Biochimica et Biophysica Acta, 975 (1989) 132–141
Elsevier

BBA 43002

Visualization of antibody binding to the photosynthetic membrane: the transmembrane orientation of cytochrome *b*-559

Olivier Vallon [1,2], Gun-Sik Tae [3], William A. Cramer [3], David Simpson [2], Gunilla Hoyer-Hansen [2] and Lawrence Bogorad [1]

[1] *Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA (U.S.A.),* [2] *Department of Physiology, Carlsberg Laboratory, Valby (Denmark) and* [3] *Department of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN (U.S.A.)*

(Received 16 November 1988)
(Revised manuscript received 21 February 1989)

Key words: Thylakoid membrane; Membrane protein topography; Photosystem II; Cytochrome *b*-559; Immunocytochemistry; Monoclonal antibody

We have used immuno-gold labeling and electron microscopy to study the topography of thylakoid membrane polypeptides. Thylakoid vesicles formed by passage through a French press were adsorbed onto a plastic film supported by an electron microscope grid and processed for single or double immuno-gold labeling. After shadowing with platinum, the inside-out and right-side-out vesicles were identified by their distinctive morphologies. Right-side-out vesicles were labeled by a monoclonal antibody recognizing an epitope located in the trypsin-cleaved, N-terminal portion of the LHC II apoprotein, and by an antibody to CF_1. A monoclonal antibody to the α-subunit of cytochrome *b*-559 reacted with a synthetic tridecapeptide corresponding to the C-terminal portion of the polypeptide. Both this antibody and a polyclonal antibody to the synthetic peptide labeled inside-out vesicles exclusively, indicating that the polypeptide C-terminus was exposed on the lumenal (exoplasmic) surface of the membrane.

Introduction

Photosystem II (PS II) is a major functional complex of the thylakoid membrane. Its primary photochemical reactions show striking analogies to those of the reaction center of purple non-sulfur bacteria [1]. Extensive sequence homology exists between the two major subunits of the PS II core, D1 and D2, and subunits L and M of the bacterial center [2,3]. Since the structure of the bacterial center is known at atomic resolution [4], predictions can be made concerning the topography of D1 and D2 in PS II [5]. One major difference between these two systems, however, resides in the presence in PS II of a component with no apparent equivalent in the bacterial membrane, namely cytochrome *b*-559. This cytochrome can function at cryogenic temperatures as an electron donor to P-680, the photochemically active chlorophyll of the reaction center of PS II, but it does not seem to participate in the normal electron transfer chain at physiological temperatures [6]. Recently, it has been proposed [7] that photoinhibition in PS II occurs via photo-oxidation by $P\text{-}680^+$ of two putative monomer chlorophylls analogous to the monomeric bacteriochlorophylls in the bacterial center. In this hypothesis, cytochrome *b*-559 protects the center by reducing the oxidized chlorophylls. The cytochrome would be re-reduced by PS II acceptors, thus functioning in a cyclic electron flow around PS II. A structural test for this hypothesis is that the heme should be close enough to both the monomer chlorophylls and the PS II acceptors for electron transfer to occur with the expected efficiency.

The purified cytochrome is composed of two polypeptide subunits, referred to as α and β (molecular weight in spinach: 9182 and 4268, respectively). The genes for both, termed *psb*E and *psb*F, respectively, have been cloned from various organisms and sequenced [8–13]. Each polypeptide chain contains a single 24–28 amino-acid hydrophobic stretch, which could span the membrane as an α-helix. In view of the

Abbreviations: CF_1, Coupling factor 1; ESs, endoplasmic surface (stacked); LHC II, Light-harvesting complex of Photosystem II; PS II, Photosystem II.

Correspondence (present address): O. Vallon, Laboratoire de Microscopie Electronique, Institut Jacques Monod, CNRS, Université Paris VII, 2 Place Jussieu, T 43, 75005 Paris, France.

bis(histidine) axial ligation of the heme [14] and of the presence of a single histidine in each polypeptide, five residues into the hydrophobic stretch, the heme must cross-link two His-containing polypeptide chains [15]. Several models have been proposed in which a heme is linked to two identical or different subunits [16]. In these models, the position(s) assigned to the heme(s) in the membrane depend upon the orientation of the polypeptide chains, i.e., on which sides of the membrane their N- and C-termini are exposed. In the present study, we have analyzed the transmembrane orientation of the α-subunit of cytochrome *b*-559, using antibodies specific for its carboxy-terminal domain, and 'on grid' immuno-gold labeling. In this technique, membrane fragments adsorbed onto a plastic film supported by an electron microscope grid are labeled with the antibody, followed by a colloidal gold probe, then shadowed to reveal the membrane surface.

Materials and Methods

Antibodies

Monoclonal antibody 6A5 was obtained using a standard protocol as described in Ref. 17. Female Balb/c mice were immunized with a barley PS II core preparation kindly provided by U. Hinz (Carlsberg Laboratory). 1.2 mg protein was administered in eight intra-peritoneal injections. Spleen cells were fused with cells of myeloma line X63-Ag8.653.

The preparation and the properties of the antibody to the synthetic tridecapeptide have been described elsewhere [18]. The MLH1 and CF_1 antibodies were generously provided by S. Darr (Michigan State University, East Lansing) and S. Merchant (Harvard University), respectively.

Immunoblot analysis

Samples were electrophoresed in 10–20% polyacrylamide gradient gels containing 6 M urea according to Ref. 19 or in 18% high-Tris gels according to Ref. 20. They were transferred to nitrocellulose filters according to Ref. 21. Immunoblot assays were carried out using peroxidase and 3-amino-9-ethylcarbazole as in Ref. 17, or alkaline phosphatase (Proto-blot, Promega Biotech) following the manufacturer's instructions.

Membrane preparations

Synechocystis sp. PCC 6803 and *Cyanophora paradoxa* (UTEX LB 55) were grown according to Refs. 22 and 23, respectively. Cells, suspended in 50 mM Tricine-NaOH (pH 7.4)/10 mM $CaCl_2$, were ruptured by passage through a French press twice at 6000 psi for *Synechocystis* and once at 3500 psi for *Cyanophora*, and the membranes were collected by centrifugation at 30 000 $\times$ *g* for 10 min. Tobacco thylakoids were kindly provided by J. Lukens (Harvard University). The barley cytochrome *b*-559 preparation [26] was a gift of F. Koenig and B. Lindberg Møller (Agricultural University, Copenhagen).

Spinach (*Spinacia oleracaea*) was maintained in a growth chamber under light-dark cycles. Leaves were homogenized at 4°C in 10 mM Tricine-NaOH (pH 7.4)/5 mM $MgCl_2$/300 mM sucrose (buffer A). The slurry was filtered through two layers of 20 μm nylon mesh, centrifuged at 1000 $\times$ *g* for 5 min and the pellet was resuspended briefly in 1 mM Tricine-NaOH (pH 7.4)/5 mM $MgCl_2$ (buffer B) and centrifuged. The pellet was washed once in buffer A, then in buffer C (1 mM Tricine-NaOH (pH 7.4)/100 mM sucrose) and resuspended in buffer C. The thylakoid suspension was passed through a French press at 3500 psi. The homogenate was centrifuged at 1000 $\times$ *g* for 10 min and the supernatant used for labeling.

Inside-out and right-side-out vesicles were obtained from stacked barley thylakoids by aqueous-polymer two-phase partitioning according to Ref. 24. The bot-

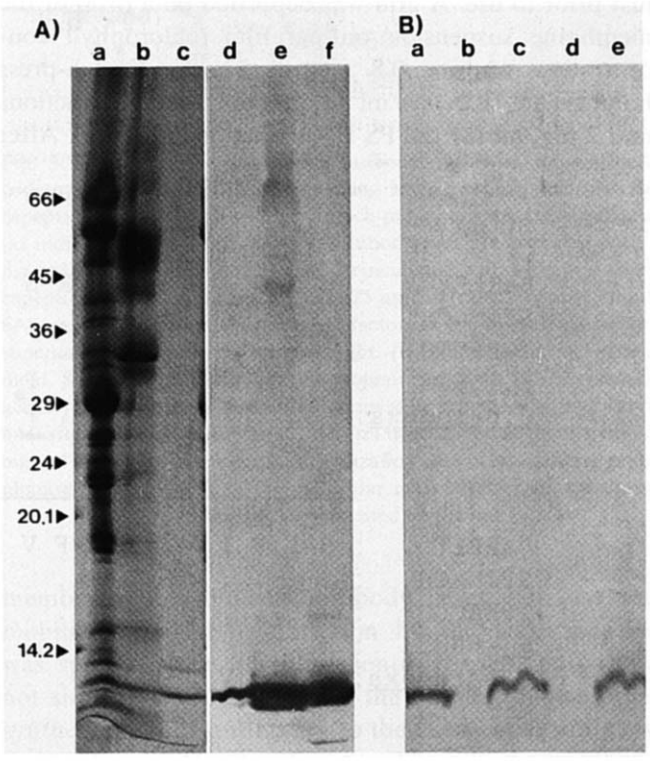

Fig. 1. Specificity of antibody 6A5. (A) Lanes a–c: Coomassie blue-stained gel (10–20% acrylamide gradient containing 6 M urea); lanes d–f: immunoblot from a duplicate gel which was reacted with 6A5 and developed with the peroxidase reaction. Lanes a and d: barley thylakoids; b and e: barley PS II core preparation; c and f: purified cytochrome *b*-559 from barley. The positions of the molecular mass markers (in kDa) are indicated on the left. (B) Immunoblot reacted with 6A5 and developed with the alkaline phosphatase reaction; lane a: barley; b: *Synechocystis* 6803; c: spinach; d: *C. paradoxa* and e: tobacco. Samples were electrophoresed in an 18% acrylamide gel [20]. 15 μg chlorophyll was loaded onto each lane for each of the higher plants and 7.5 μg for *C. paradoxa* and *Synechocystis* to avoid overloading. Positive reactions are seen only in lanes a, c and e.

tom and top phases were repartitioned four times, yielding fractions B5 (inside-out vesicles) and T5 (right-side-out vesicles), respectively. To remove extrinsic polypeptides, the B5 fraction was resuspended in 1 M $CaCl_2$ for 20 min at 4°C and recovered by centrifugation at 100000 × *g* for 30 min. PS II-enriched membranes from barley were prepared according to Ref. 19.

'On-grid' immuno-labeling

Gold solutions (approx. 10 nm diameter) were prepared according to Ref. 25 and conjugated either with protein A for the detection of polyclonal antibodies or with anti-mouse antibody for the detection of monoclonal antibodies. For double-labeling experiments, the anti-mouse gold complex was centrifuged in a microfuge for 15 min and the supernatant, which contained only small size gold (5–10 nm), was used. The goat anti rabbit-gold complex (15 nm diameter) was obtained from Janssen, Beerse, Belgium.

Electron microscope grids (300 mesh, nickel), covered with a parlodion film, were coated with a carbon film just prior to use. A grid was deposited on a drop of the membrane suspension on parafilm (chlorophyll concentration, approx. 0.8 mg/ml for the French-press homogenate, 0.2 mg/ml for the B5 and T5 fractions and 2 mg/ml for the PS II-enriched membranes). After 1–5 min, the grid was blotted by running its edge on a piece of filter paper and transferred onto a drop of the appropriate resuspension buffer (1–5 min). It was then transferred to the labeling buffer (10 mM sodium phosphate (pH 7.4)/5 mM NaCl/100 mM sucrose/0.5% bacitracin (Sigma), which was used in all following steps. After 15 min, the grid was transferred to the diluted antibody and incubated for 15 min. Typically, purified IgGs were used at a concentration of 10–50 μg/ml, sera at 1 : 1000 dilution and hybridoma supernatants at 1/40 dilution. After three rinses in buffer (5 min each), the grid was placed on the colloidal-gold marker (15 min) and rinsed in buffer (5 min). The grid was briefly rinsed on a drop of distilled water, blotted and dried. Double labeling was performed by either of two methods. In the first method, the grid was incubated successively with one antibody, followed by its specific gold probe, then with the other antibody followed by its probe. In a second method, the grid was incubated with a mixture of the two antibodies, followed by a mixture of the probes. Incubation conditions and times were as for single labeling. Best results were obtained with one method or the other, depending on the combination of antibodies used.

After labeling, the dried grids were shadowed unidirectionally at room temperature in a Balzers freeze-

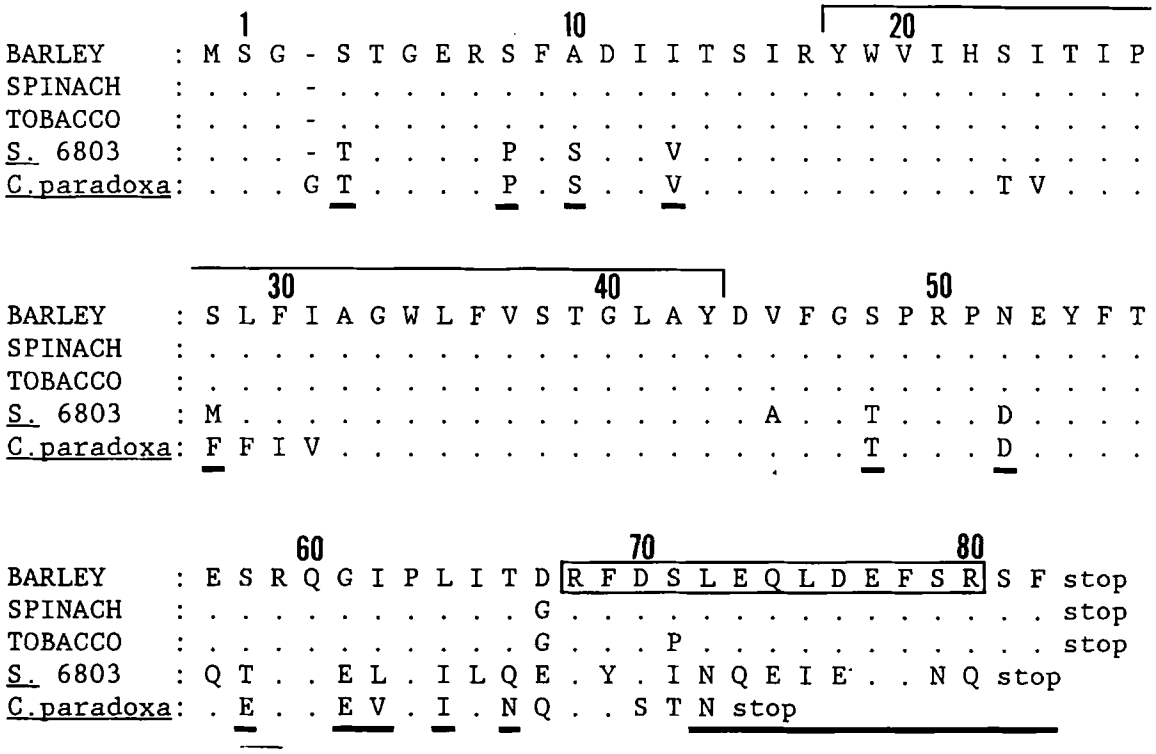

Fig. 2. Comparison of the amino-acid sequences of the *psb*E gene products of barley [12], spinach [8], tobacco [9], *Synechocystis sp.* PCC 6803 [13] and *C. paradoxa* [10]. Amino acids are indicated where different from barley. The initiator methionine is cleaved in the mature spinach protein, so that numbering starts with the next residue. —— indicates a gap introduced to maximize homology. The putative transmembrane segment is overlined, the region corresponding to the synthetic peptide is boxed. Residues which were identical in barley, spinach and tobacco but were different in *Synechocystis* 6803 and in *C. paradoxa* are underlined.

etch apparatus (either a prototype computer-operated or a conventional BAF 301 apparatus), at an 18° angle with platinum and at 90° with carbon.

Results and Discussion

6A5, a monoclonal antibody recognizing the C-terminal part of the α-subunit of cytochrome b-559.

Monoclonal antibodies were obtained from mice immunized with a barley PS II core preparation. In immunoblot assays, antibody 6A5 reacted very strongly with a polypeptide of apparent M_r 8–10 kDa present in the barley membrane (Fig. 1A). This polypeptide was identified as the α-subunit of cytochrome *b*-559, since it was enriched in the PS II core preparation and comigrated with the main Coomassie blue-stained band in a purified cytochrome *b*-559 preparation, i.e., fraction 56, obtained by the 'mild' isolation procedure in Ref. 26. As shown in Fig. 1A, additional reacting bands were observed in the 43 kDa and 70 kDa regions in the PS II preparation. They may represent oligomeric forms or aggregates. The reaction seen slightly below the main band was observed inconsistently in different preparations and is therefore thought to be due to proteolysis.

In an attempt to localize the epitope in the amino-acid sequence, we compared the reactivity of the antibody to thylakoid polypeptides from different organisms for which the sequence of the polypeptide is known. We studied three higher plants, barley (*Hordeum vulgare*), spinach (*S. oleracaea*) and tobacco (*Nicotiana tabaccum*), the cyanobacterium *Synechocystis sp.* PCC 6803 and the cyanelle of the flagellate *C. paradoxa*. Proteins from the photosynthetic membranes of these organisms were separated by SDS-PAGE, and transferred to nitrocellulose. After reaction with 6A5, bands of the expected molecular weight were observed in all three higher plants, but not with *Synechocystis* 6803 nor with *C. paradoxa* (Fig. 1B). Examination of the protein sequences (Fig. 2) revealed several regions where both the *Synechocystis* and the *C. paradoxa* sequences differ from the higher plant sequences, the last 14 residues of the higher plant sequence showing a strikingly low level of conservation.

In order to identify more precisely the epitope in the sequence, the antibody was reacted with a synthetic tridecapeptide of sequence R F D S L E Q L D E F S R [18], corresponding to residues 68–80 of the mature barley and spinach polypeptides. In ELISA tests, the antibody reacted strongly with the tridecapeptide (Fig. 3A. The reaction was inhibited by preincubating the antibody with the peptide. This treatment also inhibited the binding of the antibody to the thylakoid membrane. In control experiments, an unrelated decapeptide of sequence D K P E I P L P H E (PSI-A1.5, residues 230–239 of the spinach PSI-A1 protein) did not inhibit the binding of 6A5 to the tridecapeptide or to the

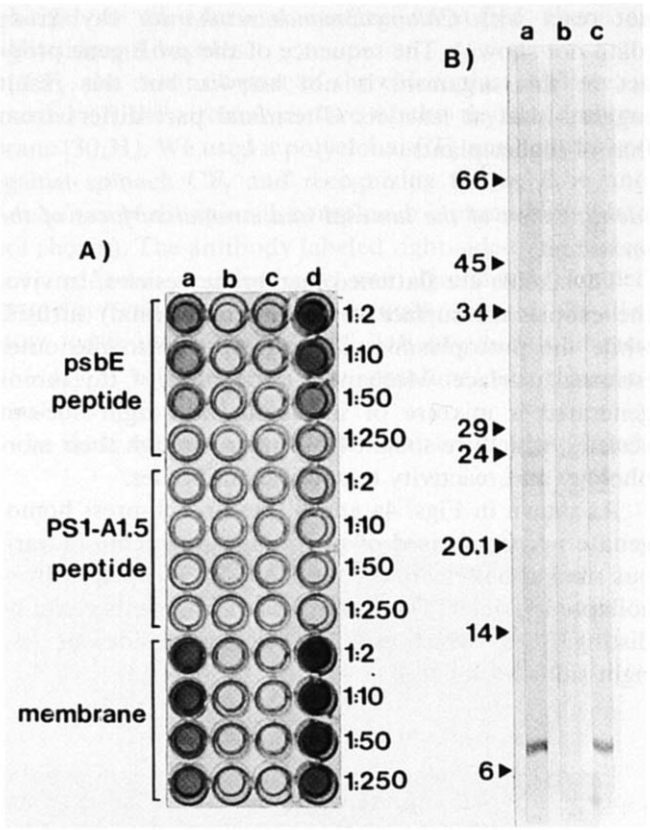

Fig. 3. Reactivity of monoclonal antibody 6A5 with the synthetic tridecapeptide. (A) ELISA tests using the tridecapeptide, the decapeptide PS1-A1.5 and spinach French-press homogenate as antigens (as indicated on the left). Lane a: antibody 6A5 preincubated with 1 mg/ml PSI-A1.5 peptide; b: 6A5 preincubated with 1 mg/ml tridecapeptide; c: 6A5 preincubated with 25 μg/ml tridecapeptide; and d: 6A5, no preincubation. The dilution factors of the hybridoma culture supernatant are indicated on the right. (B) Immunoblotting experiment. Spinach thylakoids were electrophoresed in an 18% acrylamide gel [20] and blotted. Nitrocellulose strips were reacted with 6A5 (a) or 6A5 after preincubation with 0.1 mg/ml tridecapeptide (b) or with 0.1 mg/ml PSI-A1.5 peptide (c). Visualization was with alkaline phosphatase. The positions of the molecular mass markers on the corresponding gel are indicated on the left.

membrane, nor did the antibody react with the immobilized PSI-A1.5. Inhibition by the tridecapeptide was specific since, in other control experiments (data not shown), it did not inhibit the binding of two other synthetic peptide antibodies to their respective antigens.

The reactivity of the antibody with the synthetic tridecapeptide was further demonstrated in immunoblotting experiments (Fig. 3B). Preincubation of the antibody with the tridecapeptide completely abolished the binding of 6A5 to the α-subunit band, whereas the unrelated peptide PSI-A1.5 did not compete. These results indicate that monoclonal antibody 6A5 recognizes a unique epitope located in the sequence encompassed by the tridecapeptide. The fact that the tobacco protein, in which serine-71 is replaced by a proline, reacted with the antibody suggests that this residue is not part of the epitope. In other experiments, 6A5 did

not react with *Chlamydomonas reinhardtii* thylakoids (data not shown). The sequence of the *psb*E gene product in this organism is not known, but this result suggests that at least its C-terminal part differs from that of higher plants.

Identification of the lumenal and stromal surfaces of the membrane

Thylakoids are flattened membrane vesicles. In vivo, the exoplasmic surface is the inner (lumenal) surface, while the protoplasmic side corresponds to the outer (stromal) surface. Mechanical disruption of thylakoids generated a mixture of inside-out and right-side-out vesicles, which we sought to identify through their morphology and reactivity to specific antibodies.

As shown in Figs. 4a and b, the French-press homogenate was comprised of membrane fragments of various sizes, usually with the disk-like shape expected from collapsed vesicles. Two categories of fragments could be distinguished, which we identified as inside-out and right-side-out vesicles.

Inside-out vesicles appeared as large vesicles (0.3–0.5 μm diameter), often irregular in shape, covered with large particles ranging in size between 12 and 16 nm (Fig. 4b). These vesicles represented about 60% of the material, on a surface-area basis. The surface particles were sometimes arranged in rectangular arrays (see, for example, the arrow in Fig. 6b). In some instances, especially in the arrays, the particles could be seen to have a square shape and a multimeric structure. Therefore, these particles were identified as the equivalent of the 'tetrameric' ESs particles which have been described on the lumenal surface of freeze-etched thylakoids [27], and the surface carrying them as the lumenal surface (ES).

This identification was confirmed by examination of purified barley inside-out vesicles (B5 fraction, Fig. 4c) and of PS II-membrane fragments obtained by Triton X-100 treatment (data not shown), both of which showed similar surface particles. Particles on the purified inside-out vesicles were somewhat less distinct, probably due to the presence of residual polymer, which can obscure

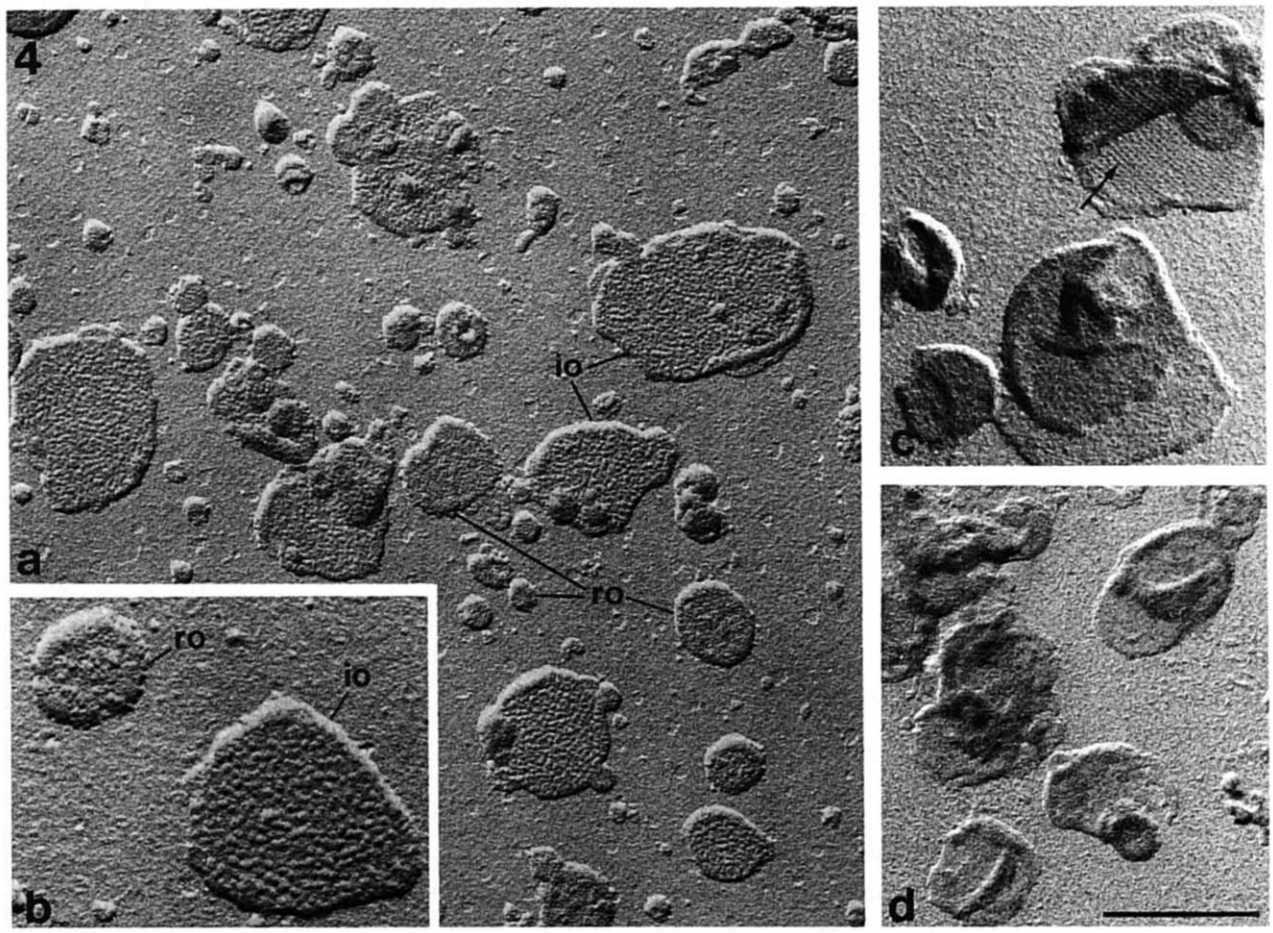

Fig. 4. 'On grid' shadowing of thylakoid membrane preparations. (a, b) French-press homogenate of spinach thylakoids. io: inside-out vesicles; ro: right-side-out vesicles. (c) Barley inside-out vesicles isolated by phase partitioning. The arrow points to a region where the large surface particles are arranged in a regular lattice. (d) Barley inside-out vesicles after treatment with 1 M $CaCl_2$. The surface particles are no longer discernible. The dimension of the bar is 0.5 μm, except for (b), where it is 0.29 μm.

the particles if the membranes are not thoroughly washed [28]. The ESs particles have been shown to contain the three extrinsic polypeptides associated with the water-oxidizing-side of PS II [28,29]. Both the ESs particles and these polypeptides can be removed by treating the membranes with 1 M $CaCl_2$ [28]. As a further control, we subjected purified inside-out vesicles to this treatment. We observed the disappearance of the large surface particles (Fig. 4d).

In addition to the inside-out vesicles, the homogenate contained a category of vesicles densely covered with smaller (8–10 nm) particles. These vesicles were generally smaller in size than the inside-out vesicles. As shown below, labeling experiments with antibodies specific to the stromal surface identified them as right-side-out vesicles.

CF_1, the extrinsic part of the chloroplast ATPase, is located on the stromal surface of the thylakoid membrane [30,31]. We used a polyclonal CF_1 antibody raised against spinach CF_1 and recognizing the α, β, τ and δ-subunits of the spinach complex in immunoblots (data not shown). The antibody labeled right-side-out vesicles, but inside-out vesicles were not significantly labeled (Fig. 5a). Some gold was occasionally seen on the film and on the inside-out vesicles, probably due to adsorption of CF_1 released from the membrane during mechanical disruption or labeling.

In these experiments, the level of background label-

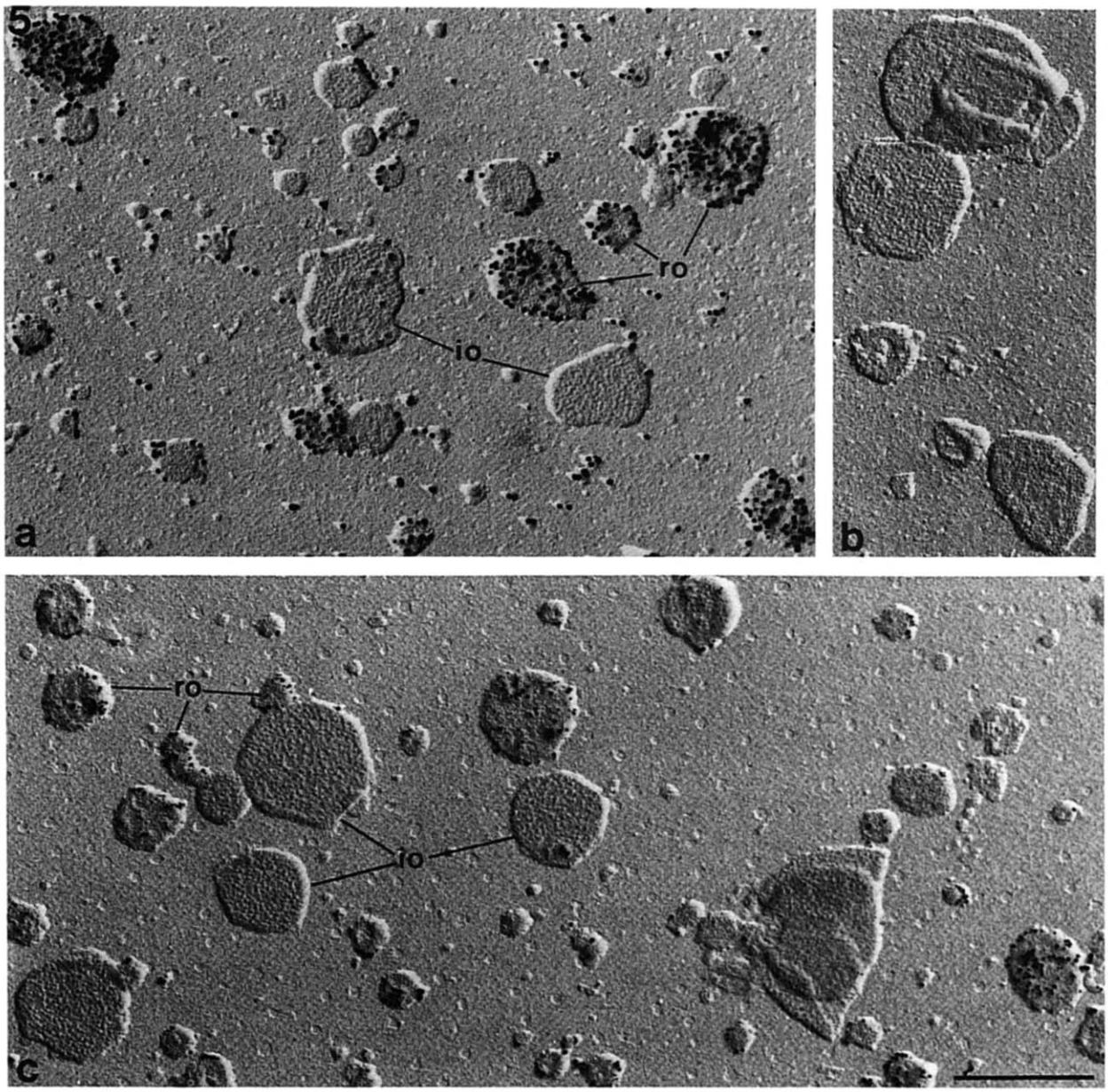

Fig. 5. 'On grid' labeling of the French-press homogenate (a) with the CF_1 antibody; (b) with a non-immune serum and (c) with monoclonal antibody MLH1. The dimension of the bar is 0.5 μm.

ing was estimated by replacing the specific antibody with non-immune sera at the same dilution from different rabbits. The background level varied from one rabbit to another but was usually very low (Fig. 5b). When noticeable, the background was usually more pronounced on inside-out than on right-side-out vesicles. When the primary antibody was omitted, scarcely any gold was seen on the grid (data not shown).

Monoclonal antibody MLH1 was also found to label specifically the right-side out vesicles (Fig. 5c). The epitope of this antibody is believed to be located on the stomal surface of the membrane, based on the following

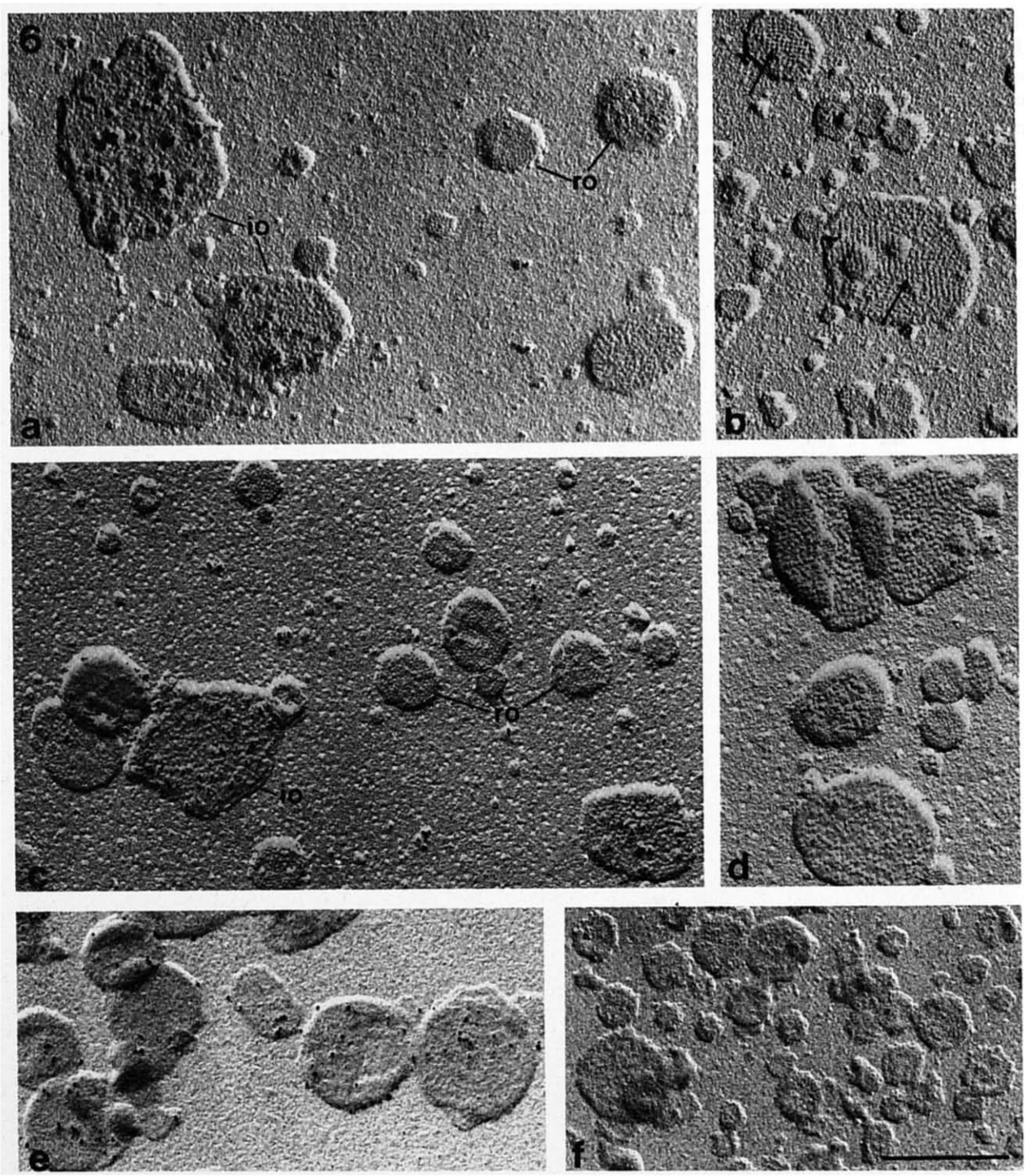

Fig. 6. 'On grid' labeling with 6A5 and the polyclonal antibody to the tridecapeptide. (a–d) French-press homogenate; (e) barley inside-out vesicles and (f) barley right-side-out vesicles. (a) (e) and (f) show grids reacted with 6A5; (b) 6A5 preincubated with the tridecapeptide; (c) the polyclonal peptide antibody; (d) the polyclonal peptide antibody preincubated with the tridecapeptide. The dimension of the bar is 0.5 μm.

observations. (i) MLH1 recognizes the major apoprotein of LHC II in pea and other higher plants [32], including spinach (immunoblotting experiments, data not shown). This 26–27 kDa chlorophyll *a/b*-binding protein (probably a mixture of products from a family of genes) is a peripheral light-harvesting antenna for PS II. A 1–2 kDa fragment of the protein is exposed on the stromal surface and can be cleaved by trypsin [33]. (ii) Trypsin treatment of the intact membrane has been shown to abolish the binding of MLH1 [34].

'On grid' labeling with antibodies to cytochrome b-559

We used 'on grid' labeling to determine the location of the C-terminal domain of the α-subunit of cytochrome *b*-559. In addition to monoclonal antibody 6A5, we have used a polyclonal antibody raised against the synthetic tridecapeptide described above. The antibody is characterized in detail elsewhere [18]. It reacts with the peptide in ELISA and with the protein in ELISA and immunoblotting experiments, these reactions being specifically inhibited by preincubation with the free peptide.

As shown in Fig. 6a and c, both antibodies labeled inside-out vesicles only. The specificity of the reactions was shown by experiments where the antibodies were reacted with the tridecapeptide prior to incubation with the grid (Fig. 6b and d). For both antibodies, this resulted in a nearly complete inhibition of the labeling. The identity of the labeled surface was confirmed by labeling experiments with 6A5 on purified inside-out and right-side-out vesicles from barley. Only the former were labeled (Fig. 6e and f). In addition, barley PS II-enriched membranes, which expose only the lumenal surface of the membrane, were also labeled by the antibody (data not shown).

In double-labeling experiments with 6A5 and the polyclonal antibody to the peptide, both antibodies were found to label the same vesicles (Fig. 7a). In experiments using 6A5 and the CF_1 antibody (Fig. 7b and c), the vesicles labeled by 6A5 (small beads) were not labeled by the CF_1 antibody (large beads) and vice-versa. This is consistent with the two antibodies recognizing two different surfaces of the membrane. The arrow in Fig. 7b points to a partly opened inside-out

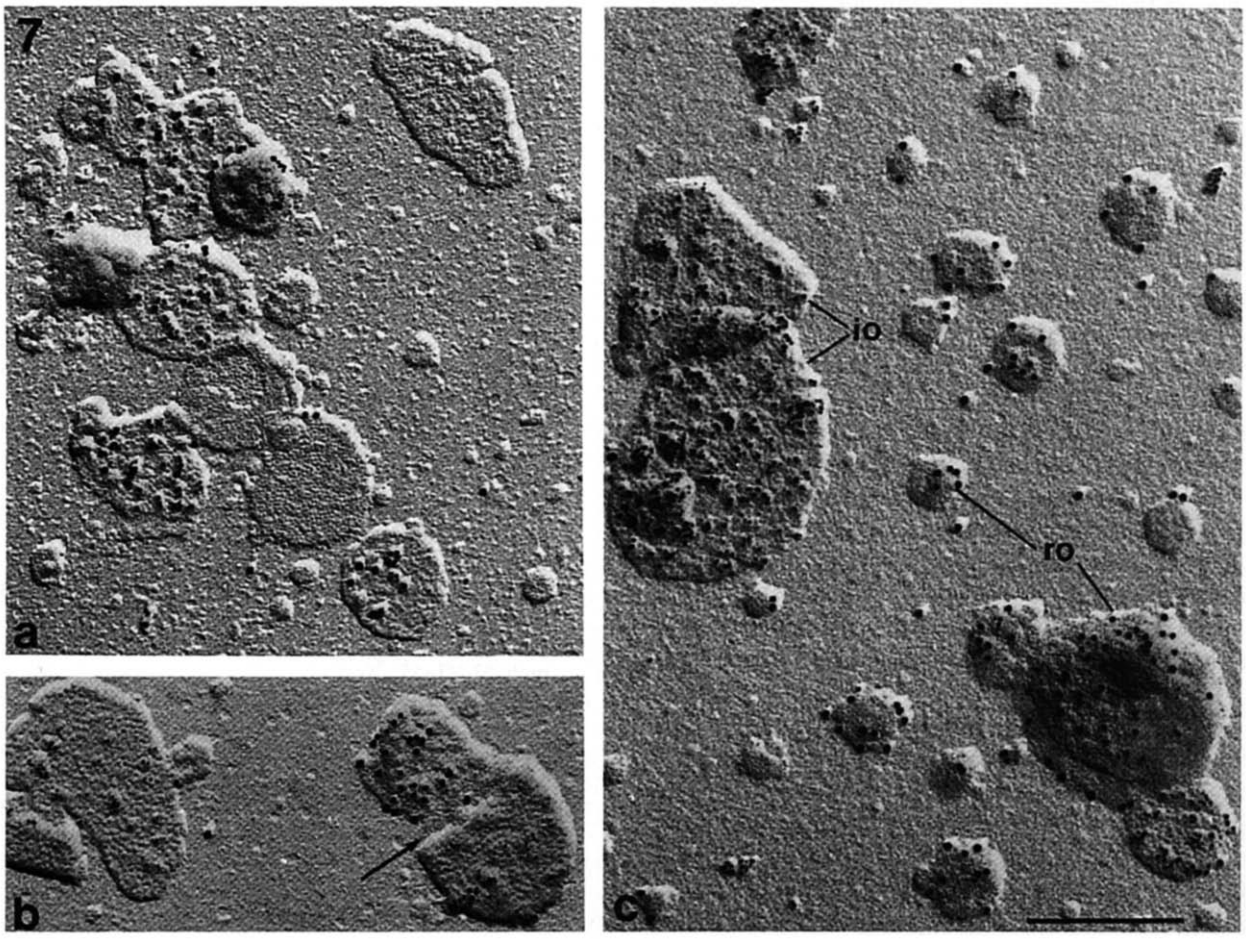

Fig. 7. French-press homogenate, double labeling (a) with 6A5 (small gold particles) and the polyclonal peptide antibody (large gold particles); (b, c) with 6A5 (small gold particles) and the CF_1 antibody (large gold particles). In (b), the arrow points to a partially opened inside-out vesicle showing both the stromal and the lumenal surfaces, which are labeled with large and small gold granules, respectively. The dimension of the bar is 0.5 μm.

vesicle, with the lumenal surface labeled by 6A5 and the stromal surface by the CF_1 antibody.

Structure of cytochrome b-559

The results above indicate that the C-terminal domain of the α-subunit of cytochrome *b*-559 (at least from residue 68 on) is exposed on the lumenal surface of the membrane. If the hydrophobic stretch between residues 18–43 spans the membrane bilayer as an α-helix, it follows that the N-terminal domain is exposed on the stromal surface. In another set of experiments using the peptide antibody to monitor proteolysis of the protein on immunoblots [18], we find that the N- and C-terminal domains of the polypeptide are exposed on the stromal and on the lumenal surfaces, respectively. The results presented here, obtained by an independent approach, confirm this conclusion.

The orientation of the β-subunit of cytochrome *b*-559 has not yet been determined. Michel et al. have suggested that it can be phosphorylated at its N-terminal Thr [36], in which case its transmembrane orientation would be similar to that of the α-subunit, i.e., N-terminus on the stromal and C-terminus on the lumenal surface. The most likely model for the protein would then be that of a hetero-dimer ($\alpha\beta$), with His-22 of the α-chain and His-17 of the β-chain both ligating the heme. The heme would be within the membrane lipid bilayer but close to the stromal surface, consistent with the rapid oxidation of cytochrome *b*-559 by the impermeable ion ferricyanide in intact thylakoids [37]. If the cytochrome structure is superimposed on that of the bacterial center [4], with Arg-17 placed at the same level in the membrane as Arg-231 of subunit L, the heme and the tetrapyrrole ring of the bacteriochlorophyll special pair will be approx. 16 Å apart. Since the histidines which ligate the accessory bacteriochlorophylls in the bacterial center are not conserved in PS II, the positions of the putative monomer chlorophylls cannot be predicted. However, if they assume a similar position in the membrane, they would be approx. 14–18 Å away from the heme.

If two hemes are present per reaction center, as suggested by measurements on various PS II preparations (Refs. 38 and 39; however, see Refs. 40 and 41), they would be in similar positions in the membrane, possibly related by the rotational symmetry which, by analogy with subunits L and M in the bacterial center, is expected in the PS II center [4]. At this stage, however, we cannot rule out that the β-chain assumes the opposite orientation, i.e., with its N-terminus on the lumenal surface. In this case, the histidines in the α- and β-subunits would be too far apart to be ligands of the same heme. A possible structure would be that of two homo-dimers ($\alpha_2\beta_2$), with one heme ligated by the histidines of two α-chains (and close to the stromal surface) and one ligated by the histidines of two β-chains (and close to the lumenal surface).

Acknowledgements

We thank Professor D. Branton and his group for making available to us their electron microscopy facility, and Professor Branton and Professor D. von Wettstein for useful suggestions and reading the manuscript. We acknowledge the help of T. Bektved in the monoclonal work. This work was supported in part by a fellowship from the Fondation pour la Recherche Medicale to O.V., by a grant from N.I.H. (GM-38323) to W.A.C., and by grants from the Competitive Research Grant Office of the U.S. Dept. of Agriculture and the National Science Foundation to L.B.

References

1 Okamura, M.Y., Feher, G. and Nelson, N. (1982) in Photosynthesis: Energy conservation by Plants and Bacteria (Govindjee, ed.), Vol. I, pp. 195–272, Academic Press, New York.
2 Zuravski, G., Bohnert, H.J., Whitfield, P.R. and Bottomley, W. (1982) Proc. Natl. Acad. Sci. USA 79, 7699–7703.
3 Williams J.C., Steiner, L.A., Ogden, R.C., Simon, M.I. and Feher, G. (1983) Proc. Natl. Acad. Sci. USA 80, 6505–6509.
4 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
5 Trebst, A. (1986) Z. Naturforsch. 41c, 240–245.
6 Cramer, W.A. and Whitmarsh, J. (1977) Annu. Rev. Plant Physiol. 28, 133–172.
7 Thompson, L.K. and Brudvig, G.W. (1988) Biochemistry 27, 6653–6658.
8 Herrmann, R.G., Alt, J., Schiller, B., Widger, W.R. and Cramer, W.A. (1984) FEBS Lett. 176, 239–244.
9 Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chungwongse, J., Obokata, J., Yamagushi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdo, N., Shimada, H. and Sugiura, M. (1986) EMBO J. 5, 2043–2049.
10 Cantrell, A. and Bryant, D.A. (1987) in Progress in Potosynthesis Research (Biggins, J., ed.), Vol. IV, pp. 659–662, Martinus Nijhoff, Dordrecht.
11 Cushman, J.C., Christopher, D.A., Little, M.C., Hallick, R.B. and Price, C.A. (1988) Curr. Genet. 13, 173–180.
12 Krupinska, K. and Berry-Lowe, S. (1988) Carlsberg Res. Commun. 53, 43–55.
13 Pakrasi, H.B., Williams, J.G.K. and Arntzen, C.J. (1988) EMBO J. 7, 325–332.
14 Babcock, G.T., Widger, W.R., Cramer, W.A., Oertling, W.A. and Metz, J.G. (1985) Biochemistry 34, 3638–3644.
15 Widger, W.R., Cramer, W.A., Hermodson, M. and Herrmann, R.G. (1985) FEBS Lett. 191, 186–190.
16 Cramer, W.A., Theg, S.M. and Widger, W.R. (1986) Photosynth. Res. 10, 393–403.
17 Hønberg, L.S. (1984) Carlsberg Res. Commun. 49, 703–719.
18 Tae, G.S., Blake, M.T., Cramer, W.A., Vallon, O. and Bogorad, L. (1988) Biochemistry 27, 9075–9080.
19 Bassi, R., Hinz, U. and Barbato, R. (1985) Carlsberg Res. Commun. 50, 347–357.
20 Thomas, J.O. and Kornberg, R.G. (1978) in Methods in Cell

Biology, (Stein, G. et al. eds.), Vol. XVIII, pp. 429–440, Academic Press, New York.
21 Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
22 Dzelzkalns, V. and Bogorad, L. (1986) J. Bacteriol. 165, 964–971.
23 Bothe, H. and Floener, L. (1978) Z. Naturforsch. 33c, 981–987.
24 Henry, L.E.A., Mikkelsen, J.D. and Lindberg-Møller, B. (1983) Carlsberg Res. Commun. 48, 131–148.
25 Horisberger, M. (1979) Biol. Cell. 36, 253–258.
26 Koenig, F. and Lindberg-Møller, B. (1982) Carlsberg Res. Commun. 47, 245–262.
27 Staehelin, L.A. (1976) J. Cell Biol. 71, 136–158.
28 Simpson, D. and Andersson, B. (1986) Carlsberg Res. Commun. 51, 467–474.
29 Seibert, M., DeWit, M. and Staehelin, L.A. (1987) J. Cell Biol. 105, 2257–2265.
30 Oleszko, S. and Moudrianakis, E.N. (1974) J. Cell Biol. 63, 936–948.
31 Miller, K. and Staehelin, L.A. (1976) J. Cell BIol. 68, 30–47.
32 Darr, S.C., Somerville, S.C. and Arntzen, C.J. (1986) J. Cell Biol. 103, 733–740.
33 Mullet, J.E. (1983) J. Biol. Chem. 258, 9941–9948.
34 Darr, S.C., Arntzen, C.J. and Somerville, S.C. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 141–144, Martinus Nijhoff, Dordrecht.
35 Andersson, B., Sundby, C. and Albertsson, P.Å. (1980) Biochim. Biophys. Acta 599, 391–402.
36 Michel, H., Hunt, D.F., Shabanowitz, J. and Bennett, J. (1988) J. Biol. Chem. 263, 1123–1130.
37 Horton, P. and Cramer, W.A. (1974) Biochim. Biophys. Acta 368, 348–360.
38 Yamamoto, Y., Tabata, K., Isogai, K., Nishimura, M., Okayama, S. and Itoh, S. (1984) Biochim. Biophys. Acta 767, 493–500.
39 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
40 Ghanotakis, D.F., Babcock, G.T. and Yocum, C. (1984) Biochim. Biophys. Acta 765, 388–398.
41 Gounaris, K., Pick, U. and Barber, J. (1987) FEBS Lett. 211, 94–98.