Volume 228, number 2, 268-272 FEB 05575 February 1988

Isolation of the intact photosystem I reaction center core containing P700 and iron-sulfur center F_X

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Received 3 December 1987

The photosystem I reaction center core containing P700 and iron-sulfur center F_X has been isolated from a *Synechococcus* photosystem I particle with 6.8 M urea at pH 10.0 followed by sucrose density ultracentrifugation. The reaction center core has retained >90% of F_X and 100% of P700 (determined by optical spectroscopy) but is totally devoid of iron-sulfur centers F_A and F_B (determined by optical and ESR spectroscopy). SDS-PAGE indicates the retention of the 57 kDa reaction center polypeptide(s) but the total absence of the 16.4 and 8.1 kDa polypeptides. The loss of F_A and F_B is further reflected in the decline of acid-labile sulfide from 11.8 ± 0.4 S^{2-}/P700 in the control particle to 4.6 ± 0.3 S^{2-}/P700 in the reaction center core. This preparation represents the first isolation of an intact reaction center core incorporating the components P700 and F_X but totally lacking F_A and F_B.

Photosynthesis; Reaction center; Photosystem I; P700; Iron-sulfur center F_X; Iron-sulfur center F_A; Iron-sulfur center F_B

1. INTRODUCTION

The photosystem I reaction center complex of plants and cyanobacteria consists 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_A, F_B and F_X (reviews [1–3]). Recently it has been suggested that A_1 and F_X are located on the P700- and A_0-containing reaction center polypeptides [4–8] and that F_A and F_B are located on a peripheral 8 kDa polypeptide [9,10].

In this paper, we report the use of 6.8 M urea in the isolation of the intact photosystem I reaction center core containing P700 and F_X. We indicated earlier [4,7,8] that iron-sulfur centers F_A and F_B are partially removed from a spinach photosystem I particle by addition of 1% LDS. We had alsc reported [11] that F_X was the least susceptible component to oxidative denaturation following treatment of a spinach photosystem I reaction center with 2 or 4 M urea. Since the denaturants LDS and urea both interfere with the patterns of ion-ion associations in proteins, we investigated the ability of high concentrations of urea to remove the low molecular mass polypeptides carrying F_A and F_B without affecting the integrity of F_X. We chose a cyanobacterial reaction center for study because it responded to urea with minimal destruction of F_X and because it contains fewer low molecular mass polypeptides than spinach [12].

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Abbreviations: Chl, chlorophyll; LDS, lithium dodecyl sulfate; DCPIP, 2,6-dichlorophenolindophenol; PAGE, polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

Photosystem (PS) I reaction center particles containing the full complement of electron acceptors (A_0, A_1, F_X, F_A, F_B) were isolated from *Synechococcus* 6301 (*Anacystis nidulans* TX-20). The cells were grown in Krantz and Myers medium C and incubated at 35–39°C under 1% CO_2 in air and harvested 24 h

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

after inoculation. The cells were centrifuged, resuspended to 0.7 mg/ml Chl in 0.05 M Tris, pH 7.5, and 2 mM EDTA, and broken in a French pressure cell (2500 on lb/inch^2 scale). The eluate was spun at 3000 rpm for 3 min and the supernatant was re-centrifuged at 19000 rpm for 60 min (SS-34 rotor). The resulting pellet was resuspended to 100 μg/ml Chl in 50 mM Tris, pH 8.3, containing 0.2 M KCl and 1% Triton X-100, and after 24 h of incubation, the suspension was centrifuged at 14000 rpm for 30 min. The supernatant was concentrated to 30 ml over a YM-100 ultrafiltration membrane (Amicon) and centrifuged for 48 h at 24000 rpm (SW-27 rotor) in a 0.1 to 1.0 M sucrose gradient containing 50 mM Tris, pH 8.3, and 0.1% Triton X-100. The lower green band was isolated, dialyzed against 50 mM Tris, pH 8.3, containing 1% Triton X-100 and loaded onto a DEAE Bio-Gel A column that had been pre-equilibrated with the same buffer. The column was washed until the eluant was colorless, and the PS I particle was removed with a pulse of 1 M NaCl in 50 mM Tris, pH 8.3, containing 0.1% Triton X-100. The PS I particle was dialyzed, concentrated over a YM-100 membrane and recentrifuged on a 0.1 to 1 M sucrose gradient in the absence of Triton X-100. The chlorophyll to P700 ratio at this stage was 92 when determined by chemical difference spectroscopy, and 91 when determined photochemically. The sample was dialyzed against 50 mM Tris, pH 8.3, and placed in 0.1 M glycine, pH 10.0, containing 6.8 M urea at 250 μg/ml Chl for 10 min. The material was dialyzed against 50 mM Tris, pH 8.3, for 24 h, concentrated over a YM-100 membrane, and centrifuged for 48 h at 24000 rpm (SW-27 rotor) in a 0.1 to 1 M sucrose gradient containing 50 mM Tris, pH 8.3, and 0.1% Triton X-100. The lower green band, which contains the reaction center in the state [P700....A_0,A_1,F_X], was removed, dialyzed against 50 mM Tris, pH 8.3, and stored at -80°C in 20% glycerol.

Chlorophyll was determined in 80% acetone [13]. Acid-labile sulfide was determined as described in [14]. Spinach ferredoxin at a purity ratio of 0.48 (A_{420}/A_{276}) served as absolute standard for acid-labile sulfide [15].

Flash-induced absorption transients were determined at 698 nm [4]. The recovery time of the DC-coupled amplifier (EG&G model 113) following a saturating laser flash was ~10 μs; photochemical P700 is defined for the purpose of this study as any 698 nm absorption transient with at least this lifetime.

ESR studies were performed on a Varian E-109 spectrometer equipped with an Air Products liquid helium transfer cryostat. The spectrometer was interfaced to a Nicolet 4094A digital oscilloscope and a Macintosh Plus computer for signal averaging and baseline subtraction. Sample temperatures were monitored with a gold chromel thermistor situated directly below the sample tube. Light-minus-dark difference spectra were obtained by illuminating the sample with a 150-W xenon lamp (Oriel).

Electrophoresis (PAGE) was performed in a 8 cm × 1.5 mm slab gel containing a linear 10–15% polyacrylamide (bis:acrylamide, 1:20) gradient (Hoefer model SE-200). The control and urea-treated PS I reaction centers (1 μg/μl protein) were incubated in 0.0625 M Tris (pH 6.8), 2% SDS, 10% glycerol and 5% β-mercaptoethanol for 24 h at 30°C [16]. The samples were applied to the stacking gel at a protein concentration of 20 μg/well. Electrophoresis was carried out at 20°C at a constant current of 12 mA for 2.5 h. Gels were stained with Coomassie brilliant blue and scanned with an LKB laser densitometer.

3. RESULTS

Fig.1A shows the flash-induced absorption change at 698 nm in a PS I reaction center particle isolated from *Synechococcus* 6301. The 30-ms half-time indicates a backreaction between the primary electron donor P700+ and the terminal electron acceptors $[F_A/F_B]^-$. Fig.1B shows the flash-induced absorption change after 10 min of incubation with 6.8 M urea at pH 10.0. The 1.2-ms half-time is typical of a reaction center in which F_A and F_B have been removed prior to flash photolysis [4]; under these conditions electron flow terminates at F_X, resulting in a characteristic backreaction with P700+. The kinetics and magnitude of the absorption change indicate that the urea protocol removes F_A and F_B entirely from the PS I reaction center core without affecting F_X.

Prolonged urea treatment does not result in the simultaneous loss of F_X relative to F_A and F_B; typically, >90% of F_X is retained after 120 min of exposure to 6.8 M urea. This relative stability makes it feasible to remove the urea by overnight dialysis without degradation of the iron-sulfur cluster. Most significantly, the dialyzed reaction center is stable in 0.1% Triton X-100. The ability to withstand detergents allows the treated reaction center to be purified by ultracentrifugation in a 0.1% Triton-containing, 0.1–1.0 M sucrose gradient; after 48 h at 48000 × g the treated reaction center separates into an upper, protein-containing band, and a lower, chlorophyll-containing band. The latter band is photochemically active in electron flow from P700 to F_X (determined optically).

Fig.2 shows the ESR spectrum of F_A and F_B in the control reaction center (A) and their absence in the 6.8 M urea-treated, ultracentrifuged reaction center (B) after reduction with 1 mM dithionite at pH 10.0. To ensure complete reduction of F_A and F_B, the urea-treated sample was illuminated while cooling to 12 K; no further indication of F_A or F_B was found.

Fig.3 shows the ESR spectrum of F_X in the urea-treated, ultracentrifuged reaction center after incubation with ascorbate at pH 10.0 and illumination at 6 K. The spectrum was resolved by subtracting four light-off scans from four light-on

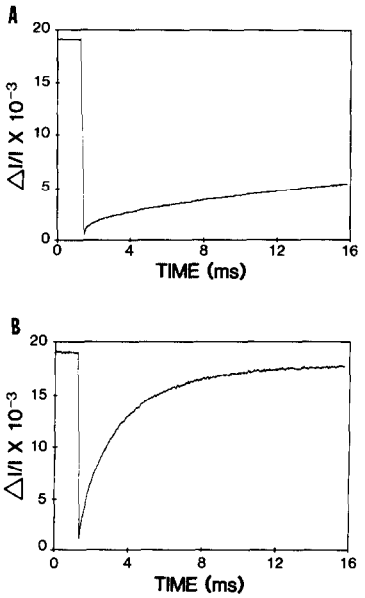

Fig.1. Flash-induced absorption change at 698 nm before (A) and after (B) addition of 6.8 M urea to a *Synechococcus* PS I reaction center. All measurements were made at 10 μg/ml Chl in 0.1 M glycine, pH 10.0, containing 1.7 mM ascorbate and 0.033 mM DCPIP. Both samples were allowed to incubate for 10 min in darkness prior to flash photolysis.

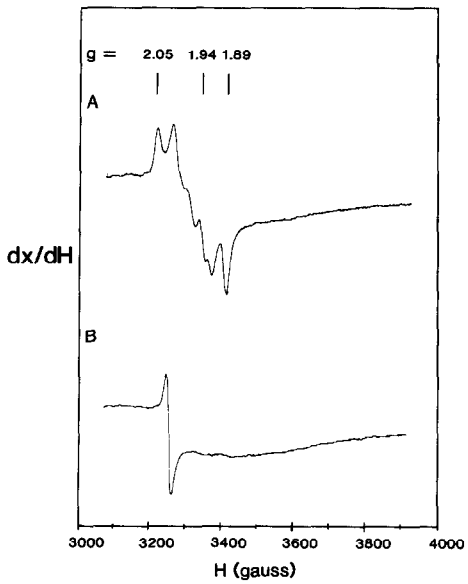

Fig.2. X-band ESR spectrum of F_A and F_B in control (A) and 6.8 M urea-treated, ultracentrifuged (B) PS I reaction centers. Both samples were prepared by addition of 1 mM sodium dithionite and 10 μM methyl viologen in 0.1 M glycine, pH 10.0. There was no significant increase in the amount of F_A or F_B upon illumination of the sample at 12 K. Spectrometer conditions: temperature, 12 K; microwave power, 10 mW; microwave frequency, 9.18 GHz; receiver gain, 5.0×10^3; modulation amplitude, 10 G at 100 kHz.

scans. The retention of the prominent high-field resonance at $g = 1.79$ and the altered (~30% broader) lineshape is characteristic of F_X in a reaction center deficient in F_A and F_B [7,8].

The loss of F_A and F_B is also reflected in the decline of acid-labile sulfide from 11.8 ± 0.4 S^{2-}/P700 ($n = 3$) in the control reaction center to 4.6 ± 0.3 S^{2-}/P700 ($n = 3$) in the reaction center core preparation. This ratio is calculated on the basis of photochemical P700; the chemical difference measurement of P700 indicates that nearly all of the available P700 is photoactive on this time scale. The negligible contamination with F_A and F_B, along with the precision of the P700 and labile sulfide measurements, permits the existence of four S^{2-}/P700 to be established in the core preparation with a high degree of confidence.

The polypeptide compositions of the control and urea-treated preparations are shown in fig.4. The control *Synechococcus* reaction center contains low molecular weight polypeptides at 16.4 and 8.1 kDa in addition to the reaction center polypeptides at 90 and 57 kDa (the 90-kDa band contains

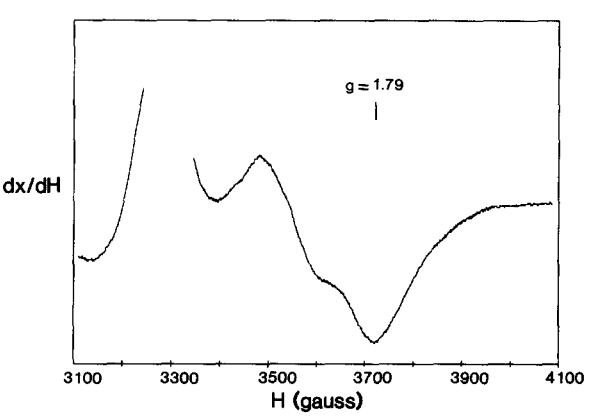

Fig.3. Light-minus-dark (before light) X-band ESR spectrum of F_X in the 6.8 M urea-treated, ultracentrifuged PS I reaction center. The sample contained 1 mM ascorbic acid and 0.8 mM DCPIP in 0.1 M glycine at pH 10.0. Spectrometer conditions: temperature, 6 K; microwave power, 40 mW; microwave frequency, 9.25 GHz; receiver gain, 5×10^3; modulation amplitude, 40 G at 100 kHz. The spectrum represents the average of four 4-min sweeps each for the light and dark samples, and a 5-fold enlargement in software.

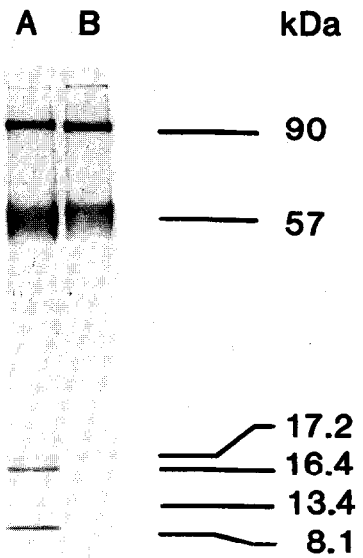

Fig.4. Polypeptide composition of the control (A) and the 6.8 M urea-treated, ultracentrifuged (B) photosystem I reaction centers. Molecular masses are calculated on the basis of the R_f values of soluble proteins from 6.21 kDa to 66.0 kDa treated identically to the PS I particles but run in alternate wells. Details of the electrophoresis are given in section 2.

chlorophyll, and under more rigorous treatment conditions, degrades to the diffuse 57 kDa band). There are also minor bands at 13.4 and 17.2 kDa. In the urea-treated and ultracentrifuged reaction center the 90 and 57 kDa bands are present in the same amount as the control, but the 16.4 and 8.1 kDa bands are totally missing. The minor polypeptides are still present to some degree; the 13.4 kDa band is depleted to 35–50% of the control and the 17.2 kDa band is 75–85% retained. If the 8.1 kDa polypeptide corresponds to the 8 kDa polypeptide that has been identified as an [8Fe-8S] protein in spinach, the loss of ~8 mol of labile sulfide per mol of P700 may be understood as the complete removal of the polypeptide carrying F_A/F_B.

4. DISCUSSION

We suggest that the cyanobacterial reaction center reported in this paper represents the intact PS I core incorporating the components P700 and F_X but totally lacking F_A and F_B. The data indicate that little deterioration of the reaction center has taken place throughout the course of purification; even after extensive sample handling we find nearly the entire complement of F_X present in the core preparation as was present in the control particle. The retention of four S^{2-}/P700 in the core preparation agrees with our previous estimate of the labile sulfide content of F_X [8] and indicates the existence of four Fe-S pairs in PS I distinct from that present in F_A and F_B. This is enough labile sulfide for one [4Fe-4S] cluster or two [2Fe-2S] clusters. Since F_X has been proposed to consist of [2Fe-2S] clusters [17,18], the data are consistent with earlier suggestions [17–19] that two molecules of F_X exist per molecule of P700.

The polypeptide composition is more problematical since there are differences reported in the number as well as molecular masses of the polypeptides in various cyanobacterial PS I preparations [12,20–22]. Most reaction centers contain at least two low molecular mass polypeptides and a diffuse protein band at about 55–70 kDa. Our data indicate that the 6.8 M urea protocol removes the 16.4 and 8.1 kDa polypeptides entirely from a *Synechococcus* PS I reaction center. Even though the isolated reaction center core retains, to a minor degree, two additional low molecular mass polypeptides, we suspect that these polypeptides may not represent integral components of PS I. As such, we suggest that the data agree with our earlier assessment that F_X resides on the high molecular mass polypeptides in PS I.

Acknowledgements: This material is based upon work supported by the Cooperative State Research Service, US Department of Agriculture under Agreement no. 87-CRCR-1-2382 (to J.H.G.), and by a grant from the Robert A. Welch Foundation, no. F-867 (to J.J.B.).

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