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Correlation of some published amino acid sequences for photosystem I polypeptides to a 17 kDa LHCI pigment-protein and to subunits III and IV of the core complex

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Photosystem I (PSI) in barley consists of at least 11 polypeptides of which three have apparent sizes of 15–19 kDa. Two of these polypeptides (subunits III and IV) are constituents of the core complex (CCI), the third is a component of the light-harvesting complex (LHCI). After fractionation of PSI into its CCI and LHCI components, each of the polypeptides has been isolated and its N-terminal region sequenced. We conclude that the gene sequence published for subunit IV of spinach [(1988) FEBS Lett. 237, 108–112] is not that of subunit IV but rather that of the 17 kDA LHCIc pigment protein. We confirm that the published sequence for subunit III [(1988) Curr. Genet. 14, 511–518] is indeed that of subunit III; seemingly conflicting identifications, based on apparent sizes on SDS-PAGE, of which polypeptides are subunits III and IV are probably explained by subunit III's electrophoretic migration rate being dependent on the solvent.

Photosynthesis; Photosystem I subunit; Light-harvesting complex; Protein sequence; Pigment protein

1. INTRODUCTION

Photosystem I can be thought of as being composed of two pigmented multi-protein components, a core complex (CCI) and a light-harvesting complex (LHCI) [1,2]. The primary structures of all of the CCI polypeptides, except that of subunit VI, have been deduced from gene sequences: the *psaA* and *psaB* gene products (subunit I), the 68 kDa apoproteins of the P700-chlorophyll *a* protein [3]; the *psaD* gene (subunit II) product (e.g. [4,5]), the putative ferredoxin-binding 21 kDa polypeptide [6]; the *psaE* gene product (subunit III), the putative plastocyanin-binding polypeptide of 17 kDa [5,7]; the *psaF* and *psaG* gene products (subunits IV and V) of as yet undetermined function [8]; and the *psaC* gene product, the 8 kDa Fe-S containing polypeptide [9]. For LHCI, however, the data are not so advanced. In fact, the subunit composition of LHCI is not unequivocally established; nevertheless, it is apparent that at least four polypeptides of 24, 21, 17 and 11 kDa are present ([10–13], see also [2] for review). It is probable that all of these subunits are apoproteins that bind both carotenoids and chlorophylls [10,11,13]. Three putative LHCI gene sequences have been published [14–16]; it has been proposed that these sequences might be those of the major chlorophyll *a/b* binding constituent (the 21 kDa subunit), but this view remains to be substantiated.

We have purified the 17 kDa component of barley and maize LHCI and determined their N-terminal amino acid sequence. The derived sequences aligned particularly well with that published for CCI subunit IV of spinach [8] and, more recently, with that for the P21 polypeptide of *C. reinhardtii* thylakoids [17], as well as with a 17 kDa PSI polypeptide of pea, a partial N-terminal sequence of which was determined by

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Dunn et al. [18]. We present evidence here that the sequences reported for spinach and pea are that of a 17 kDa LHCI polypeptide and not, as was proposed for spinach [8], that of subunit IV of CCI. Furthermore, we confirm that the subunit III sequence determined by Munch et al. [5] is that of subunit III; differences in the identification of subunits III and IV on SDS-PAGE are probably explained by subunit III's relatively large variation in electrophoretic mobility in different systems (cf. [19]).

2. MATERIALS AND METHODS

Barley (*Hordeum vulgare* var. Prato) and maize (*Zea mays* W273) seedlings were grown in a greenhouse under normal growth conditions.

The preparation of barley thylakoid membranes and PSI vesicles was essentially the same as described for *C. reinhardtii* [20], with the following important modifications: after removal of the proton-ATPase complexes from the thylakoids by octyl glucoside/sodium cholate solubilization and high speed centrifugation [19], the membranes were resuspended in 25 mM Tricine pH 8.0 buffer, but, unlike previous procedures, Triton X-100 was not added for membrane solubilization since it disrupted the integrity of the LHCI complex. Rather, the membrane suspension was loaded onto a DEAE-cellulose column, which was washed with 0.1% (w/v) dodecyl maltoside. A PSI-enriched fraction was subsequently eluted from the column with a buffered 0–0.3 M NaCl gradient containing 0.1% dodecyl maltoside. The eluted material was loaded onto the top of a 5–25% (w/v) sucrose density gradient and centrifuged at 80000 × *g* for approx. 24 h. The heaviest pigmented band proved to be the PSI complex.

Purified PSI isolated from sucrose gradients was incubated at 37°C for 20 min and fractionated into its LHCI and CCI components by electrophoresis on non-denaturing Deriphat-PAGE [10,21]. For the isolation of the LHCIc apoprotein and subunits III and IV, polyacrylamide strips containing LHCI or CCI, respectively, were soaked in denaturing buffer containing 1% SDS and 100 mM dithiothreitol, and the proteins were separated by SDS-PAGE, using the buffers of Laemmli [22] to which 4 M urea (recrystallized) was added to the separating gel buffer and 1 mM sodium thioglycolate to the electrode buffer [23]. Protein bands were transferred to PVDF membranes [24], and sequenced on an Applied Biosystems model 470A gas phase sequenator. Maize LHCIc pigment protein was obtained as described in Vainstein et al. [11], and its apoprotein prepared for sequencing as described above.

All chemicals and reagents were obtained from Sigma Chemical Company, except those used in the preparation of proteins for sequencing which were purchased from Bio-Rad. PVDF (Immobilon-P) membrane was obtained from Millipore Corp.

3. RESULTS

In fig.1, SDS-PAGE has been used to show the subunit compositions of PSI, CCI and LHCI fractions of the photosynthetic apparatus of barley. The PSI preparation was obtained by a modification of the procedure of Schuster et al. [20]. Essentially, the solubilizing surfactant for the thylakoids was changed from Triton X-100 to dodecyl maltoside, and the density gradient centrifugation was through sucrose solution containing dodecyl maltoside. The PSI fraction was then heated to 37°C and the resulting solution electrophoresed through a non-denaturing PAGE system using the conditions developed by Peter et al. [10,21] which maintain the complexes in as native a state as possible. The PSI material was fractionated into two smaller pigmented bands (CCI and LHCI); some of the original PSI material remained (fig.1A) (see also [12]). In fig.1B the subunit compositions of these bands can be compared. It is clear that a combination of the polypeptides in the CCI and LHCI fractions sums to those that are present in PSI. Further, the CCI component contains the multiple polypeptides (i.e. subunits I–VII), as expected from previous studies [10,11,25,26], and LHCI contains subunits of 24, 21, 17 and 11 kDa (cf. [10–12]). The 21 kDa component can be seen occasionally, at least in barley and maize, as a doublet. Note that the 11 kDa component stains much less than the others, and that the 21 kDa component(s) have a similar electrophoretic mobility as that of subunit II of CCI (the *psa*D gene product), and that the 17 kDa subunit of LHCI migrates between subunits III and IV of CCI.

The 17 kDa LHCI polypeptide was isolated (see section 2) from the denatured LHCI complex by layering it across the entire width of an SDS-polyacrylamide slab gel. After electrophoresis the protein bands in the gel were electroblotted onto a PVDF sheet [24] which was subsequently lightly stained with Coomassie blue. The 17 kDa polypeptide band was excised and later placed in the reaction vessel of a gas-phase amino acid sequencer. The sequence obtained is shown in table 1 in which it is compared with the sequences of the equivalent 17 kDa polypeptide isolated from maize by a slightly different procedure (cf. [11]), with those of subunit IV of spinach [8] and of P21 of *C. reinhardtii* [17] and with the partial N-terminal sequence of a 17 kDa subunit of photosystem I of pea [18]. It is apparent that the sequences are

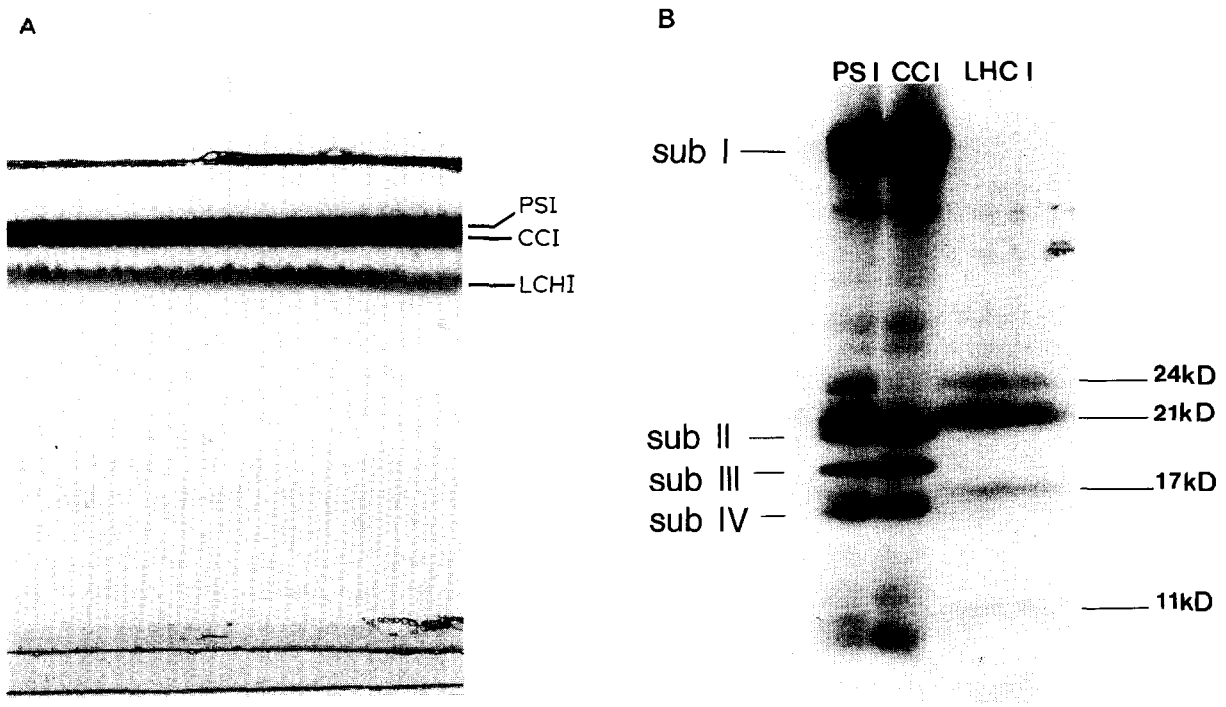

Fig.1. (A) Non-denaturing Deriphat 160-PAGE of isolated PSI after treatment at 37°C for 20 min; note that some of the original PSI contributes the uppermost band. The gel is not stained. (B) Denaturing SDS-PAGE of each of the green bands dissected from the gel in part A.

Table 1

Amino acid sequences of the N-terminal region of some PSI polypeptides

Component	Species	N-terminal sequence	Ref.
LHCIc[a]	Barley	DI AGLTPAKEEKAAAKHEQD.	
		* * * * * * * * * * * * *	
LHCIc[a]	Maize	AI AGLTPPKESKAFAK? EKN.	
		* * * * * * * * * * * * *	
Subunit IV[b]	Spinach	DI AGLTPCKESKQFAKREKQALKK.	[8]
		* * * * * * * * * * * * * * * *	
P21[b]	*C. reinhardtii*	DI AGLTPCSESKAYAKLEKKELKT.	[17]
		* * * * * * * * * * * * * * *	
17 kDa[a]	Pea	D/ AI SGLTPCKESKQFAKREKQ.	[19]
Subunit III[a]	Barley[c]	AEEPT AAAPAEPAPAAD.	
		* * * * * * * * *	
Subunit III[b]	Spinach	AAEEA AAAPAAASPEGEAPK.	[5]
		* * * * * * * *	
13 kDa[a]	Pea	ASEDTAEAAAP.	[18]
Subunit IV[a]	Barley	Blocked	

[a] Sequence determined by amino acid sequencing
[b] Sequence deduced from DNA sequencing
[c] Compare with sequence in [29]

almost certainly those of the homologous polypeptide in the five species.

Since our partial sequences for the barley and maize proteins were obviously related to that published as subunit IV of CCI [8], it behooved us to confirm that the sequences for those polypeptides that migrate most closely to the 17 kDa polypeptide in PSI (i.e., subunits III and IV), differ from that of the 17 kDa component, even though the 17 kDa polypeptide had been prepared from LHCI and not CCI. By definition, subunits III and IV are the third and fourth largest subunits in CCI [25,26]; furthermore, we used an antibody against subunit III [27] to confirm that the polypeptide labeled subunit III in fig.1 was indeed subunit III. The same procedures as described above were used to isolate the subunit III and IV polypeptides from fractionated barley CCI and to obtain their sequences. These sequences are shown in the lower half of table 1 together with those for subunit III of CCI of spinach and a 13 kDa polypeptide of pea PSI [18].

4. DISCUSSION

The amino acid sequences of six of the seven CCI subunits have apparently been deduced from their gene sequences (e.g. [3–5,8,9]). Some ambiguity remains, however, because identification of subunits III and IV might have been inverted in some instances (cf. the work on spinach and pea sequences [5,8,18]). During our studies on LHCI, in particular on its 17 kDa component, it became necessary to obtain a partial sequence of this polypeptide so that we could construct a probe for screening purposes. The partial N-terminal sequences that we obtained for the 17 kDa component from two different plant species looked remarkably like that of a published sequence for subunit IV of CCI. It was unlikely that we had sequenced a CCI polypeptide rather than an LHCI polypeptide in both cases, and furthermore we obtained the barley polypeptide from an LHCI fraction that lacked the CCI subunits (fig.1). Nevertheless, to confirm that we had not sequenced one of the CCI subunits, we determined the sequences of the two closest migrating CCI subunits in barley PSI; i.e., subunits III and IV (table 1). Our sequence for subunit III agreed well with that obtained for subunit III of spinach [5], whereas that of subunit IV was blocked. Hence our partial sequences for the 17 kDa polypeptide (table 1) were those of an LHCI component. A possible explanation for Steppuhn et al. [8] isolating a clone for the 17 kDa LHCI polypeptide rather than for subunit IV, is that the antibody used to obtain the subunit IV clone reacts with both subunit IV and the 17 kDa LHCI polypeptide, which can comigrate on SDS-PAGE in some species; in addition, the 17 kDa polypeptide has been shown to be the most difficult of the LHCI components to remove from CCI preparations [10]. Furthermore, if they do comigrate and if the N-terminus of subunit IV in spinach is also blocked, the N-terminal sequence obtained for this protein band [8] will be that of the 17 kDa component. Interestingly, Rheil and Bryant's [28] partial amino acid sequences of a 17 kDa polypeptide of PSI (*psaF* gene product) in two cyanobacteria revealed that in one (*Nostoc* sp. PCC 8009) the N-terminus was blocked while in the other (*Synechococcus* sp. PCC 7002) a partial sequence could be obtained which does not match any of the other PSI 13–17 kDa sequences that have been obtained. It is therefore quite possible that in this one cyanobacterium, its *psaF* gene product is subunit IV of CCI.

There are some other complete or partial sequences for 17 kDa polypeptides in PSI. In *C. reinhardtii*, a clone for the P21 polypeptide was obtained by Franzen et al. [17] who pointed out that the deduced protein sequence matched very well with that reported for subunit IV of spinach [8]. We would therefore propose that the *C. reinhardtii* P21 polypeptide is the LHCIc apoprotein in this organism. Dunn et al. [18] obtained partial N-terminal sequencs of six PSI polypeptides of pea which were designated by their apparent sizes on SDS-PAGE in a gradient of urea. The sequence of their 17 kDa component aligns well with that of the LHCIc apoprotein, and, based on very short partial sequences, that of their 13 kDa polypeptide bears considerable resemblance to the N-terminal region of subunit III (table 1); note that each N-terminal sequence is alanine-rich. We would assign the six pea partial sequences as follows: the 21 kDa sequence is that of subunit II, the 17 kDa is that of LHCIc, the 13 kDa is that of subunit III, the 11, 9 and 8 kDa are those of subunits V, VI and VII, respectively; we would predict that subunit IV of pea is blocked

and therefore was not sequenced. Why subunit III in the Dunn et al. [18] work migrates with an apparent size somewhat smaller than in other reports, including the work described here, may reflect the influence of the electrophoretic conditions on the folding of the polypeptide during electrophoresis (cf. [19]). In this connection, Okkels et al. [29] have obtained a cDNA clone for a barley polypeptide with an apparent size of 16 kDa, the first 17 amino acid residues of which are identical to those we determined for barley subunit III (table 1). However, the true size of the mature polypeptide is only 10.8 kDa [29].

If, as we propose, the published gene sequences [8,17] are those of the LHCIc apoprotein, then it is pertinent to compare its possible folding with respect to the membrane with that of a much studied and equivalent light-harvesting component of PSII, LHCIIb [30]. It is obvious from the 17 kDa sequence that it does not belong to the same gene family as do most of the sequences assigned in the literature to LHCIIb or the other LHCs [31]. Furthermore, it contains relatively less chlorophyll *b* than LHCIIb but nevertheless our preliminary studies indicate that it probably has the same number of transmembrane helices as LHCIIb (Takeuchi, Anandan and Thornber, in preparation).

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