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Cytochrome b_6-f complex : The carburettor of exciton distribution in oxygenic photosynthesis

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Abstract. Efficient oxygenic photosynthesis not only requires synchronous turover and operation of photosystem I (PS I) and photosystem II (PS II) but also the preferential turnover of PS I for cyclic photophosphorylation to maintain required ATP and NADPH ratio during carbon dioxide reduction. Ohe initial higher rate of turnover of PS II *in viva* is accounted by the fact that (i) PS I contains only about one-third of total chlorophylls, (ii) about 90% of light harvesting a/b protein (LAC) which accounts for about 50% of the total chlorophylls, remains associated with PS II as PS II-LHC II complexes (PS II_α) and (iii) the ratio of PS II/PS I is always greater than unity, in the range of 1-2 : 1 under different environmental regimes. Ohe initial preferential feeding of PS II, due to its larger antenna, is bound to result in faster rate of turn over of PS II than PS I, leading to higher rate of reduction of an intersystem carrier than the rate of its oxidation by PS I. Ohe light dependent phosphorylation of a 'mobile' and small pool (– 20%) of LHC II of PS II_α (possibly located at the edge of appressed regions of the membranes) increases the repulsive forces of LHC II resulting in its migration to non-appressed region associating itself with PS 1. Ohe phosphorylation itself is controlled by the redox state of an intermediate of electron transport.

Several experimental approaches have provided evidence which suggest that (i) phosphorylation of LAC II involves interaction of cyt b_5-f complex with LAC II kinase and the interaction of QA with cyt b_5-f complex and (ii) different kinases may be involved in phosphorylation of LHC II *versus* PS II polypeptides. Ohe major purpose of light dependent LAC II phosphorylation and its consequent migration close to PS I appears to balance the rate of cyclic *versus* non-cyclic photophosphorylation. Ohe mechanism by which cyt b_5-f complex controls the activation of LAC II is not known. Ohe role of membrane bound ealmodulin, electron transfer through cyt b_6-f complex in activation of LAC II kinase should be explored.

Keywords. Lateral asymmetry; exciton distribution; LHC phosphorylation; cytochrome b_6-f complex.

1. Introduction

The asymmetric distribution of PS ll, LHC II and PS I in the appressed *versus* non-appressed regions of the thylakoids (Andersson and Akerlund 1978; Andersson *et al* 1980; Andersson and Anderson 1980; Anderson and Melis 1983; Akerlund and Andersson 1983) results in functional imbalance in the distribution of exciton between the two photosystems, requiring quick action to avoid over-reduction of PS ll components (and consequent photoinhibition). This review though not exhaustive, attempts to correlate the changes in the dynamics of exciton distribution between the two photosystems involving light mediated interaction of Q_z, site of

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PQ with cyt b_6f complex in activation of thylakoid membrane bound LHC Il kinase with lateral asymmetric distribution of pigment-protein complexes. An attempt has been made here to point out the controlling element(s), the differences in phosphorylation of polypeptides by possibly different kinases and finally the areas of gaps which need to be explored.

2. Molecular asymmetry of chioroplast membranes

lt is now well established that the components of electron transport are asymmetrically distributed between appressed and non-appressed regions of thylakoids (see Murphy 1986).

On chlorophyll (Chl) basis, about 85% of PS II and LHC II are present in the appressed regions of thylakoids, while about 80-90% of the PS I is present in the non-appressed regions. The PS II–LHC II complexes localized in the appressed region together form PS II_α centres (Melis and Homann 1975). Thus, PS II_α centres have a larger antenna and show a fast non-exponential fluorescence induction kinetics (Melis 1985). About 15% of PS II in association with LHC II is known to be present in the non-appressed regions of the thylakoid forming PS II_β centres. PS II has a small antenna and shows a slow exponential fluorescence induction (Melis and Hornann 1975).

Besides lateral asymmetric distribution of PS II, LHC II and PS I a high degree of heterogeneity in the lateral distribution of acyl lipids has been observed. The appressed regions are rich in proteins while poor in acyl lipids and contain more anionic lipid species relative to non-appressed regions (Murphy 1986). The lipids have a high degree of restriction of motion in appressed regions as determined by spin labelling.

About 90% of LHC II, which accounts for 50% of total Chl, is present in the appressed regions. The apoprotein of LHC II has been shown to form three transmembrane helices in *Leinna* (Karlin–Neumann *et ai* 1985). The N-terminal end of apoprotein, having four positive charges, protrudes towards stroma. The amino acid sequence of the exposed N-terminal portion of pea LHC II cleaved by trypsin has been identified as Lys-Arg-Ser-Thr-Thr-Lys-Lys (Mullet 1983). The removal of surface exposed portion of LHC II has been shown to disrupt the stacking (Bennet 1983). The surface exposed hexapeptide with four positive charges is thus responsible for screening of negative charges and for bringing about appression of the membranes.

3. Light dependent phosphorylation of membrane proteins

The light dependent phosphorylation of membrane polypeptides of chioroplast was first demonstrated by Bennet (1977, 1979). The principle polypeptides which were radiolabelled are components of LHC II and they included a doublet with 26 kDa. The protein phosphorylation was shown to be dependent on light (electron transport) and photophosphorylation (Bennet 1977). The fact that DCMU inhibited LHC II phosphorylation, in the presence of exogeneously added [γ-^{32}P] ATP and an electron donor of PQ to the isolated thylakoid preparations, demonstrated that photosynthetic electron transport beyond Q is essential for protein phosphorylation (Bennet 1979).

A membrane bound protein kinase phosphorylates LHC II (Bennet 1977, 1979). The threonine residue of N-terminal exposed portion in the hexapeptide region of LHC II was shown to be phosphorylated (Mullet 1983). The increase in the negative charges on LHC II due to its phosphorylation is responsible for its migration from appressed regions to non-appressed regions and the phosphorylated LHC II gets functionally attached with PS I (Kyle *et ai* 1984).

A decrease in the yield of chlorophyll fluorescence of PS II was caused by the addition of ATP to the isolated thylakoids under conditions which permitted protein phosphorylation (Bennet 1979). The decrease in PS II fluorescence at room temperature was directly correlated with phosphorylation of LHC II (Allen and Horton 1981). Under the same conditions, there is an increase in relative fluorescence emission from PS I at 77°K. These studies clearly established that LHC II phosphorylation is mediated by a protein kinase which is activated by light through |an intersystem redox carrier.

The light mediated phosphorylation of LHC ll decreased the stacking of thylakoids by 20-25% and increased the proportion of LHC II in the non-appressed regions (Black and Horton 1985) and in PSa,, centres (Black and Horton 1985). Most of the experimental data do not demonstrate movement of PS II along with phosphorylated LHC II.

Redox titration of ATP dependent quenching of PS Il fluorescence (Horton and Black 1980) and of LHC lI phosphorylation (Horton *et al* 1981) under the conditions of kinase activation established that these processes are controlled by the redox state of two electron carrier with a mid-point potential (pH 7·8) of 0 to + 50 mV representing plastoquinone (PQ).

At the same time, inhibitor studies using DBMlB as an antagonist of PQ oxidation (K_i, < 50 nM) (Allen and Horton 1981) and using TMQH2, (which was found to be specific electron donor of PQ) as the electron donor of PQ established that LH II phosphorylation occurs even in dark in the presence of $TMQH_2$, and [γ–^{32}P]ATP (Allen and Aorton 1981).

These results point out that the redox state of PQ controls the activation of LHC II kinase and thus phosphorylation of LHC II and consequent alteration in the distribution of excitation energy in favour of PS I. A membrane bound protein phosphatase is responsible for dephosphorylation under steady state conditions (Bennet 1980). The recent experimental data no longer support the contention that the redox state of PQ directly controls the activation of LHC H kinase. Cytochrome b_6-f complex appears to play the major role in activation of LHC II kinase.

A mutant of *Lemna perpusiiia* lacking cyt b_6-f complex though possessing normal PSI and PS II, LHC II and PQ did not show state 1-2 transitions; also, the LHC II phosphorylation does not occur in this mutant (see Gal *et ai* 1988). On the contrary, PS Il polypeptides were phosphorylated in this mutant (Gal *et ai* 1988). Similarly, in maize mutant (*hcf 6*) which lacks cyt b_6-f complex but possesses PS II, LHC II and PQ, phosphorylation of LHC II polypeptides and two minor phosphoproteins (15 and 54 kDa) does not take place (Bennet *et ai* 1988). lmmunological data suggest that LHC Il kinase was present in normal amounts in maize and *Leinan* mutants.

One of the most significant developments in this area has been the discovery of LHC II phosphorylation in dark in *Acetabuiaria mediterranea* thylakoids (Gal *et al* 1988). These thylakoids retain the ability of LHC II phosphorylation *in vitro*

in dark without added reductants but the kinase was reversibly inactivated by the oxidants *in vitro* or by far-red light *in vivo.* lt has further been shown in *A. mediterranea,* that the LHC II phosphorylation is inhibited by the inhibitors of the Q_z site of cyt b_6-f complex even when PQ pool is fully reduced (Gal *et al* 1988).

The functional relationship between cyt b_6-f complex and phosphorylation of small pool of LHC II would suggest that LAC II kinase may be present possibly at the 'end margins' and in association with cyt b_6-f complex. The LHC II upon light dependent activation phosphorylates small pool of LHC II present at the 'end margins'. The purification studies on cyt b_6-f complex suggest physical association of small pool of cyt b_6-f complex which always copurifies with LHC II kinase.

The cyt b_6-f complex is uniformly distributed in appressed and non-appressed regions (see Hope 1993). Immunochemical localization studies also suggest its uniform distribution (Gal *et al* 1988, 1990).

Preparations enriched in cyt b_6-f complex obtained from spinach thylakoids by detergent extraction and precipitation with ammonium sulphate followed by different procedures of purification, were found to possess LAC lI kinase activity. Analysis of cyt b_6-f content and kinase activity of fractions obtained by histone-sepharose and immunoaffinity columns, immunoprecipitation and sucrose density centrifugation, indicate functional association of kinase and cyt b_6f (Gal *et ai* 1990). Plastoquinol-1 addition enhanced the phosphorylation in these fractions. The phosphorylates activity was associated with a 64 kDa polypeptide (Gal *et ai* 1990). This LHC II kinase was localized almost exclusively in the grana region of the thylakoids. A higher concentration of immunogold-labelled 64 kDa LHC ll kinase at the edge of grana stacks is well in agreement with the fact that only a small pool of LHC II is phosphorylated (Gal *et al* 1988).

The electron transfer between PS lI and PS I, due to heterogeneity in the localization, requires a mobile carrier. Inspite of uniform distribution of cyt b_6-f, only 2/3 of cyt b_6-f is reduced by plastoquinol (Joliot and Joliot 1992). ln the grana region, cyt b_6-f complex is the electron carrier while for long range electron transfer involving grana stacks, plastocyanin acts as a carrier (Joliot and Joliot 1992). The diffusion of PQ in the grana is restricted. Thus, diffusional limitation of plastoquinol may be a reason for the involvement of cyt b_6-f complex in the process of activation of LAC II kinase.

The direct involvement of PQ in LHC II kinase activation can be ruled out on the basis of the studies with mutants and inhibitor. Aowever, the components of cyt b_6-f complex *viz.* cyt f and Rieske Fe-S are one electron carrier and their mid-point redox potentials are much higher (> + 290 mV) while the regulator of LAC II phosphorylation is a two electron carrier with mid-point redox potential of 0 to + 80 (Horton and Black 1980; Millner *et al* 1982). On the contrary, mid point redox potential of cyt b_6 shows variable behaviour.

From the foregoing account, it is becoming increasingly clear that LAC II phosphorylation is dependent on the interaction of cyt b_6-f complex with LAC II kinase. Knaff (1991) has reached the same conclusion. Aowever, the mechanism by which cyst b_6-f complex 'communicates' to activate LHC II kinase remains largely unclear.

ln summary these studies suggest that (i) different protein kinases may be involved in phosphorylation of LHC II *versus* PS II polypeptides, (ii) these kinases may be activated by different mechanisms and (iii) the phosphorylation of LHC II is

mediated by a protein kinase whose activation is controlled by cyt b_6-f complex. LHC II phosphorylation possibly plays dual role, not only balancing the synchronous turnover of both photosystems by physically moving phosphorylated LHC II to non-appressed regions of the thylakoids to bring about NADPH production along with ATP synthesis through non-cyclic photophosphorylation but may control relative rates of cyclic *versus* non-cyclic photophosphorylation for efficient carbon reduction.

The evidence in support for the role of LHC II phosphorylation in cyclic photophosphorylation comes from the studies of Fernyhough *et al* (1984). Their studies also provide some evidence which suggests that electrogenic Q cycle may be the key event, controlling LHC II phosphorylation. With isolated maize protoplasts, it has been shown that it is the decrease in transthylakoid pH that causes the increased LHC II phosphorylation. Further, the results suggest that the decrease in LHC II phosphorylation at high light intensity is caused by an effect of pH on LHC II phosphorylation. Under conditions of low Δ pH (due to high rate of proton release through ATP synthase) when ATP utilization exceeds its production (thus lowering of ATP levels), LHC II phosphorylation should be maximal; under these conditions the higher rate of cyclic photophosphorylation will lead to efficient carbon reduction through glyceraldehyde-3-phosphate dehydrogenase step requiring NADPH (Fernyhogh *et al* 1984).

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