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Amino-terminal sequence of the 21 kDa apoprotein of a minor light-harvesting pigment-protein complex of the Photosystem II antenna (LHC IId/CP24)

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The 21 kDa apoprotein of LHC IId, a minor light-harvesting antenna component of Photosystem II, has been isolated and subjected to N-terminal protein sequencing. A sequence of 66 residues was obtained which contains regions of considerable homology to both those reported for LHC II and LHC I, but which is obviously distinct from them. The proposed occurrence of an identical 21 kDa LHC subunit in both photosystems I and II is shown to be incorrect.

Light-harvesting complex; Pigment-protein; Photosystem II subunit; Protein sequence

1. INTRODUCTION

Efficient light capture by photosynthetic organisms involves an intricate cooperation between the pigments in a light-harvesting antenna complex (LHC) and those in a core complex (CC) of a photosystem [1]. Each LHC is made up of several photosynthetic pigment-protein complexes. In higher plant photosystem II (PS II), the LHC contains at least four distinct pigment-binding antenna protein components (LHC IIa, b, c and d; reviewed in [2]), which bind approx. 55% of the total chlorophyll in a chloroplast [1]. LHC IId (or CP 24) is a relatively minor antenna constituent, binding approximately 3% of the total chlorophyll, and was first identified by Dunahay et al. [3]. It is thought to contain one (Peter and Thornber, manuscript in preparation) or at least two apoproteins of 21-25 kDa ([3,4], see [1] for review]. LHC IId typically has a relatively low chlorophyll *a/b* ratio of 0.8-1.0 and a room temperature red absorbance peak at 668 nm [3,4] or 674 nm (Peter and Thornber, manuscript in preparation). A 77K fluorescence maximum has been reported for LHC IId at 680-690 nm [3,4]. This pigment-protein complex has been postulated to serve with other minor LHC IIs as a 'linker' between the core complex II and the major LHC II complex, LHC IIb ([3], Peter and Thornber, manuscript in preparation).

Similar to LHC II, the light-harvesting antenna system of PS I contains several different pigment-protein complexes [1]. Lam and coworkers [5,6] have observed two different LHC I complexes LHC Ia and LHC Ib. LHC Ia was found to be composed of two apoproteins of about 22 and 23 kDa with a 77K fluorescence maximum at 680 nm. These authors reported that the second antenna complex, LHC Ib, contained one apoprotein of 20 kDa and had a 77K fluorescence maximum at 680 nm. In contrast Bassi and collaborators [7] have characterized two LHC I complexes with a slightly different subunit composition than those described by Lam et al. They have shown that LHC I-730 contains two major proteins of 21.5 and 24 kDa and has a 77K fluorescence maximum at 730 nm, while LHC I-680 is composed of one 21 kDa protein and has a 77K fluorescence maximum at 690 nm. The relationship between the LHC I complexes described by the two groups is unclear. Furthermore, LHC I-680 has been postulated to be the same pigment-protein complex as LHC IId due to their similar spectroscopic characteristics and slight antigenic cross-reactivity [4], although exact homology between the apoproteins of the two complexes has yet to be conclusively demonstrated.

While many primary structures have been deduced for apoproteins of LHC IIb [8] and LHC I [2,9], none has yet been knowingly obtained for the minor LHC IIs. To this end we have isolated the LHC IId apoprotein from barley and subjected it to N-terminal amino acid sequencing. A sequence of 66 residues was obtained which displays some homology to those of the LHC IIb and LHC I polypeptides; nevertheless, it is a distinct LHC apoprotein which is obviously related to other proteins derived from the *Cab* gene family. It is hoped that the derived sequence will be used to obtain a full length sequence from a clone or will enable an already

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existing clone to be identified as that of LHC IId's apoprotein.

2. MATERIALS AND METHODS

Barley (*Hordeum vulgare* var. Prato) seeds were imbibed in distilled water overnight and then grown in vermiculite in a greenhouse for seven days under natural lighting conditions. PS II-enriched thylakoid membrane fractions were isolated by the methods of Dunahay et al. [10] but with an additional wash with 1 M $CaCl_2$ to remove the polypeptides of the oxygen-evolving complex [11]. For isolation of the LHC IId apoprotein, PS II-enriched membranes were first solubilized in octyl glucoside and SDS and then the individual chlorophyll-binding components were separated on a non-denaturing PAGE system, according to Dunahay et al. [3]. The green band corresponding to the LHC IId pigment-protein complex was excised and stored in distilled water at -20°C prior to further processing. The LHC IId complex in the polyacrylamide gel strips was fully denatured and electrophoresed on a 10–16% polyacrylamide gel using the buffer system of Laemmli [12] but with 0.75 M Tris-HCl, pH 8.8 and 4 M urea in the separating gel. The gel was lightly stained [13] and the LHC IId apoprotein electroeluted. The electroeluted protein was dialyzed overnight against 10 mM ammonium bicarbonate, 0.05% sodium dodecyl sulfate and then concentrated using Centricon 10 microconcentrators (Amicon, Danvers, MA, USA). The protein was sequenced using an Applied Biosystems 470A gas-phase protein sequenator.

Western blots were performed according to Towbin et al. [14]. LHC I monoclonal antibody (CMpLHCI; a kind gift of Dr Gunilla Hoyer-Hansen [15] was used at a 1:200 dilution. PS I was isolated on sucrose gradients as described by Anandan and Thornber [16].

All electrophoresis chemicals were ultra-pure grade. Isolation of the various pigment protein complexes was carried out using reagent-grade chemicals and detergents.

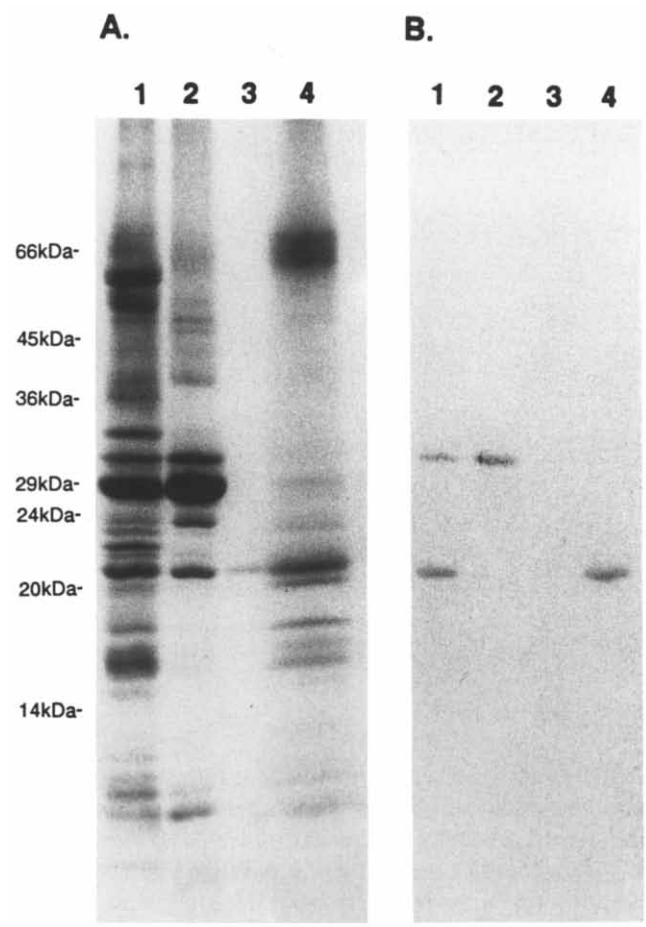

Fig. 1. Polypeptide analysis of the different pigment-protein complexes separated by fully denaturing SDS-PAGE. (A) Stained with Coomassie blue and (B) Western blot with the anti-LHC I antibody. Fractions in each lane are: (1) whole thylakoids; (2) PS II-enriched membrane preparation; (3) isolated LHC IId; (4) PS I.

RESULTS AND DISCUSSION

We have purified the LHC IId apoprotein to homogeneity by fractionation of PS II-enriched membrane preparations on non-denaturing followed by fully denaturing PAGE (Fig. 1). In barley the LHC IId apoprotein has an apparent size of 21 kDa (Fig. 1A, lane 3). Several steps were taken to minimize contaminants co-migrating with LHC IId, particularly the 23 kDa oxygen-evolving complex protein and LHC I apoproteins. To this end the thylakoid membranes were washed with 1 M $CaCl_2$ prior to preparation of the PS II-enriched membranes to remove the extrinsic oxygen-evolving complex proteins. Furthermore, the use of a PS II-enriched fraction as the starting material greatly reduced or eliminated the LHC I pigment-proteins of PS I, which are the only other major polypeptides in thylakoids with an almost identical size (Fig. 1A, lane 4). Lastly, we used the LHC I antibody CMpLHCI [15] to probe a Western blot of various thylakoid membrane protein preparations (Fig. 1B). The monoclonal antibody cross-reacts with the 31 kDa LHC IIa apoprotein (Fig. 1B, lanes 1 and 2) and a 21 kDa polypeptide of photosystem I, i.e. the LHC Ib apoprotein (LHC I-680; Fig. 1B, lanes 1 and 4), but not with the LHC IId apoprotein (Fig. 1B, lanes 2 and 3). This observation contradicts the interpretation of Bassi et al. [4] on the cross-reactivity of the LHC Ib and LHC IId polypeptides. The cross-reactivity observed by Bassi and coworkers could possibly be due to small amounts of LHC Ib in their LHC IId preparations. We therefore conclude that: (i) since the antibody does not cross-react with the polypeptide we isolated, we did not have the LHC Ib apoprotein present in our LHC IId preparations; and (ii) the LHC Ib (LHC I-680) apoprotein is a different polypeptide than that of LHC IId, and therefore there are two different (cf. [4]) 21 kDa pigment-proteins in photosystems I and II. In support of this latter conclusion we stress that in barley the LHC IId apoprotein differs in having an open N-terminus (see below), while that of LHC Ib is blocked (Anandan and Thornber, manuscript in preparation). Additionally, an N-terminal sequence has been obtained for the 21 kDa LHC Ib apoprotein from pea which displays essentially no homology in the first 25 residues to the sequence obtained for LHC IId (Anandan and Thornber, manuscript in preparation).

Approximately 1.4 nmol of the purified LHC IId 21 kDa protein were initially loaded into the sequenator

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                        10        20        30        40        50        60
LHCIId    NH2-AAAGKKSWIP AFKSDAEFIN PSWLDGSLPG DFGFDPLGLG KDPAFLKWYG WAELI---WA M-AVLG

LHCI-15   (13).VSAVAADPD RPLWFPGSTP .E..... ..... ...ES...NA Q...VHSR.. .LGAA.
CAB6-A              NH2-SADWMPGQPR ..Y....A.. ..... .V..N.ER.K ES...HCR.. .L..P.
LHC IIb   (10)GSPWYGPDRV KYLGPFSGES ..Y.T.EF.. .Y.W.TA..S A..ETFAKNR EL.V.HGR.. .LGA..
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Fig. 2. Sequence of the first 66 amino-terminal residues of the LHC IId apoprotein and comparison with two deduced LHC I sequences, LHCI-15 [20] and Cab6-A [22] and a LHC IIb sequence [19]. A (.) indicates homology to the LHC IId sequence. A (–) indicates an undetermined amino acid residue. Numbers in parentheses before the LHCI-15 and LHC IIb sequences indicate the number of residues from the putative processing site for the mature protein.

and the sequence of the first 66 residues of the LHC IId apoprotein was obtained (Fig. 2). The sequence contains predominantly hydrophilic residues, indicating its probable exposure to the chloroplast stroma or to the thylakoid lumen. Residues 21–66 of the LHC IId sequence have regions of distinct similarity to the amino acid sequence of LHC IIb from various plant species (Fig. 2). This might indicate that the LHC IId apoprotein resides in the thylakoid membrane in a similar conformation as proposed for the LHC IIb apoprotein [17,18] with its N-terminus exposed to the stroma. Within the first 20 residues of the LHC IId apoprotein, no homology to the LHC IIb protein sequence is observed. The amino terminal residue is an alanine and not a methionine residue as has been proposed to be the initial amino acid for the mature LHC IIb apoprotein [17]. Additionally, residue no. 4 of the LHC IId amino acid sequence produced equal amounts of Asp and Gly during sequencing, possibly indicating a small multigene family is being expressed for this apoprotein.

Recently a cDNA clone, LHCI-15 [20], has been isolated from petunia which putatively codes for a 24 kDa apoprotein of LHC I. Genomic and cDNA clones, both termed *Cab*-7, have also been isolated from tomato and are similar in sequence to LHCI-15 [21]. Although the petunia LHCI-15 clone was identified as coding for a polypeptide associated with the PS I complex, it was termed a gene coding for the CP24 (LHC IId; i.e. the component we have studied in this paper) apoprotein mainly on the basis of the gene product's antigenicity to antibodies made against various chlorophyll-binding apoproteins of which the anti-CP24 and anti-LHC I reacted the most strongly. However, comparison of the first 22 residues of the LHC IId sequence with the derived LHCI-15 sequence reveals no obvious sequence homology (Fig. 2) and probably indicates that the LHCI-15 gene product is not equivalent to the CP24 apoprotein. The high amount of cross-reactivity with the LHC antibodies used to characterize the LHCI-15 clone could be attributed to high degrees of epitope similarity found within areas of the LHC IId and LHCI-15 polypeptides (see Fig. 2). The sequence from residues 23–43 of LHC IId shows absolute homology to the LHCI-15 sequence of petunia and partial homology to the LHC IIb sequence, indicating a possible conserved function or secondary structure in this region among different *Cab* gene products. The LHCI-15 gene also codes for a mature protein that is putatively 13 amino acids longer at the N-terminus than the LHC IId apoprotein. Similarly, *Cab*-6A, another putative LHC I cDNA clone from tomato coding for a polypeptide of about 24 kDa [22], contains similar regions of homology, although overall it does not appear to be homologous to either LHC IId or LHCI-15 (Fig. 2).

In summary, we have purified the apoprotein of LHC IId to homogeneity and subjected it to N-terminal protein sequencing. The resulting sequence displays regions of high homology to both LHC IIb and LHC I sequences, although it is definitely a different protein. Additionally, it is evident that LHC IId (CP24) and LHC Ib (LHC I-680) are not the same pigment-protein complex as has been previously postulated. Work has been initiated to isolate a gene for the LHC IId apoprotein in order to determine its full sequence.

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