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IDENTIFICATION OF POLYPEPTIDES OF PHOTOSYSTEM I REACTION CENTER AS THE PRODUCTS OF CHLOROPLAST GENES PS1A1 AND PS1A2

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The Photosystem I Reaction Center of spinach was found to contain two polypeptides of approximate apparent Mr of 56,000 and 64,000. The 56 kDa polypeptide was identified as the product of chloroplast gene PS1A1 using an antibody specific for the PS1A1 gene product of corn. Presumably the 64 kDa polypeptide is the product of gene PS1A2.

Photosystem I Reaction Center (PS I RC), also called chlorophyll protein complex I (CP I) can be obtained from thylakoids by preparation of photosystem I particles and further fractionation, or by treatment of thylakoids with SDS and electrophoresis on acrylamide gels (1). Prepared by the latter procedure PS I RC has usually been called CP 1. PS I RC prepared by either procedure has P700 activity and predominantly a polypeptide(s) of about 60 kDa (1). The 60 kDa band has been reported as either a single band, or a doublet, sometimes requiring special gel conditions, e.g. the presence of urea for resolution (2, 3, 4, 5). We found that when spinach thylakoids were heated under reducing conditions

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Abbreviations: CP I (see PS I RC), CP II- chlorophyll protein complexes I and II, respectively; FC- free chlorophyll; LDS and SDS- lithium and sodium dodecyl sulfate, respectively; PS I RC- photosystem I RC (see CP I).

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with LDS and electrophoresed, the PS I RC band found without heat treatment, was replaced by two bands of approximately 56 and 64 kDa. The recent finding that chloroplast DNA contains two highly homologous PS I RC genes (6,7) which are transcribed into a polycistronic mRNA (7, 8, 9) suggested that the two polypeptides we had been observing were distinct products of the two PS I RC genes. This conjecture appears to be correct.

MATERIALS AND METHODS

Spinach was grown in vermiculite, (10) except that the light intensity was 500 μE/m2 x min, and one plant was grown per 4 in pot suspended in nutrient solution. Leaves were harvested repeatedly between the sixth and twelfth weeks from sowing. Thylakoids were isolated from gradient-purified chloroplasts (10), and stored at -80°C. Electrophoresis was carried out on 10 % acrylamide gels, as described (10), except where indicated otherwise, using 3 mm thick gels for preparation of PS I RC, and 1.5 mm gels for analytical purposes. Samples of thylakoids were prepared for electrophoresis by treatment with LDS at 0°C, or they were heated 2 min at 80°C, except where noted otherwise. Gels were stained with Coomassie Brilliant Blue (10) or with silver (11), or were left unstained. Unstained gels were photographed with a blue filter (Tiffen 80A), and Coomassie-stained with a green filter (Tiffen 58).

Antibody was raised (12) to a PS I RC preparation containing only the A and B polypeptides (13). Otherwise, to prepare PS I RC, thylakoids containing 2.5 mg of chlorophyll were treated with LDS and electrophoresed in the cold on 10 % acrylamide gels with a slot 240 x 0.3 mm. The green band was cut out, ground in a mortar and pestle and extracted 3 X with 50 mM Tris, 0.1 % SDS, pH 8.0. Acrylamide was removed by centrifugation, extracts were dialyzed against buffer without SDS, and the dialyzed extract was centrifuged 18 h in a Beckman 60 Ti rotor at 50,000 rpm to pellet PS I RC. The supernatant was removed from the noncompacted pellet with a syringe, PS I RC resuspended in residual supernatant, SDS added to 0.1%, and was stored at -80°C. The PS I RC was reelectrophoresed on an 8 % gel, about 0.3 mg chlorophyll per 240 x 0.3 mM well, and the complex was recovered as just described for 10 % gels. Antibody (sp66) to a synthetic peptide corresponding to amino acids 414 to 423 of corn PS1A1 was obtained from Dr. L. Fish (4). Polypeptides in acrylamide were transferred to nitrocellulose (14), reacted with antibody and the reaction with antibody detected with goat anti-rabbit coupled to horse radish peroxidase (15).

To estimate protein in PS I RC and in bands A and B, thylakoids were heated at different temperatures, electrophoresed, and stained with Coomassie. Lanes were cut, placed in a silica boat, and scanned at 596 nm with a Gilford Model 240 spectrophotometer and gel scanning attachment. Relative amounts of protein were estimated by peak heights.

RESULTS

When spinach thylakoids are heated two min at 80°C the green PS I RC found on electrophoresis of unheated thylakoids appears to be replaced by two colorless bands, a sharp band (A) which migrates just behind the α subunit of CF 1, and a diffuse band (B) which migrates between the α and β bands of CF 1 (Fig. 1, lanes 7 and 8, and Fig. 2, lanes 5 and 6). This apparent reciprocal relationship was quantitated by plotting the disappearance of PS I RC and the appearance of polypeptides A and B as a function of temperature at which thylakoids were heated (Fig. 3). To test further the presence

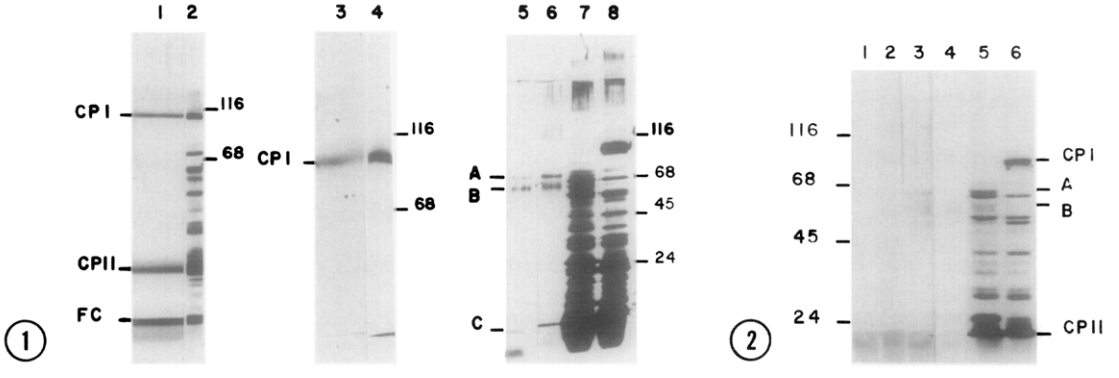

Figure 1. Purification of PS I RC. A) 10 % gel: lane 1 unstained; lane 2, stained with Coomassie. B) PS I RC (CP 1) isolated from 10 % gels and rerun on an 8 % gel: lane 3, unstained; lane 4, stained with Coomassie. C) Analysis of PS I RC from 10 % and 8 % gels on a 10 % gel, stained with silver: lane 5, PS I RC from 10 % gel, heated; lane 6, PS I RC from 8 % gel, heated; lane 7, thylakoids, heated; lane 8, thylakoids, unheated. CP I and CP II are chlorophyll protein complexes I (PS I RC) and II, respectively. FC is free chlorophyll. Numbers to the right of each panel are Mr in kDa.

Figure 2. Identification of the product of gene PS1A1. Samples of thylakoids, containing 25 ug chlorophyll, were treated with LDS in the cold (lane 6), or were heated 2 min at 80 C (lanes 1-5). Lanes 5 and 6 were stained with Coomassie. Lanes 1-4 were blotted to nitrocellulose. The nitrocellulose was reacted with anti-PS I RC (lane 3) and its corresponding preimmune serum (lane 1), or with anti-sp66 (lane 4) or with its corresponding preimmune serum (lane 2). Numbers to the left are kDa. CP 1 is PS I RC, and A and B are PS I RC polypeptides A and B. The only materials on the nitrocellulose that reacted with antibody were in the region of A and B. Other bands are colored material not related to the immunological reaction.

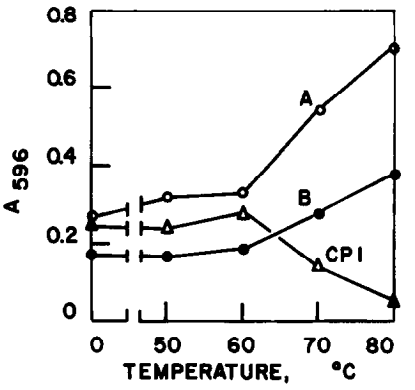

Figure 3. Polypeptide bands A and B arise in parallel from PS I RC (CP 1) on heating of thylakoids. Replicate samples of thylakoids, containing 25 ug chlorophyll, were heated with SDS for 2 min at the temperatures indicated. They were electrophoresed, gels stained, scanned, and the relative amounts of PS I RC, and PS I RC polypeptides A and B were determined.

of bands A and B in PS I RC, PS I RC was isolated. Thylakoids were treated with LDS at 0 C and electrophoresed at 4°C on 10 % acrylamide gels (Fig. 1, lanes 1 and 2). The PS I RC band (CP I) was recovered, and a portion was reelectrophoresed under the same conditions, except on 8 % acrylamide gels, to remove contaminating non-pigmented polypeptides (Fig. 1 lanes 3 and 4)(16), and the complex recovered as above. PS I RC from the 10% gel (Fig 1 lane 5), and PS I recovered from the 8 % gel (Fig. 1 lane 6) were heated and electrophoresed on a 10 % gel. PS I RC polypeptides A and B were present in both preparations, indicating that both are components of the pigment protein complex. In addition, a polypeptide band of approximately 10 kDa was also present, as has been observed previously (5). It was concluded that 56 kDa and 64 kDa polypeptides are components of PS I RC.

The possibility that PS I RC polypeptides A and B are the products of the PS1A1 and PS1A2 genes was tested in an immunodecoration experiment (Fig. 2) using 1) an antibody to a PS I RC preparation which contained polypeptides A and B,

but not C, and 2) an antibody (anti-sp66) to the derived sequence (amino acid numbers 414 to 423) of the PS1A1 gene of corn. This sequence is also present in spinach PS1A1 (amino acids 413 to 422)(7, and Margulies, M., unpublished). As expected antibody 1 reacted with both the A (sharp) and B bands (diffuse) of PS I RC of spinach thylakoids (lane 3). In contrast, antibody 2 reacted with only the B band (lane 4). The corresponding preimmune sera did not react (lanes 1 and 2). It was concluded that the B band corresponds to the product of the PS1A1 gene. Deductively, the A band is probably the product of the PS1A2 gene.

DISCUSSION

PS I RC, prepared as described here contains, in addition to the two high Mr polypeptides, a polypeptide of low Mr. This result has been observed previously (5). Spinach PS I RC has been shown here to contain two polypeptides of apparent Mr 56,000 and 64,000. A similar observation has been made for corn (4, 6). However, good separation and definition of the two bands was obtained without the addition of urea to gels, as was required with corn PS I RC (4). The lower band was identified as the product of the PS1A1 gene, even though its predicted Mr from the DNA sequence of the gene is lower than the product of gene PS1A2 (7)(Fig. 2). In contrast, in corn, when PS I RC polypeptides are separated on acrylamide containing urea, the upper band is the PS1A1 product (4). The reason for this difference is not known, but could possibly be due to the composition of the SDS used for separation of the polypeptides (17), the presence of urea, or it might be due to an intrinsic property of the polypeptides. However, this difference in order of migration of PS1A1 and PS1A2 gene products in corn and spinach is not regarded as important

because these polypeptides behave anomalously. The apparent Mr of the corn gene products is considerably lower than the Mr indicated from the DNA sequence, even though the polypeptide products do not seem to be processed (4). The reason for this behaviour is not known.

It is unlikely that PS I RC polypeptide A is a derirative of PS I RC polypeptide B, the product of gene PS1A1, since the peptide, sp66, to which antibody was prepared, is present in the middle of the molecule both in corn, and in spinach (7, Margulies, M., unpublished). Since polypeptide A reacts with antibody to PS I RC, which contains both the A and B polypeptides (Figs. 1 and 2), but not with anti-sp66, which is specific for the ps1a1 gene product (4), it seems likely that polypeptide A is the product of gene PS1A2. However, positive identification of the PS1A2 gene product has not been made.

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