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POLYPEPTIDE COMPOSITION OF THE PURIFIED PHOTOSYSTEM II PIGMENT-PROTEIN COMPLEX FROM SPINACH

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Summary

The Photosystem II pigment-protein complex, the chlorophyll *a*-protein comprising the reaction center of Photosystem II, was prepared from EDTA-treated spinach chloroplasts by digitonin extraction, sucrose-gradient centrifugation, DEAE-cellulose column chromatography, and isoelectrofocussing on Ampholine.

The dissociated pigment-protein complex exhibits two polypeptide subunits that migrate in SDS-polyacrylamide gel with electrophoretic mobilities corresponding to molecular weights of approximately 43 000 and 27 000. The chlorophyll was always found in the free pigment zone at the completion of the electrophoresis. Heat-treatment of the sample (100°C, 90 s) for electrophoresis caused association of the two polypeptides into large aggregates. It is concluded that these two polypeptides, 43 000 and 27 000, are valid structural or functional components of Photosystem II pigment-protein complex.

Introduction

Two types of chlorophyll-protein complex of chloroplast thylakoids, the Photosystem I pigment-protein complex and the light-harvesting chlorophyll *a/b*-protein complex, have been reasonably well characterized on the basis of their constituent polypeptides [1—3]. The Photosystem II pigment-protein complex, the chlorophyll *a*-protein comprising the reaction center of Photosystem II, has been extensively purified in several laboratories [4—6]. Solubilization of chloroplast thylakoids by digitonin and subsequent successive application of sucrose-gradient centrifugation, DEAE-cellulose column chromatog-

Abbreviation: CF_1, chloroplast coupling factor 1.

raphy [5], and isoelectrofocusing to the solubilized extracts have led to the successful isolation and the purification of the Photosystem II pigment-protein complex [6]. The polypeptide composition of the purified Photosystem II preparations from different laboratories [6—8], however, was not consistent, although their spectral and photochemical properties were similar to some extent.

In the present study, some improvements were made both on the preparation of the pigment-protein complex and on the analysis of polypeptides of Photosystem II. The results strongly suggest that two polypeptides of molecular weights of about 43 000 and 27 000 are valid structural or functional components of the Photosystem II pigment-protein complex.

Materials and Methods

Preparation of Photosystem II pigment-protein complex

The purified preparations of Photosystem II pigment-protein complex were obtained from spinach leaves as previously described [6]. The method included the following steps; (a) Digitonin treatment (1.25%, 3 g digitonin per g chlorophyll, 60 min) to obtain Photosystem II enriched particles; (b) additional digitonin treatment (1.25%, 4.3 g digitonin per g chlorophyll, 20 h) in the presence of 0.35 M NaCl to solubilize the particles followed by sucrose-gradient centrifugation (10—30% sucrose, 0.4% digitonin, 90 000 × *g*, 45 h) of the solubilized supernatnat; (c) two successive DEAE-cellulose column chromatographies of the Photosystem II band from step b, in the presence of 0.1% digitonin; (d) isoelectrofocusing of the DEAE-cellulose eluates in Ampholines (LKB) within the pH range of 4—6 in the presence of 0.1% digitonin.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was carried out on slab gel (12.5% acrylamide) by the method of Laemmli [9]. In the standard procedure, the sample containing 10% glycerol, 2.3% SDS, 5% mercaptoethanol, 62.5 mM Tris-HCl (pH 6.8), 0.005% bromophenol blue was heated (100°C, 90 s) [10]. The polypeptide bands were stained with 0.05% Coomassie blue, 10% acetic acid and 25% isopropanol for 2 h and destained in 10% acetic acid and 10% isopropanol overnight. Densitometric tracings were made at 563 nm on the stained gel with a microdensitometer (Toyo, DMU-2). The amounts of a polypeptide component in different fractions were compared with the intensities of A_{563}. For comparison, the absorbance was normalized to 1.0 at the highest fractions for each polypeptide in Figs. 3 and 5.

Preparation of chloroplasts deficient in chloroplast coupling factor 1

Preparation of chloroplast coupling factor 1 (CF_1)-deficient chloroplasts from spinach leaves was made by the method described by McCarty [11] which involves washing of chloroplasts with 10 mM NaCl followed by the treatment with dilute solution of EDTA (0.75 mM, pH 8.0). Usually this procedure was repeated twice to eliminate the trace of remaining CF_1 in the preparation.

Results and Discussion

In a previous paper [6], we reported evidence for the existence of six polypeptide components (upper trace of Fig. 1) in highly purified preparations of Photosystem II pigment-protein complex obtained from spinach by isoelectrofocusing. However, further study was required because appreciable amount of materials stained with Coomassie blue remained at the origin of the running gel in the SDS-polyacrylamide gel electrophoresis. It was found that the omission of heat treatment from the standard procedure of sample preparation for SDS-polyacrylamide gel electrophoresis brought about a complete dissociation of the materials at the origin into two polypeptide bands (lower trace of Fig. 1). The two bands were relatively diffuse as compared with the other six bands, and the molecular weights were estimated by comparison with marker proteins to be approx. 43 000 and 27 000.

In order to decide which one (or more) of the eight polypeptides is a valid component of the Photosystem II pigment-protein complex, the polypeptide pattern of each alternate fraction from the Ampholine column was examined without heat-treatment of the sample preparation for SDS-polyacrylamide gel electrophoresis (Fig. 2). Evidently three groups of polypeptides are eluted from the column succession (Fig. 2): (a) A single polypeptide of the molecular weight of about 24 000 (III) which is ascribable to the light-harvesting chlorophyll *a/b*-protein reported by Thornber (fractions 18—26); (b) Two polypeptides of about 43 000 (IIa) and 27 000 (IIb) (fractions 22—34); (c) Polypeptides of about 55 000 (Ca), 52 000 (Cb) and 34 000 (Cc) together with three other faint bands (they are not clear in Fig. 2) in the low molecular weight region. The six polypeptides were regarded as the components of Photosystem

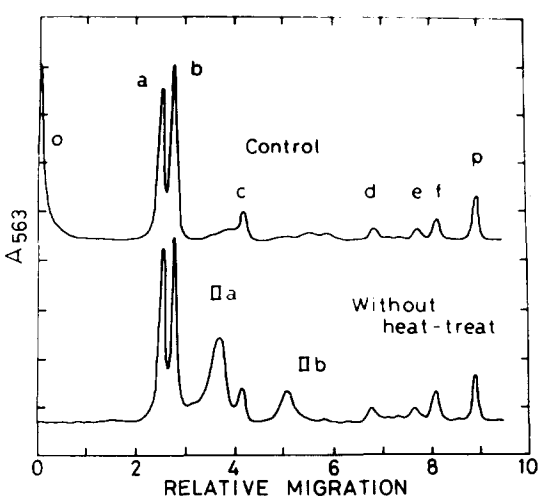

Fig. 1. Densitometric traces of polyacrylamide gels of the electrophoretically separated polypeptides of the purified Photosystem II pigment-protein complex. Upper trace, electrophoresis performed after heat-treatment of the sample (100°C, 90 s). Lower trace, electrophoresis performed without heat-treatment. Sample solution containing approx. 1 μg chlorophyll was added to each slot on the slab gel. The polypeptides shown by the upper trace were designated Ca (a), Cb (b), Cc (c), Cd (d), Ce (e), and Cf (f) in the order of decreasing molecular weight; o, Origin of the running gel; p, Pigment front.

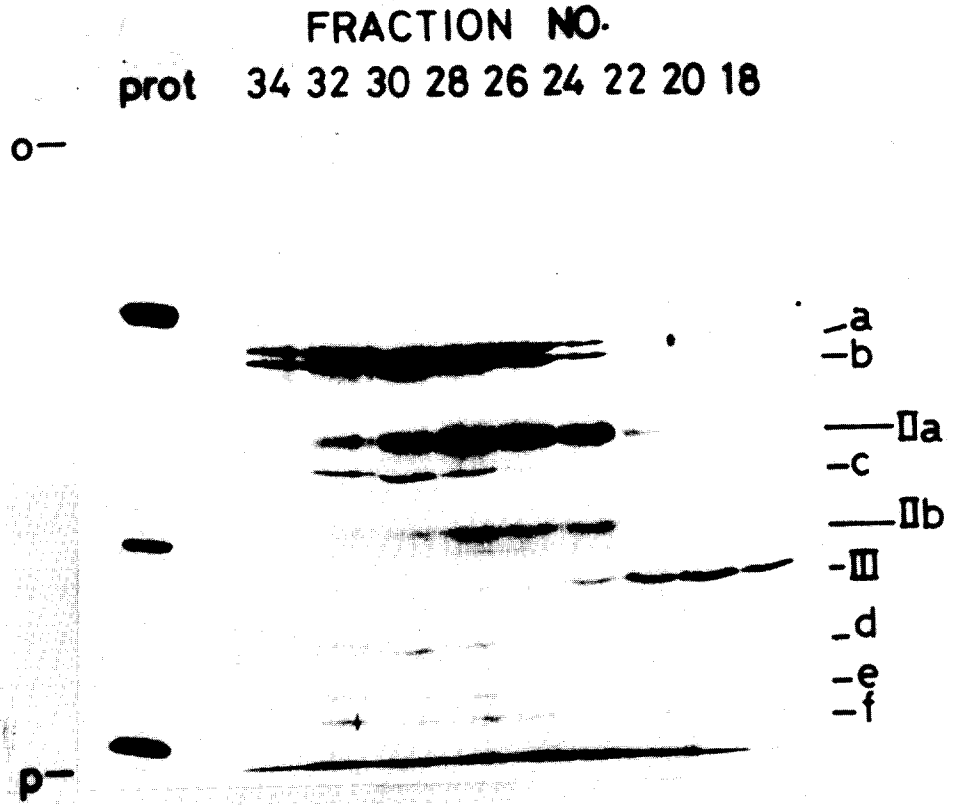

Fig. 2. SDS-polyacrylamide gel electrophoretic patterns of the fractions from Ampholine column. The fraction eluted from DEAE-cellulose column was dialyzed against a 0.5% ampholite solution with 0.1% digitonin. The dialyzed material was then placed in the middle of Ampholine column (LKB ampholite, 0.5—1.5%). The electrofocusing on the column was run at about 1.2 W for 45 h at 4°C. The fractions (each 1.5 ml) were taken from the bottom of the column (acid side). A 40-μl sample from each alternate fraction from Ampholine column was mixed with an equivalent amount of concentrated sample buffer, then applied to the slab gel. Purified samples of serum albumin (67 000), chymotrypsinogen A (25 000), and cytochrome *c* (12 400) from Boehringer-Mannheim were used as molecular weight markers. See legend of Fig. 1.

II pigment-protein complex in the previous paper [6] (fractions 24—36).

Densitometric tracing at 563 nm of the stained gel estimated the relative amount of each polypeptide in the different fractions from Ampholine column. The relative amounts of three polypeptides, III, IIa and Cb, for each fraction from Ampholine column, together with the pH, the chlorophyll absorbance at the red maximum at room temperature ($A_{\mathrm{red\ max}}$), and the relative contribution of chlorophyll *b* absorption in the red region of spectrum ($A_{645}/A_{\mathrm{red\ max}}$) are shown in Fig. 3. The high content of chlorophyll *b* when polypeptide III is eluted supports the previous interpretation that the 24 000 component is the polypeptide of light-harvesting chlorophyll *a*/*b*-protein [1,2]. A single chlorophyll *a*-protin apparently accounts for the main elution peak, because absorption and emission spectra in the red region measured at 77 K, and the photochemical activity of 2,6-dichlorophenolindophenol reduction by 1,5-diphenylcarbazide on a chlorophyll basis were practically identical for the fractions on

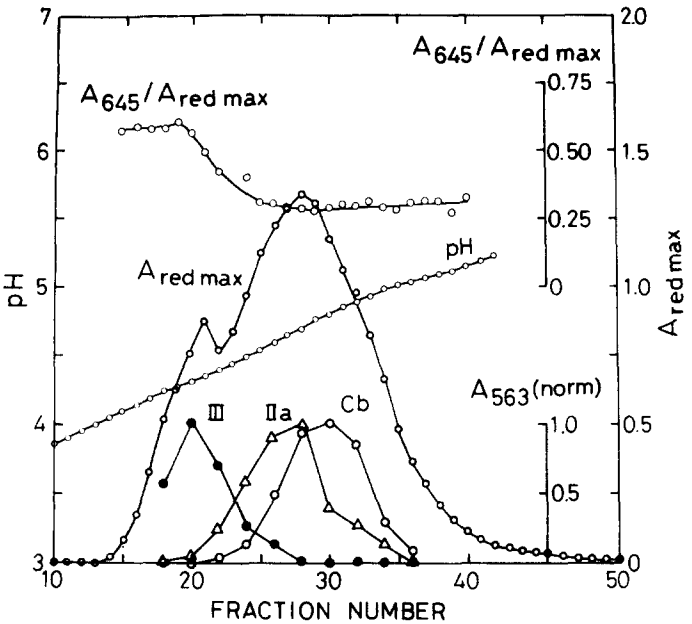

Fig. 3. Fractions obtained from Ampholine column. The data corresponded exactly to those shown in Fig. 2; For each fraction, the pH, the absorbance at the red maximum at room temperature ($A_{red\ max}$), the contribution of chlorophyll *b* absorption to the red region of spectrum as shown by the ratio of A_{645} to $A_{red\ max}$ ($A_{645}/A_{red\ max}$), and the relative amounts of three polypeptides (III, ●; IIa, △; Cb, ○). See legend of Fig. 2.

the either side of this elution peak of chlorophyll *a*. Thus it is probably that the component eluted in parallel with the main chlorophyll *a* band is Polypeptide IIa rather than Polypeptide Cb, although the isoelectric points for these two polypeptides are very close (separated only about 0.05 pH): Therefore polypeptides of C-group (Ca to Cf) are most probably contaminants of some other protein(s) with similar density and isoelectric point to the Photosystem II pigment-protein complex rather than Photosystem II proteins. It was also noted that the SDS-polyacrylamide gel electrophoretic profile obtained with the standard procedure of the purified Photosystem II pigment-protein complex (upper trace of Fig. 1) is very similar to that of the CF_1 [12], except for some slight differences in the estimated molecular weights and for the presence of one additional polypeptide band in the 13 000—17 000 range in Photosystem II preparations.

To clarify this point further, purification of Photosystem II pigment-protein complex was started from chloroplasts deficient in CF_1. Chloroplast preparations treated once with dilute solution of 0.75 mM EDTA (pH 8.0) [11] still retained appreciable amount of CF_1 protein as shown by the SDS-polyacrylamide gel electrophoresis. Thus the EDTA-treatment was repeated twice and satisfactory preparations with little CF_1 protein were obtained. However, the activity of 2,6-dichlorophenolindophenol photoreduction of the preparations was usually reduced to about one-half of that of the control chloroplasts by the second treatment. Subsequent purification steps proceeded almost identically with those for control chloroplasts, except for the relatively rapid decay of the

activity during the purification. The final preparations of Photosystem II pigment-protein complex obtained exhibited the photochemical activity of 2,6-dichlorophenolindophenol reduction by 1,5-diphenylcarbazide comparable to that of the original EDTA-treated chloroplasts (60—120 μmol DCIP reduced/mg chlorophyll per h). The preparations also showed essentially the same absorption and emission spectra measured at 77 K, chlorophyll *a* to chlorophyll *b* ratios (greater than 50), and isoelectric points (4.6 ± 0.05) as those of preparations from control chloroplasts (see ref. 6). SDS-polyacrylamide gel electrophoresis of the preparations obtained from EDTA-treated chloroplasts exhibited none of the six polypeptide bands shown in the upper trace of Fig. 1. Thus we can conclude that the six polypeptides reported previously [6] in purified Photosystem II preparations are attributable to contamination by CF_1 (and probably one other polypeptide). The sample for SDS-polyacrylamide gel electrophoresis prepared by standard procedure (with heat-treatment) gave no appreciable polypeptide band, except for one large spot at the origin of the gel. SDS-polyacrylamide gel electrophoretic analysis of the sample prepared without heat-treatment revealed that the purified Photosystem II pigment-protein complex is composed of two polypeptides, namely IIa and IIb (Figs. 4 and 6). In the same type of experiment as depicted in Fig. 3, but conducted on preparations obtained from EDTA-treated chloroplasts, polypeptides IIa and IIb were eluted from the Ampholine column in parallel with the main chlorophyll *a* absorption (Fig. 5). In SDS-polyacrylamide gel electrophoresis, however, all the chlorophyll was found in the free pigment zone at

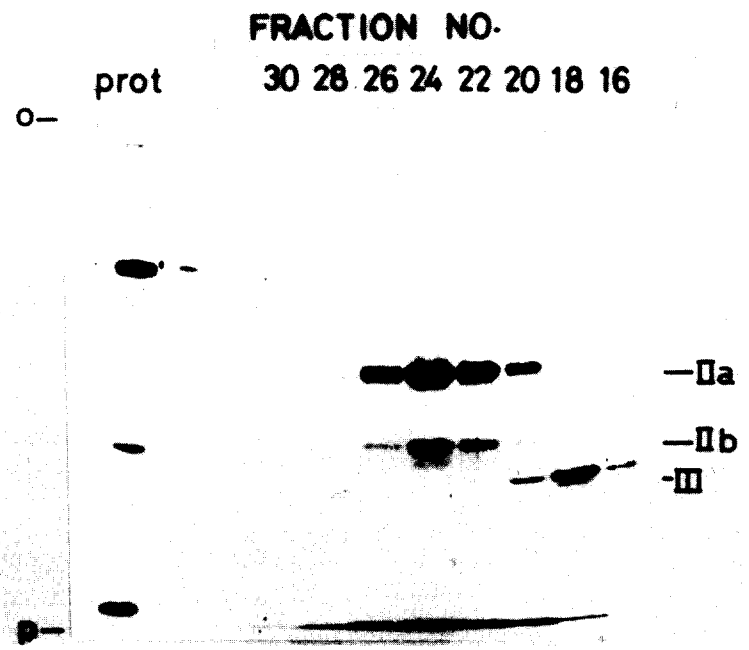

Fig. 4. SDS-polyacrylamide gel electrophoretic patterns of the fractions from Ampholine column. The preparations were obtained from EDTA-treated chloroplasts. See legends of Figs. 1 and 2.

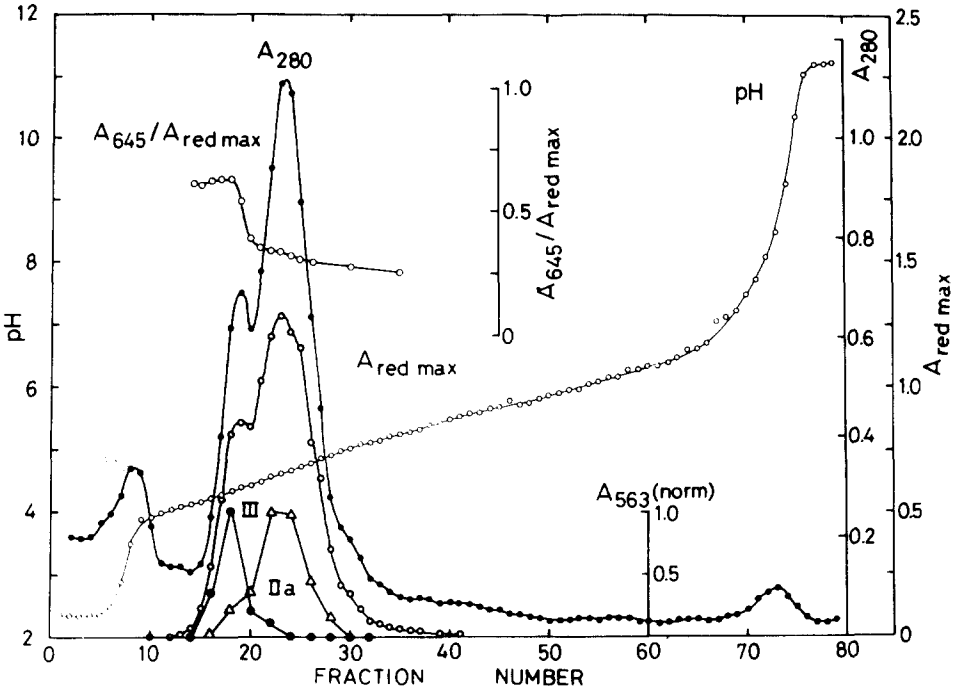

Fig. 5. Fractions obtained from Ampholine column. The data corresponded exactly to those shown in Fig. 4. A_{280}, absorbance at 280 nm. See legends of Figs. 3 and 4.

the completion of electrophoresis. Some attempts (for example, lowering the SDS concentration or using urea instead of SDS) were made to find condition under which one or both polypeptides carried chlorophyll *a* during the electrophoresis, but without success. Based on the following facts, I believe polypeptides, IIa and IIb, are valid structural or functional components of Photosystem II pigment-protein complex: (a) The presence of the bands in the profile of SDS-polyacrylamide gel electrophoresis of the original chloroplasts, and their presence in the purified Photosystem II complex; (b) The elution from Ampholine column in parallel with the elution of chlorophyll *a* which comigrates with the Photosystem II activity; (c) The absence of any other noticeable polypeptide band, even in the stacking gel, in the SDS-polyacrylamide gel electrophoresis of the purified preparations.

The purified preparations of Photosystem II pigment-protein complex obtained by Wessels et al. [7] and by Vernon et al. [8] have much more complex polypeptide profiles than that of this preparation. However, a polypeptide band of the molecular weight of about 43 000 is one of the common, major component of all of these three preparations [6—8]. The location of the reaction center polypeptide of Photosystem II in the electrophoretic patterns of solubilized chloroplast thylakoids has been investigated using mutants of *Chlamydomonas* [13] and barley [14] lacking Photosystem II activity. Both mutant studies showed that the absence of a polypeptide of the size corresponding approximately to the 43 000 band (47 000 in *Chlamydomonas* and 46 000 in barley) was correlated with lack of Photosystem II activity. The

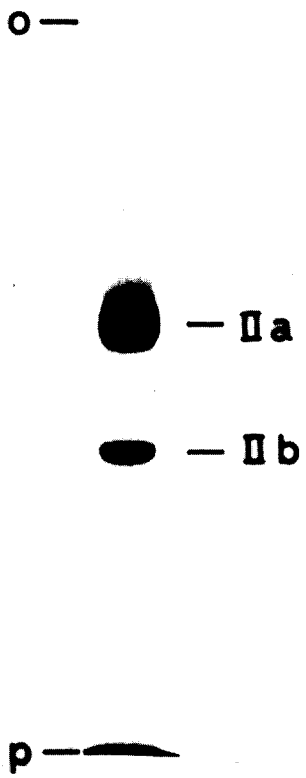

Fig. 6. SDS-polyacrylamide gel electrophoretic pattern of the purified Photosystem II pigment-protein complex prepared from EDTA-treated chloroplasts. Sample containing approx. 7 μg chlorophyll was added to the gel. See lengend of Fig. 1.

mutant of barley (vir-c^{12}) deficient in Photosystem II activity was shown to be lacking both a 46 000 component and a polypeptide of a size, in my estimate, of about 27 000 [14]. The evidence which indicates the presence of a second chlorophyll *a*-protein complex which appears in SDS-polyacrylamide gel electrophoresis between the Photosystem I chlorophyll *a*-protein complex (CPI) and the light-harvesting chlorophyll *a/b*-protein (CPII), and which may be correlated with Photosystem II activity, are accumulating recently [15—22]. Among them, band IId of Herrmann and Meister [16], complex IV of Hayden and Hopkins [18], band C of Wessels and Borchert [19], band A of Henriques and Park [20], and band CPa of Anderson et al. [21] were well characterized on the basis of the spectral properties, although there was no direct proof for the activity. Polypeptide analysis of the band A of Henriques and Park [20] obtained from lettuce chloroplasts demonstrated the presence of a polypeptide component of about 42 000 (the size almost identical with that of IIa) in the second chlorophyll *a*-protein complex.

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