Volume 203, number 2 FEBS 3879 July 1986

Immunological studies on the light-harvesting polypeptides of photosystems I and II

R.S. Williams and R.J. Ellis

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England

Received 15 June 1986

Monoclonal and polyclonal antibodies have been raised against the three apoproteins of the peripheral light-harvesting complex of photosystem I (LHC I) from *Pisum sativum* L. These antibodies have been used to study the immunological relatedness of the light-harvesting polypeptides of photosystems I and II. The results suggest that there is no immunological/structural relationship between the two light-harvesting systems. The apoproteins of the LHC I fall into two distinct groups corresponding to the two chlorophyll *a/b* complexes comprising the PS I antenna.

Photosystem I *Photosystem II* *Thylakoid protein* *Monoclonal antibody* *Light-harvesting complex* *Chlorophyll antenna*

1. INTRODUCTION

In higher plant chloroplasts, the photosynthetic electron transport chain contains a number of pigment-protein complexes consisting of the reaction centres of photosystem (PS) I and PS II plus their associated light-harvesting antennae. The light-harvesting system of PS II, (LHC II), which binds both chlorophyll *a* and *b*, and particularly its major 26 kDa polypeptide constituent (LHCP), has been well characterised in terms of both its molecular biology and its role in regulating photosynthetic electron transport [1]. The light-harvesting system associated with PS I, however, is much less well studied.

Haworth et al. [2], working with *Pisum sativum*, were the first to isolate a native chlorophyll *b*-containing antenna complex from a PS I preparation. This complex, designated LHC I, contains 3 major polypeptides of 19–24 kDa and displays a chlorophyll *a/b* ratio of 3.5–4.0. The LHC I was shown to retain the low-temperature fluorescence maximum at 735 nm characteristic of PS I. Lam et al. [3] were able to fractionate further a LHC I preparation from spinach into two chlorophyll *b*-containing fractions termed LHCPIa and LHCPIb. LHCPIa was found to contain two polypeptides of 23 and 22 kDa, while LHCPIb was enriched in a 20 kDa polypeptide. This latter polypeptide and its associated pigments have been identified [4] as the origin of the long-wavelength 77 K fluorescence emission maximum of PS I. In addition to differences in protein composition, the two complexes were found to differ in various spectral characteristics which the authors attributed to a greater susceptibility of the LHCPIa complex to perturbation by detergent.

These results suggest that the LHC I is a heterogeneous complex consisting of at least two distinct antenna species containing different complements of polypeptides, and in which the environment of the pigment molecules also differs. However, evidence concerning the structural relationships of the polypeptide constituents of the light-harvesting complexes is lacking. Lam et al. [5] showed that antibody raised against the LHCP of PS II does not cross-react with the 20 kDa polypeptide of the LHC I, but White and Green [6] have reported immunological cross-reactivity between chlorophyll *a/b* complexes associated

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

with the two photosystems from barley. Evidence concerning the immunological relationships of all three LHC I polypeptides with one another and with the polypeptides of the LHC II is presented here from the use of both monoclonal and polyclonal antibodies. The polypeptides of the two photosystems in *P. sativum* are immunologically distinct.

2. MATERIALS AND METHODS

PS I particles were prepared from the leaves of *P. sativum* essentially as described in [8] except that all steps following thylakoid unstacking were carried out in the presence of 6.2 mM Tris, 48 mM glycine (pH 8.3). In addition, to minimise the chances of proteolytic degradation, phenylmethylsulphonyl fluoride (PMSF) was added to all buffers to a concentration of 200 μM. To purify the LHC I polypeptides, PS I samples containing 250–300 μg chlorophyll were solubilised in 2% SDS, 50 mM dithiothreitol, 50 mM Na_2CO_3, 7.5% glycerol, 0.01% bromophenol blue and the constituent polypeptides were resolved by SDS-PAGE using a 40 cm, 10–16% acrylamide gradient to maximise separation of the low-molecular-mass LHC I proteins. Gels were stained with Coomassie brilliant blue and the LHC I polypeptides recovered from the gel matrix by excision and electroelution. Purified proteins were stored at −80°C.

Polyclonal antibodies were raised in rabbits using individual purified LHC I polypeptides as antigens. Sera were treated with 40% saturated ammonium sulphate, and the resulting precipitates resuspended and dialysed against phosphate-buffered saline (0.8% NaCl, 0.02% KCl, 0.115% Na_2HPO_4, 0.02% KH_2PO_4, pH 7.4, anhydrous salts). The method used to raise antibody against the LHCP has already been described [7].

To raise monoclonal antibodies to LHC I polypeptides, a new method of PS I purification was used. This method was devised in order to recover a fraction containing PS I particles associated with the LHC II; details of this method will be published separately. Thylakoids were unstacked as in [8], then solubilised with 0.5% Triton X-100 in the presence of 25 mM Tris-HCl (pH 8.3) for 30 min at 22°C. The material was then centrifuged at 30000 × *g* for 30 min. The resulting green pellet was dialysed against water, boiled and injected directly into female mice (strain Balb/c). After immunization, total serum from each mouse was screened by immunoblotting. Hybridoma cultures were raised as in [9] and supernatants from these cultures were screened for the presence of antibody according to [10].

Subfractionation of PS I into core and antenna particles was performed using the method of Haworth et al. [2]. Purified LHC II was made as described in [11]. SDS-PAGE was carried out using the system of Laemmli [12]. The method of Vaessen et al. [13] was followed for immunoblotting, with the minor modification that the concentration of BSA used to block the cellulose nitrate filters was decreased to 4%.

Peptide mapping using staphylococcal V8 protease was carried out essentially as described in [7]. The LHCP was isolated for this purpose from a sample of purified LHC II by electrophoresis on a 20% acrylamide gel.

All chlorophyll determinations were carried out using the method of Arnon [14].

3. RESULTS AND DISCUSSION

Fig.1 shows a stained SDS-polyacrylamide gel of the 3 purified LHC I polypeptides, together with the complete PS I preparation from which they are derived. For comparison, a sample of purified LHC II is also shown. The LHC I proteins have been designated P1 (24 kDa), P2 (23 kDa) and P3 (21 kDa). It is evident that the preparations are very pure. Even upon silver staining (not shown) no trace of contamination with other polypeptides could be seen. Close visual examination of tracks reveals a slight contamination of the original PS I preparation with the polypeptides of the LHC II. This contamination could be minimised by careful manipulation of conditions during the original solubilisation of the membrane, but in no preparation was it ever completely removed.

The specificities of polyclonal antibodies raised against P1–P3 were checked by immunoblotting and the results are shown in fig.2. It is clear that a complex set of cross-reactions exists. Three main points can be noted. Firstly, antibodies raised against P1 and P3 but not P2 both cross-react with the proteins of the LHC II (tracks 5 of fig.2a,c). Similarly, there is cross-reactivity between the an-

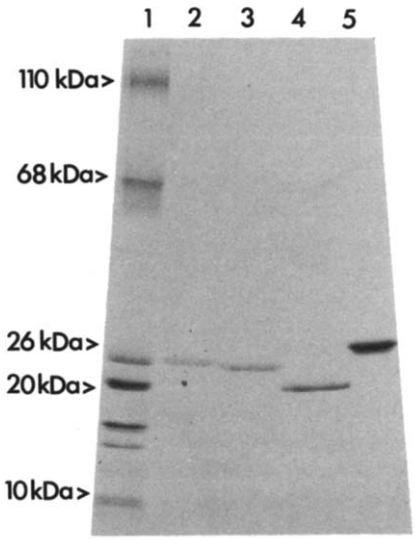

Fig.1. SDS-polyacrylamide gel analysis of purified LHC I proteins: (1) complete PS I particle, (2) P1, (3) P2, (4) P3, (5) purified LHC II. The gel is stained with Coomassie brilliant blue.

tibody raised against the LHCP and the three polypeptides of the LHC I (fig.2d). Secondly, antibodies raised against P1 and P2 react strongly with both these proteins, but relatively poorly with P3, while anti-P3 antiserum reacts strongly with P3 but not at all with either P1 or P2. Finally, none of the antisera recognise any PS I polypeptides other than the light-harvesting proteins, thus confirming the conclusion reported in [5] that the LHC I polypeptides are distinct from the PS I reaction centre proteins.

The immunological cross-reactivity between the preparations of LHC I and the LHC II polypeptides is of interest since it is at variance with the results reported in [5] but agrees with those in [6], where the authors suggest that the cross-reactivity may be attributable to possible sequence similarities resulting from evolution from a common ancestral sequence. For this reason it was important to determine whether the cross-reactivity observed was real or due to contamination of the original LHC I antigens with either LHC II or its breakdown products. One approach to this problem is to produce a preparation containing LHC I polypeptides completely free from LHC II contamination. This can then be probed with the anti-LHCP serum to determine whether cross-reactivity can still be observed. In order to do this, the method of Haworth et al. [2] for producing a purified LHC I preparation was employed. This method results in a sucrose gradient containing three green bands, as described by Ortiz et al. [15]. Fig.3a shows the polypeptide composition of each band analysed by SDS-PAGE. It can be clearly seen that the lightest fraction (band I) on the gradient represents the LHC I fraction described in [2], containing all 3 LHC I polypeptides. There is also a considerable amount of 26 kDa LHCP present. Band II represents a PS I core complex equivalent to the PS I-65 fraction in [2] and contains no LHC I polypeptides. Band III, the heaviest band on the gradient, is a complete PS I preparation containing polypeptides from both the core and the light-harvesting complexes. This fraction differs from the original PS I preparation in that it contains no stainable LHCP, and thus represents a PS I particle uncontaminated by LHCP.

Fig.3b shows the same fractions after immunoblotting with the anti-LHCP serum. The LHCP contaminant in the original PS I preparation reacts with the antibody (track 1). In addition, in some preparations, the LHCP antiserum also recognises several smaller bands migrating in similar positions to the LHC I polypeptides. The LHC I fraction (track 2) also reacts strongly with this antiserum which recognises both the major LHCP contaminant and its dimer, plus a number of smaller peptides. Similarly, a reaction can be seen with the core complex (track 3), the antibody picking up the LHCP and polypeptides of similar size to those of the LHC I, while no reaction is seen with the clean PS I fraction in lane 4.

To check the identity of the LHC I polypeptides on this blot, the same filter was re-probed with the antiserum raised against P2 (fig.3c). This antiserum recognises all 3 LHC I polypeptides, but is the only one which does not recognise the polypeptides of the LHC II (fig.2b). From a comparison of lane 4 in fig.3b,c it is clear that not only does the antiserum to the LHCP fail to detect any 26 kDa LHCP in the clean PS I fraction, but it also fails to recognise any of the LHC I proteins, which are present in this fraction in significant amounts. Furthermore, the large number of low-molecular-mass bands detected by the LHCP antibody in lane 2 of fig.3b indicates that a significant degree of pro-

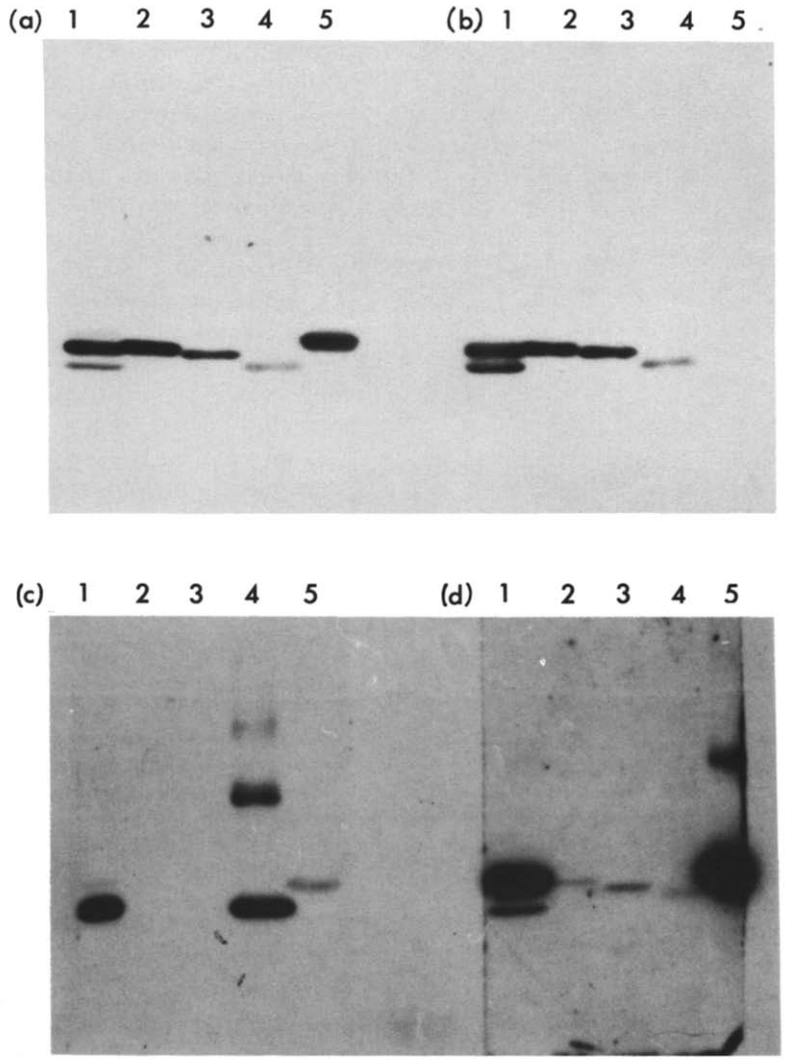

Fig.2. Autoradiographs of immunoblots using (a) anti-P1, (b) anti-P2, (c) anti-P3 and (d) anti-LHCP antisera respectively. The tracks on each blot contain: (1) complete PS I particle, (2) P1, (3) P2, (4) P3, (5) purified LHC II.

teolysis of the LHCP contaminant has taken place during the LHC I isolation procedure. The products of this proteolysis appear to co-purify chiefly with the light LHC I fraction, leaving the heavy PS I fraction free from contamination. These results suggest very strongly that the observed immunological cross-reactivity between polypeptides of the two light-harvesting systems is a direct result of contamination of the LHC I polypeptides P1 and P3 with proteolytic breakdown products of the LHC II, and is therefore spurious.

Immunological cross-reactivities between the polypeptides of the LHC I reveal an interesting pattern. From the results shown in fig.3 it appears that no detectable proteolysis of the LHC I polypeptides takes place during the PS I isolation procedure, suggesting that the observed cross-reactivities are not due to cross-contamination between LHC I proteins and their proteolytic products. Furthermore, the results shown in fig.2b using the anti-P2 serum indicate that intra-LHC I cross-reactivities are not due to contamination with LHC II breakdown products, since this an-

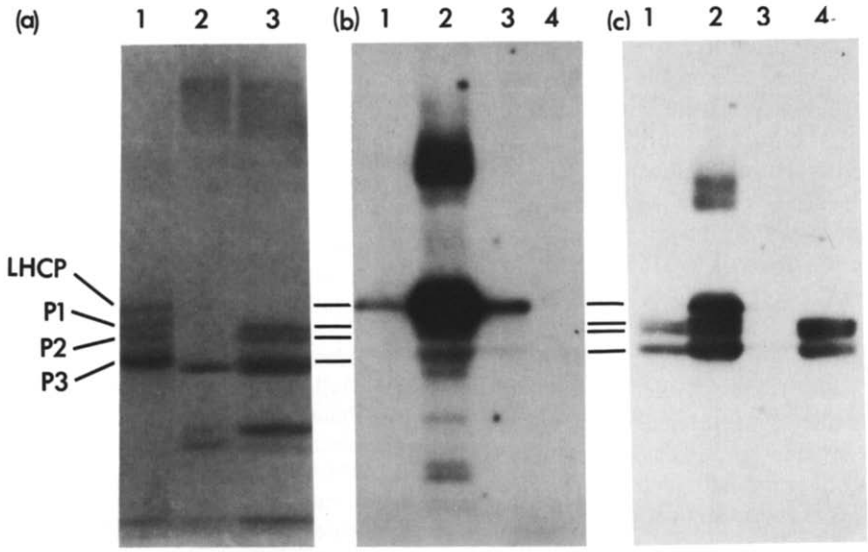

Fig.3. (a) SDS-polyacrylamide gel analysis of bands I–III from the sucrose density gradient. The gel is stained with Coomassie brilliant blue. (1) Band I-purified LHC I, (2) band II-PS I core, (3) band III-uncontaminated PS I. (b) Autoradiograph of immunoblot using anti-LHCP antiserum: (1) complete PS I particle, (2–4) as tracks (1–3) in (a). (c) Autoradiograph of immunoblot using anti-P2 antiserum: tracks (1–4) as in (b).

tibody does not recognise the LHC II polypeptides. We conclude that the polypeptides of the LHC I share immunological similarities. However, anti-P3 serum does not recognise either P1 or P2 while anti-P1 and anti-P2 both recognise P3, although not very strongly (fig.2a–c). These observations indicate that P1 and P2 are more similar to each other than either are to P3. Comparison of the proteolytic cleavage patterns generated by applying increasing concentrations of staphylococcal V8 protease to the 3 LHC I polypeptides and to the LHCP confirm the relationships suggested by the immunological data (not shown).

Three monoclonal antibodies raised against the LHC I polypeptides were screened for specificity by immunoblotting and the results are summarised in table 1. It can be seen that two of the antibodies react strongly with P2, and one of these also reacts with P1, indicating the presence of at least one epitope common to these two proteins. A third antibody is specific for P3 only. None of the monoclonals react with the polypeptides of the LHC II.

The results presented here suggest two main conclusions. Firstly, the immunological similarities observed between the polypeptides of the LHC I and LHC II are a result of cross-contamination and the two complexes must therefore be regarded as distinct entities. Since the antisera were raised against SDS-denatured proteins it is unlikely that the proteins of these complexes will be found to share any major sequence homologies. This conclusion is of interest in view of the fact that these two complexes both bind similar pigments and perform a similar light-harvesting function [15]. In addition, the LHC I apoproteins are encoded by nuclear genes [16] and must be imported into the chloroplast, in common with those of the LHC II.

Table 1

Summary of specificities of three monoclonal antibodies tested

Monoclonal antibody	P1	P2	P3	LHC II
2A1	–	++	–	–
1C4	+	++	–	–
3C2	–	–	++	–

(++) Strong reaction, (+) limited reaction, (–) no detectable reaction

Secondly, the LHC I is heterogeneous in terms of its polypeptide constituents. It appears from both the antibody work and from the peptide mapping that P1 and P2 are quite similar, while P3 is relatively unique. However, the fact that polyclonal antibodies raised against P1 and P2 both react to some extent with P3 suggests that the 3 proteins may share some similarities in sequence. Work reported by the authors in [3] indicates that P1 and P2 are restricted to the LHCP1a while P3 is unique to the LHCP1b. The results reported here reflect this dichotomy. Since the protein constituents of the LHC I fall so clearly into two groups it is reasonable to suggest that they are encoded by distinct nuclear genes or gene families. Current work is concerned with determining how and at what level the accumulation of the LHC I proteins in the thylakoid membrane is regulated.

ACKNOWLEDGEMENTS

This work was supported by funds from the UK Science and Engineering Research Council (SERC).

REFERENCES

[1] Bennett, J. (1983) Biochem. J. 212, 1–13.
[2] Haworth, P., Watson, J.L. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 151–158.
[3] Lam, E., Ortiz, W. and Malkin, R. (1984) FEBS Lett. 168, 10–14.
[4] Kuang, T.Y., Argyroudi-Akoyunoglou, J.H., Nakatani, H.Y., Watson, J. and Arntzen, C.J. (1984) Arch. Biochem. Biophys. 235, 618–627.
[5] Lam, E., Ortiz, W., Mayfield, S. and Malkin, R. (1984) Plant Physiol. 74, 650–655.
[6] White, M.J. and Green, B.R. (1985) in: First International Congress of Plant Molecular Biology Abstracts (Galau, G.A. ed.) p.131, University of Georgia Centre for Continuing Education, Athens, GA.
[7] Bennett, J. (1981) Eur. J. Biochem. 118, 61–70.
[8] Mullett, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822.
[9] Kohler, G. and Milstein, C. (1975) Nature 256, 495–497.
[10] Bennett, J., Williams, R. and Jones, E. (1984) in: Proceedings of the VIth International Congress on Photosynthesis (Sybesma, C. ed.) vol.3, pp.99–106, Nijhoff/Junk, The Hague.
[11] Burke, J.J., Ditto, C.J. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263.
[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Vaessen, R.T.M.J., Kreike, J. and Groot, G.S.P. (1981) FEBS Lett. 124, 193–196.
[14] Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
[15] Ortiz, W., Lam, E., Ghirardi, M. and Malkin, R. (1984) Biochim. Biophys. Acta 766, 505–509.
[16] Mullet, J.E., Grossman, A.R. and Chua, N.-H. (1982) Cold Spring Harbor Symp. Quant. Biol. 46, 979–984.