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Spatial organization of the cytochrome b_6-f complex within chloroplast thylakoid membranes

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The spatial distribution of the chloroplast thylakoid protein complex comprised of cytochromes *f* and *b*-563, and the Rieske iron-sulfur protein (Cyt b_6-f) has been controversial because of conflicting results obtained by different techniques. We have combined the following biochemical and immunochemical techniques to approach this question: (1) French press disruption of thylakoids, followed by repeated two-phase aqueous polymer partitioning to separate inside-out grana from right-side-out stroma membrane fragments; (2) electrophoretic analysis followed by the 3,3′,5,5′-tetramethylbenzidine stain for cytochrome hemes; (3) electroblot analysis with anti-Cyt b_6-f antibodies; (4) agglutination of membrane fragments with anti-Cyt b_6-f antibodies; and (5) post-embedment thin-section immunolabeling of chemically fixed or ultrarapidly frozen chloroplasts with anti-Cyt b_6-f antibodies. Our results indicate that the complex is present in both of the isolated membrane fragment populations in similar amounts, with the bulk of the immunoreactive sites exposed to the thylakoidal lumen. Direct immunolabeling of thin-sectioned chloroplasts resulted in localization of the complex throughout the thylakoids, without specialized compartmentation. These results provide both the temporal and spatial resolution necessary for accurate localization of the complex. We concur with models proposing distribution of Cyt b_6-f throughout all thylakoid membranes.

Introduction

Asymmetry at all levels is crucial to the specialized functions carried out by membranes, including electron transport reactions. Therefore, it is pivotal to our understanding of the mechanisms of electron transport in the chloroplast to know the spatial distribution of the various components which are involved. Chloroplast thylakoid membranes contain five major membrane protein complexes: (i) the Photosystem II reaction center and 'bound' antenna pigment-protein complex (PS II), (ii) the photosystem I reaction center and 'bound' antenna pigment-protein complex (PS I), (iii) the mobile chlorophyll (*a*/*b*)-containing light-harvesting protein complex (mobile LHC II), (iv) the CF_0-CF_1 coupling factor ATP synthase complex, and (v) the complex comprised of cytochromes *f* and *b*-563, and the Rieske iron-sulfur protein (Cyt b_6-f) which mediates electron-flow between PS II and PS I [1–4]. Of these five complexes, the first four have been demonstrated convincingly to partition laterally in an unequal, non-random fashion between grana and stroma membrane regions of stacked thylakoids. Thus, PS II and much of the mobile LHC $_{II}$ are present primarily within grana membranes, whereas PS I and CF_0-CF_1 are present

Abbreviations: Cyt, cytochrome; PS, Photosystem; TMBZ, tetramethylbenzidine; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl) ethyl]-glycine.

almost entirely within the stroma membrane region (see Refs. 5–7 for reviews). However, a consensus has not been reached regarding the lateral distribution of the Cyt b_6-f complex.

Three possible major distribution patterns have been proposed for Cyt b_6-f, and each has received experimental support: (1) random distribution between grana and stroma membranes [8,9]; (2) localization in the membrane region interfacing the grana and stroma membranes, perhaps with migration of Cyt b_6-f into the stroma membranes [10,11]; and (3) localization entirely within stroma membranes [5,12]. Since these conflicting hypotheses are based on data acquired by widely different techniques, it is probable that they reflect artifactual or interpretative rather than real biological differences.

Using physical fractionation and redox difference spectra techniques, Cox and Andersson [8] and Anderson [9] demonstrated that cytochromes *b*-563 and *b*-559 (low potential) are randomly distributed between stroma and grana membranes, but that cytochrome *b*-559 (high potential) is found exclusively in grana membranes. Consideration of the electron-transfer kinetics between plastoquinone, Cyt b_6-f and P-700$^+$ led Haehnel [13,14] to suggest that Cyt b_6-f is randomly distributed, but that only those complexes in stroma membranes are involved in linear electron transport, an idea shared by Anderson [9]. Alternatively, the suggestion has been made that Cyt b_6-f resides only in stromal membranes [5,12]. This hypothesis is based upon the stoichiometric parity between Cyt *f* and chlorophyl P-700 in grana and stroma membranes [1,15] and in mesophyl and agranal bundle sheath chloroplasts of *Zea mays* [10]. Further circumstantial evidence was provided by co-isolation of Cyt b_6-f and PS I during preparation of a Cyt b_6-f complex [3], and the absence of Cyt b_6/f from a purified granal membrane preparation [12]. In an attempt to reconcile the divergent results obtained by different laboratories, Ghirardi and Melis [10] proposed that Cyt b_6-f is found in the region interfacing grana and stroma membranes and is therefore co-fractionated with both membrane populations upon physical disruption of thylakoids in this region. This idea was supported by Barber [11], based upon theoretical considerations of membrane surface properties.

To circumvent certain of the problems inherent to deducing in vivo organization based upon in vitro observations of protein complex activities, we have combined physical fractionation of grana and stroma membrane fragments (by two-phase aqueous polymer partitioning of French press-disrupted thylakoids) with immunochemical and biochemical analyses of the fractions. In addition, we have directly visualized Cyt b_6-f distribution by post-embedment thin-section immunolabeling of intact and broken chloroplasts which were prepared by chemical fixation, or ultrarapidly frozen and directly embedded. Ultrarapidly frozen material provided the temporal resolution required to ensure that no artifactual redistributions of the complex could occur, as is possible with chemical fixation. Our results, obtained by the various techniques, are all internally consistent and are in agreement with our previously published work [16]. We support models proposing localization of Cyt b_6-f throughout the thylakoid membranes. The significance of these results is discussed.

Materials and Methods

Thylakoid fractionation. Chloroplast isolation was carried out as described previously [16]; local commercially grown spinach (*Spinacia oleracea*) or peas (*Pisum sativa* var. Laxtons progress No. 9), grown under constant light, were used as the source of chloroplasts. Thylakoid disruption and two-phase aqueous polymer partitioning techniques were performed essentially as described by Andersson, Åkerlund and co-workers [17,18], with subsequent modifications [19]. Chloroplasts were disrupted osmotically by suspension in 10 mM NaCl/50 mM sodium phosphate (pH 7.4). The lysed chloroplasts were sedimented at $2000 \times g_{max}$ for 10 min (4°C) and washed once in the same manner. The pellet was resuspended in 5 mM NaCl/100 mM sucrose/10 mM sodium phosphate/5 mM $MgCl_2$ (pH 7.4) and passed twice through a French press (Aminco, Silver Spring, MD) under 10.3 MPa, at a flow-rate of approx. 5–8 ml/min. To the pressate was added 0.1 M EDTA (Sigma Chemical Co., St. Louis, MO) to a final concentration of 5 mM. The chelated pressate was passed twice more through the press at 20.7 MPa. The final pressate was clarified of large

debris by sedimentation at $1000 \times g_{max}$ for 10 min at 4°C. Grana and stroma membrane fragments were fractionated by two-phase aqueous polymer partitioning using 5.77% (w/v) poly(ethylene glycol) 4000 (Sigma Chemical Co.) and 5.60% (w/v) Dextran T-500 (Pharmacia Fine Chemicals, Uppsala, Sweden) in 10 mM sodium phosphate/5 mM NaCl/20 mM sucrose (pH 7.4) (polymer buffer) [19]. The top and bottom phases were further repartitioned at least twice against fresh bottom and top phases, respectively. The final top (primarily stroma membrane fragments) and bottom (grana membrane fragments) phases were collected, diluted at least 3-fold with polymer buffer and sedimented at 100000 *g* for 90 min at 4°C. In certain experiments, the partitioned bottom fraction was resuspended in polymer buffer, or the same buffer without $MgCl_2$, sonicated for up to 90 s (on ice) with a Branson Sonic Power sonifier (model S125, Heat systems Co., Great Neck, NY) equipped with a probe tip (power setting approx. 5), then re-partitioned and used for subsequent analyses.

Biochemical techniques. Isolated membrane fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins using a 4.5% (w/v) acrylamide stacking gel, 12.5% acrylamide analytical gel, with or without 4 M urea, and the discontinuous buffer system of Laemmli [20]. Gels were stained for heme-associated peroxidase activity by the method of Thomas et al. [21], with 3,3′,5,5′-tetramethylbenzidine (TMBZ) as substrate. TMBZ-stained gels were de-stained with 100 mM sodium sulfite in 30% (v/v) isopropanol, washed in 30% isopropanol, and re-stained with Coomassie brilliant blue R-250 for visualization of polypeptide banding patterns [21]. Band intensities were estimated visually. Electroblot analysis, based on the technique of Towbin et al. [22], was performed with rabbit anti-cytochrome b_6-f, antibodies directed against the α- and β-subunits of coupling factor CF_1 complex (anti-CF_1), or pre-immune antibodies (all of which are described in Ref. 23), using horseradish peroxidase-conjugated goat anti-rabbit immunoglobulins for visualization (Bio-Rad Laboratories, Richmond, CA).

Agglutination assay. Agglutination assays were set up by dilution of the separated membrane fractions into 10 mM sodium phosphate buffer (pH 8.0), containing 10 mg/ml bovine serum albumin (Sigma Chemical Co., essentially fatty acid-free). Serial dilutions of antibody were made in the same buffer and an equal volume of the membrane materials was added. The round-bottomed tubes were incubated overnight at 4°C and scored both macroscopically and microscopically for agglutination.

Immunocytochemical labeling. Chloroplasts were either chemically fixed or ultrarapidly frozen without cryoprotectants in preparation for embedment. Routine fixation, dehydration, embedment in Lowicryl K4M (Polysciences, Warrington, PA), and resin polymerization were carried out as described elsewhere [16]. In separate experiments, broken chloroplasts were unstacked on ice in 0.4 M sucrose/50 mM Tricine/10 mM NaCl/5 mM EDTA (pH 8.0) for 1 h, prior to fixation (in the absence of Mg^{2+}). Aliquots of unstacked chloroplasts were sedimented, then placed in the same buffer containing 5 mM $MgCl_2$ instead of EDTA, for restacking of thylakoids, Samples were then fixed (2.5% (w/v) glutaraldehyde/10 mM sodium phosphate (pH 7.4)) after either 2 min or 2 h of restacking. Alternatively, unfixed, non-cryoprotected chloroplast samples were ultrarapidly frozen in a Gilkey-Staehelin propane jet freezer [24] between two thin copper specimen holders held 15 μm apart by a copper electron microscope grid (200 mesh). The specimens, held within the grid's mesh, were placed in a Coulter-Terracio freeze-drying unit (Ladd Research Industries, Burlington, VT) and freeze-dried overnight at a temperature rising slowly from -90 to -45°C. Samples were warmed to ambient temperature and directly infiltrated under vacuum for 45 min with complete Lowicryl K4M. They were then transfered into Beem capsules and polymerized as above.

Thin sections (silver-gray to silver-gold) were picked up on nickel grids. Immunostaining was carried out as described by Allred and Staehelin [16].

Results

Membrane fractionation experiments

Isolated inside-out grana and right-side-out stroma thylakoid membranes contained the ex-

pected groups of distinct polypeptides when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue (Fig. 1). This demonstrates that the two membrane regions were effectively separated by French press treatment and two-phase aqueous polymer partitioning of the disrupted chloroplasts. In contrast to the results of Henry and Möller [12], however, both cytochromes *f* and b_6 were present in each fraction (Fig. 2A), as detected by use of the TMBZ gel stain for heme-associated peroxidase activity. Approximately equal activities of cytochrome *f* and b_6 hemes were present in both fractions, when loaded on a chlorophyll basis. This result was supported by western blot analysis of the gel-separated fractions using anti-Cyt b_6-*f* antiserum (Fig. 2B), indicating that approximately equal amounts of Cyt *f* and b_6 polypeptides were also present. This is in contrast to the results obtained with anti-CF_1 (Fig. 2B). Thus, whereas most other polypeptide components of thylakoid membranes are non-randomly distributed between grana and stroma membranes (see Refs. 5,6,7 and 11 for reviews), these fractionation studies indicate that the Cyt b_6-*f* complex is distributed throughout the thylakoid membranes, in similar quantities. The differential presence of the high-molecular-weight doublet in lanes 6–8 (Fig. 2B) appears to reflect variable

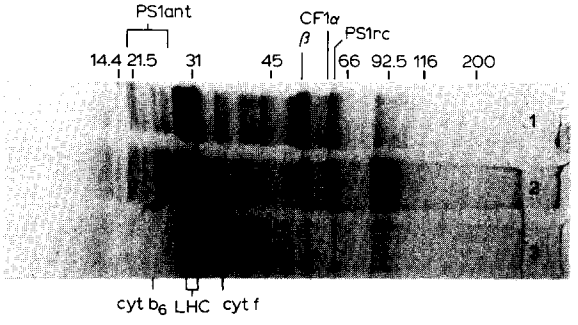

Fig. 1. Coomassie blue-stained polypeptide profiles of (1) total spinach thylakoids, (2) right-side-out stromal thylakoid membrane-derived vesicles, and (3) inside-out grana membrane-derived fragments. Note the differences in lanes 2 and 3, and the 'average' composition of lane 1. CF1α, β = coupling factor subunits CF1α and CF1β; Cyt *f* and Cyt b_6 = cytochromes *f* and *b*-563; LHC = polypeptides of the chlorophyl *a*/*b* light-harvesting antenna complex; PSIrc = PS I reaction center polypeptide; PSIant = PS I antenna polypeptides.

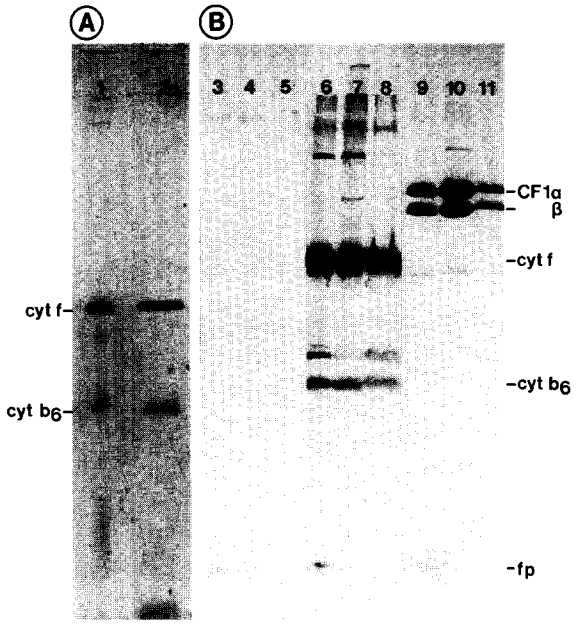

Fig. 2. Demonstration of cytochromes *f* and b_6 in stroma membrane- and grana membrane-derived fragments of spinach thylakoids. (A) TMBZ stain for heme-associated peroxidase activity. (B) Electroblot analysis with pre-immune (3–5), anti-Cyt b_6-*f* (6–8), or anti-CF_1 (9–11) antibodies. This gel was more heavily loaded than is optimal for electroblotting, exaggerating the level of contamination of grana by stoma membranes when labeled with anti-CF1. Lanes 3,6 and 9 are total thylakoids, 1,4,7 and 10 are stroma membrane-derived, and 2,5,8 and 11 are of grana membrane origin. Gel B contained 4 M urea, which markedly affects the migration and apparent size of cytochrome *f*. fp = free pigment; all others are as indicated in Fig. 1.

dissociation of the Cyt b_6-*f* complex under different solubilization conditions, rather than an asymmetric distribution of components (Allred, D.R. and Staehelin, L.A., unpublished data). We are currently pursuing the significance of this behavior.

When isolated inside-out grana and right-side-out stroma membrane fractions were treated with anti-Cyt b_6-*f* antibody to assay for agglutination reactions (Fig. 3), only the inside-out fragments were significantly agglutinated (see Table I, Fig. 3A, B). Right-side-out stroma vesicles were midly agglutinated, but only at very high concentrations of antibody (Fig. 3C, D). The poor agglutinability of the the right-side-out vesicles, in conjunction with the data indicating similar amounts of Cyt

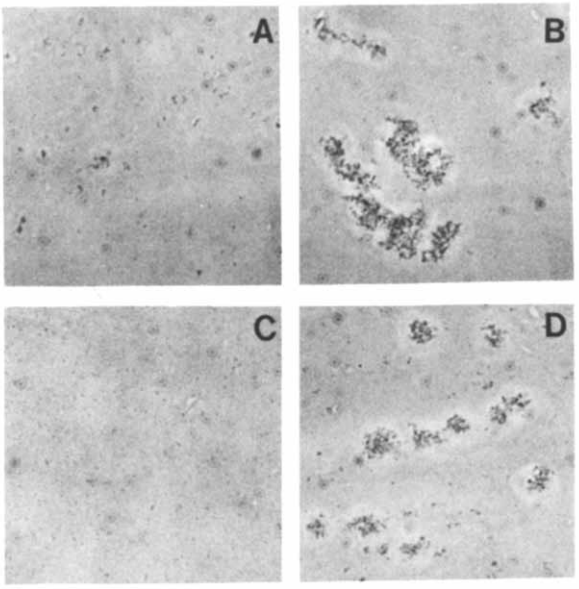

Fig. 3. Agglutination of grana- or stroma membrane-derived fragments from spinach thylakoids with pre-immune or anti-Cyt b_6-f antibodies. Inside-out grana membrane fragments treated with (A) pre-immune antibodies (1/32 dilution) or (B) anti-Cyt b_6-f antibodies (1/32 dilution). Right-side-out stroma membrane vesicles treated with (C) pre-immune antibodies (1/2 dilution), or (D) anti-Cyt b_6-f antibodies (1/2 dilution). Right-side-out vesicles required very high concentrations of specific antibody to be agglutinated. ×2500.

TABLE I

AGGLUTINATION BY ANTI-CYTOCHROME b_6-f ANTIBODIES, AND PRESENCE OF CYTOCHROME f AND b_6 IN ISOLATED THYLAKOID MEMBRANE FRAGMENTS

r.o.v., right-side-out stroma membrane-derived vesicles; i.o.v.-top, inside-out grana membrane-derived vesicles which re-partitioned to the top phase after sonication; i.o.v.-bottom, sonicated i.o.v. which re-partitioned to the bottom phase, in the presence or absence of 5 mM $MgCl_2$. Titer: Immune = anti-Cyt b_6-f complex antibodies (IgG fraction); pre-immune = from the same rabbit, IgG fraction.

Fraction	Titer immune/ preimmune	TMBZ reaction Cyt f-b_6
r.o.v.	4/0	3+/1+
i.o.v.-top (+Mg^{2+})	256/ < 4	2+/+/−
i.o.v.-top (no Mg^{2+})	256/0	2+/+/−
i.o.v.-bottom (+Mg^{2+})	256/ < 4	2+/+/−
i.o.v.-bottom (no Mg^{2+})	256/ < 4	2+/+/−

b_6-f in both membrane fractions would suggest that the major immunoreactive portion of the Cyt b_6-f complex, and perhaps the bulk of the exposed polypeptides, faces the thylakoid lumen rather than the stroma.

To determine whether the Cyt b_6/f complex might be located within the stroma/grana membrane interface region, we attempted to shear-off the margins of the inside-out grana membrane fragments using sonication, then to fractionate the fragments by two-phase aqueous polymer partitioning. We reasoned that any materials which re-partitioned to the top phase might include both everted (i.e., to normal sidedness) vesicles, as indicated by Sundby et al. [25], as well as any membrane fragments which might have been released from the margins. The polypeptide profiles of membrane fragments collected from both the top and bottom phases of sonicated and re-partitioned inside-out grana membranes were essentially identical (Fig. 4), as were their TMBZ-staining characteristics (data not shown). Similarly, both re-partitioned fractions showed identical agglutination behavior with anti-Cyt b_6-f antibody (Table I). Thus, no evidence for lateral differentiation of the grana membranes was obtained. When viewed by freeze-fracture electron microscopy (Fig. 5), control and sonicated grana membrane fragments retained the same inside-out orientation, consistent with images obtained by Simpson and co-workers [26]. Therefore, the differential partitioning into the two phases without a similar discrepancy in

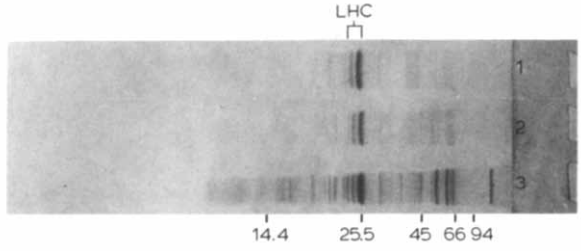

Fig. 4. Electrophoretic profiles of Coomassie blue-staining polypeptides from inside-out grana membrane fragments of spinach thylakoids which were sonicated, then re-partitioned with fresh two-phase aqueous polymers. (1) Top phase; (2) bottom phase; (3) stroma membrane-derived materials, without sonication. The inside-out fragments normally partition to the bottom phase, without sonication. LHC, light-harvesting chlorophyll a/b complex.

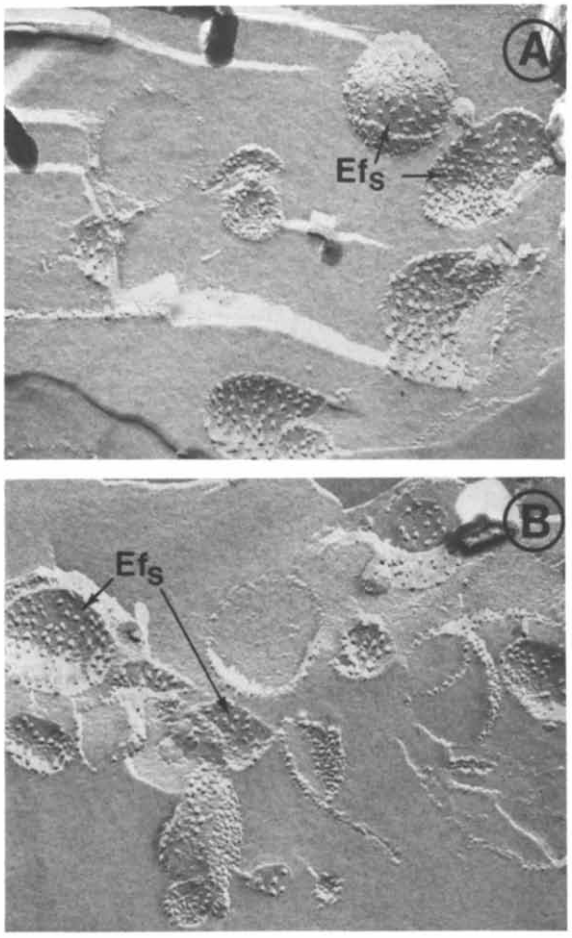

Fig. 5. Freeze-fracture electron micrographs of phase-partitioned inside-out grana membrane fragments of spinach thylakoids which were sonicated, then processed for freeze-fracture electron microscopy. (A) Unsonicated control, (B) identical material sonicated for 90 s with a probe tip sonicator. No apparent eversion of the vesicles occurred. EF_s = exoplasmic fracture face, originally from a stacked membrane region. ×50000.

the major polypeptides and without loss of Cyt b_6-f implies that Cyt b_6-f is distributed throughout the plane of the grana membrane fragments. The differential partitioning is presumably due to minor differences in the two fragment populations with regard to the composition or configuration (after sonication) of the major polypeptides/complexes, or perhaps to the loss of a minor polypeptide. Why Sundby et al. [25] found eversion of sonicated vesicles and we did not is unclear to us, but is probably related to differences in sonication conditions.

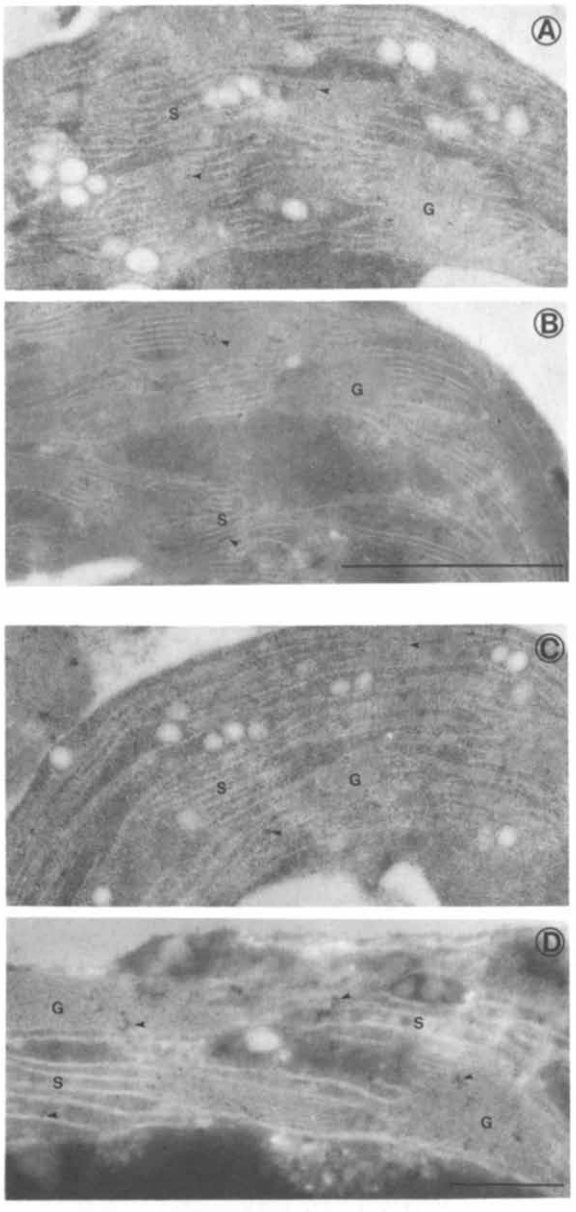

Fig. 6. Spinach chloroplasts immunolabeled with the IgG fraction of either pre-immune or anti-Cyt b_6-f antibodies, by a post-embedment, thin-section labeling technique. Chloroplasts in A–C were fixed in glutaraldehyde prior to embedment, whereas that shown in D was ultrarapidly frozen, freeze-dried and directly embedded without chemical fixation. (A) Pre-immune antibodies; (B) anti-Cyt b_6-f pre-adsorbed against purified Cyt b_6f; (C) and (D) anti-Cyt b_6-f antibodies (dilution approx. 1/100). The rabbit antibodies were localized with ferritin-conjugated goat anti-rabbit IgG antibodies. S = stroma membranes; G = grana stacks; arrowheads indicate ferritin granules. A–C, ×30500; D, ×61000; the bars represent 1 μm (B) or 0.2 μm (D).

Immunolabeling of thylakoids

To confirm the indirect data presented above, we immunolabeled thin-sectioned intact chloroplasts using rabbit anti-Cyt b_6-f antibodies; the results are shown in Fig. 6. Buffer control (not shown) and pre-immune antibody controls (Fig. 6A) showed a distinct paucity of labeling and non-specificity of ferritin localization, whereas the Cyt b_6-f-adsorbed antibody control showed a very significant reduction of labeling density but specific localization (Fig. 6B). Samples which were exposed to non-adsorbed anti-Cyt b_6-f, however, were labeled to much higher densities over both grana stacks and stroma membranes (Fig. 6C and D), even though much lower antibody concentrations were used than for the control experiments. These data indicate very strongly that Cyt b_6-f is present in both stroma- and grana-derived membranes. This result is in complete agreement with our previously published observations of chloro-

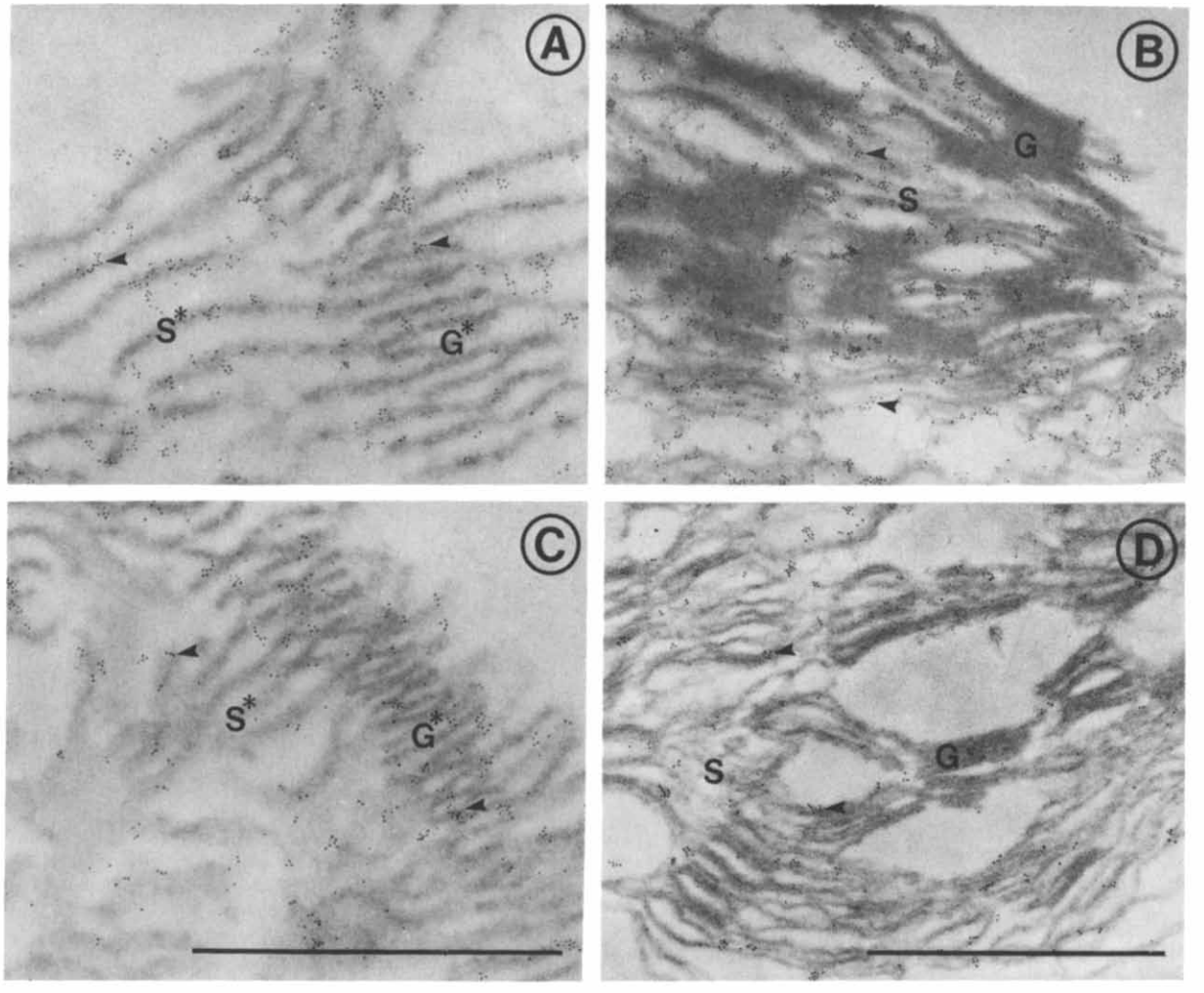

Fig. 7. Pea thylakoids which were unstacked, then re-stacked (or not) prior to fixation and embedment. A and B were exposed to anti-CF_1, C and D to anti-Cyt b_6-f. A and C show unstacked thylakoids, whereas B and D show thylakoids which were first unstacked, then re-stacked in 5 mM $MgCl_2$ for 2 h. The CF_0/CF_1 complex randomizes upon unstacking and re-segregates upon re-stacking, whereas Cyt b_6-f remains randomly distributed throughout the manipulations. Control thylakoids (data not shown) which were not unstacked maintained their normal distributions during the experiment. A and C, ×50000; B and D, ×40000; the bars equal 1 μm. G*, former grana membranes; S*, former stromal membranes; G and S, newly formed grana and stromal membrane regions, respectively. Arrowheads indicate ferritin granules.

plasts fixed exclusively with glutaraldehyde [16], as well as those of Goodchild et al. [27] and Shaw and Henwood [28] regarding the distribution of cytochrome *f*. Significantly, the finding of Cyt b_6-*f* in both membrane regions in unfixed, ultrarapidly frozen and freeze-dried chloroplasts (Fig. 6D) argues strongly that artifactual randomization of the complex did not occur during chemical fixation or dehydration. Thus, we have now provided the necessary temporal resolution to state unambiguously that Cyt b_6-*f* is distributed throughout the thylakoids of intact chloroplasts, without specialized lateral compartmentation. What appears to be clustered antigen (i.e., ferritin clusters) may be a reflection of microheterogeneity in the exposure of antigens which may or may not have a uniform distribution within a membrane area. Controls performed using ferritin in differing states of aggregation, varying reagent dilutions, surface etching of sections and varying incubation times all lead to a similar appearance (data not shown).

Broken chloroplasts which were unstacked under low-salt conditions prior to fixation showed randomization of the CF_0-CF_1 ATP synthase complex when immunolabeled with anti-CF_1 (Fig. 7A). Restacking of the membranes with 5 mM MgCld2 caused the CF_0-CF_1 complex to resegregate into unstacked membrane regions (Fig. 7B). Similar migrations of Cyt b_6-*f* were not observed during these manipulations (Fig. 7C and D). This implies (1) that Cyt b_6-*f* is not tightly associated with any of the other thylakoidal protein complexes which do migrate during unstacking and restacking (see Ref. 7 for review), or (2) that similar quantities of Cyt b_6-*f* are associated with different complexes in granal and stromal membranes and any migrations are therefore not apparent upon randomization.

Discussion

Of the studies which have been published regarding Cyt b_6-*f* localization, several have relied upon kinetic measurements of the oxidation or reduction of various components of the electron-transport pathways. Whereas such an approach yields valuable information regarding the activities of the complexes at the molecular level, it suffers from the drawbacks that the data reflect the reaction of the complex within a particular microenvironment and that information on large-scale distributions can only be obtained by extrapolation. On the other hand, physical fractionation techniques such as those used in this study and others, while giving direct data regarding the composition of separated fractions, suffer from the drawback that all such data are inherently 'averaged'. This caveat led Ghirardi and Melis [10] and Barber [11] to propose that Cyt b_6-*f* may be located in the membrane region interfacing grana and stroma membranes (fret), and would therefore co-fractionate with both grana and stroma membranes during disruption. This hypothesis seemed to provide a rationale for the inconsistency between studies involving Yeda press [8,9], French press [12,15] and detergent-induced [29–32] disruption of thylakoids with regard to the localization of Cyt b_6-*f*.

Our findings, however, do not support this hypothesis nor a localization of Cyt b_6-*f* in any one membrane region. Instead, in agreement with results presented by certain other groups [8,9], our data clearly indicate that Cyt b_6-*f* is laterally distributed throughout all regions of the thylakoid membranes. Our immunochemical detection of transverse asymmetry in the orientation of the polypeptides of the Cyt b_6-*f* complex is in agreement with the proteinase digestion experiments of Mansfield and Bendall [33], as well as the structure proposed for cytochrome *f* by Willey et al. [34]. At a superficial level , our data do not appear to be consistent with the results of Ortiz and Malkin [35], who, using the impermeant probes, trinitrobenzene sulfonate and pronase E, found all polypeptides of the Cyt b_6-*f* complex to be exposed to the stroma. Although the reasons for this apparent discrepancy are unclear, the results of the two studies are obviously not mutually exclusive.

Our confirmation of the 'random' lateral distribution of Cyt b_6-*f* has several ramifications concerning the electron transport functions of this complex. First, Cyt b_6-*f* may be involved as a non-biased electron carrier in both cyclic and non-cyclic electron transport, with the actual function served being determined in large part by the physical location of the complex and, therefore, its accessibility to components of cyclic transport. If the Cyt b_6-*f* populations of both granal and

stromal membrane regions were functional in linear transport, then the implication would be that both plastocyanin and plastoquinone perform some short-range as well as long-range electron transport. This may help to explain the biphasic nature of electron transport from P-680 to P-700$^+$ [36]. Second, should only the stroma membrane Cyt b_6-f population function in linear electron transport, as suggested by Haehnel [14], then the grana pool of Cyt b_6-f would be essentially non-functional in vivo and only plastoquinone would be a long-range carrier. However, this seems unlikely, given the large amount of Cyt b_6-f present in grana membranes relative to the total Cyt b_6/f population (i.e., approximately one-half to two-thirds). Third, it is possible that the function of Cyt b_6-f in cyclic and non-cyclic electron transport is regulated by some factor other than its physical location, such as, for example, the redox poise of plastoquinone/dihydroplastoquinol. Alternatively, Hind et al. [37] have suggested that feedback regulation of ferrodoxin:NADP$^+$ reductase occurs, dependent upon stromal NADPH/NADP$^+$ ratios. This could directly modulate the direction of electron shuttling between linear and cyclic transport pathways without Cyt b_6-f exerting a direct regulatory role.

Interaction between two or more regulatory events with differing kinetics, at separate points in the pathway, could provide a very sensitive balance between cyclic and linear electron transport based upon fluctuating physiologic demands. Regulation of Cyt b_6-f activity by some mechanism, such as those mentioned above, might explain how this pivotal complex with an ubiquitous distribution could behave as though it were distributed asymmetrically.

Acknowledgements

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