Monoclonal antibodies specific for the light-harvesting chlorophyll *a*/*b*-protein complex (LHC)

Detection of conserved antigenic determinants in LHC from different species

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Monoclonal antibodies have been raised against the light-harvesting chlorophyll *a*/*b*-protein complex (LHC) of pea and characterised using the enzyme linked immunosorbent assay (with purified LHC or intact thylokoids) or immunoblotting using chloroplast proteins transferred from SDS-PAGE gels. Several clones showed strong binding to the two major polypeptides of pea LHC, even after trypsin or proteinase K treatment. The two antibodies with the most efficient binding to pea LHC were shown to cross-react with polypeptides from green algae and higher plants; indicating sequential similarities and the presence of several closely related polypeptides between phylogenetically distant species.

Monoclonal antibody *Light-harvesting chlorophyll a/b-protein complex* *Enzyme-linked immunosorbent assay* *Protein A-^{125}I immunoblot* *Protease treatment* *Cross-reaction*

1. INTRODUCTION

The light-harvesting chlorophyll *a*/*b* protein complex (LHC) is the major integral protein complex of thylakoid membranes from green algae and higher plants [1]. Apart from its antennae function, the LHC is involved in thylakoid adhesion [2] and regulates the distribution of excitation energy from the complex to the photosystems I and II [3]. Native LHC is an asymmetrical transmembrane complex [4] and probably exists as an oligomer of identical subunits [5].

Investigations on the genetics of the LHC polypeptides of pea [6] and petunia [7] have indicated that the LHC polypeptides are products of a small family of nuclear genes which are nearly identical within one species, as well as between pea and petunia. The LHC of different species is itself known to be heterogeneous with respect to its polypeptide composition [4,8–11]. Different immunochemical studies using polyclonal antibodies [4,12–15] have compared the orientation, function and structure of individual thylakoid membrane complexes from different species. Immunological similarity has been shown between the LHC polypeptides of spinach and *Chlamydomonas reinhardii* [12] and between components of the green algae *Acetabularia mediterranea* [13], barley [15] or spinach [12,16]. The latter studies were carried out using polyclonal antibodies [12], or monoclonal antibodies [16].

Recently the relatedness of membrane components has been investigated using monoclonal antibodies directed against thylakoid proteins [16–18] or thylakoid lipids [19]. Here we describe the production and characterisation of monoclonal antibodies against pea LHC and report on cross-reactivity between LHC polypeptides of several phylogenetically distant species.

Abbreviation: PAGE, polyacrylamide gel electrophoresis

Dedicated to Professor Kurt Mühlethaler on the occasion of his 65th birthday

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2. MATERIALS AND METHODS

Peas (*Pisum sativum* var. Carnosa cv. sugar snap) and beans (*Vicia faba*) were cultivated as described [8]. Spinach (*Spinacia oleracea*) was purchased from a local market. 2 week-old barley plants (*Hordeum vulgare*, wt) were provided by P. Frick, ETH-Zurich. Fern (*Nephrolepsis*) was obtained from horticultural suppliers and *C. reinhardii* cells (arg^-, mt^+, 137c) were a gift from Dr H. Michel and Professor A. Boschetti, University of Bern. All materials used for the production of monoclonal antibodies are described elsewhere [20]. Trypsin TRTPCK was purchased from Millipore, Freehold; proteinase K from Boehringer Mannheim and the protease inhibitor phenylmethylsulphonyl fluoride (PMSF) from Serva, Heidelberg.

LHC was isolated from pea leaves as described [8]. For the production of monoclonal antibodies Balb c/J mice were immunised intraperitoneally using monthly injections of 100 μg purified LHC. 3 days after the last injection the fusion was carried out using standard procedures [21]. After 2 weeks, supernatants of macroscopic clones were screened for LHC-specific antibodies using an enzyme linked immunosorbent assay (ELISA), carried out as in [22] using chloroplast thylakoids or purified LHC. After limiting dilution, positive clones were additionally tested for their binding ability to intact thylakoid membranes, purified pigments or total lipid extract (purified according to [23] and kindly provided by Dr Kleoniki Gounaris, Imperial College, London). The immunoglobulin class of the antibodies was determined using a modified ELISA assay. For cross-reactions, whole chloroplasts, or cells, were separated on either 15, 17.5 or 20% acrylamide gels according to [24], transferred to nitrocellulose using the method described by Towbin et al. [25] and subjected to protein A-^{125}I immunoblot analysis (for experimental details see [26]).

The digestion of thylakoid membranes with trypsin and proteinase K was performed as described [4,27] at a chlorophyll concentration of 0.5 mg/ml for 40 min at room temperature.

3. RESULTS

In total 65 antibody-secreting clones were detected as specific for isolated LHC using an ELISA assay. 10 were selected as showing both strong binding and an immunoglobulin class suitable for protein A-mediated screening; these were subcloned by limiting dilution. Thereafter each clone was screened for binding ability to either isolated pea LHC, intact pea thylakoids, purified pigments or total lipid extract. By means of this exclusion technique, 5 clones were shown to recognise protein determinants on the LHC (these clones bound to isolated LHC and thylakoids but not to pigments or lipids). All other clones were either directed against pigment- or lipid-determinants.

The results obtained by the ELISA assay were confirmed using the protein A-^{125}I immunoblot system using chloroplast proteins transferred from SDS-PAGE gels. All 5 clones shown to be capable

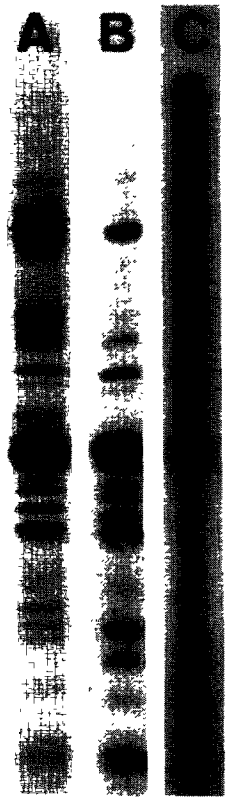

Fig.1. Pea thylakoid separated by SDS-PAGE (17.5% acrylamide) shown in (A), transferred into nitrocellulose and amido black stained (B) and protein A-^{125}I immunoblot assay carried out with monoclonal antibodies against pea LHC (C).

of binding to the protein of LHC in the ELISA assay also exclusively recognised LHC from SDS-denatured chloroplast polypeptides. Pea LHC migrates as 2 predominant bands with apparent molecular masses of 25 and 27 kDa [28] when electrophoresis is carried out under fully denaturing conditions. In the immunoblot, irrespective of whether isolated pea LHC or total thylakoid membranes were used, each monoclonal antibody recognised both polypeptide components of the peak LHC (fig.1). To obtain more information regarding the nature of the binding sites of our antibodies, the hydrophilic portion of native LHC was proteolytically degraded. Proteolysis of LHC releases small polypeptide segments which are exposed on the outer thylakoid surface [29]. Digestion with proteinase K appears to remove slightly more of the polypeptide chain of the apoprotein than trypsin (as revealed by SDS-PAGE, fig.2a). After proteolysis the binding of the anti-LHC sera to degraded LHC was unmodified, demonstrating that the binding sites involved do not correspond to the cleavable segments (fig.2b).

To investigate the immunological relationship between LHC polypeptides from different species we tested the binding ability of these monoclonal antibodies to other thylakoid membranes. In addition to pea, thylakoids from spinach, bean, barley, maize, fern and *C. reinhardii* were analysed on SDS-PAGE (fig.3a) and transferred onto nitrocellulose. Cross-reactions were detected using the immunoblot (fig.3b). This indicates identical antigenic determinants among the LHC polypeptides of all species tested. The anti-LHC antisera cross-reacted with 3 spinach LHC polypeptides (15.1; 15.2 and 16, nomenclature according to [12]). Bean LHC was resolved on SDS-PAGE into 3 polypeptides which showed cross-reaction with pea LHC. These 3 bands cannot be clearly resolved in the autoradiograph shown, due to overlapping of the bands. The same problem arose with the components of barley LHC which can be resolved into 2 major bands on SDS-PAGE. The LHC of maize was resolved into at least 4 polypeptides, 3 of which cross-reacted with pea LHC antibodies. Fern LHC polypeptides showed poor immunological relatedness to pea LHC. The antibodies were capable of binding to the fern LHC polypeptide with the highest apparent M_r provided heat treatment prior to electrophoresis was used. If heat denaturation was omitted no cross-reaction was observed. Structural relatedness has also been found between pea LHC and the phylogenetically most distant species tested, namely *C. reinhardii* LHC. The antibodies bound with the highest affinity to polypeptides 11 and 16 (nomenclature according to [11]) whereas polypeptide 17, which is reported to be immunologically related to polypeptides 11 and 16 [12], only exhibited weak binding with the monoclonal antibodies used.

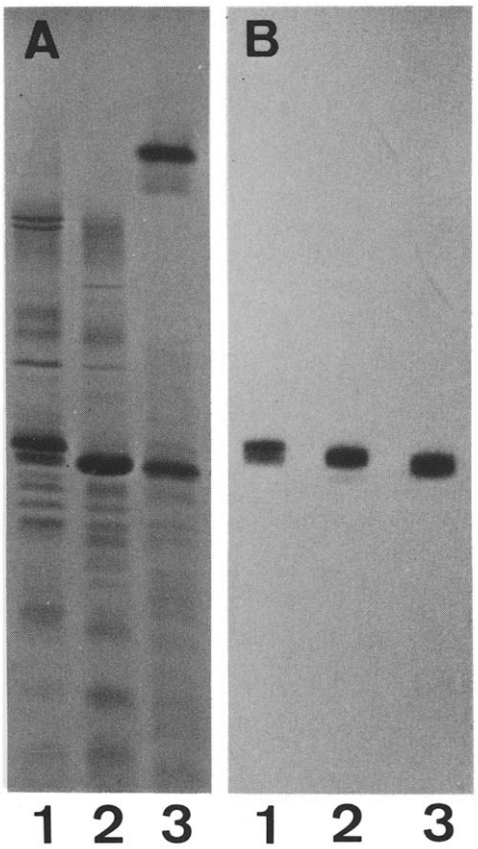

Fig.2. SDS-PAGE (17.5% acrylamide) of proteolytically degraded pea thylakoids (A) and the corresponding immunublot assay (B). Control (1), trypsin digestion (2) and proteinase K digestion (3).

All these cross-reactions detected by immunoblotting could also be confirmed using the ELISA assay with native thylakoid membranes prepared from each species. This indicates strong structural similarities in the hydrophilic portion of the light harvesting polypeptides amongst the species tested.

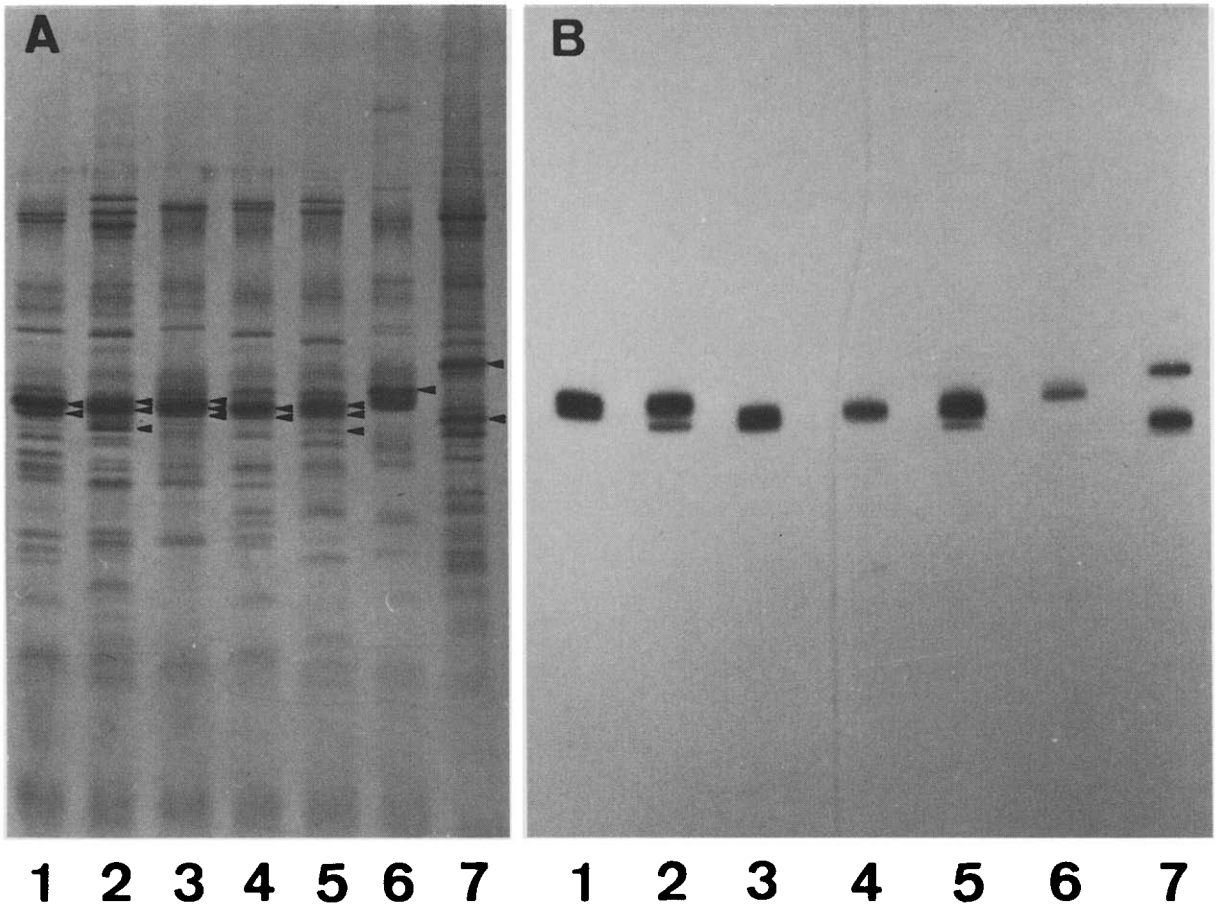

Fig.3. Polypeptide pattern of chloroplasts, respectively cells, obtained by SDS-PAGE (20% acrylamide) (A) from pea (1), spinach (2), bean (3), barley (4), maize (5), fern (6) and *C. reinhardii* (7). Cross-reacting polypeptides on the protein A-^{125}I immunoblot assay (B) detected with monoclonal anti-pea LHC serum. The arrows indicate the cross-reacting polypeptides.

4. DISCUSSION

Several monoclonal antibody-producing clones, specific for pea LHC, have been established. Since isolated LHC consists of protein, pigments, and lipids [5], antibodies can be expected specific for each component. Using the ELISA and immunoblot assays monoclonal antibodies specific for the proteinaceous determinants were clearly characterised. All clones tested by immunoblotting recognised both LHC polypeptides which, once again, points out the similarity of the two polypeptides [28,30,31]. The fact that the anti-LHC sera still bound to the polypeptides, even if heat denatured prior to SDS-PAGE, suggests that the antigenic determinants represent sequential rather than conformational determinants. Protease treatment of the external surface of thylakoid membranes removes surface-exposed peptides of 1–1.5 kDa from the N-terminus of LHC apoproteins, which is paralleled by an inhibition of thylakoid adhesion [29] and changes in excitation energy distribution [3]. Since proteolytic degradation of the thylakoids did not influence antibody binding, the surface-exposed antigenic determinants do not appear to correspond to the proteolytically cleavable N-terminus segments. Since the LHC is a transmembrane complex with considerable portions buried within the lipid bilayer only a limited amount of the sequence is accessible to antibodies.

Although small fragments of LHC can be cleaved by wide-range proteases the antigenic determinants are retained, even after prolonged incubations with proteases. This may reflect hydrophilic segments which may contain antigenic determinants but lack protease-suspectable sites.

The two antibodies showing the most efficient binding to pea LHC were shown to cross-react with polypeptides from green algae and high plants. The cross-reactions were observed with native membranes, isolated protein (in the ELISA assay), as well as with denatured polypeptides (detected in the immunoblot assay), which indicates sequential homologies in the hydrophilic domains accessible on the stromal-orientated portion of LHC. Our results confirmed earlier studies carried out with polyclonal antibodies using crossed immunoelectrophoresis, where similarity between spinach and *C. reinhardii* LHC polypeptides was reported [12] except that, in our studies, we only found weak binding to polypeptide 17. In the case of barley the 28.5 kDa polypeptide could not be verified as a constituent of LHC as has been described [15].

The use of monoclonal antibodies provides a specific detection system for sequence similarity of hydrophilic protein regions. In our studies all the monoclonal antibodies tested recognised 2 or more LHC polypeptides in each species (with the exception of fern, where only one polypeptide reacted). These common features, namely sequential similarities and the presence of several closely related polypeptides between phylogenetically distant species indicate a functional relevance.

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