) restoration of the 30 ms optical transient between $P\text{-}700^+$ and $[F_A/F_B]^-$ after anaerobic incubation of the combined YM-100 filtrate and the Photosystem I core protein with inorganic iron and sulfide in the presence of β-mercaptoethanol, (iii) regeneration of the characteristic light-induced ESR spectrum of interacting F_A and F_B after anaerobic incubation of the combined YM-100 filtrate and the Photosystem I core protein with inorganic iron and sulfide in the presence of β-mercaptoethanol, and (iv) SDS-PAGE indicating the rebinding of the 8.9 kDa polypeptide to the Photosystem I core protein after anaerobic incubation with inorganic iron and sulfide in the presence of β-mercaptoethanol.

Reconstitution of soluble apoferredoxins has been reported with yields typically greater than 60% (i.e., 60% [39], 75% [40], 70–90% [30], and approx. 100% [32]). This is in general agreement with the yields we obtain for F_A/F_B and F_X. However, since reconstitution of F_A and F_B is concurrent with rebinding to the Photosystem I core protein, an interesting situation arises. In situ reconstitution with equimolar ratios of Photosystem I core protein and F_A/F_B apoprotein (both at 50 pmol/ml) results in a yield of approx. 70%, whereas reconstitution with 10-fold molar excess F_A/F_B apoprotein results in a yield approaching 100%. Furthermore, SDS-PAGE analysis after incubation of the YM-100 filtrate with the Photosystem I core protein in the absence of $FeCl_3$, Na_2S and β-mercaptoethanol followed by ultrafiltration shows no rebinding of the 8.9 kDa polypeptide. This indicates that the binding affinity for the Photosystem I core protein is extremely high in the presence of the F_A/F_B clusters but low in the absence of intact clusters. Perhaps the presence of intact iron-sulfur clusters confers a favorable charge or conformation to the F_A/F_B protein which allows interaction with the Photosystem I core protein. While the

F_A/F_B protein can be reconstituted apart from the Photosystem I core protein [41], the iron-sulfur clusters in the isolated protein are extremely sensitive to heat and/or oxygen. However, when the F_A/F_B holoprotein is rebound to the Photosystem I core protein, the iron-sulfur clusters become as stable as the control preparation. We suspect that the rebuilding of the F_A/F_B clusters reported in this paper is, in part, driven by the efficient rebinding of the F_A/F_B holoprotein to the Photosystem I core protein.

With the successful reconstitution of the F_A and F_B iron-sulfur clusters, we questioned whether the F_X iron-sulfur cluster within the Photosystem I core protein could also be reconstituted. This involved first the oxidative denaturation of F_X with 3 M urea and 5 mM potassium ferricyanide to produce a reaction center core containing the psaA and psaB polypeptides and electron transfer components P-700, A_0 and A_1. Optical analysis of this minimal reaction center shows a 3 μs, room temperature optical transient and a 600 μs, 77 K optical transient diagnostic of the relaxation of the P-700 triplet state. However, the room-temperature quantum yield of P-700 triplet formation is 0.96 in the apo-F_X preparation (Fig. 7a) vs. 0.58 in the SDS-CP1 preparation (data not shown). The former quantum yield is therefore significantly higher than the 0.3 [42] to 0.8 [43] yield observed resulting from the $P\text{-}700^+ A_0^-$ backreaction. Recently, the $P\text{-}700^+ A_1^-$ backreaction has been reported to occur through the triplet state of P-700 with a quantum efficiency greater than 0.9 [44]. Perhaps the F_X denaturation protocol preserves electron flow to A_1; certainly reconstitution of electron flow to F_X presupposes an intact and functional A_1. Reconstitution of the apo-F_X containing Photosystem I core protein after anaerobic incubation with $FeCl_3$ and Na_2S in the presence of β-mercaptoethanol is shown by: (i) restoration of the 1.2 ms charge recombination between $P\text{-}700^+$ and F_X^- and (ii) regeneration of the characteristic light-induced ESR spectrum of F_X.

We have also attempted the reconstitution of F_X in the SDS-CP1 preparation, but without success. The SDS-CP1 reaction center preparation (also known as P-700-Chl-*a*-protein or Complex I) has been extensively studied and is considered the most basic functional Photosystem I preparation (for a more thorough discussion see Ref. 2). It is composed of the 82 and 83 kDa reaction center core polypeptides and contains the electron transfer components P-700 and A_0. Phylloquinone is found in this preparation but the photoreduction of A_1 has not been observed. One explanation for our lack of success in reconstituting CP1 is that SDS-treatment damages the reaction center in a manner which prevents reconstitution of the F_X iron-sulfur cluster. A good possibility is that SDS may bind to the 'pocket' that once contained the F_X cluster and may not be easily displayed by an incoming artificial iron-sulfur cluster. Indeed, the presence of SDS precludes formation of the F_X iron-sulfur cluster in the Photosystem I core protein. It should be noted that the presence of Triton X-100 at concentrations of 0.05% or greater during the reconstitution protocol also prevents reconstitution of F_X in the Photosystem I core protein (data not shown). A second explanation is that since the secondary electron acceptor A_1 has not been observed to be photoreducible in SDS-CP1, electron transfer might not occur even after the reconstitution of F_X. In this case reconstitution of a functional A_1 may be required. In any event, this result should be considered preliminary and we are continuing to investigate this problem.

Another relevant issue is the number of low-molecular-mass polypeptides in the cyanobacterial Photosystem I complex and Photosystem I core protein. Our analysis by SDS-PAGE of the *Synechococcus* Photosystem I complex had indicated only about 5 low molecular mass subunits, while Wynn et al. [45] have identified as many as seven low molecular mass polypeptides in cyanobacterial Photosystem I complexes. Our current assessment of the number of low-molecular-mass polypeptides released with 6.8 M urea from *Synechococcus* Photosystem I is consistent with the presence of at least seven polypeptides. We had thought previously that either cyanobacteria contained fewer low-molecular-mass subunits than higher plants [10], or that several of the low-molecular-mass subunits became partially depleted during the course of purification [28]. We now suspect that as the ionic detergent SDS begins to unfold the hydrophobic reaction center polypeptides, the latter entrap some of the low-molecular-mass polypeptides in a cross-linked or entangled matrix that cannot enter the resolving gel. This is suggested by the finding that when the low-molecular-mass polypeptides are dissociated from the reaction center polypeptides with urea and separated by ultrafiltration, no material remains unresolved between the stacking and resolving gels (not shown). The entrapment of low-molecular-mass polypeptides by the unfolding reaction center polypeptides could be the reason for the observed variability in the stoichiometry and number of Photosystem I polypeptides between cyanobacterial and higher plant reaction centers (see Ref. 11).

In conclusion, the reconstitution protocol outlined here allows complete experimental control over iron-sulfur cluster denaturation and reconstitution in both the Photosystem I core protein and the F_A/F_B polypeptide, and rebinding of the reconstituted-F_A/F_B polypeptide to the native- or reconstituted-Photosystem I core protein to yield the intact Photosystem I complex. It is significant that the Photosystem I complex is fully functional in electron flow from P-700 to F_A/F_B at both cryogenic and room temperatures. Studies which include chemical or genetic modification of the apoproteins followed by cluster reconstitution can now

be considered prior to in vitro reassembly of the Photosystem I complex.

Acknowledgments

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