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Transbilayer organization of the main chlorophyll *a*/*b*-protein of photosystem II of thylakoid membranes

Jan. M. Anderson and D.J. Goodchild

CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

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To probe the location of the carboxyl-terminus of the 28 kDa apoprotein of the light-harvesting chlorophyll *a*/*b*-protein complex of PS II (LHCII), an antibody was generated against a synthetic octapeptide corresponding to the C-terminal region of LHCII. The high specificity of the octapeptide antiserum was demonstrated by immunoblots and immunogold labelling. The octapeptide antiserum agglutinated destacked thylakoid membranes, but no significant agglutination occurred with inside-out vesicles suggesting that the COOH-terminus is located at the outer, stroma-exposed surface where the NH_2-terminus is also located [(1983) J. Biol. Chem. 258, 9941–9948]. Our results support a model for LHCII with four transmembrane-spanning domains.

Chlorophyll *a*/*b*-protein; Carboxyl-terminal localization; Immunogold labeling; Light-harvesting complex; Photosynthesis; Thylakoid membrane

1. INTRODUCTION

The light-harvesting chlorophyll *a*/*b*-protein complex of PS II (LHCII) is the main protein complex of higher plant and green algal thylakoids. LHCII occurs mainly in the appressed grana membranes where it transfers light excitation energy to the PS II reaction center and mediates thylakoid stacking [1–3]. The chlorophyll *a*, chlorophyll *b* and xanthophylls of LHCII are noncovalently associated with several heterogeneous apoproteins of 24–30 kDa, but most plant species have two major apoproteins (28 and 26 kDa) of similar primary structure [2]. Both the 28 and 26 kDa proteins of LHCII span the membrane [4,5] and their NH_2-terminal regions are located at the outer membrane surface [6]. The LHCII apoproteins are encoded by a nuclear multigene family [7–11] and the 28 kDa apoprotein gene has been cloned and sequenced [7,11–14]. The three-dimensional structure of LHCII has been determined by image reconstruction from electron micrographs [15,16]. A model with three transmembrane domains has been proposed for a *Lemna* Chl *a*/*b*-protein from hydropathy plot analysis [12]. To test this model experimentally, we have used antiserum generated against a synthetic octapeptide corresponding to the C-terminus of LHCII. Our agglutination studies suggest that the carboxyl-terminus is located at the outer surface and we propose a model for LHCII with four membrane-spanning domains.

Correspondence address: J.M. Anderson, CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Abbreviations: Chl, chlorophyll; LHCII, light-harvesting chlorophyll *a*/*b*-protein complex of PS II; PS, photosystem; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; BSA, bovine serum albumin

2. MATERIALS AND METHODS

Spinach thylakoid membranes [4] were destacked by incubation in 10 mM Tricine buffer

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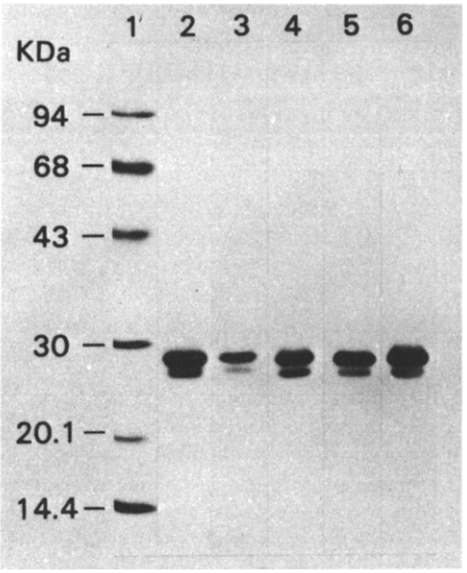

Fig.1. Specificity of the octapeptide antiserum. SDS-PAGE of standard markers (lane 1) and LHCII (lane 2). Western immunoblots of thylakoids (lanes 3,5) and LHCII (lanes 4,6) with polyvalent LHCII antisera (lanes 3,4) and octapeptide antiserum (lanes 5,6).

(pH 8.0), 100 mM sucrose for 1.5 h at 4°C [4]. LHCII was isolated by Triton X-100 solubilization of thylakoids [17]. Right-side-out and inside-out thylakoid vesicles of uniform composition were isolated by aqueous polymer two-phase partition [4].

For use as an immunogen, the synthetic octapeptide, NH_2-Ala-Thr-Asn-Phe-Val-Pro-Gly-Lys-COOH (Dr J. Young, Children's Hospital, Boston, MA), was coupled (in 20 mM excess) to BSA with glutaraldehyde [18]. The free peptide was removed by dialysis. The octapeptide-BSA conjugate (1 mg/ml) in 0.1 M phosphate buffer (pH 7.5) was emulsified with Freund's complete adjuvant (day 0) or incomplete adjuvant (days 28, 56) and injected into rabbits at multiple sites intradermally. Blood was taken 1 week after each booster injection, and the serum frozen at −20°C. Polyvalent antisera against the 28 and 26 kDa proteins were raised in rabbits as in [4]. Proteins were electrophoretically transferred from SDS-PAGE slab gels [4] to nitrocellulose sheets [5], probed with octapeptide antibodies, and the antibody binding was detected with 5-bromo-4-chlor-3-indolyl phosphate and nitroblue tetrazolium (Promega Biotec., Madison). Immunogold labelling was carried out as in [19,20].

3. RESULTS AND DISCUSSION

The octapeptide, NH_2-Ala-Thr-Asn-Phe-Val-Pro-Gly-Lys-COOH, was synthesized as a probe for the localization of the carboxyl-terminal region

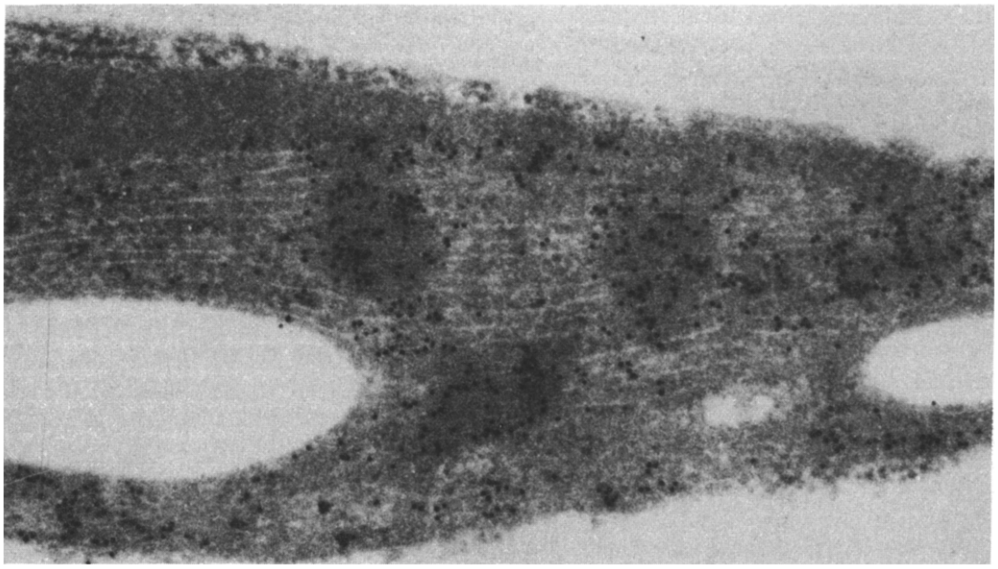

Fig.2. Immunogold labelling of LHCII with octapeptide antiserum. Electron micrograph of a spinach leaf section embedded in LR gold resin and treated with octapeptide antibodies followed by goat anti-rabbit 10 nm gold.

of LHCII [7,11–14]. The octapeptide was coupled to BSA and injected into rabbits. The specificity of the antiserum obtained was tested by immunoblots of spinach thylakoid proteins separated by SDS-PAGE. The octapeptide antiserum cross-reacted with only the 28 and 26 kDa Chl *a/b*-proteins with thylakoid membranes or isolated LHCII (fig.1), as did polyvalent LHCII antiserum. Further, if the antiserum was pretreated with destacked thylakoids or LHCII, it no longer cross-reacted with LHCII on immunoblots. These results show that the octapeptide antiserum is highly specific for LHCII.

Immunogold labelling of chloroplasts in spinach leaf sections with the octapeptide antiserum showed that about 85% of the gold label occurred in the appressed membranes (fig.2). Since this immunogold labelling pattern was identical to that observed with polyvalent LHCII antisera [20], it is clear that the octapeptide serum is reacting with the total pool of LHCII, and not a specific subpopulation.

As LHCII is located mainly in the appressed membrane region [1] and hence is inaccessible to antibodies, it was first necessary for the agglutination studies to induce destacking of the thylakoid membranes. Suspension of thylakoids in a low-salt medium leads to destacking and mixing of all thylakoid components including LHCII in the membrane plane [21]. Intact, destacked thylakoids treated with octapeptide antiserum showed a marked, rapid agglutination (fig.3A) that was almost as strong as that obtained with polyvalent LHCII, whereas they were not agglutinated with control preimmune serum (fig.3B). There was no agglutination with the pretreated LHCII-absorbed octapeptide antiserum (fig.3C) showing that the agglutination observed with destacked thylakoids was indeed specific for LHCII. Significant agglutination was observed also with right-side-out vesicles of uniform composition but not with inside-out vesicles. Hence our results suggest that the carboxyl-termini of the main Chl *a/b*-proteins of LHCII are exposed to the chloroplast stroma at the outer membrane surface.

3.1. *Model for the transmembrane arrangement of the main Chl* a/b-*proteins of LHCII*

Our experimental finding that the carboxyl-terminus of the main LHCII apoprotein is located at the outer membrane surface, as is the amino-terminus [6], suggests that LHCII must contain an

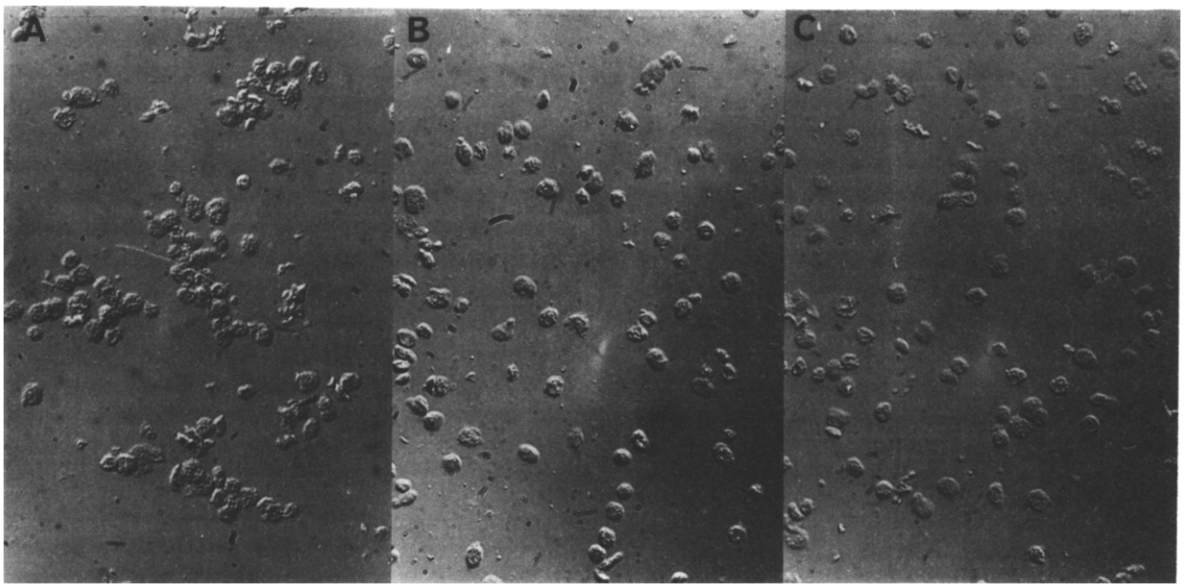

Fig.3. Agglutination of spinach thylakoid membranes. (A) Destacked spinach thylakoids mixed with octapeptide antiserum at a 1:5 dilution. (B) Destacked thylakoids plus preimmune serum. (C) Destacked thylakoids plus pretreated LHCII-absorbed octapeptide antiserum. × 390.

even number of transmembrane domains. Consequently, these results are inconsistent with the three transmembrane domain model of Karlin-Neumann et al. [12] based on hydropathy plot analysis which has the carboxyl-terminus located at the inner, lumenal surface.

We used a modified algorithm of Chou and Fasman [22] to predict the secondary structure of the LHCII polypeptide sequence deduced for a *Lemna* Chl *a/b*-protein [12]. Five putative α-helical regions (residues 59–82, 87–111, 117–140, 161–177 and 183–207) were observed in the Chl *a/b*-protein sequence. Since our data support a model with an even number of membrane-spanning domains, and four of the putative α-helical regions that each contain 24 or 25 amino acid residues are sufficiently long to extend across the membrane, our model has four transmembrane domains (fig.4). The shorter α-helical region (residues 161–171) that contains four negatively charged and two positively charged amino acid residues is unlikely to be buried within the membrane core. The proposed four transmembrane domains contain 43% of the total amino acids and with α-helix V, the Chl *a/b*-protein has an α-helical content of 50%, consistent with the ultraviolet circular dichroic results of 44 ± 7% α-helical content [23]. Although the four transmembrane helices contain twelve charged amino acids, six can form three salt bridges on the α-helix (residues 59–62, α-helix I; 136–139, α-helix III; 200–204, α-helix IV); two glutamate residues are in α-helix I, and one aspartate, two glutamate and one lysine residue are in α-helix II (fig.4). Consistent with their preferred location in intrinsic proteins, all four methionine and two of the three histidine residues of the Chl *a/b*-protein are in the transmembrane domains, while only two of the sixteen proline residues are buried in the membrane. Thus, there is a significant difference between the amino acids that are buried in the transmembrane domains from those located at the membrane surfaces.

The proposed model (fig.4) is consistent with structural analyses of two-dimensional LHCII crystals [15,16]. First, some 37% of the amino acids of the Chl *a/b*-protein are exposed at the outer membrane surface, but only 20% at the inner surface (fig.4). This proposed distribution is consistent with structural analyses [15,16] which show

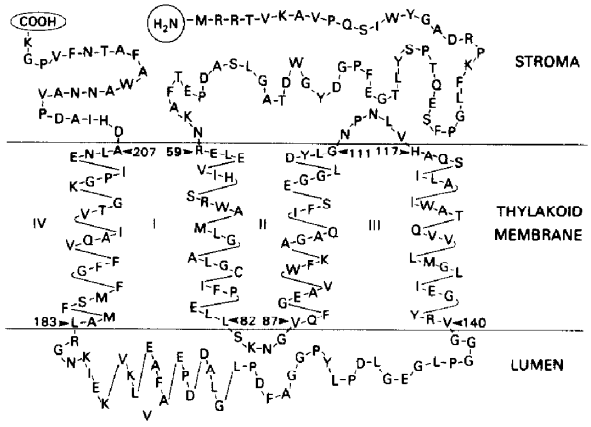

Fig.4. Model of the polypeptide chain organization of the main Chl *a/b*-protein of LHCII.

LHCII protruding 10–15 Å from one side of the membrane, but only 7–10 Å on the other. Second, Kühlbrandt [15] shows LHCII as a trimer, with two trimeric units per unit cell (fig.3 of [15]); each of the three subunits appears to have four cylindrical domains, 3 prominent outer domains, A–C (15–10 Å diameter) and a smaller, central bridging domain, D (11 Å diameter). The lower side contains two prominent domains that match up with domains A and D. We suggest that the bridging domains of the two-dimensional LHCII crystals [15] represent the interaction of 3 similar α-helices of each LHCII unit acting in series to form a parallel, tilted super α-helical coil interacting closely together at the top (10 Å center to center) and angled out at the bottom of the crystal (30 Å, center to center). The tilted transmembrane α-helical structure of LHCII with compact globular domains at both membrane surfaces and a charged α-helix located at the inner membrane surface is analogous to that of the photosynthetic bacterial reaction center complex [24]. We suggest that the 28 kDa apoprotein of LHCII has four transmembrane domains with the carboxyl- and amino-termini located at the outer membrane surface, but further studies are needed to verify this model.

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