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Micro-organisation of thylakoids and the control of whole chain photosynthetic electron transport.

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Introduction

Photosynthetic electron transport in thylakoids from higher plants is mediated by four integral protein complexes: photosystem (PS) II, light-harvesting (LHC) complexes, cytochrome (cyt) *bf* complexes, and PS I. Some of the proteins are inhomogeneously distributed between the granal thylakoids and stroma lamellae (Anderson 1982, Anderson 1992). Most of PS II and LHC II are located in the granal stacks whereas PS I with LHC I, together with the ATP-synthase, are entirely excluded from stacked membranes. In contrast to the more or less strict compartmentation of these complexes, cyt *bf* complexes are distributed throughout all membrane regions (Wollenberger et al. 1994, Albertsson 1995). The electron flow between the protein complexes is thought to be managed by two mobile redox carriers: plastoquinone (PQ) which transfers electrons from PS II to cyt *bf* migrates within the lipid phase of the membrane. Plastocyanin is a small copper protein which mediates electron transfer from cyt *bf* to PS I. It diffuses within the aqueous space of the thylakoid lumen.

The diameter of a granal stack is in the range of 400-500 nm (Staehelin and Van der Staay, 1996). As PQ is thought to diffuse rapidly within a lipid bilayer (1.3 to $3.5 \cdot 10^{-7}$ cm^2/s for pure phosphatidylcholine vesicles (Blackwell et al. 1994), it seems to be an excellent candidate for long distance shuttling throughout the thylakoid membrane. However it has been argued that the diffusion may be restricted due to the abundance of integral proteins that may act as obstacles. At least 50% of the membrane area is covered by proteins (Lavergne et al 1992).

Micro-domain concept: Joliot and co-workers (Joliot et al. 1992, Lavergne et al 1992) have presented good evidence that indeed no global redox equilibrium between Q_A, cyt b_6 and the mobile PQ is achieved. They proposed the existence of small local PQ diffusion micro-domains created by random distribution of densely packed proteins within the lipid space. PQ is `trapped´ in the micro-domains within which it can diffuse rapidly. In contrast to that, diffusion across the borders of a micro-domain is slow. Kirchhoff et al (2000) demonstrated that restriction of PQ diffusion occurs in grana stacks only. After moderate destacking, rapid redox equilibration throughout the membrane is achieved. As the electron flow through the cyt *bf* complex is known to be the slowest step in the electron transport chain, PS II should exert little control on the whole chain flux ($H_2O \rightarrow$ PS I) under saturating light. This was indeed what Kirchhoff et al. found in destacked thylakoids, which exhibit a non-linear control curve: if PS II activity was titrated to 50% of its total activity, the whole chain flux, J_e, decreased only about 25% (Kirchhoff et al. 2000). In contrast to that, their flux control curve for stacked thylakoids was nearly linear, indicating that PS II exerts strong control on the overall electron flux. In stacked membranes, the sum of flux control coefficients for PS II and

cyt *bf* exceeds the value of one ($Cj_{PS\ II} + Cj_{cyt\ bf} > 1$; Kirchhoff et al. 2000). This is in conflict with the control theory (Kacser and Burns 1973) according to which the sum of all individual control coefficients in a linear sequence of enzyme reactions should not exceed one, provided all substrates can migrate within the reaction space and interact with a large number of enzymes. Therefore the data were interpreted by assuming that only in destacked membranes do a large number of PS II share a pool of diffusing PQ (Fig. 1A). In stacked thylakoids (Fig. 1B and C), rapid PQ diffusion to cyt *bf* is restricted to small domains in the close vicinity of individual PS II centres (Kirchhoff et al. 2000). Kirchhoff et al. proposed a dynamic protein network formed in grana stacks by specific interactions between PSII and LHCII. In this arrangement, cyt *bf* complexes may have access to photo-reduced PQ in the close vicinity of PS II only (Fig. 1B). According to their calculations, roughly 70% of PS II are located in membrane areas where the access to cyt *bf* complexes by PQ rapid diffusion is highly restricted, such that an average number of only 1-2 PS II centres share cyt *bf* in their close vicinity. These domains, however, should be regarded as flexible structures as all proteins move somewhat. Restriction of fast PQ diffusion to micro-domains in the grana could prevent an excess of electrons at PS I, and strong control by PS II of electron transport might optimise adaptation processes.

In this study, we present flux control analyses for linear electron flow in thylakoids from tobacco and spinach. The control theory (Kacser and Burns 1973) describes how control in a sequence of enzyme-substrate reactions can be shared between individual enzymes. We found that the two species investigated show complex, s-shaped control by PS II of whole chain transport. This is explained by an extended micro-domain concept and fitted with a mathematical model.

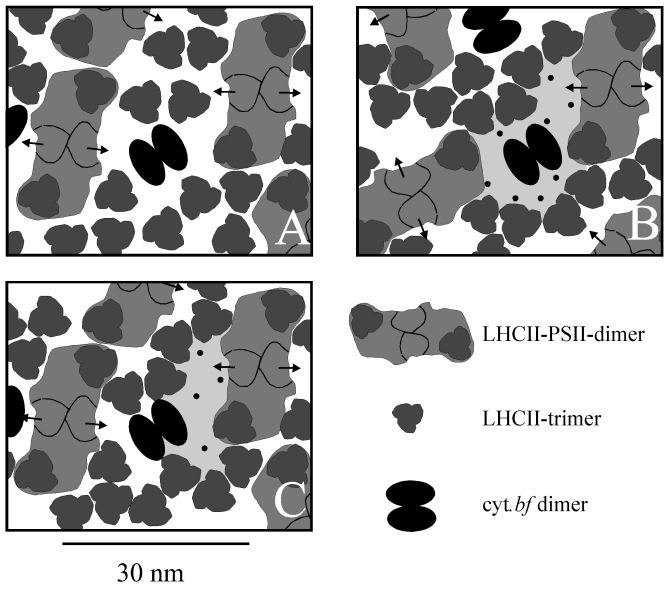

Fig. 1: Tentative assembly of proteins in the thylakoid membrane. Destacked (**A**), and stacked (**B** and **C**). The lightly shaded area represents one selected example of a PQ diffusion domain. **B**: a cyt *bf* dimer associated with a micro-domain. **C**: a cyt *bf* monomer associated with a micro-domain. Small dots represent PQ. According to Kirchhoff (2000), modified

Materials and methods

Growth conditions: spinach was grown in a climate chamber (14-15 °C, 200 µmol quanta/(m^2s). Tobacco was grown in a glasshouse.

Thylakoids from spinach: Intact chloroplasts were isolated and stored on ice. Before each measurement thylakoids were obtained freshly by osmotic shock (30 s) in 7 mM $MgCl_2$, 80 mM KCl, and 30 mM HEPES (pH 7.6). After the shock thylakoids were stored in 7 mM $MgCl_2$, 80 mM KCl, 30 mM HEPES (pH 7.6), and 330 mM sorbitol. Destacked thylakoids were prepared in the same way except that $MgCl_2$ was omitted and the concentration of KCl was 15 mM.

Thylakoids from tobacco: Chloroplasts of tobacco were osmotically broken during the isolation procedure. Thylakoids were stored in a medium containing 7 mM $MgCl_2$, 80 mM KCl, 30 mM HEPES (pH 7.5), and 300 mM sorbitol.

Electron transport and PS II activity: electron flow from H_2O to methylviologen and from H_2O to DMBQ, respectively, were measured in the presence of 1µM nigericin using an oxygen electrode (Hansatech). The light intensity was saturating (6000 µmol quanta/(m^2·s). PS II activity was titrated with DCMU.

Results and Discussion

Control measurements

We carried out a large number of flux control experiments with stacked thylakoids from different plants. It turned out that the shape of control functions was variable. Apart from more or less linear control curves (as an example see Fig. 2), s-shaped control function were found (Fig. 3). The s-shape can be more or less pronounced as can the curvature. Usually, control functions from spinach are more linear while those from tobacco exhibit more curvature, but some variability was also seen for different plants from one species. It may reflect physiological adaptation of the thylakoid structure. It should be noted that tobacco was grown in a glass house and hence under slightly varying irradiance and temperature.

The complex control functions cannot be explained by only assuming that PQ diffusion is restricted to small micro-domains, leading to a more or less linear flux control. So far, the control curves have been interpreted assuming a linear sequence of reactions within the electron transport chain. The complex, s-shaped control behaviour may reflect a non-linear sequence of reactions. It is often discussed that cyt *bf* activity might be controlled by the redox state of PQ. Such a control would lead to a non-linear sequence of reactions, as the cyt *bf* activity would depend on the PS II activity.

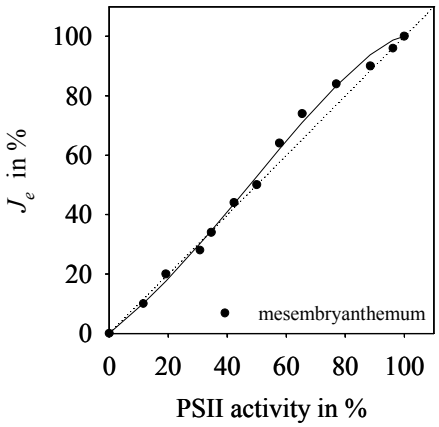

Fig. 2: Nearly linear flux control curve from *Mesembryanthemum crystallinum* (L.). Data from Mark Schöttler, unpublished. The solid line shows a fitted curve (n=1.5, r=0.54). The dotted line represents a 1:1 relationship For explanation see section `Model of flux control´ and appendix.

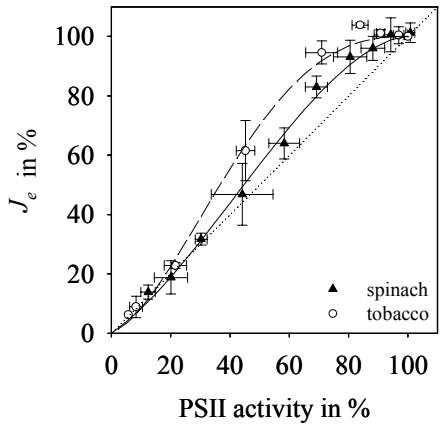

Fig. 3: Complex relationships between whole chain electron flux (J_e) and PS II activity. Data are from spinach and tobacco. Lines show fitted curves. Solid: spinach (n=1.7, r=0.40), dashed: tobacco (n=2.5, r=0.17).

Model of flux control

The control curves were fitted with a mathematical model incorporating interacting adjacent plastoquinone diffusion domains that are functionally coupled by dimeric cytochrome *bf* complexes (see appendix). Two parameters were introduced to characterise the domain size (n) and the redox control of cyt *bf* (r). The parameter n reflects how many PS II share a PQ pool with access to cyt *bf*. It should be stressed, however, that n may not simply be interpreted as a stoichiometric number. Micro-domains should be regarded as a temporal structure and each domain will rearrange. Thus a micro-domain only reflects the local and temporal stochiometry of proteins functionally connected to each other. It can be regarded as relatively stable if its turnover time exceeds that of fast electron transport (10 to 20 ms). As the stability decreases, the relative PQ diffusion space increases and a larger number of PS II share a pool with access to cyt *bf*. To some extent lateral PQ exchange may also be influenced by the relative density of cyt *bf*: if cyt *bf* is located in the close vicinity of PS II centres, photo-reduced PQ is rapidly trapped by cyt *bf* binding sites and consequently the exchange area for PQ decreases. This might somewhat smooth out local PQH_2 gradients and reduce the lateral PQH_2/PQ exchange. In this sense n should be taken as a general exchange parameter. It reflects the probability for each individual PS II centre to share a diffusion domain for rapid access to cyt *bf* with other PS II centres. For n = 1 there is no lateral PQ exchange. Each PS II has separate access to cyt *bf*, and consequently the control function becomes linear (Fig. 4, left). A linear flux control relates to a strong control by PS II of J_e. With increasing n the curvature increases and hence flux control by PS II decreases.

S-shaped control curves could be obtained by introducing a second fit-parameter, r (with $0 \le r \le 1$) (Fig. 4, middle and right). r describes a negative co-operativity between domains, as could be mediated by cyt *bf*. It is now generally assumed that cyt *bf* occurs as a dimer (see Cramer et al. 1996). The functional significance of the dimer is not yet clear. In a manner similar to that we assume for dimeric PS II complexes (see Fig. 1), the two monomers of a dimeric cyt *bf* complex could temporarily participate in different PQ diffusion domains, as depicted in Fig. 1C. With partially inhibited PS II, the PQH_2 binding sites of the cyt *bf* dimer could then be occupied non-equally. A value of r = 1 means that both monomers of the cyt *bf* dimer operate independently. In this case inactivation of one monomer (PQH_2 site not occupied) does not influence PQH_2 oxidation on the other monomer. In contrast r < 1 means

that the two monomers of a dimer operate in a co-operative way (see for example Graan and Ort 1986, Pan et al 1987, Gopta et al 1998). Thus inactivation of one monomer leads to complete ($r = 0$) or partial inhibition ($0 < r < 1$) inhibition of the other monomer (r = `residual activity´). This kind of a co-operativity leads to a negative curvature of the control function (Fig. 4, middle). It should be pointed out that n and r derived from data fitting represent average values. Inhomogeneous distribution of these parameters throughout the membrane is likely, but is not described by our model. Best fits for data from different experiments are shown in Figs. 1 and 2 (see legends).

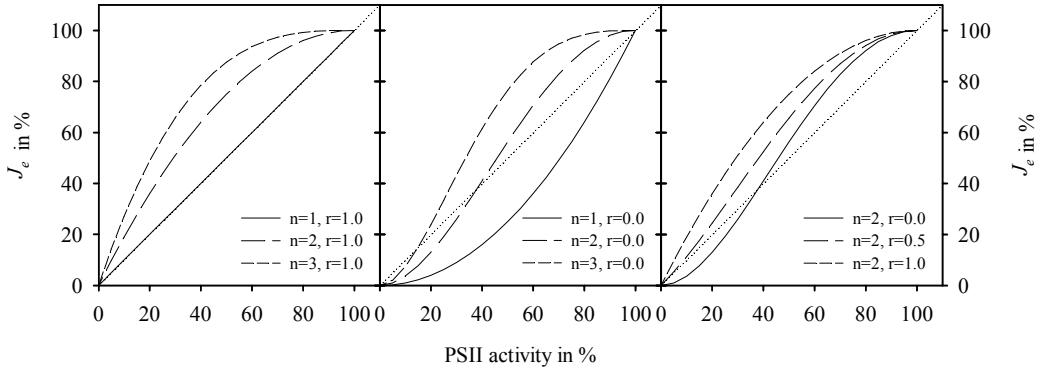

Fig. 4: Calculated curves. Two fit-parameters were varied: The number, n, of PS II accessing the same diffusion domain over a certain time period, and the `residual activity´, r, of cytochrome *bf*. Some of the theoretically possible combinations (e. g. n=1 together with r=0) are not found in our experiments.

In Fig. 5, the light–saturated, whole chain electron transport rate is plotted against the parameters n and r obtained as best fits for individual flux control experiments. Only trends can be detected as there is considerable scatter in the data. Interestingly, despite the scatter, there seems to be a negative correlation between n and J_e, indicating that the relative size of domains decreases in plants with high electron transport capacity. If fast lateral PQ migration is restricted to the grana, the capacity of J_e may be controlled by cyt *bf* located in the grana stacks (close to PS II) only (Kirchhoff et al. 2000). Hence, in grana from plants with high photosynthetic fluxes, the ratio PS II/cyt *bf* should be relatively low, i.e. each PS II may be located in the close vicinity of a cyt *bf* complex. This increases the probability of photo-reduced PQ being trapped rapidly by a cyt *bf* binding site near each individual PS II centre. In a densely packed membrane area, this effect could further decrease the exchange radius of PQ (decrease in n). A lower exchange radius on the other hand would lead to a greater control by PS II of J_e and consequently be of adaptational significance. It has to be noted, however, that the measurements of PS II activity include both PS II_α and PS II_β but only PS II_α are located in the grana and can consequently influence the structure of the micro-domains. There is also a slight tendency for r to increase with J_e, but this correlation is less significant. No significant correlation was seen between the fit-parameters and chl a/b and PS II capacity, respectively (not shown).

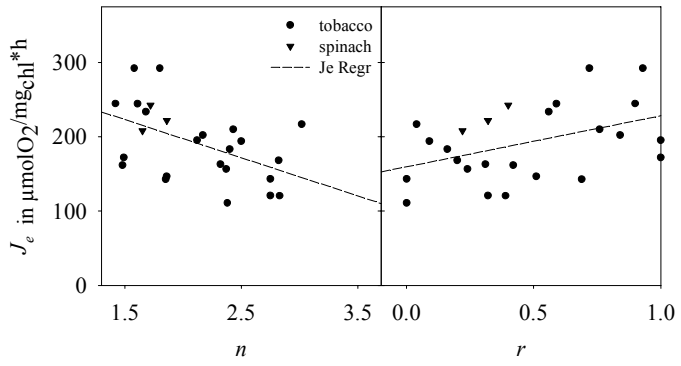

Fig. 5: Relationship between J_e and the fitted parameters n and r.

Appendix:

Model of how whole chain transport decreses with increasing PS II inhibition by DCMU

J_e = whole chain electron transport in relation to the unihibited control,
c = number of cyt *bf* monomers in a micro-domain,
n = number of PS II in a micro-domain,
r = residual cyt *bf* activity,
PS = proportion of active PS II,
I = proportion of inhibited domains with all contributing PS II inhibited,
$P(i)$ = probability of a domain to have as neighbours i inhibited domains with which it is sharing a cyt *bf* complex,

It is assumed that J_e is limited by cyt *bf* activity. Each micro-domain contains c cyt *bf* monomers. Thus, J_e from all micro-domains should add to:

$$J_e = \cdot \sum_{i=0}^{c} P(i) \cdot \left(\frac{2(c-i)}{2c} + \frac{r \cdot i}{c} \right) \tag{1}$$

with,

$$P(i) = (1-I) \cdot I^i \cdot (1-I)^{c-i} \cdot \frac{c!}{(c-i)! \cdot i!} \quad , \tag{2}$$

$$I = (1-PS)^n \quad . \tag{3}$$

In the case of a micro-domain with inhibited cyt *bf* its activity is diminished by $2(c-i)$ as the inhibition of a monomer (i) leads to the inhibition of the whole dimer. The factor $\frac{1}{2c}$ is to normalize the activity of a (partially) inhibited domain to that of an undisturbed domain. In the equation for $P(i)$, the first term takes into account that only domains which are active themselves are contributing to J_e.

$$J_e = \sum_{i=0}^{c} I^i \cdot (1-I)^{c-i+1} \cdot \frac{c!}{(c-i)! \cdot i!} \cdot \left(\frac{2(c-i)}{2c} + \frac{2r \cdot i}{2c} \right) \quad , \tag{4}$$

which simplifies to:

$$J_e = (1-I) \cdot [(1-I) + r \cdot I] \quad . \tag{5}$$

Inserting eq. (3) leads to:

$$J_e = (1-(1-PS)^n) \cdot [(1-(1-PS)^n) + r \cdot (1-PS)^n] \quad . \tag{6}$$

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