Journal of Bioenergetics and Biomembranes, Vol. 18, No. 5, 1986

Implications of Cytochrome b_6/f Location for Thylakoidal Electron Transport

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Received February 10, 1986

Abstract

The cytochrome b_6/f complex of higher plant chloroplasts is uniformly distributed throughout both appressed and nonappressed thylakoids, in contrast to photosystem II and photosystem I, the other major membrane protein complexes involved in electron transport. We discuss how this distribution is likely to affect interactions of the cytochrome b_6/f complex with other electron transport components because of the resulting local stoichiometries, and how these may affect the regulation of electron transport.

Key Words: Cytochrome b_6/f; membrane topography; electron transport; spatial distribution.

Introduction

Photosynthetic membranes (thylakoids) of higher plant chloroplasts are structurally differentiated into two very distinct but continuous domains—appressed (grana) and nonappressed (stroma) membranes. This structural differentiation is matched by a functional differentiation which is reflected in the highly segregated distribution of virtually all thylakoid membrane components except the cytochrome b_6/f complex (cyt b_6/f). Photosystem II (PSII) and associated chlorophyll a/b light-harvesting antenna complexes (LHCII) reside primarily (ca. 80–85 mol %) in the appressed thylakoids, whereas photosystem I (PSI) and associated light-harvesting complexes (LHCI) reside mostly (ca. 90–100 mol %) within nonappressed membrane regions (see reviews by Anderson, 1981; Anderson and Andersson, 1982; Staehelin and Arntzen, 1983; Staehelin and DeWit, 1984; Barber, 1984; Staehelin, 1986). On the other hand, the third major protein complex involved in photosynthetic

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0145-479X/86/1000-0419$05.00/0

electron transport, the cyt b_6/f complex, appears to be distributed throughout all regions of the thylakoids (Akerlund *et al.*, 1976; Cox and Andersson, 1981; Anderson, 1982; Akerlund and Andersson, 1983; Allred and Staehelin, 1985, 1986; Goodchild *et al.*, 1985; Shaw and Henwood, 1985), although this view is not uniformly accepted (Henry and Moller, 1981; Ghirardi and Melis, 1983; Crofts and Wraight, 1983; Barber, 1984; Whitford *et al.*, 1984). However, far from simplifying our understanding of electron transport in thylakoid membranes, the roughly uniform distribution of cyt b_6/f makes it difficult to envision how this complex can fulfill its postulated roles in linear and cyclic electron transfer reactions. The purpose of this minireview, then, is to (a) provide a description of our knowledge to date concerning the makeup of the complex, (b) critique the information concerning its distribution in thylakoidal membranes, (c) discuss its interactions with the other major electron transport complexes as well as with the small diffusible carriers, plastoquinone and plastocyanin, and (d) attempt to provide ideas and perhaps insights into how the distribution of cyt b_6/f and changes in its local stoichiometries with other electron transfer components may play a role in regulating different activites of the electron transport chain.

I. Identity and Organization of the Cytochrome b_6/f Complex

A. Components of cyt b_6/f. The cyt b_6/f complex was first isolated from higher plant thylakoids by Nelson and Neumann (1972) and later by others (Anderson and Boardman, 1973; Ke *et al.*, 1975; Cox, 1979). However, it was not until the work of Hurt and Hauska (1981) that the complex was found to retain demonstrable plastoquinol-plastocyanin oxidoreductase activity, thereby identifying its probable position in the electron transfer pathway.

As isolated by Hurt and Hauska (1982b), the complex contains four major polypeptides which, in different species, fall into size classes of 33–34 kDa (subunits 1a and 1b = cytochrome *f*, which migrates as a doublet and was first thought to be two separate polypeptides), 23–24 kDa (subunit 2 = cytochrome b_6, i.e., cytochrome b_{563}), 20–21 kDa (subunit 3 = the Rieske Fe-S protein), and a 17–18 kDa polypeptide (subunit 4). In addition, one or more small polypeptides in the size range of 5–6 kDa and of unknown function also appear to be constituents. A strongly bound quinone, probably plastoquinone-9 (Lam and Malkin, 1985), may be a further integral part of the complex, serving to enhance both the oxidant-induced reduction of cyt b_6 (a universal activity of all cyt $b \cdot c$ complexes; see Hauska *et al.*, 1983) and plastoquinol-plastocyanin oxidoreductase activities (Hurt and Hauska, 1982a). Similar results have been reported for the involvement of ubiquinone in the mitochondrial cyt bc_1 complex, although there is disagreement whether it is an integral component (von Jagow *et al.*, 1984) or merely feeds electrons

into the complex (Pasquali *et al.*, 1985). Clark *et al.* (1984) have suggested that the 37 kDa enzyme, ferredoxin-$NADP^+$ oxidoreductase (FNR; EC 1.18.1.2), should be considered part of the complex, as it copurifies stoichiometrically with the cyt b_6/f complex when isolated from EDTA-washed thylakoids. FNR appears to bind electrostatically to the complex since washing the isolated complex with 2 M LiBr, or intact thylakoids with 2 M NaBr, quantitatively removes this protein (Clark *et al.*, 1984). Although FNR is not necessary for plastoquinol-plastocyanin oxidoreductase activity, its association with cyt b_6/f may play an important role in the regulation of cyclic electron transport; these reactions will be discussed in later sections.

B. Stoichiometry of Components. Based upon Coomassie blue- and amido black-staining of cyt b_6/f complex polypeptides resolved by sodium dodecylsulfate–polyacrylamide gel electrophoresis, the four major polypeptides (i.e., subunits 1a + b, 2, 3, and 4) were concluded to isolate with a stoichiometry of 1 : 1 : 1 : 1 (Hurt and Hauska, 1982b). At first, this was confusing because two heterogeneous hemes were detected for cyt b_6 by redox titration assays (Hurt and Hauska, 1982b), an enigma that has only recently been explained (Hurt and Hauska, 1983; Clark and Hind, 1983; Saraste, 1984; Widger *et al.*, 1984). The equimolar stoichiometries of the four major subunits of cyt b_6/f is in general agreement with that of the analogous cytochrome bc_1 complex from mitochondria, although in that case the subunit structure is more complicated and some confusion surrounds cyt *b* (see, e.g., Hauska *et al.*, 1983). One assumption made in these stoichiometric determinations is that the complex functions in a monomeric configuration; some recent findings have led to a reevaluation of this assumption (see Section I.D).

C. Topography of Subunits within the Membrane. Numerous studies have attempted to determine the general topography of the cyt b_6/f complex. The approaches taken have included proteolytic digestion (Mansfield and Bendall, 1984; Ortiz and Malkin, 1985; Bricker and Sherman, 1982; Mansfield and Anderson, 1985) or antibody agglutination of thylakoid vesicles of known orientation (Allred and Staehelin, 1986) or of reconstituted cyt b_6/f complexes in liposomes (Morschel and Staehelin, 1983), and labeling of intact stacked thylakoids with impermeant chemical probes (Ortiz and Malkin, 1985). Depending on the method used, these approaches have indicated some degree of exposure of from one to all four major polypeptides to the stroma, whereas all four appear to be exposed to the lumenal compartment. Based upon sequence analysis of the transcripts encoding each of the polypeptides (Heinemeyer *et al.*, 1984; Willey *et al.*, 1984; Alt and Herrmann, 1984; Phillips and Gray, 1984), topographical models have been proposed in which all four polypeptides span the bilayer of the thylakoid membrane (Willey *et al.*, 1984; Hauska, 1986), with the great bulk of the cytochrome *f*

polypeptide exposed to the lumen (Willey *et al.*, 1984; Alt and Herrmann, 1984). It was further suggested that three of the polypeptides have their N-terminal ends exposed to the lumen, but that the Rieske Fe-S protein resides in the opposite orientation (Hauska, 1986). However, the results of carboxypeptidase treatment of right-side-out vesicles suggest that all four proteins expose their C-terminal ends to the stroma (Mansfield and Anderson, 1985) (Fig. 1).

At least two of the polypeptides, cyt *f* and the Rieske Fe-S protein, are processed from larger precursors prior to or during insertion into the membrane and association with the complex (Alt *et al.*, 1983); the same appears to be the case for the mitochondrial equivalents (Kolarov and Nelson, 1984). Cytochromes *f* and b_6 and subunit 4 are all plastid-encoded proteins, whereas the Rieske Fe-S protein is nuclear-encoded (Alt and Herrmann, 1984; Alt *et al.*, 1983; Phillips and Gray, 1984; Willey *et al.*, 1983). The assembly mechanism of cyt b_6/f has not yet been elucidated, but in the mitochondrion cyt *b* may be necessary as a nucleus for organizing the other proteins into the complex (Sen and Beattie, 1985). In the mitochondrial inner membrane the Rieske Fe-S protein appears to be present in excess of that needed for the cyt bc_1 complex, although the excess protein may lack the Fe_2S_2 cluster (Nishikimi *et al.*, 1985).

In the context of membrane function, the location of the prosthetic groups of cyt *f* and b_6 and the Rieske Fe-S protein is of major importance. Cyt *f* contains only one heme (Hurt and Hauska, 1982b), which is accessible to exogenous reagents only in inside-out grana thylakoid vesicles (Cox and Andersson, 1981; Atta-Asafo-Adjei and Dilley, 1985). On the other hand, cyt b_6 carries two hemes (Hauska *et al.*, 1983) which are distinguishable spectrally (Hurt and Hauska, 1983; Clark and Hind, 1983) and by their midpoint redox potentials (Hurt and Hauska, 1982b, 1983). Evidence from EPR studies also suggests that the two hemes reside in different microenvironments (Case and Leigh, 1976; Bergstrom *et al.*, 1983), perhaps near the opposite headgroup regions of the thylakoid bilayer. Both hemes of cyt b_6 are sensitive to shifts in pH, in contrast to cyt *f* (Hurt and Hauska, 1982b, 1983), consistent with the involvement of cyt b_6 in the electrogenic activity of the cyt b_6/f complex (Hurt *et al.*, 1982, 1983; Selak and Whitmarsh, 1982). The Fe_2S_2 cluster of the Rieske Fe-S protein appears to be near the lumenal side of the thylakoid membrane (Prince, 1983), in keeping with its proposed vectorial role in cyt b_6/f electrogenic activity (Fig. 1). A more complete discussion of the topography of cyt b_6/f polypeptides can be found in the recent reviews of Hauska (1986) and Cramer *et al.* (1985).

D. Stoichiometry of the Cyt b_6/f Complex. A subject of importance but considerable uncertainty is the stoichiometric configuration of the cyt b_6/f complex relative to itself. Several lines of evidence tend to suggest a dimeric

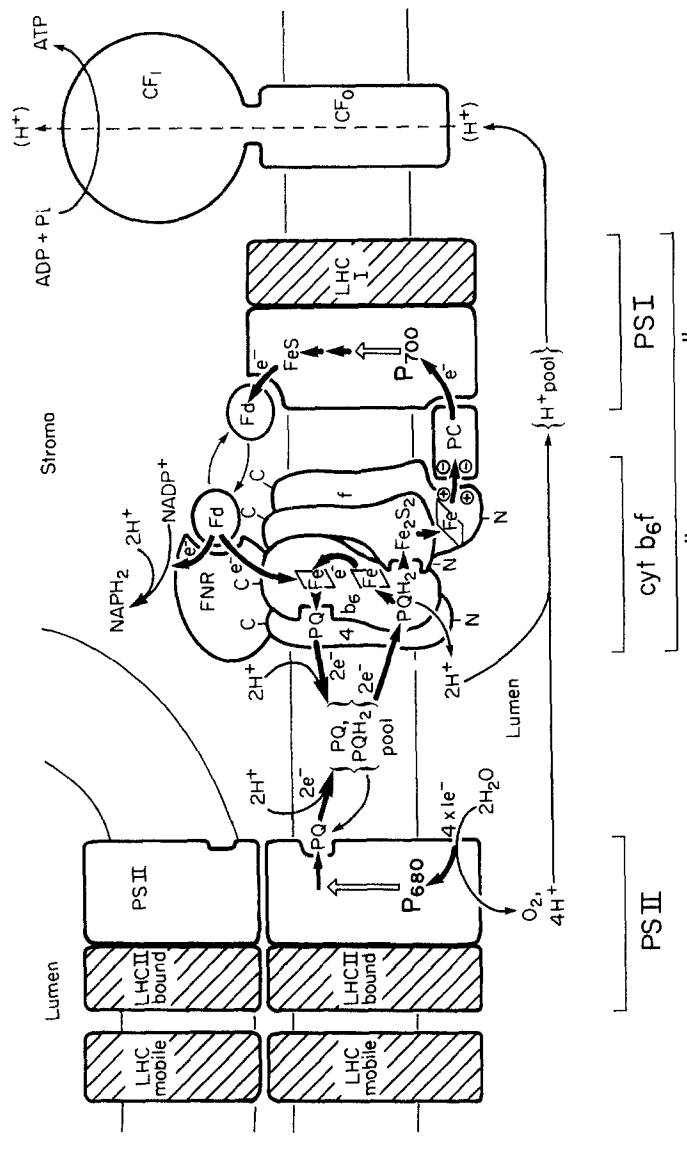

Fig. 1. Schematic diagram showing the probable flow of electrons from PSII through PQH_2 to the cyt b_6/f complex, and hence to PSI via PC. We have shown the general topography of cyt b_6/f subunits, their heme orientations, association with FNR, and the formation of a "supercomplex" between cyt b_6/f, PC, and PSI. For further details see the text. Fd, ferredoxin; Fe_2S_2, Rieske Fe-S protein; b_6, cyt b_6; *f*, cyt *f*; 4, subunit 4; P_{680}, PSII reaction center chlorophyll; P_{700}, PSI reaction center chlorophyll; -C and -N, carboxy- and amino-terminal ends of proteins; heavy arrows, electron flow; light arrows, proton flow or reaction direction; CF_0/CF_1, coupling factor ATP synthase; all other abbreviations are as used in the text.

configuration, including calculations of complex mass based on freeze-fracture particle sizes (Morschel and Staehelin, 1983), and stoichiometric inhibition of two cyt b_6/f complexes by one molecule of 2,5-dibromo-3-methyl-5-isopropylbenzoquinone (DBMIB) (Hurt and Hauska, 1981; Graan and Ort, 1985), a plastoquinone analogue which interacts with the Rieske Fe-S protein (Malkin, 1982). However, using detergent column chromatography techniques, Hurt and Hauska (1981) also found that complex/detergent mixed micelles migrated with an apparent mass of ca. 185 kDa, consistent with a monomeric form of the complex. Unfortunately, the validity of all these findings can be questioned. Indeed, technical artifacts affecting the apparent stoichiometry of the complex cannot be excluded, and the use of inhibitors and kinetic analyses on intact thylakoids must assume equal accessibility of the complexes to all reagents, a condition which may not hold. On the other hand, there is evidence that the analogous mitochondrial cytochrome $b \cdot c_1$ complex exists *in vivo* as a dimer (Leonard *et al.*, 1981), which is functional (Capaldi, 1982). A further possibility is that the thylakoid cyt b_6/f complex may exist in an equilibrium between monomeric and oligomeric forms, which may be related to the function of the complex within the different membrane regions. Suggestive of this is the variable $H^+/2e^-$ stoichiometries observed in the electrogenic operation of cyt b_6/f (Hurt *et al.*, 1982; Rich, 1984; Rich and Heathcote, 1984). Obviously, further study is needed to clarify the situation and explain its biological significance.

II. Reactions of the cyt b_6/f Complex in Electron Transfer

A. Position of cyt b_6/f in the Redox Hierarchy. The cyt b_6/f complex occupies a redox niche roughly midway between reduced pheophytin of the PSII reaction center and P_{700+} of the PSI reaction center. In this position, cyt b_6/f is an integral partner in essentially all forms of electron transport activity between PSII and PSI (Haehnel, 1984a; Rich, 1984; Ort, 1986; Whitmarsh, 1986; Anderson, 1986; Joliot and Joliot, 1986).

The electron hole created in P_{700+} by absorption of a photon is reduced by acceptance of an electron from the $1e^-$ carrier, plastocyanin (PC) (Bottin and Mathis, 1984; Haehnel *et al.*, 1980, 1982, 1984a, b; Olsen, 1982). PC, a small (ca. 10.5 kDa) copper protein, resides in the thylakoidal lumen (Haehnel *et al.*, 1981) and has a midpoint redox potential of ca. +370 mV (Katoh, 1960; Katoh *et al.*, 1962). This enables it to receive an electron from cyt *f*, of midpoint redox potential +340 mV (Hurt and Hauska, 1982b), whose sole heme is also in the lumen where it is readily accessible to oxidized PC (see Section I.C). Oxidized cyt *f* is, in turn, reduced by an electron from the Rieske Fe-S protein (Whitmarsh *et al.*, 1982), the Fe_2S_2 cluster of which has a midpoint redox potential of ca. +290 mV (Malkin and Aparicio, 1975).

Reduction of oxidized Rieske Fe-S protein is accomplished by plastoquinol (PQH_2), a $2e^-$ carrier with a midpoint redox potential of ca. +117 mV (reviewed by Rich, 1984, 1985). Finally, plastoquinone (PQ) which was oxidized by the Rieske Fe-S protein is reduced by electrons generated by P_{680} during light excitation of PSII reaction centers. A more comprehensive discussion of the whole electron chain can be found in the review by Ort (1986).

B. Pathway of Electrons through the cyt b_6/f Complex. The $2e^-$ transfer by PQH_2 poses logistical problems for the cyt b_6/f complex because the carriers in this complex involved in linear transport, i.e., the Rieske Fe-S protein and cyt *f*, are of the $1e^-$ type. However, in addition to these $1e^-$ carriers, cyt b_6 (with two heme groups) is also an integral and functional part of the complex, and provides a potential storage buffer for the "extra" electron. The hemes on cyt b_6 each have distinct midpoint redox potentials of −50 and −170 mV (Hurt and Hauska, 1982b, 1983) and, as previously mentioned, appear to reside in different microenvironments within the membranes. In this context, the observation that a PQ analogoue binds to both the Rieske Fe-S protein and cyt b_6 (Oettmeier *et al.*, 1982) is of particular importance for understanding the movement of electrons through the complex. Support for involvement of cyt b_6 has come in part from the following observations: (1) DBMIB decreases the labeling of both proteins by an azide derivative of PQ, but affects cyt b_6 to a greater extent; and (2) DBMIB alters the EPR signal of the Rieske protein Fe_2S_2 cluster in the isolated cyt b_6/f complex but not in isolated Rieske Fe-S protein (Hurt *et al.*, 1981). These results have been interpreted to imply that two or more subunits—presumably the Rieske Fe-S protein and cyt b_6—together are necessary for binding of PQH_2 to the complex.

These results are consistent with a concerted two-step oxidation of PQH_2 by two $1e^-$ carriers in the cyt b_6/f complex, cyt b_6 and the Rieske Fe-S protein, and are consistent with the general scheme of the Q cycle proposed by Mitchell (1975, 1976). In the Q cycle scheme, the two-step oxidation of PQH_2 by cyt b_6/f provides the mechanism for the electrogenic activity of this complex. For discussions of this topic and the fine points relating to the variations which have been proposed, the reader is refered to the following recent reviews (Rich, 1985; Joliot and Joliot, 1986; Anderson, 1986; Whitmarsh, 1986; Ort, 1986; Rich, 1984; Haehnel, 1984a; Hauska, 1986; Hendler *et al.*, 1985).

C. Binding of Plastocyanin to cyt b_6/f. PC must be able to bind to both cyt *f* and P_{700+} for electron transfer. However, it is unclear whether PC is a freely diffusible carrier, as has been proposed (Olsen, 1982; Selak and Whitmarsh, 1984; Wood, 1974). Three lines of evidence suggest that it may not be: (1) the thylakoid lumenal width during illumination is only about 4 nm

(Murakami and Packer, 1970)—about the same as the longest dimension of PC (Coleman *et al.*, 1978)—and may therefore physically restrict its free diffusion (Haehnel, 1984b); (2) the viscosity of the lumenal aqueous phase is quite high (Berg *et al.*, 1979); and (3) from kinetic studies it has been suggested that PC may, in fact, be a part of a "supercomplex" which also includes cyt b_6/f and PSI (Haehnel *et al.*, 1980; Haehnel, 1982, 1984b; Bottin and Mathis, 1984). In addition, Nanba and Katoh (1985) have shown that although cyt c_{553}, the analogue of PC in the cyanobacterium *Synechococcus* (Wood, 1977), serves as a diffusible carrier to connect one cyt *f* with several PSI reaction centers, diffusion is not rapid enough to transfer electrons over large distances. Fast kinetic studies have indicated that PC may, in fact, be present as two pools, one bound and one freely diffusible (Haehnel *et al.*, 1980; Bottin and Mathis, 1984). This result is not surprising, given the probable overall stoichiometry of 1:2:1 between cyt b_6/f:PC:PSI in thylakoids (Andersson and Haehnel, 1982; Whitmarsh and Ort, 1984; Graan and Ort, 1984a, b; Whitmarsh, 1986; but see also Melis and Brown, 1980; Melis and Anderson, 1983; Anderson and Melis, 1983).

One should not think of such a "supercomplex," if it indeed exists, as being a static one; the opposite is probably true. Instead, it merely requires that the lifetime of association of the components be long relative to the half-time for transfer of electrons between components for it to behave as a "solid-state" system (see Rich, 1984 for a more complete discussion). One could envision that the lifetime of association might depend on such factors as the electrostatic charge of PC near its negative patch as well as conformational changes brought about by changes of oxidation state (Gross *et al.*, 1984; Anderson *et al.*, 1985). PC binds to the heme edge of horse cyt *c* by its negative patch and, in doing so, affects the electronic structure of the heme (King *et al.*, 1985). Chemical modification of carboxyl groups on PC (Takabe *et al.*, 1984), or amino groups on cyt *f* (Takenaka and Takabe, 1984), both greatly reduce the rate of electron transfer from cyt *f* to PC. On the other hand, transfer from PC to P_{700+} was far less dependent upon electrostatic interactions (Takabe *et al.*, 1984). Alternatively, Joliot and Joliot (1984b) have suggested that cyt b_6 and cyt *f* each affect the redox properties of the other upon reduction, thereby altering the equilibrium constants between cyt *f*, PC, and P_{700+}. In addition, it has been reported that cyt *f* becomes "disconnected" from PC for from 1 to 100 msec after a flash (Bouges-Bouquet, 1977). Thus, it may be that electrostatic interactions within the cyt b_6/f complex directly affect the lifetime of any potential "supercomplex."

III. Distribution of cyt b_6/f within Thylakoids

A. Localization of cyt b_6/f. It is now well established that both PSII and PSI are nonrandomly distributed between appressed and nonappressed

thylakoid membranes (see Introduction, but see also Atta-Asafo-Adjei and Dilley, 1985). The distribution of cyt b_6/f, however, has been more difficult to determine. Early information concerning localization of cyt b_6/f relied upon its co-isolation with either PSII or PSI during detergent-induced fragmentation of thylakoid membranes. Thus, digitonin extraction of thylakoids for PSI isolation resulted in co-isolation of cyt f and b_6 with PSI (Boardman and Anderson, 1967; Anderson and Boardman, 1966; Cox, 1979); indeed, the tehniques for isolation of cyt b_6/f are derived from PSI isolation schemes (Nelson and Neuman, 1972; Hurt and Hauska, 1981; Anderson and Boardman, 1973; Ke *et al.*, 1975; Hurt and Hauska, 1982b; Clark *et al.*, 1984). On the other hand, PSII preparations made by Triton X-100 extraction (Whitford *et al.*, 1984; Vernon *et al.*, 1971) or by French press treatment (Sane *et al.*, 1970; Henry and Moller, 1981) of thylakoids failed to provide evidence for a significant association of cyt b_6/f with PSII. Because of these results, and the apparently equal stoichiometry of cyt b_6/f and PSI in intact thylakoids, it was proposed that cyt b_6/f is bound only in nonappressed membranes (Henry and Moller, 1981; Whitford *et al.*, 1984), together with most PSI.

Other workers, utilizing physical disruption of thylakoids by Yeda press or French press treatment followed by aqueous two-phase polymer partitioning techniques, found cyt b_6/f associated with both right-side-out vesicles enriched in PSI (derived from nonappressed membranes) and with inside-out membrane fragments derived from appressed membranes (Cox and Andersson, 1981; Anderson, 1982; Akerlund *et al.*, 1976; Akerlund and Andersson, 1983; Allred and Staehelin, 1986; Albertsson, 1985). These results suggested a random distribution of cyt b_6/f throughout both appressed and nonappressed membranes. Haehnel (1984b) proposed a functional differentiation of cyt b_6/f by suggesting that only those complexes present in nonappressed membranes function in linear electron transport. To our knowledge, no direct evidence to support or refute this contention has yet been presented.

Ghirardi and Melis (1983) reported that a stoichiometric parity exists between cyt f and P_{700} in both mesophyll and agranal bundle sheath cell chloroplasts of *Zea mays*. To reconcile this finding with studies suggesting the presence of cyt b_6/f in both appressed and nonappressed regions, they proposed that cyt b_6/f is present only in intergranal membranes, and more specifically in the region interfacing appressed and nonappressed membranes (i.e., the "fret" region). Since Yeda and French press treatments are thought to break thylakoids in this region (Barber, 1984) this would have neatly resolved the conflict between the two models. This hypothesis was supported by Barber (1984), based on considerations of thylakoid membrane surface properties in the two regions. However, a different conclusion was reached by Crofts and Wraight (1983) who, after consideration of PQ diffusion times

and electron transfer rates, suggested that cyt b_6/f should be enriched in appressed membrane areas.

As an attempt to resolve this problem, the authors (Allred and Staehelin, 1985, 1986) and several others (Goodchild *et al.*, 1985; Shaw and Henwood, 1985) have applied the technique of post-embedment immunolabeling of thin-sectioned chloroplasts to directly localize the cyt b_6/f complex (Allred and Staehelin, 1985, 1986; see Fig. 2), cyt f (Goodchild *et al.*, 1985; Shaw and Henwood, 1985), or the Rieske Fe-S protein (F. -A. Wollman, personal

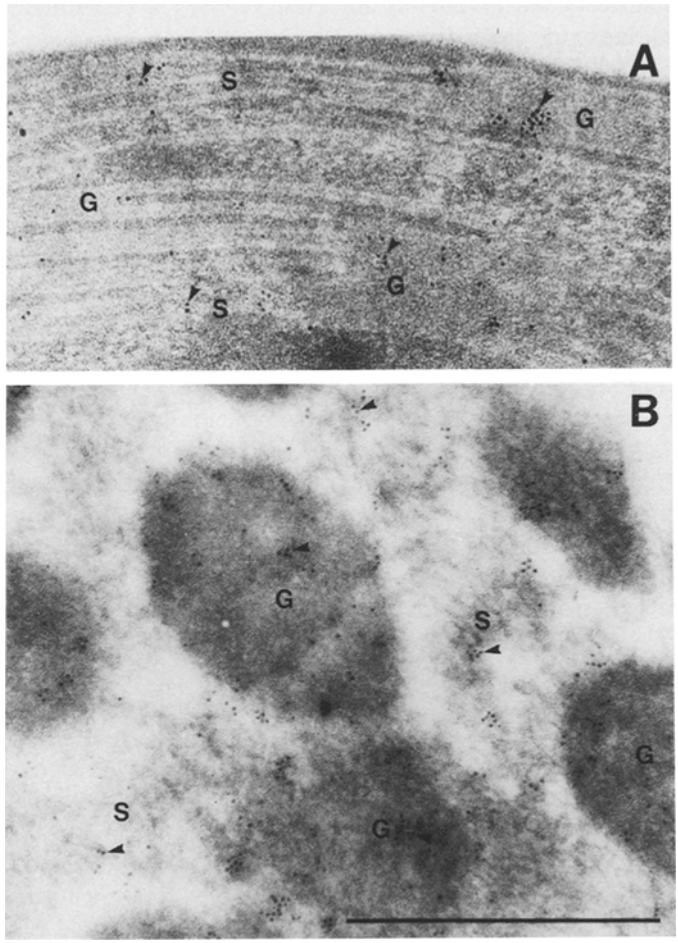

Fig. 2. Electron micrographs of both an intact chloroplast (A) and thylakoids (B; shown *en face*) in which the cyt b_6/f complex was localized by the thin-section immunolabeling technique. Sections were treated first with anti-cyt b_6/f antibodies, then with ferritin-conjugated goat anti-rabbit IgG antibodies. G, appressed grana membranes; S, nonappressed stroma membranes; arrowheads indicate ferritins in either region. Bar equals 0.5 μm; both $\times$ 110,000.

communication). In each of the studies, when using chemically fixed chloroplasts, cyt b_6/f was localized throughout both appressed and nonappressed membranes at similar densities (Fig. 2). However, the caveat to this technique is that, during the time necessary for chemical fixation (very slow compared to the probable diffusion rate of the complex), artifactual diffusion of cyt b_6/f from its *in vivo* location might occur. As a test of this possibility, the authors (Allred and Staehelin, 1986) prepared unfixed spinach chloroplasts in a buffered osmoticum by ultrarapid freezing followed by freeze-drying and direct infiltration and embedment into plastic resin (all under vacuum). In this case, too, cyt b_6/f distribution matched that of the chemically fixed samples. Using the complementary technique of freeze-fracture electron microscopy, evidence has also been found for the presence of cyt b_6/f in all regions of the thylakoid membrane (F. -A. Wollman, personal communication).

Because of these results and the preponderance of complementary biochemical data, we will assume for all subsequent discussions that cyt b_6/f is distributed throughout the thylakoidal membranes at approximately equal densities. Whether this distribution is truly random, or not, remains to be seen, but a specific localization in, for example, the interface between appressed and nonappressed membranes is not supported. However, nonrandom distributions at the molecular level are not unlikely (see, e.g., Albertsson, 1985). In retrospect, it appears that the digitonin- or Triton X-100-extracted thylakoids gave the results they did because membrane protein complexes are differentially soluble in detergents. For example, we have found that, using a mixture of octyl-β-*D*-glucopyranoside and deoxycholate, as little as 8–10% of the total chlorophyll may be solubilized from thylakoids and yet most (ca. 60–80%) of the cyt b_6/f is removed from the remaining membrane material (Allred and Staehelin, unpublished data). Also, in a comparison of PSII preparations, Dunahay *et al.* (1984) found quite variable associations of cyt *f* with samples derived by different detergent treatments.

B. Population Heterogeneity of cyt b_6/f. Little is currently known regarding similarities or differences of cyt b_6/f complexes in appressed and nonappressed membranes. Thylakoid membrane lipids exhibit a very high degree of "fluidity" (discussed by Haehnel, 1984a), and yet thylakoids are able to maintain domains with very different component distributions by means of membrane appression. Nevertheless, even large protein complexes such as mobile LHCII can move reversibly between stacked and unstacked membrane regions under certain conditions, as evidenced by the structural membrane changes associated with state I/state II transitions (Staehelin and DeWit, 1984; Barber, 1983; Kyle *et al.* 1983; Miller and Lyon, 1985). Thus, it is not unreasonable to postulate that a free exchange of cyt b_6/f between nonappressed and appressed membranes may occur, although on a time scale too slow to provide for electron transport (Whitmarsh, 1986).

If the "supercomplex" of cyt b_6/f, PC, and PSI suggested by the results of Haehnel and colleagues (Haehnel *et al.*, 1980; Haehnel, 1982, 1984b) and Bottin and Mathis (1984) is correct, then cyt b_6/f complexes involved in such a higher-order structure would presumably be unable to freely exchange with cyt b_6/f in appressed membranes, or would do so only very slowly. This association would be expected to shift the equilibrium distribution of cyt b_6/f between appressed and nonappressed regions, depending upon a number of physiologic factors (see Sections IV.B and V). It may be that there are subtle differences in the organization of cyt b_6/f complexes within the various regions which might affect their association with PC (Allred and Staehelin, unpublished data). Should the postulated heterogeneity of cyt b_6/f exist *in vivo*, then one must question the function of cyt b_6/f in the appressed regions where it lacks free access to FNR (Jennings *et al.*, 1979; Berzborn, 1969) or stoichiometric amounts of PSI (the probable stoichiometries are discussed in Section IV.B). It is unknown at present whether plastocyanin is found throughout the thylakoidal lumen.

IV. Communication of cyt b_6/f with other complexes

A. Receipt of Electrons from PSII. We know of no data indicating formation of "supercomplexes" between cyt b_6/f and PSII, although the suggestion has been made that direct electron transfer from PSII to cyt b_6/f can occur (Joliot and Joliot, 1981). On the other hand, the consensus seems to be that PQH_2 shuttles electrons from PSII to cyt b_6/f. Thus, PQ has been shown to functionally link PSII and cyt b_6/f in a reconstituted system (Lam and Malkin, 1982), probably by diffusion (reviewed by Rich, 1984; Ort, 1986; Whitmarsh, 1986; Joliot and Joliot, 1986; Rich, 1985; Ragan and Cottingham, 1985). The problem which remains, however, is a kinetic one: can PQ diffuse rapidly enough to account for electron transfer rates, when PSII and PSI are physically segregated over long distances? The answer seems to be "yes," since, as pointed out by Whitmarsh (1986), electron transport has yet to be shown to be diffusion-limited. Although questions of how PQH_2 and PQ can diffuse over long distances within the requisite time period to prevent electron transport from becoming diffusion-limited are crucial to an overall understanding of electron transport in thylakoids, this topic is beyond the scope of this review. However, if diffusion of PQ/PQH_2 is sufficiently rapid to maintain free communication between PSII in the appressed regions and cyt b_6/f in nonappressed regions, it may be largely irrelevant to linear electron transport that the activity of cyt b_6/f in appressed regions is in question (see Section III.B).

B. Local Stoichiometries of cyt b_6/f and Other Participants Involved in Electron Transport. If, for the sake of argument, we assume that the ratios of electron transport participants reported by several laboratories (Andersson

and Haehnel, 1982; Whitmarsh and Ort, 1984; Graan and Ort, 1984a, b) are correct, then the *overall* stoichiometries of PSII : PQ/PQH_2: cyt b_6/f: PC : PSI in total thylakoids are very close to 1 : 6 : 1 : 2 : 1. However, if one takes into account the relative surface areas accounted for by appressed and nonappressed membranes in normally stacked thylakoids (ca. 60%/40%, respectively), the nonrandom distributions of PSII and PSI in each, and the areas occupied by complexes in each area, these ratios change significantly. Thus, if PQ/PQH_2 and PC were to diffuse freely within the bilayer and the lumen, respectively, then the stoichiometries (with cyt b_6/f taken to be unity) would be: (1) in appressed membranes, 1.3 : 4.6 : 1.0 : 2.0 : 0.3; and (2) in nonappressed membranes, 0.5 : 8.1 : 1.0 : 2.0 : 2.5 (see Fig. 3). However, this fails to consider information suggesting that cyt b_6/f, PC, and PSI may participate in a transient "supercomplex" (see Section II.C). If one assumes a 1 : 1 : 1 stoichiometry of cyt b_6/f: PC : PSI in "supercomplexes," a sufficiently avid association between them to cause a "trapping" of PC and cyt b_6/f in nonappressed membranes and free diffusion of the remaining PC and cyt b_6/f, then the local stoichiometries become significantly altered. Thus, if one were to assume (for purpose of example) that ca. 40% of the cyt b_6/f were to be trapped in nonappressed membranes in this way, then local stoichiometries (again relative to cyt b_6/f) would become: (1) in appressed regions, 2.2 : 8.8 : 1.0 : 2.7 : 0.5; and (2) in nonappressed regions, 0.3 : 4.4 : 1.0 : 1.6 : 1.6 (Fig 3). Although a trapping effect has not been observed during immunolocalization (see Section III.B), the failure to do so may relate more to the minimal photosynthetic activity of isolated chloroplasts during the time of fixation. Obviously, the actual stoichiometries would be dependent upon the binding constants of cyt b_6/f, PC, and PSI for one another, the rate of PSI turnover, restrictions to diffusion of cyt b_6/f and PC, and other such variables.

V. Implications of cyt b_6/f Location

Obviously, many of the possibilities discussed above are largely hypothetical; however, they do highlight points that need to be considered in the context of the functional regulation of cyt b_6/f activities. These are: (1) cyt b_6/f appears to be distributed throughout appressed and nonappressed thylakoid membranes at an approximately uniform density; (2) since PSI is largely excluded from appressed membrane regions, then cyt b_6/f, PC, and PSI cannot form "supercomplexes" in this region; (3) the exclusion of FNR from appressed membrane regions would imply that ferredoxin-mediated cyclic electron transport should also be minimal in this region; and (4) the local stoichiometries of cyt b_6/f with other reactants is widely different in appressed and nonappressed regions, and probably varies with changes in physiological conditions.

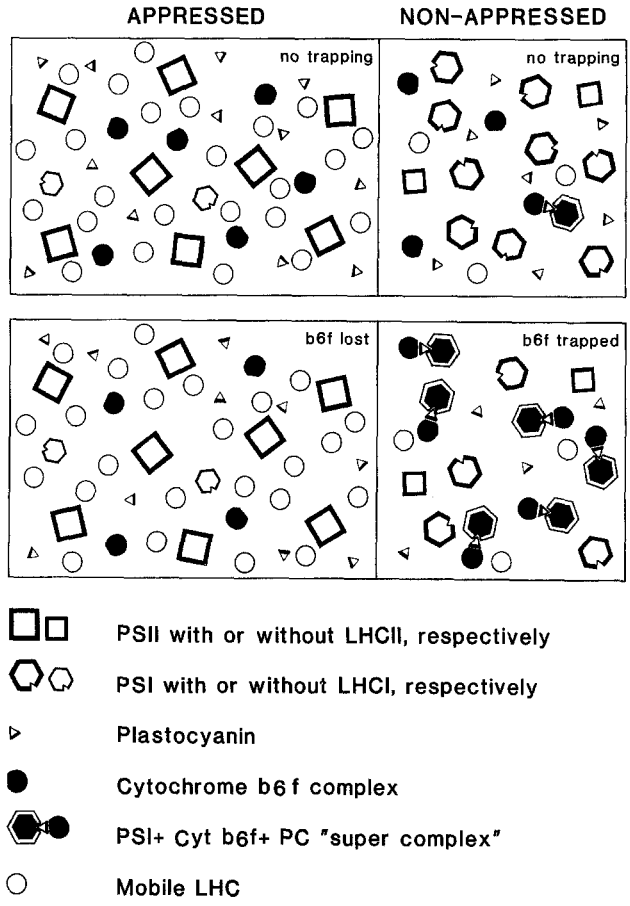

Fig. 3. Schematic diagram graphically illustrating the local stoichiometries of cyt b_6/f relative to the other participating components of electron transport, either without any trapping of PC or cyt b_6/f, or with ca. 40% of the cyt b_6/f trapped in nonappressed membranes by "supercomplex" formation. PQ has been ommitted to avoid confusion. The small proportion of "supercomplexes" shown in the top panels is to imply that the lifetime of such higher-order structures is too low under these conditions to trap PC and cyt b_6/f in nonappressed regions.

Taken as a whole, the impression one gets is that cyt b_6/f complexes in appressed membranes may be largely inactive, perhaps serving as a pool to help thylakoids maintain optimal function. For example, a transition from state I to state II, with decreased appressed membrane area due to migration of some LHCII into nonappressed membranes (see Staehelin and DeWit, 1984; Kyle *et al.*, 1983) might also expose more of the "stored" cyt b_6/f to nonappressed regions. This would allow them to associate with PC and PSI, and perhaps to bind FNR to become competent in ferredoxin-mediated

cyclic electron transfer. This could be of significant regulatory value since PSI is thought to turn over more rapidly under these conditions, and the activity of FNR for $NADP^+$ reduction is greatly enhanced by membrane binding (Forti and Bracale, 1984). Thus, migration and trapping of cyt b_6/f complexes, with the resulting changes in local stoichiometries—analogous to LCHII in state I/state II transitions—may represent yet another subtle form of electron flow regulation. In this way, chloroplasts might be able to respond rapidly to environmental fluctuations with increased competence for both linear and cyclic electron flow.

The preceding discussions have outlined some of the considerations about the effects of cyt b_6/f location on its interactions with other electron transfer complexes and carriers. We have tried to point out alternative ways of viewing long-standing questions so that new, testable avenues of research might be developed. If we have succeeded in providing the reader with new insights or ideas, then we will consider our goal to have been achieved.

Acknowledgements

This work was supported by NIH grant No. GM22912. The authors thank Dr. T. G. Dunahay for helpful comments on the manuscript.

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