Reconstitution of the photosystem I complex from the P700 and
Fx-containing reaction center core protein and the FA/FB
polypeptide

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Complete restoration of electron flow from P700 to FA/Fs was achieved by incubating a P700 and Fx-containing photosystem (PS) I core protein from Synechococns sp. 6301 with the 8.9 kDa, FA/Fs polypeptide from spinach. The ESR spectrum of the reconstituted PS I complex shows nearly equal photochemical reduction of FA and Fs when frozen in darkness and illuminated at 16 K. When illuminated during freezing, both FA and Fs are quantitatively reduced and the spectrum is nearly indistinguishable from FS and FA in the control PS I complex. In the reconstituted PS I complex Fx is photochemically reduced only in the presence of FS and FA, and the high-field resonance appears indistinguishable from Fx in the control PS I complex. Optical flash photolysis after extensive washing confirms the complete restoration of the P700⁺ [Fx/Fa] 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, FA/Fs polypeptide, and makes possible independent manipulation of the two subunits that carry the entire electron acceptor system of PS I.

1. INTRODUCTION

The PS I complex of plants and cyanobacteria is a chlorophyll-containing, light-driven, plasto-
tremely effective in removing the low molecular mass proteins, including the FA/FB 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 Fx. 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\textsubscript{A} and F\textsubscript{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\textsubscript{A}/F\textsubscript{B} polypeptide from spinach. Electron flow from P700 to F\textsubscript{A}/F\textsubscript{B} at both 16 K and room temperature is completely restored, and F\textsubscript{X} photoreduction occurs only in the presence of prerduced F\textsubscript{A} and F\textsubscript{B}. Most significantly, the ESR spectra of F\textsubscript{A}, F\textsubscript{B} and F\textsubscript{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\textsubscript{0}, A\textsubscript{1}, F\textsubscript{X}, F\textsubscript{A}, F\textsubscript{B}) was isolated from *Synechococcus* sp. 6301 (*Anacystis nidiulans* 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 psa\textsubscript{A} and psa\textsubscript{B}, and the electron acceptors A\textsubscript{0}, A\textsubscript{1} and F\textsubscript{X} [15,16]. The F\textsubscript{A}/F\textsubscript{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\textsubscript{A}/F\textsubscript{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\textsubscript{A} and F\textsubscript{B} confirms the loss of the low molecular mass polypeptides, including the 8.9 kDa, F\textsubscript{A}/F\textsubscript{B} polypeptide [15,16], from the cyanobacterial reaction center (the broad, shallow resonance of F\textsubscript{X} is barely visible under the conditions optimal for observation of iron-sulfur centers F\textsubscript{A} and F\textsubscript{B}; see fig.4A). The ESR spectrum of the chemically reduced 8.9 kDa, F\textsubscript{A}/F\textsubscript{B} polypeptide from spinach is shown in fig.1B. When compared with the control PS I complex, the resonances of F\textsubscript{A} and F\textsubscript{B} are significantly broader, and all but the g\textsubscript{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\textsubscript{A}/F\textsubscript{B} polypeptide and frozen in darkness, the low-temperature photoreduction of F\textsubscript{A} (g = 2.056, 1.956, 1.872) and F\textsubscript{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 re-bound to the reaction center core.

When the reconstituted PS I complex is illuminated during freezing, the low- and high-field resonances of \( F_A \) \((g = 2.056, 1.949, 1.999)\) 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 re-bound 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 \( F_A/F_B \) 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

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**Figure 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 \( \mu g/ml \) Chl. Spectrometer conditions as in fig.1.

**Figure 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 \( \mu g/ml \) Chl. Spectrometer conditions as in fig.1.
of iron-sulfur center Fx. 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 Fx in a reaction center deficient in FA and FB [8]. When the spectrum of the reconstituted PS I complex is measured under identical conditions, the low-temperature photoreduction of Fx 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 Fx 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 Fx in the reconstituted PS I complex (fig.4B) (note that the Fx resonances are distorted under the conditions optimal for Fx). The ESR signal of Fx, especially in the high-field region, is indistinguishable from that of Fx in the untreated PS I complex (see [3]). This behavior is also consistent with efficient binding of the FA/FB 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 FA and FB have been removed prior to flash photolysis [7]; under these conditions electron flow terminates at Fx. The P700 absorption transient after addition of the spinach FA/FB 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 FA/FB has been reestablished. The
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+ Fx back-reaction at the expense of the 30 ms, P700+ [FA/FB]− back-reaction (fig.3C).

4. DISCUSSION

The protocol described here represents the first reconstitution of the PS I complex from the P700 and Fx-containing PS I core protein and the FA/FB polypeptide. The restoration of cryogenic and room-temperature electron flow from P700 to the terminal iron-sulfur clusters FA/FB, and the near-normal ESR spectrum of FA and FB 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 FA/FB 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 FA and/or FB. The change is reversible, since we obtained completely normal ESR signals after reconstitution of FA/FB onto the PS I core protein (figs 2A,3A). This proves that the isolated FA/FB polypeptide is not denatured even though it has an abnormal ESR spectrum. The ESR signal of Fx, which was rather broad in the PS I core protein (cf. fig.4A), narrowed to resemble the control spectrum after reconstitution with the FA/FB polypeptide (fig.4B), suggesting that the microenvironment around the iron-sulfur cluster of Fx also recovers. There might be discrete binding sites for FA/FB on the PS I core protein, and the binding of FA/FB at this site allows recovery of the normal configuration of both FA/FB and Fx. The observation that Fx as well as FA was reduced on illumination at 16 K in the reconstituted PS I complex (cf. fig.2A,B) suggest that the FA/FB polypeptide rebinds with an orientation resulting in a closer location of Fx to Fx than in the control. The ease of reconstitution shows that the binding affinity of the FA/FB 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 FA and FB at 16 K, and the slightly different g values of FA and FB. These differences are minor and may be due to the use of a spinach rather than a *Synechococcus* FA/FB protein. However, there are no significant differences in the ESR or EXAFS spectra of FA, FB and Fx 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 psaC polypeptides differ in only 8 of 80 amino acid residues – 6 of which are extremely conservative (Rhiel, E. and Bryant, D.A., unpublished). The psaA and psaB 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 Fx. We would expect that this region would be important for the interaction with the FA/FB protein, especially since the FA/FB and Fx iron-sulfur clusters are probably in very close contact. It may not be surprising, therefore, that reconstitution of the spinach FA/FB polypeptide with the *Synechococcus* sp. 6301 PS I core protein occurs with demonstrated high efficiency.

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