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D1-D2 complex of the photosystem II reaction center from spinach

Isolation and partial characterization

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A pigment-protein complex consisting of D1 and D2 proteins, but depleted in the two lower molecular mass components of photosystem II, i.e. cytochrome *b*-559 and *psbI* gene product, has been isolated by octyl-β-D-glucopyranoside treatment of the purified photosystem II reaction center complex from spinach [(1987) Proc. Natl. Acad. Sci. USA 84, 109–112], followed by separation by high performance liquid chromatography using a gel-permeation column (TSK G3000 SW). The isolated complex is photochemically active in the photoreduction of intrinsic pheophytin *a* under steady-state illumination, in the presence of dithionite and methyl viologen, and exhibits pigment stoichiometries similar to those in the untreated reaction center, indicating that the D1-D2 complex provides the site of primary photochemistry in photosystem II, as well as the principal binding sites of pigments in the reaction center.

Charge separation; Cytochrome *b*-559; D1-D2 complex; Octyl-β-D-glucopyranoside; Photosystem II reaction center

1. INTRODUCTION

The primary charge separation in photosynthesis takes place in membrane-bound pigment-protein complexes called the reaction center (RC). The RC complex of photosystem II (PSII) of oxygenic photosynthesis, so far isolated [1–5], consists of 5 polypeptides, i.e. D1 and D2 proteins, α- and β-subunits of cytochrome b-559 and *psbI* gene product, and anchors the following cofactors: 4–6 chlorophylls, 2 pheophytins, 1–2 β-carotenes and 1–2 cytochrome *b*-559 hemes (see, however, [6]). The D1 and D2 proteins are partially homologous, in their primary structure, to the L and M subunits, respectively, of purple bacterial RCs whose structure has recently been elucidated by X-ray crystallographic analysis [7–9]. This fact, together with recent isolation of a photochemically active PSII RC complex consisting of D1 and D2 proteins as the principal components [1], led us to conclude that these two proteins are responsible for binding the cofactors engaged in the primary photochemistry of PSII, in a similar manner to the L and M subunits forming the purple bacterial RCs [1,7,10]. However, PSII RC complexes so far isolated contain 3 additional polypeptides other than D1 and D2 proteins [5,11,12] and thus no experimental evidence has been provided yet to substantiate this conviction in the strict sense; no preparation equivalent to the LM complex in purple bacteria [13] has been isolated for PSII.

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The partial disintegration of the isolated PSII RC complex from spinach has been attempted in this study, in order to elucidate the organization of PSII and to examine the functional role(s) of the lower molecular weight components of the RC. A photochemically active pigment-protein complex consisting of D1 and D2 proteins, but completely depleted in cytochrome *b*-559 and *psbI* gene product, has been isolated by octyl-β-D-glucopyranoside treatment of the purified PSII RC [1] followed by separation by a high performance liquid chromatography using a gel-permeation column, and thus it is concluded that only D1 and D2 proteins are needed as protein components in PSII RC, for the primary charge separation.

2. MATERIALS AND METHODS

The PSII RC complex was purified from Triton X-100 treated spinach grana thylakoids by the method of Nanba and Satoh [1], followed by substitution of digitonin for Triton X-100 as described in [5]. In order to concentrate this, the purified materials were adsorbed onto a small DEAE-Toyopearl column and then eluted with 50 mM Tris-HCl buffer (pH 8.5) containing 0.2% digitonin and 500 mM NaCl. The materials in the elution buffer were treated with 5% octyl-β-D-glucopyranoside at 0°C at 500 μg Chl · ml^{-1} and then subjected to the high-performance gel-permeation chromatography as in [14], using a prepacked TSK-gel column (G3000 SW XL, Tosoh, Tokyo, Japan). 50 mM Tris-HCl buffer (pH 7.2) containing 0.1% digitonin and 100 mM NaCl was used for chromatographic development and the elution profile was monitored at 673 nm.

Pigment analysis was carried out for 80% acetone extracts by a reverse-phase high performance liquid chromatography using a Zorbax-ODS column (Shimadzu, Kyoto, Japan). Methanol/isopropanol, 3:1 (vol/vol), was used for chromatographic development according to the procedure of Eskins et al. [15], and the elution of the

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components was monitored by the absorption at 255 nm (pheophytin *a*) and at 440 nm (chlorophyll *a* and β-carotene). The spectrophotometry of the components was based on the absorption coefficients determined by Eskins et al. [15] for chlorophyll *a* and β-carotene and by Vernon [16] for pheophytin *a*. The amounts of cytochrome b-559 were estimated by the method of Hind and Nakatani [17]. Polypeptides were analyzed by SDS-polyacrylamide gel electrophoresis as described by Chua [18] followed by silver staining [22]. 6 M urea was included in the analyzing gel.

The absorption spectrum at room temperature was measured with a dual-beam spectrophotometer (model 557, Hitachi, Tokyo, Japan). The photochemical activity in terms of accumulation of reduced pheophytin *a* under steady-state illumination was measured at 25°C in a 10-mm cuvette with a Hitachi-356 spectrophotometer equipped with a cross-illumination system. A filter combination was used for protecting photomultiplier from strong actinic illumination as follows: two Corning 4-96 filters for the measuring beam; a red cut-off filter (VR-67, Toshiba, Tokyo) and heat- and UV-absorbing filters for the actinic beam. Saturating actinic light was provided by an incandescent lamp (1 kW). The sample (1.2 μg Chl · ml^{-1}) in 100 mM Tris-HCl buffer (pH 8.0), containing 1 μM methyl viologen, 2 mg · ml^{-1} of sodium dithionite and 0.2% digitonin was incubated for a few minutes before each measurement.

Octyl-β-D-glucopyranoside was purchased from Sigma Chemical Co. (MO, USA) and digitonin was from BDH Chemicals (UK). All other chemicals were of reagent grade.

3. RESULTS AND DISCUSSION

Fig. 1A shows an elution profile of the untreated PSII RC complex, obtained by a gel-permeation chromatography monitored at 673 nm. The complex exhibits a single symmetrical peak which probably corresponds to the monomeric form of PSII RC surrounded by detergent molecules. The particle size of the complex was estimated by this procedure to be 180–160 kDa using soluble proteins of known molecular weight in the same buffer system as calibration standards.

Treatment of the complex with 5% octyl-β-D-glucopyranoside for 5 min, as described in section 2, resulted in the appearance of 3 chlorophyll-containing peaks designated as F-I, F-II and F-III, in the order of decreasing particle size (Fig. 1B); the molecular masses of these components were estimated to be 500–470, 170–150 and 80–70 kDa, respectively (see later). To analyze polypeptide profile of the 3 fractions obtained above, we utilized SDS-polyacrylamide gel electrophoresis in the presence of 6 M urea (Fig. 2). From Fig. 2, it is evident that both F-I and F-II fractions predominantly contain D1 and D2 proteins, but practically no lower molecular weight components of PSII RC, i.e. cytochrome *b*-559 and *psbI* gene product, although the β-subunit of cytochrome and *psbI* gene product are not clearly resolved under the electrophoretic condition. In contrast, polypeptides of cytochrome *b*-559 were enriched in fractions between F-II and F-III (Fig. 2), where the absorbance difference at 559 nm between dithionite-reduced and ferricyanide-oxidized samples is predominant (Fig. 1). No protein component was detected by SDS-polyacrylamide gel electrophoresis in the F-III fraction, indicating that

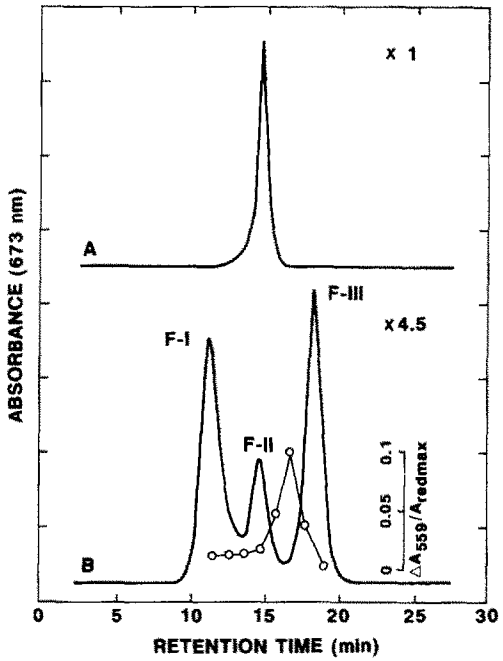

Fig. 1. Elution profile from TSK G3000 SW column of the untreated (A) and octyl-β-D-glucopyranoside-treated (B) photosystem II reaction center complexes. Open circles represent the ratio of reduced-*minus*-oxidized absorbance difference at 559 nm to the absorbance at the red maximum ($\Delta A_{559}/A_{red.max}$). The number beside each trace represents the relative amount of reaction centers applied to the column.

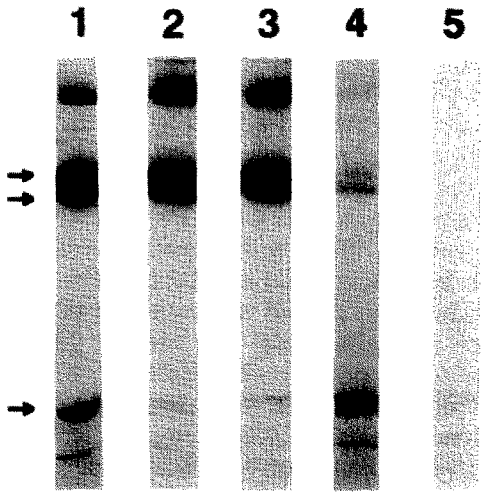

Fig. 2. SDS-polyacrylamide gel electrophoresis of the fractions obtained by octyl-β-D-glucopyranoside treatment. Polyacrylamide concentration, 15%. Polypeptide profiles of the untreated photosystem II reaction center prepared as in [5] (1), and of F-I (2), F-II (3), fraction no. 17, between F-II and F-III (4) and F-III (5) in Fig. 1 are shown. Arrows on the left side indicate the positions of D-2 protein, D-1 protein, and α-subunit of cytochrome *b*-559, respectively, from top to bottom, as indicated by Western blot analysis [5].

solubilized pigments are incorporated into detergent micelles in this fraction.

The retention time for the F-I corresponds to the exclusion limit of the column and thus the fraction can be ascribed to the aggregated form(s) of F-II, based on the polypeptide profile. The pigment-to-protein ratio, however, was much lower in this fraction than in F-II, suggesting that most of the pigments are released from proteins in the F-I fraction. Based on the polypeptide profiles of the fractions obtained, it is evident that F-II is formed by dissociation of cytochrome *b*-559 (and *psbI* gene product) from the native complex. However, it is reasonable to expect that the undissociated complex also contributed to this peak, since the difference in the molecular mass between the native and the dissociated complexes is too small to be separated under the experimental condition. Thus, the amounts of cytochrome *b*-559 α-subunits present in the F-II fraction probably represent the amounts of contaminated native complex; the proportion of cytochrome subunits to D1/D2 proteins on SDS-polyacrylamide gel electrophoresis decreased by increasing incubation time as well as by increasing concentration of detergent used for solubilization.

The F-II fraction was concentrated by a small DEAE-Toyopearl column and then subjected to the second gel-permeation chromatography after a brief treatment (5 s) with 5% octyl-β-D-glucopyranoside as in the first treatment. A single predominant peak corresponding to the F-II was obtained together with small amounts of F-I and F-III fractions. The purified F-II was relatively stable as long as digitonin is supplemented in the medium and was completely devoid of cytochrome *b*-559 and *psbI* gene product as shown in Fig. 3.

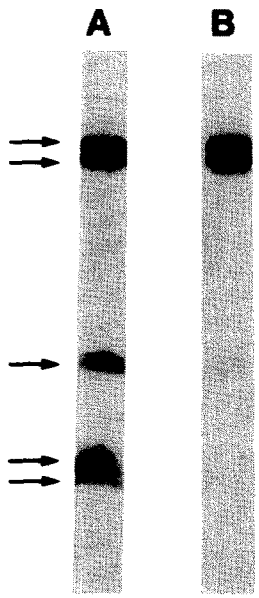

Fig. 3. SDS-polyacrylamide gel electrophoresis of the purified D1-D2 complex. For comparison, the profile of untreated photosystem II reaction centers is shown in lane (A). A linear gradient of polyacrylamide of 10–20% was used. Arrows on the left side indicate the positions of D-2 protein, D-1 protein, α-subunit of cytochrome *b*-559, *psbI* gene product and β-subunit of cytochrome *b*-559, respectively, from top to bottom.

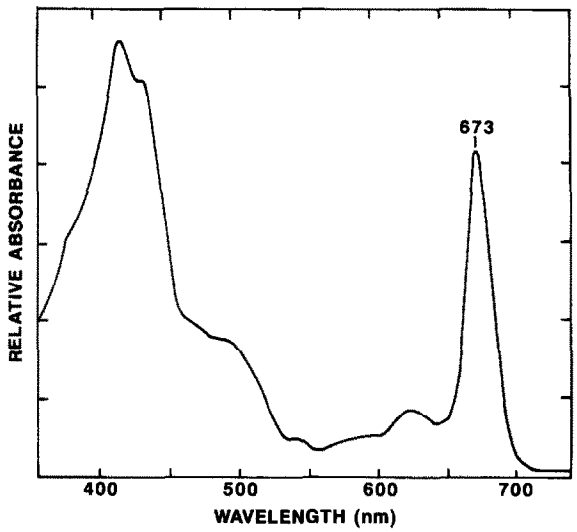

Fig. 4. Absorption spectrum of the purified D1-D2 complex measured at 25°C.

The absorption spectrum at room temperature of the purified D1-D2 complex shown in Fig. 4 is essentially

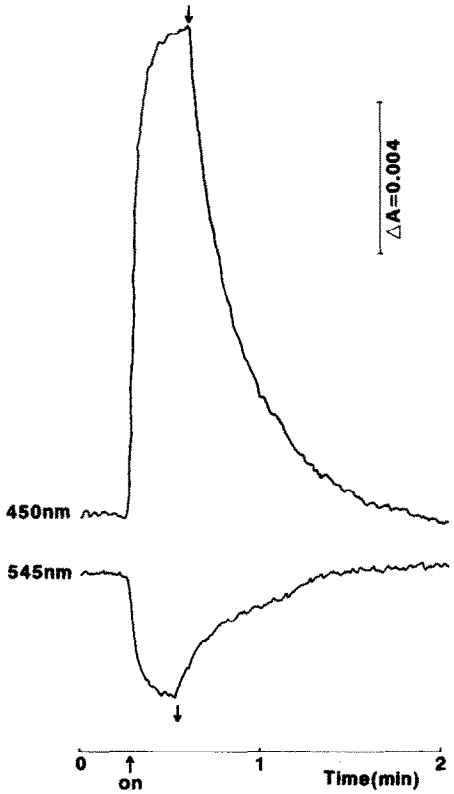

Fig. 5. Kinetic traces of light-induced absorbance changes measured at 25°C at 450 and 545 nm. Arrows, downward and upward, indicate the time when the actinic light was switched on and off, respectively.

similar to that of the untreated PSII RC complex [1], but with some small noticeable differences. The ratio of the absorption at the blue maximum to the red maximum is slightly lower in the D1-D2 complex and the shoulder around 435 nm is more remarkable than that in the untreated RCs [5]. These changes could be ascribed to the removal of cytochrome *b*-559 from the complex.

In accordance with the spectroscopic properties described above, the pigment composition of the purified D1-D2 complex was similar to that of the untreated RC complex [19], except for carotenoid; the complex contains, in molar ratio, 5.8 chlorophyll *a* and 0.9 β-carotene per two chemically estimated pheophytin *a*. This can be taken as evidence which indicates that the small subunits of PSII RC, i.e. cytochrome *b*-559 and *psbI* gene product, are not involved in the pigment-binding.

The D1-D2 complex isolated here exhibits a reversible absorbance change originating from photoaccumulation of reduced pheophytin *a* [20] as shown by the light-induced increase at 450 nm and the decrease at 545 nm (Fig. 5). Using millimolar difference absorption coefficient of 8 at 545 nm [21], the amount of photoactive pheophytin *a* was estimated to approximately one pheophytin molecule (0.85) out of two chemically estimated molecules in the complex, as in the intact RC [5].

The results obtained here clearly indicate that site of primary charge separation in photosystem II is located in the heterodimer consisting only of D1 and D2 proteins, as in LM complex in purple bacterial RCs, and that both cytochrome *b*-559 and *psbI* gene product are not directly involved in the primary photochemistry, as well as in the pigment binding. This conclusion further supports the similarity in the molecular architecture between PSII RC and purple bacterial RCs. Further analysis on the structure and function of the isolated D1-D2 complex is evidently needed to elucidate the organization of PSII RC as well as the function of smaller molecular weight components of the RC.

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