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Reconstitution of the photosystem I complex from the P700 and F_X-containing reaction center core protein and the F_A/F_B polypeptide

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Complete restoration of electron flow from P700 to F_A/F_B was achieved by incubating a P700 and F_X-containing photosystem (PS) I core protein from *Synechococcus* sp. 6301 with the 8.9 kDa, F_A/F_B polypeptide from spinach. The ESR spectrum of the reconstituted PS I complex shows nearly equal photochemical reduction of F_A and F_B when frozen in darkness and illuminated at 16 K. When illuminated during freezing, both F_A and F_B are quantitatively reduced and the spectrum is nearly indistinguishable from F_A and F_B in the control PS I complex. In the reconstituted PS I complex F_X is photochemically reduced only in the presence of $F_{\bar{A}}$ and $F_{\bar{B}}$, and the high-field resonance appears indistinguishable from F_X in the control PS I complex. Optical flash photolysis after extensive washing confirms the complete restoration of the $P700^+$ $[F_A/F_B]^-$back-reaction, indicating quantitative rebinding of the 8.9 kDA polypeptide. This procedure represents the first reconstitution of the PS I complex from a purified PS I core protein and an isolated 8.9 kDa, F_A/F_B polypeptide, and makes possible independent manipulation of the two subunits that carry the entire electron acceptor system of PS I.

Photosystem I; Reaction center; Reconstitution; Iron-sulfur center; Core protein; F_A/F_B protein

1. INTRODUCTION

The PS I complex of plants and cyanobacteria is a chlorophyll-containing, light-driven, plastocyanin:ferredoxin oxidoreductase. The bound electron-transport components consist of a chlorophyll primary electron donor, P700, a chlorophyll primary electron acceptor, A_0, a quinone intermediate electron acceptor, A_1, and three iron-sulfur centers, F_X, F_B, and F_A (reviews [1–3]). According to current understanding, a singlet exciton migrates from an antenna chlorophyll to the reaction center trap, bringing about charge separation between P700 and A_0. The electron is then passed through the intermediate acceptors A_1 and F_X to the terminal electron acceptors, F_B/F_A.

The polypeptide location of these electron-transport components is now known: A_1 [4–6] and F_X ([7–9], see also [10,11]) are associated with the P700 and A_0-containing [12] reaction center proteins, and F_A and F_B are located on a peripheral 8.9 kDa polypeptide [13,14]. In a recent series of papers we showed that chaotropic agents, such as urea, NaI, NaBr, $NaClO_4$ and NaSCN were ex-

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Abbreviations: PS, photosystem; Chl, chlorophyll; DCPIP, 2,6-dichlorophenolindophenol

Definitions: 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 heterodimer of psaA and psaB isolated from the photosystem I complex with chaotropes, containing P700 and acceptors A_0, A_1 and F_X. F_A/F_B polypeptide: 8.9 kDa polypeptide isolated by acetone precipitation, containing iron-sulfur centers F_A and F_B. Reconstituted photosystem I complex: photosystem I core protein containing rebound F_A/F_B polypeptide

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tremely effective in removing the low molecular mass proteins, including the F_A/F_B polypeptide, from a cyanobacterial PS I complex [15,16]. In the resulting PS I core protein, transient charge separation and recombination occurred between P700 and iron-sulfur center F_X. At about the same time, Wynn and Malkin [14] confirmed an earlier procedure [13] for the purification of the 8.9 kDa polypeptide that carries the electron acceptors F_A and F_B. The isolated polypeptide showed an ESR spectrum characteristic of an iron-sulfur cluster, but with significant differences from the spectrum found in an intact PS I complex.

Here, we report that the PS I complex can be reconstituted from the isolated PS I core protein from *Synechococcus* sp. 6301 and the isolated F_A/F_B polypeptide from spinach. Electron flow from P700 to F_A/F_B at both 16 K and room temperature is completely restored, and F_X photoreduction occurs only in the presence of prereduced F_A^- and F_B^-. Most significantly, the ESR spectra of F_A, F_B and F_X revert to their original characteristics and are nearly indistinguishable from a control PS I complex.

2. MATERIALS AND METHODS

The PS I complex containing the full complement of electron acceptors (A_0 A_1 F_X F_A F_B) was isolated from *Synechococcus* sp. 6301 (*Anacystis nidulans* TX-20) membranes [15]. Treatment with 6.8 M urea or 2 M NaI results in loss of the low molecular mass polypeptides and in purification of the PS I core protein containing only the reaction center heterodimer psaA and psaB, and the electron acceptors A_0A_1 and F_X [15,16]. The F_A/F_B polypeptide was isolated according to [13,14] except that excess dithionite was present throughout the isolation procedure. The reconstitution protocol consisted of mixing the PS I core with the F_A/F_B polypeptide in an approx. 1:1 molar ratio and incubation in the presence of 0.1% β-mercaptoethanol for 3 min.

Chlorophyll concentration was determined in 80% acetone [17]. Flash-induced absorption transients were determined at 698 nm [15] and ESR studies were performed on a Varian E-109 spectrometer as described in [8].

3. RESULTS

3.1. *Characterization of the reconstituted PS I complex by ESR spectroscopy*

The ESR spectrum of the PS I core protein from *Synechococcus* sp. 6301 is shown in fig.1A. The absence of ESR resonances characteristic of F_A and F_B confirms the loss of the low molecular mass

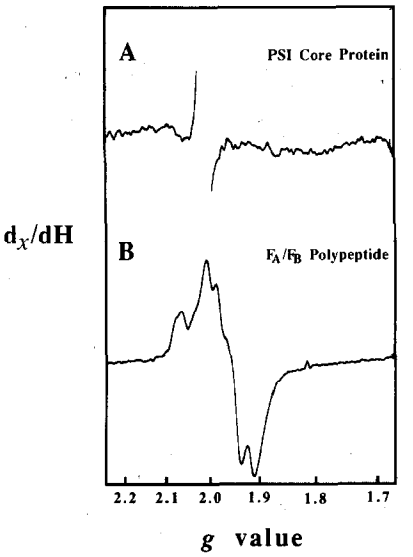

Fig.1. ESR spectrum of the *Synechococcus* sp. 6301 PS I core protein and the spinach F_A/F_B polypeptide. (A) Spectrum of the PS I core protein after illumination at 16 K. The sample was suspended in 50 mM Tris, pH 8.3, containing 1 mM ascorbate and 0.3 mM DCPIP at 500 μg/ml Chl. The spectrum was resolved by subtracting the light-off from the light-on spectrum and amplifying 50-fold in software. The broad dip in the high-field region represents the weak signature of F_X under conditions optimal for observation of F_A and F_B. (B) Spectrum of the F_A/F_B polypeptide after 4 min incubation with sodium dithionite and 0.033 mM methyl viologen in 0.1 M glycine, pH 10.0. The spectrum was resolved by subtracting the oxidized from the (chemically) reduced spectrum and amplifying 12-fold in software. Spectrometer conditions: temperature, 16 K; microwave power, 20 mW; microwave frequency, 9.140 GHz; receiver gain, 5.0×10^3; modulation amplitude, 10 G at 100 kHz.

polypeptides, including the 8.9 kDa, F_A/F_B polypeptide [15,16], from the cyanobacterial reaction center (the broad, shallow resonance of F_X is barely visible under the conditions optimal for observation of iron-sulfur centers F_A and F_B; see fig.4A). The ESR spectrum of the chemically reduced 8.9 kDa, F_A/F_B polypeptide from spinach is shown in fig.1B. When compared with the control PS I complex, the resonances of F_A and F_B are significantly broader, and all but the g_x resonances are shifted to a slightly lower field (cf. fig.3B).

When the PS I core protein is incubated for 3 min at an approx. 1:1 molar ratio with the unreduced F_A/F_B polypeptide and frozen in darkness, the low-temperature photoreduction of F_A (g = 2.056, 1.956, 1.872) and F_B (g = 2.072,

1.934, 1.892) is observed with nearly equal spin concentrations (fig.2A). In contrast, when the control PS I complex is illuminated under identical conditions, iron-sulfur center F_A (g = 2.056, 1.949, 1.865) is predominantly photoreduced (fig.2B). There are additional small differences in the peak positions, but the most significant feature is that the spectrum of the reconstituted F_A/F_B polypeptide has narrowed and appears quite similar to the control. The integrated signal size induced on illumination at 16 K is the same in the reconstituted PS I complex as in the control PS I complex. This and the absence of diffusion-controlled reactions at 16 K suggest that the F_A/F_B polypeptide is rebound to the reaction center core.

When the reconstituted PS I complex is illuminated during freezing, the low- and high-field resonances of F_A (g = 2.056, 1.949, 1.899) and F_B (g = 2.056, 1.934, 1.899) merge and show the characteristic interaction between the two clusters (fig.3A). When the control PS I complex is illuminated during freezing under identical conditions (fig.3B), the spectrum also shows the full extent of interacting iron-sulfur centers F_A (g = 2.048, 1.949, 1.892) and F_B (g = 2.048, 1.927, 1.892), but the peak positions are slightly shifted. The narrow F_A and F_B resonances (cf. figs 3A,1B) indicate that the majority of the 8.9 kDa polypeptide is not photoreduced 'in solution' but is rebound to the PS I core protein. However, the slight difference between the spectrum of the reconstituted PS I complex and the control PS I complex (cf. mid-field regions of fig.3A,B) may be due to a small contribution of 'free' photoreduced $F_{\bar{A}}/F_{\bar{B}}$ that may be present in excess over the PS I core protein (see also fig.3 in [13]).

A further indication of reconstitution can be found by observing the low-temperature behavior

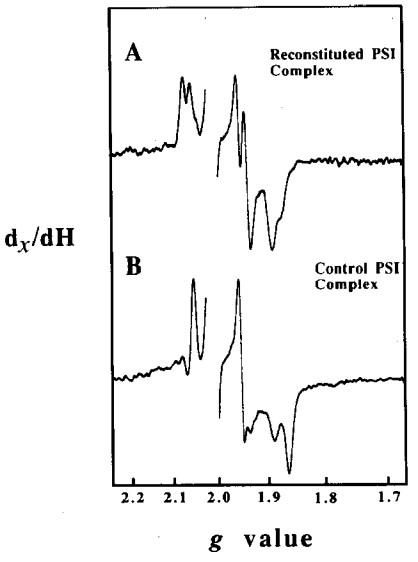

Fig.2. ESR spectrum of the reconstituted and the control PS I complexes after freezing in darkness and illumination at 16 K. (A) Light-minus-dark ESR spectrum after addition of the spinach F_A/F_B polypeptide to the *Synechococcus* sp. 6301 PS I core protein. The spectrum was resolved by subtracting the light-off (before light on) from the light-on spectrum and amplifying 50-fold in software. (B) Control PS I complex isolated from *Synechococcus* sp. 6301. The spectrum was resolved by subtracting the light-off (before light on) from the light-on spectrum and amplifying 30-fold in software. Both samples were suspended in 50 mM Tris buffer, pH 8.3, containing 1 mM sodium ascorbate and 0.3 mM DCPIP at 500 μg/ml Chl. Spectrometer conditions as in fig.1.

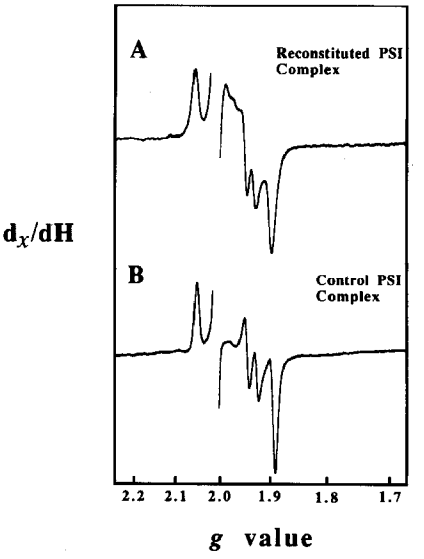

Fig.3. ESR spectrum of the reconstituted and the control PS I complexes after illumination during freezing. (A) Light-minus-dark ESR spectrum after addition of the spinach F_A/F_B polypeptide to the *Synechococcus* sp. 6301 PS I core protein. The spectrum was resolved by subtracting the light-off (before light-on) from the light-on spectrum and amplifying 15-fold in software. (B) Control PS I complex isolated from *Synechococcus* sp. 6301. The spectrum was resolved by subtracting the light-off (before light-on) from the light-on spectrum and amplifying 6-fold in software. Both samples were suspended in 50 mM Tris buffer, pH 8.3, containing 1 mM sodium ascorbate and 0.3 mM DCPIP at 500 μg/ml Chl. Spectrometer conditions as in fig.1.

of iron-sulfur center F_X. The ESR spectrum of the PS I core protein after freezing in darkness with sodium ascorbate and DCPIP at pH 8.3 and illumination at 6 K shows the characteristic g values (2.05?, 1.876, 1.782) and broader resonances typical of iron-sulfur center F_X in a reaction center deficient in F_A and F_B [8]. When the spectrum of the reconstituted PS I complex is measured under identical conditions, the low-temperature photoreduction of F_X does not occur (not shown). When the PS I core protein is illuminated during freezing in the presence of sodium ascorbate and DCPIP at pH 8.3, the full reduction of F_X occurs (fig.4A). However, the presence of sodium dithionite and methyl viologen at pH 10 is required, as in the control PS I complex, for photoreduction of F_X in the reconstituted PS I complex (fig.4B) (note that the

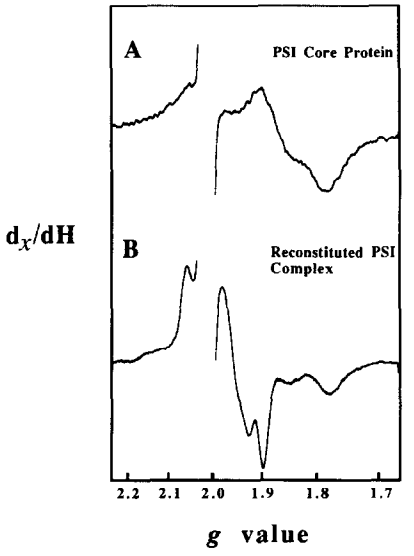

Fig.4. ESR spectrum of F_X in the PS I core protein and the reconstituted PS I complex after illumination during freezing. (A) Light-minus-dark ESR spectrum of the *Synechococcus* sp. 6301 PS I core protein. The sample was suspended in 50 mM Tris, pH 8.3, containing 1 mM ascorbate and 0.3 mM DCPIP at 500 μg/ml Chl. The spectrum was resolved by subtracting the light-off (before light-on) from the light-on spectrum and amplifying 5-fold in software. (B) Light-minus-dark ESR spectrum of the reconstituted PS I complex. The sample was incubated for 4 min with sodium dithionite and 0.033 mM methyl viologen in 0.1 M glycine, pH 10.0. The spectrum was resolved by subtracting the light-off (before light-on) from the light-on spectrum and amplifying 6-fold in software. Spectrometer conditions: temperature, 6 K; microwave power, 40 mW; microwave frequency, 9.100 GHz; receiver gain, 5×10^3; modulation amplitude, 40 G at 100 kHz.

F_A/F_B resonances are distorted under the conditions optimal for F_X). The ESR signal of F_X, especially in the high-field region, is indistinguishable from that of F_X in the untreated PS I complex (see [3]). This behavior is also consistent with efficient binding of the F_A/F_B polypeptide to the PS I core protein.

3.2. *Characterization of the reconstituted PS I complex by optical spectroscopy*

The flash-induced absorption transient at 698 nm in a PS I core protein from *Synechococcus* sp. 6301 is shown in fig.5A. The 1.2 ms optical absorption transient is typical of a reaction center in which F_A and F_B have been removed prior to flash photolysis [7]; under these conditions electron flow terminates at F_X. The P700 absorption transient after addition of the spinach F_A/F_B polypeptide is shown in fig.5B. The restoration of the ~30 ms optical transient occurs within the mixing time of 1 min and indicates that efficient electron flow from P700 to F_A/F_B has been reestablished. The

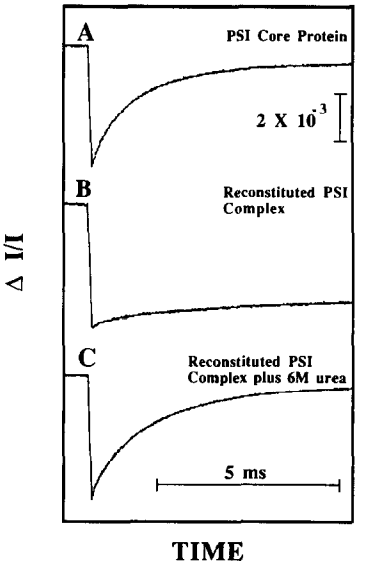

Fig.5. Flash-induced absorption changes in the PS I core protein and in the reconstituted PS I complex. (A) Absorption transient at 698 nm in the *Synechococcus* sp. 6301 PS I core protein. (B) Absorption transient 1 min after addition of the spinach F_A/F_B polypeptide. (C) Absorption transient 10 min after addition of 6 M urea to the reconstituted PS I complex. All measurements were performed at 5 μg/ml Chl in 50 mM Tris buffer, pH 8.3, containing 1.7 mM ascorbate and 0.033 mM DCPIP.

extent and kinetics of the ~30 ms transient remain intact despite extensive washing over an Amicon YM-100 ultrafiltration membrane, implying tight rebinding of the 8.9 kDa polypeptide. Addition of 6 M urea reversed the effect, causing restoration of the 1.2 ms, $P700^+ F_X^-$ back-reaction at the expense of the 30 ms, $P700^+ [F_A/F_B]^-$ back-reaction (fig.5C).

4. DISCUSSION

The protocol described here represents the first reconstitution of the PS I complex from the P700 and F_X-containing PS I core protein and the F_A/F_B polypeptide. The restoration of cryogenic and room-temperature electron flow from P700 to the terminal iron-sulfur clusters F_A/F_B, and the near-normal ESR spectrum of F_A and F_B in the reconstituted PS I complex indicate that the 8.9 kDa polypeptide and the PS I core protein reconstitute to their native configuration. It is important to note that the isolated F_A/F_B polypeptide shows a severely distorted ESR signal (fig.1B). This suggests that after isolation, the iron-sulfur clusters might be exposed to a hydrophilic environment, which may cause a structural alteration of F_A and/or F_B. The change is reversible, since we obtained completely normal ESR signals after reconstitution of F_A/F_B onto the PS I core protein (figs 2A,3A). This proves that the isolated F_A/F_B polypeptide is not denatured even though it has an abnormal ESR spectrum. The ESR signal of F_X, which was rather broad in the PS I core protein (cf. fig.4A), narrowed to resemble the control spectrum after reconstitution with the F_A/F_B polypeptide (fig.4B), suggesting that the microenvironment around the iron-sulfur cluster of F_X also recovers. There might be discrete binding sites for F_A/F_B on the PS I core protein, and the binding of F_A/F_B at this site allows recovery of the normal configuration of both F_A/F_B and F_X. The observation that F_B as well as F_A was reduced on illumination at 16 K in the reconstituted PS I complex (cf. fig.2A,B) suggest that the F_A/F_B polypeptide rebinds with an orientation resulting in a closer location of F_B to F_X than in the control. The ease of reconstitution shows that the binding affinity of the F_A/F_B polypeptide to the PS I core protein must be high and that the binding sites have not been irreversibly altered by the isolation procedures.

The only features that distinguish the reconstituted PS I complex from the control PS I complex are the different ratios of photoreduced F_A and F_B at 16 K, and the slightly different g values of F_A and F_B. These differences are minor and may be due to the use of a spinach rather than a *Synechococcus* F_A/F_B protein. However, there are no significant differences in the ESR or EXAFS spectra of F_A, F_B and F_X between spinach and *Synechococcus* sp. despite the nearly two billion year evolutionary gap separating these organisms [18]. Recent sequence analysis has also shown that the *Synechococcus* sp. 7002 and tobacco *psa*C polypeptides differ in only 8 of 80 amino acid residues – 6 of which are extremely conservative (Rhiel, E. and Bryant, D.A., unpublished). The *psa*A and *psa*B genes are remarkably similar between *Synechococcus* sp. 7002 and spinach [2], especially in the region of (putative) helix VIII, which contains the cysteine residues that are postulated to coordinate iron-sulfur center F_X. We would expect that this region would be important for the interaction with the F_A/F_B protein, especially since the F_A/F_B and F_X iron-sulfur clusters are probably in very close contact. It may not be surprising, therefore, that reconstitution of the spinach F_A/F_B polypeptide with the *Synechococcus* sp. 6301 PS I core protein occurs with demonstrated high efficiency.

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