Photosynthesis Research **46:** 141–149, 1995.

Minireview

The structure and function of the chloroplast photosynthetic membrane – a model for the domain organization

Per-Åke Albertsson
Department of Biochemistry, P.O. Box 124, S-221 00 Lund, Sweden

Received 24 March 1995; accepted in revised form 25 May 1995

Key words: photosynthesis, chloroplast thylakoid, Photosystem I, Photosystem II, linear and cyclic electron transport, plastocyanin, plastoquinone

Abstract

Recent work on the domain organization of the thylakoid is reviewed and a model for the thylakoid of higher plants is presented. According to this model the thylakoid membrane is divided into three main domains: the stroma lamellae, the grana margins and the grana core (partitions). These have different biochemical compositions and have specialized functions. Linear electron transport occurs in the grana while cyclic electron transport is restricted to the stroma lamellae. This model is based on the following results and considerations. (1) There is no good candidate for a long-range mobile redox carrier between PS II in the grana and PS I in the stroma lamellae. The lateral diffusion of plastoquinone and plastocyanin is severely restricted by macromolecular crowding in the membrane and the lumen respectively. (2) There is an excess of 14 ± 18% chlorophyll associated with PS I over that of PS II. This excess is assumed to be localized in the stroma lamellae where PS I drives cyclic electron transport. (3) For several plant species, the stroma lamellae account for 20 ± 3% of the thylakoid membrane and the grana (including the appressed regions, margins and end membranes) for the remaining 80%. The amount of stroma lamellae (20%) corresponds to the excess (14–18%) of chlorophyll associated with PS I. (4) The model predicts a quantum requirement of about 10 quanta per oxygen molecule evolved, which is in good agreement with experimentally observed values. (5) There are at least two pools of each of the following components: PS I, PS II, cytochrome *bf* complex, plastocyanin, ATP synthase and plastoquinone. One pool is in the grana and the other in the stroma compartments. So far, it has been demonstrated that the PS I, PS II and cytochrome bf complexes each differ in their respective pools.

Abbreviations: PS I and PS II – Photosystem I and II; P 700 – reaction center of PS I; LHC II – light-harvesting complex II

The photosynthetic membrane, the thylakoid, of higher plant chloroplasts is probably the most complex and ingeniously constructed of all biological membranes. Its main function, to capture light quanta and drive a series of redox reactions whereby oxygen and ATP are produced and ferredoxin reduced, is carried out under varying conditions of light and temperature. Despite extensive biophysical, biochemical and genetic research over the last decades we still know very little about how the photosynthetic process works in vivo at the membrane level.

The photosynthetic membrane is highly asymmetric. In addition to the transverse asymetry lateral inhomogeneities can also be distinguished between different lateral membrane domains which have specialized functions. In this review I shall summarize work from our laboratory where we have studied the structure-function relationship of the thylakoid at the membrane level. In short, our strategy is to fragment the thylakoid into subthylakoid vesicles, isolate these and study their properties, and from the data so obtained design a model describing the construction of the intact thylakoid

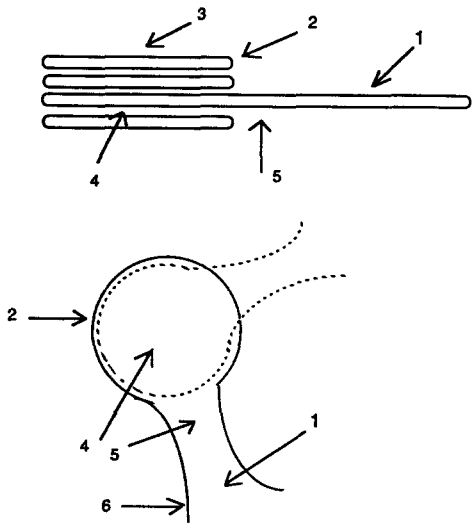

Fig. 1. Domains of the thylakoid membrane. Upper: cross section. Lower: Viev from above. (1) flat stroma lamellae; (2) margin of grana; (3) end membrane; (4) grana core (appressed partitions); (5) neck-like zone between grana and stroma lamellae; (6) margin of stroma lamellae. The dashed curve indicates a thylakoid below the plane.

system and its function. Such a model is presented here, according to which the grana of the chloroplast are specialized to carry out linear electron transport from water to ferredoxin, while the cyclic electron transport around PS I is restricted to the stroma lamellae. More than one stroma lamella can be connected to each grana sheet.

Structure

The photosynthetic membrane of the chloroplasts consists of a system of paired membranes, the thylakoids, and constitutes a single, complex membrane enclosing the lumen which is separate from the surrounding stroma where CO_2 fixation takes place. The thylakoid membrane consists of two main compartments, the grana and the stroma lamellae. The grana in turn consist of a central core with appressed membranes (partitions) forming the grana stack, and a peripheral domain, the margins, and two end membranes (Fig. 1). In contrast to the appressed grana core membranes, the margins, the end membranes and the stroma lamellae are stroma exposed. In addition, one can distinguish between the planar membrane domains (appressed grana core membranes, grana end membrane, stroma lamellae) and curved domains, such as the margins of both the grana and stroma lamellae. The neck-like connections between grana and stroma lamellae may also be considered a separate domain. The intricate three-dimensional relationship between grana and stroma lamellae has been studied by several electron microscopists and each have presented a structural model (see review by Staehelin 1986).

Despite the complexity and plasticity of the thylakoid membrane, there is a certain regularity in the structure of the thylakoid system of plants grown under normal conditions. Viewed from above, the grana stacks are circular in shape with diameters that generally fall between 0.4 and 0.5 m. The number of paired membranes per granum is 5–20. The chlorophyll *a/b* ratio is 3.0 ± 0.5. A remarkable consistency is found in the relative amount of grana membranes (including margins and end membranes) and stroma lamellae (Table 1). For 10 different plants, the amount of stroma lamellae is 20 ± 3%. This consistency must be of functional significance and will be discussed below (see section on the model).

The membrane-enclosed lumen space is narrow, particularly in light (Murakami and Packer 1970), since there are attractive forces between the two opposing inner surfaces (Albertsson 1982) such that protruding pieces of membrane proteins are in contact and occupy the lumen space. This crowded lumen space can be visualized by electron microscopy (Weibull and Albertsson 1988).

Isolation of subthylakoid vesicles

Four different types of subthylakoid vesicles, originating from different domains of the thylakoid can be isolated by a combination of sonication and aqueous two-phase partitioning (Albertsson et al. 1994). They originate from the grana, stroma lamellae, grana margins and grana core (partitions).

Isolation of grana and stroma lamellae vesicles

Through a suitable choice of medium and sonication time, selective fragmentation can be achieved such that essentially two types of vesicles, one derived from the grana and the other from the stroma lamellae, are obtained. These can then be quantitatively separated by countercurrent distribution. The left-and right-hand peak in Fig. 2 represent the grana (α) and the stroma lamellae vesicles (β), respectively. The two types of

Table 1. The amount of each membrane type, (expressed as percentage of total) in the thylakoids from chloroplasts of different plants. The amount of each type of membrane domain was measured by drawing lines at regular intervals perpendicular to the chloroplast axis and counting the number of membrane intersections (counting two membranes for the intersection of an appressed partition). About one thousand intersections were counted. The variation in the frequency of end membranes correlates with the number of thylakoids in the grana stacks. Despite this variation there is a fairly constant percentage of stroma lamellae and total grana

	Appressed + margins	End membrane	Grana total	Stroma lamellae	Reference
Spinach	70	8	78	22	Miller (1988)
Spinach	69	11	80	20	Park (1965)
Spinach	63	19	82	18	Weibull, unpub.
Spinach	70	10	80	20	Staehelin (1986)
Spinach	63	17	80	20	Greenwood (1993)
Lettuce	70	11	81	19	Arntzen (1983)
Sugar cane (mesophyll)	69	8	77	23	Laetsch (1972)
Tobacco	60	22	82	18	Staehelin (1986)
Barley	73	7	80	20	Simpson (1979)
Oat	69	11	80	20	Hanchey (1985)
Corn (mesophyll)	77	4	81	19	Shumway (1973)
Antirrhinum majus	73	4	77	23	Menke (1980)
Phleum pratense	69	11	80	20	Ledbetter and Porter (1970)
Peperomia	69	10	79	21	Juniper (1978)

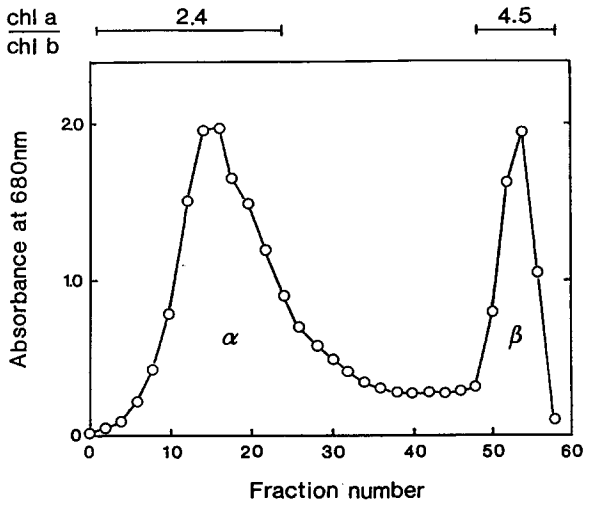

Fig. 2. Separation of grana (α) from stroma lamellae (β). Stacked thylakoids from peas were sonicated and then subjected to countercurrent distribution according to the method described by Andreasson et al. (1988). 70 ± 5% of total chlorophyll, 85% of total PS II activity and 40 ± 5% of total P 700 is found in the α peak. The fraction of the β peak probably also contains fragments from the end membranes.

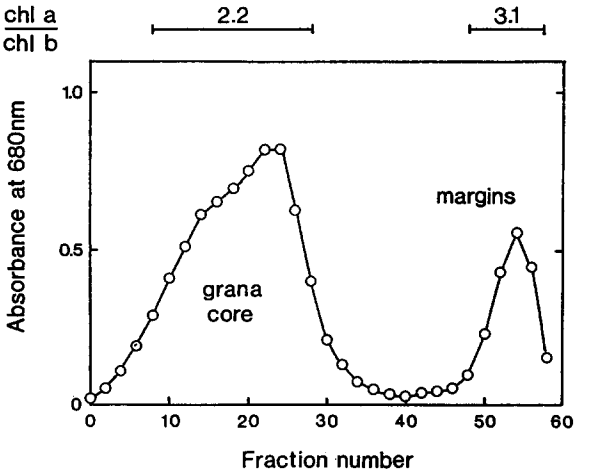

Fig. 3. Separation of grana margins from the grana core. Grana vesicles were first isolated from sonicated thylakoids by a batch partition procedure. The grana vesicles were then further sonicated and subjected to countercurrent distribution. From Wollenberger et al. (1994).

vesicles can also be separated by a three-step batch procedure (Albertsson et al. 1994).

The grana vesicles have a low chlorophyll *a/b* ratio and are enriched in PS II while the stroma lamellae vesicles have a higher chlorophyll *a/b* ratio and are enriched in PS I. The distributions of chlorophyll, P700, PS II activity and cytochrome *f* between the two types of vesicles for spinach have been published (Andreasson et al. 1988; Albertsson et al. 1994).

Results from several experiments similar to those in Fig. 2 show that the amounts of membrane vesicles, on a chlorophyll basis, in the α and β fractions are 70 $\pm$ 5 and 30 $\pm$ 5%, respectively. From the data in Table 1, one would expect only 20% in the β fraction if this consisted of only stroma lamellae vesicles. According to Sane et al. (1970) the end membranes are rippped off the grana by mechanical (pressure) treatment. We therefore assume that the β fraction illustrated in Fig. 2 contains end membranes (about 10% of the thylakoid, Table 1) in addition to stroma lamellae, which would explain why the yield of the β fraction is increased to 30%.

Fragmentation of grana vesicles – isolation of grana margins and grana core

The grana vesicles can be further fragmented by sonication and separated into smaller vesicles originating from the grana margins and the grana core (partitions), Fig. 3. The margin vesicles are enriched in PS I and have a chlorophyll *a/b* ratio of 2.8–3.2, i.e. lower than the stroma vesicles (Wollenberger et al. 1994). This is due to LHC II attached to the PS Iα of the grana margins (Svensson et al. 1991; Andreasson and Albertsson 1993). The margins are enriched in the 64 kDa protein claimed to be a kinase (Yu et al. 1994).

The grana core vesicles, i.e. grana vesicles minus margins, are highly enriched in PS II (Svensson and Albertsson 1989). They also have the highest concentration of cytochrome *bf* complex. The molar ratio of PS II to cytochrome *bf* is 2:1, which might indicate that these two components form a functional complex (Yu et al. 1993).

Heterogeneity among the photosystems

Several studies have shown that there is heterogeneity among both PS II and PS I. The grana PS II (PS IIα) has antennae which are about twice as large as the stroma lamellae PS II (PS IIβ) (Anderson and Melis 1983). In addition, there also seem to be differences in the redox properties. Both types can evolve oxygen with PPBQ as electron acceptor, but PS IIα is more efficient in reducing ferricyanide and duroquinone (Henrysson and Sundby 1990). There is also heterogeneity within PS IIα. (Albertsson and Yu 1988; Albertsson et al. 1990c). It has been suggested that the PS IIα units with the largest antennae are localized in the center of the partition regions (Albertsson et al. 1990a).

Photosystem I is also heterogeneous (Andreasson et al. 1988; Svensson et al. 1991). The grana PS I (PS Iα) which is located in the periphery of the grana, (margins) has an antenna which is 30–40% larger than the stroma lamellae PS I (PS Iβ). The additional antennae of grana PS I form a special pool of LHC II which is attached to PS Iα and functionally coupled to it (Andreasson and Albertsson 1993). Recently, it has also been shown that grana and stroma lamellae PS I differ in their ability to reduce ferredoxin (Wollenberger and Albertsson 1995). PS I in isolated stroma lamellae, but not that from grana margin vesicles, could reduce ferredoxin with ascorbate and DCIP as electron donors. However, if NADP and FNR were added both types of PS I could reduce NADP via ferredoxin. It appears from these experiments that the presence of NADP and FNR is necessary for the reduction of ferredoxin by grana margin PS I. This is consistent with the notion that the two types of PS I have specialized functions, the grana PS I in linear, and stroma lamellae PS I in cyclic electron transport.

The cytochrome *bf* complex is distributed all over the thylakoid membrane, although at different concentrations in the different domains (Albertsson et al. 1991; Albertsson 1994) The stacked grana domain has the highest concentration and the grana margins the lowest.

Lack of a candidate for a long range mobile carrier

It has been suggested that PS II in grana and PS I in stroma lamellae can cooperate in linear electron transport via a long-range (micron-scale) mobile carrier, such as the cytochrome bf complex, plastoquinone or plastocyanin (Sane et al. 1970; Andersson and Anderson 1980). The main problem is that the diffusion of these components in the membrane may be severely restricted by molecular crowding of the membrane proteins. For a molecule to act as a mobile carrier at a distance of 0.5 μm on the ms time scale, its diffusion coefficient should be of the order of at least 10^{-8} cm^2 s^{-1} (Ort 1986; Whitmarsh 1986). Below follows an evaluation of the candidates for a mobile carrier between the grana and the stroma lamellae.

The cytochrome bf complex occurs in the form of a dimer both in the grana and the stroma lamellae (Romanowska and Albertsson 1994). Its molecular mass is about 230 kDa. Its diffusion coefficient is expected to be of the order of 10^{-10} cm^2 s^{-1}, i.e.

two orders of magnitude too small to to act as a mobile carrier and to account for the maximum observed rates of electron transfer between the two photosystems.

Plastoquinone is a small, lipid-soluble, molecule which would be expected to diffuse rapidly in the middle of the membrane bilayer. Because of this, it has been the favourite candidate as a long- range mobile carrier. However, the results of three recent independent experimental results speak against the possibility that plastoquinone is a mobile carrier over long distances.

(1) Joliot et al. (1992) concluded from kinetic experiments on the reduction of plastoquinone that only a small local domain of about 4–6 molecules of plastoquinone is available for rapid reduction by each PS II unit. They proposed that, because of the close packing of the membrane proteins, the mobility of plastoquinone is sterically hindered and hence the diffusion coefficient reduced.

(2) Blackwell et al. (1994) showed that the diffusion coefficient of plastoquinone in thylakoids, (0.1–3) 10^{-9} cm^2 s^{-1}, is about two orders of magnitude smaller than the diffusion coefficient in liposomes, probably as a result of macromolecular crowding of the thylakoid membrane proteins.

(3) The third result is from the determination of the plastoquinone pool available to PS II for intact thylakoid compared with subthylakoid vesicles (Yu et al. 1993). The plastoquinone pool was found to be the same, 6–7 molecules per PS II, for the whole thylakoid, and for grana vesicles. If the plastoquinone were present and freely mobile over the entire thylakoid membrane, then the plastoquinone pool per PS II centre would be significantly reduced when the PS II-poor stroma lamellae were removed in the preparations of grana vesicles. That this is not the case is a strong argument against plastoquinone being a mobile carrier over long distances (0.1 μm and longer) in the thylakoid membrane. These results also exclude the possibility that plastoquinone could act as a long-range mobile carrier by self-redox exchange along the membrane or that plastoquinone is moved along the membrane by some active process.

Plastocyanin is a water-soluble 10.5 kDa protein and has been suggested as a mobile carrier by diffusion in the lumen from the grana to the stroma lamellae. However, as discussed above, the lumen of the thylakoid is not a free volume for the unrestricted diffusion of proteins. The two inner opposing membrane surfaces come closer together in light due to attractive forces (Albertsson 1982). The protruding hydrophilic loops of the membrane proteins are in close contact and fill the lumen space. The diffusion of plastocyanin would therefore be expected to be severely retarded by macromolecular crowding. From studies on the rate of electron transfer from P700 to cytochrome *f* Haehnel (1984) also concluded that plastocyanin is immobilized in light due to the narrow space available in the lumen. The self-exchange reaction of plastocyanin in solution is too slow for it to act as a mobile carrier (Beattie et al. 1975).

In all, the experiments described above do not support the notion that there is a long-range mobile redox carrier between the grana and stroma lamellae functioning by passive diffusion, by self redox exchange or by any active transport along the membrane.

Distribution of chlorophyll and quanta between the photosystems

A fundamental question is how much light is absorbed by the photosystems PS I and PS II, respectively. In the literature it is usually claimed that the two photosystems absorb about equal amounts of light or that there is an imbalance in favour of PS II. We have found, however, that more chlorophyll is associated with PS I than with PS II (Table 2). The calculations behind the data presented in Table 2 are based on the quantitative separation of grana and stroma lamellae (Andreasson et al. 1988) see Fig. 2, which allows the determination of the amount of chlorophyll associated with each of the four photosystems, PS Iα, PS Iβ, PS IIα and PS IIβ (Albertsson et al. 1990b).

I wish to point out the following points concerning the results in Table 2.

(1) In all three cases, there is more chlorophyll associated with PS I than with PSII, the excess being 14–18%. This is in contradiction to previous data on the chlorophyll distribution between PS I and PS II based on gel electrophoresis (Evans 1986; Melis et al. 1987). Estimations of chlorophyll in different protein bands indicated that a greater fraction of chlorophyll is associated with PS II than with PS I. Such data are uncertain, however, because part of the chlorophyll is extracted from the protein by the detergent used for solubilization. In addition, all LHC II chlorophyll was assigned to PS II which is not justified since part of the LHC II is attached to PS Iα in the grana (Svensson et al. 1991; Andreasson and Albertsson 1993). The calculation method used to obtain the data given in

Table 2. Excess of chlorophyll associated with PS I. The distribution of chlorophyll between the different four photosystems, PS IIα, PS Iα, PS IIβ and PS Iβ was calculated according to 'alternative A' in Albertsson et al. (1990b) and based on separation experiments such as that in Fig. 2. The values under the heading 'thylakoid' are the sum of PS II and PS I respectiveley. Spinach (1) and peas were grown in light from dysprosium lamps (300 m^2 s^{-1}) with a light period of 12 h. (Chlorophyll *a*/*b* = 3.1 and 3.0, respectively). Spinach (2) was grown in greenhouse with daylight (chlorophyll *a*/*b* = 3.1). The α-fraction originates from grana (appressed and margin domains). The β-fraction originates from stroma lamellae and probably also end membranes

	α-fraction		β-fraction		Thylakoid		
	PS IIα	PS Iα	PS IIβ	PS Iβ	PS II	PS I	Excess of PS I (PS I − PS II)
Spinach	38	25	3	34	41	59	18
Spinach	40	30	3	27	43	57	14
Peas	38	35	3	24	41	59	18

Table 2 is robust in that the results are not sensitive to small experimental errors.

That there is an excess of chlorophyll associated with PS I (Table 2) means that PS I will absorb more quanta than PS II. Quantification of this excess is difficult. The chlorophyll compositions of the antennae of the photosystems are different; the PS II antennae containing more chlorophyll *b*. The absorbance of chlorophyll *a* is stronger than that of chlorophyll *b* in the red region of the spectrum, the opposite is true for the blue region. Red light is more important since the flux of quanta from sunlight which strikes the ground is higher in the red region. If these facts are taken into account, one finds that there is not much difference in the absorption of quanta per chlorophyll molecule (*a*+*b*) of the different subthylakoid vesicles, i.e. the amount of chlorophyll associated with each photosystem is a fairly good indicator (within a few per cent) of the quanta absorbed by each photosystem, with a slight advantage for chlorophyll *a* over chlorophyll *b*, (Melis et al. 1987). This conclusion is supported by a comparison of the absorption spectra of the isolated domains from the thylakoid which shows that the absorption of stroma lamellae and grana vesicles in the red region (600–700 nm) differs only by a few per cent (Wollenberger et al. 1995). There is also a qualitative difference in carotenoid composition between the two Photosystems; PS I being enriched in β-carotene and PS II in lutein (Juhler et al. 1993). However, for all subthylakoid vesicles there is about 1 carotenoid molecule per 4 chlorophyll molecules independent of the type of chlorophyll or carotenoid.

In conclusion, an excess of quanta is absorbed by PS I compared with PS II, according to the data in Table 2 and the considerations described above. This is convincing evidence for the existence of cyclic electron transport around PS I in vivo. The 14–18% excess of chlorophyll associated with PS I can be compared with the 20% of the thylakoid membrane which is made up of stroma lamellae, and is consistent with the notion that this membrane domain, dominated by PS Iβ, the cytochrome *bf* complex and ATP synthase, is indeed the site of cyclic photophosphorylation.

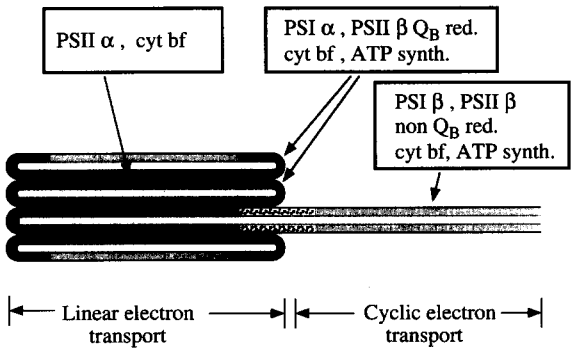

Fig. 4. Model of the thylakoid membrane from grana containing chloroplasts. Linear electron transport occurs in the grana where PS IIα, localized in the central appressed domain, cooperates with PS Iα, localized in the periphery. The border between the PS Iα and PS IIα domains is not sharp as depicted. One should rather envisage a peripheral annulus where PS Iα and PS IIα are partly intermixed and cooperate in local domains. Cyclic electron transport takes place in the single paired membrane of the stroma lamellae. The two grana photosystems PS Iα and PS IIα have larger antenna than the respective stroma lamellae systems (PS Iβ and PS IIβ). The width of the annulus is 60 nm for a circular grana disc with a diameter of 0,5 μm. 20% of the membrane is in the form of stroma lamellae. The remaining 80% is grana and distributes, approximately, amongst appressed membrane (40%) margins (30%) and end membrane (10%). These percentages for the grana domains varies somewhat depending on the size of the grana stacks.

(2) A large fraction (40–43% in the case of spinach) of the chlorophyll of the grana vesicles (α) is associated with PS Iα. The question is then whether the two grana photosystems, PSIα and PS IIα can cooperate in linear electron transport. In each granum there is more chlorophyll associated with PS IIα than PS Iα (ratio about 60 to 40). This would mean overexcitation of PS II even at low light intensities. However, although there is more chlorophyll associated with PS II than PS I in the grana vesicle fraction (Table 2) this calculation does not include the end membranes. If we assume that the end membranes contain mainly PS I then a complete granum would have a more equal distribution of chlorophyll between PS II and PS I. In addition one should consider that the number of quanta reaching the PS II of the grana may be reduced relative to the number reaching PS I in margins for the following reasons. The PS IIα domain is located in the interior of the granum. It is surrounded by the PS Iα-rich margins which have a lateral thickness of about 60 nm. Light has to pass through this layer before reaching PS IIα which will thus be exposed to a lower light intensity than PS Iα. This attenuation of the light intensity can be approximately quantified. The concentration of chlorophyll in the thylakoid membrane is 90 mM (Flores et al. 1983) and the absorptivity is, on average, 70 mM^{-1} cm^{-1} at 678 nm. Light illuminating a granum parallel to the membrane will be attenuated by about 10% after passing through a 60 nm thick PS Iα layer (it is assumed that Beers law is applicable). Calculation of the absorption of light, at 678 nm, parallel to the grana membrane, by the entire disc shows that the absorption of quanta per chlorophyll molecule, is on average, 20–30% less for the PS IIα domain than the PS Iα domain, i.e. the margin. In conclusion it appears that PS I and PS II of grana may well carry out balanced linear electron transport.

The model

A model for the structure and function of the thylakoid membrane of higher plants is presented in Fig. 4. It is a further development of a previous model (Albertsson et al. 1990a,b; Svensson et al. 1991) and resembles that of Park and Sane (see Sane 1977) in that the grana are specialized for linear electron transport and the stroma lamellae for cyclic transport but differs in that the PS II and PS I of the grana are segregated. Our model resembles that of Andersson and Anderson (1980) in that the two photosystems are segregated, but differs from theirs in that it takes into account the heterogeneity of the photosystems and that no long-range mobile redox carrier between grana and stroma lamellae is needed. According to our model, 80% of the membrane is in the form of grana and the remaining 20% consists of stroma lamellae (based on the data in Table 1). Linear electron transport occurs in the grana where PS IIα, localized in the core of the grana, cooperates with PS I in the periphery, i.e. an annulus surrounding the grana core, the margins, and the two end membranes. The annulus covers 40% of the circular grana disc in order to accomodate the 40% of the grana chlorophyll which is associated with PS Iα (Table 2). It is assumed that the chlorophyll is distributed evenly over the thylakoid membrane, which is reasonable since the membrane protein composition is dominated by the antenna protein complexes. For a circular grana membrane which is 0.5 μm in diameter, the width of the PS I α annulus would have to be 60 nm in order to constitute 40% of the area. The size of a PS Iα complex is about 20 nm. This means that three PS Iα complexes, placed radially, can be accomodated in a 60 nm wide annulus. The circular border between the PS IIα and PS Iα domains is not sharp. Rather, it is assumed that there is some intermixing between the two photosystems. This means that electron transport between them via plastoquinone, cytochrome *bf*, and plastocyanin at a short distance, 30–60 nm, is possible. The situation will be more like that between the protein complexes in the mitochondrial inner membrane. In the case of the end membrane, plastocyanine may act as a carrier across the lumen between cytochrome *bf*, in the adjacent appressed membrane and PS I in the end membrane.

The stroma lamellae, in which PSIβ, cytochrome *bf*, and PS IIβ are localized, carry out cyclic electron transport. The role of PS IIβ, which is a minor part of the stroma lamellae, is not known. Several alternatives have been suggested: that PS IIβ poises the cyclic electron flow around PS I, that PS IIβ is a precurser to PS IIα and that it is a stage in the repair cycle of PS II.

According to the model there are two pools (one in the grana and one in the stroma lamellae) of each of the following components, PS II, PS I, cytochrome *bf* complex, ATP synthase, plastoquinone and plastocyanin. It has been shown that PS II (Anderson and Melis 1983; Andreasson et al. 1988), PS I (Svensson et al. 1991) and cytochrome *bf* (Romanowska and Albertsson 1994) each differ in the two compartments. It remains to be seen whether the same holds for the oth-

er three components. It is a common phenomenon that when an enzyme is localized in different compartments of a cell, there is also a difference in some property of the two pools of the enzymes (isoenzymes).

The model takes into account the heterogeneity of the photosystems and their location as deduced from fragmentation and separation studies (Albertsson et al. 1994) and electron microscopy (Vallon et al. 1985). That cyclic electron transport is confined to the stroma lamellae is consistent with the agreement between the 14–18% excess of chlorophyll associated with PS I and the 20% abundance of stroma lamellae.

The model is also consistent with the results from quantum yield measurements (Björkman and Demmig 1987; Evans 1987). For a large number of C3 plants, the quantum requirement of oxygen evolution was between 9 and 10 quanta of light per oxygen molecule evolved. The quantum requirement one would expect from the model, would be 10–11 quanta per oxygen molecule if it is assumed that the quanta of light which are absorbed by the grana (80% of the thylakoid membrane) are efficiently (90–95% efficiency) utilized for oxygen evolution, i.e. 8–9 quanta per oxygen molecule, and that 2 quanta per oxygen molecule are absorbed by the stroma lamellae (20% of the thylakoid membrane) for cyclic elctron transport. The little variation in quantum requirement found among different C3 plants corresponds to the little variation in the relative amount of grana and stroma lamellae (Table 1).

According to the model, it is the single paired membranes, the stroma lamellae, and not the grana, which carry out cyclic photophosphorylation. Consequently those chloroplasts which have only single paired thylakoid membranes would be expected to contain mainly PS I and carry out cyclic photophosphorylation. This is indeed the case with chloroplasts from some specialized cells, such as the bundle sheath cells of some C4 plants or cells from the labellum of the orchid *Aceras anthropophorum* (Schmid et al. 1976). These have only single paired thylakoid membranes, contain mainly PS I and carry out cyclic, but not linear photophosphorylation. The same holds for the first thylakoids which appear during the greening of a leaf before complete PS II units are synthesized and grana are formed.

Acknowledgements

I wish to thank Eva Andreasson, Agneta Persson, Hreinn Stefansson, Ingun Sundén, Per Svensson, Claes Weibull, Louie Wollenberger and Shi-gui Yu for fruitful collaboration and Maria Svensson for typing the manuscript. This project has been supported by grants from the Swedish Natural Science Foundation.

References

Albertsson P-Å (1982) Interaction between the lumenal sides of the thylakoid membrane. FEBS Lett 149(2): 186–190

Albertsson P-Å and Yu S-G (1988) Heterogeneity among Photosystem II. Isolation of thylakoid membrane vesicles with different functional antennae size of photosystem II. Biochim Biophys Acta 936: 215–221

Albertsson P-Å, Andreasson E and Svensson P (1990a) The domain organization of the plant thylakoid membrane. FEBS Lett 273(1,2): 36–40

Albertsson P-Å, Andreasson E, Persson A and Svensson P (1990b) Organization of the thylakoid membrane with respect to the four photosystems, PS Iα, PS Iβ, PS IIα and PS IIβ. In: Baltscheffsky M (ed) Current Research in Photosynthesis, Vol II, pp 923–926. Kluwer Academic Publishers, Dordrecht

Albertsson P-Å, Yu S-G and Larsson UK (1990c) Heterogeneity in Photosystem II. Evidence from fluorescence and gel electrophoresis experiments. Biochim Biophys Acta 1016: 137–140

Albertsson P-Å, Andreasson E, Svensson P and Yu S-G (1991) Localization of cytochrome *f* in the thylakoid membrane: Evidence for multiple domains. Biochim Biophys Acta 1098: 90–94

Albertsson P-Å (1994) Domain structure of biological membranes obtained by fragmentation and separation analysis. Meth Enzymology 228: 503–511

Albertsson P-Å, Andreasson E, Stefansson H and Wollenberger L (1994) Fractionation of thylakoid membrane. Methods Enzymology 228: 469–482

Andersson B and Anderson JM (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. Biochim Biophys Acta 593: 427–440

Anderson JM and Melis A (1983) Proc Natl Acad Sci USA 80: 745–749

Andreasson E, Svensson P, Weibull C and Albertsson P-Å (1988) Separation and characterization of stroma and grana membranes – evidence for heterogeneity in antenna size of both Photosystem I and Photosystem II. Biochim Biophys Acta 936: 339–350

Andreasson E and Albertsson P-Å (1993) Heterogeneity in Photosystem I – the larger antenna of Photosystem I is due to functional connection to a special pool of LHC II. Biochim Biophys Acta 1141: 175–182

Arntzen C (1983) In: Zubay G. Biochemistry. Addison Wesley Publ Co Reading, Mass, London, Amsterdam

Beattie JK, Fenson DJ, Freeman HC, Woodcock E, Hill HAO and Stokes AM (1975) An NMR investigation of electron transfer in the copper-protein, plastocyanin. Biochim Biophys Acta 405: 109–114

Björkman O and Demmig B (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170: 489–504

Blackwell M, Gibas C, Gyqax S, Roman D and Wagner B (1994) The plastoquinone diffusion coefficient in chloroplasts and its mechanistic implications. Biochim Biophys Acta 1183: 533–543

Evans JR (1986) A quantitative analysis of light distribution between the two photosystems, considering variation in both the relative amounts of the chlorophyll-protein complexes and the spectral quality of light. Photobiochem and Photobiophys 10: 135–147

Evans JR (1987) The dependence of quantum yield on wavelength and growth irradiance. Aust J Plant Physiol 14: 69–79

Flores S, Graan T and Ort DR (1983) Photobiochem Photobiophys 6: 293–304

Fork DC, and Herbert SK (1993) Electron transport and photophosphorylation by Photosystem I in vivo in plants and cyanobacteria. Photosynth Res 36: 149–168

Greenwood AD (1993) In: Lawlor DW, Photosynthesis: Molecular, Physiological and Environmental Processes, 2nd ed. Longman Group, UK

Haehnel W (1984) On the lateral electron transport between the two light reactions in spinach chloroplasts. In: Sybesma C (ed) Advances in Photosynthesis Research, Vol 1, pp 545–548. Martinus Nijhoff, Dordrecht

Hanchey P (1985) In: Salisbury FB and Ross CW. Plant Physiology, 3rd ed. Wadsworth Publ Co. Belmont, CA

Henrysson T and Sundby C (1990) Characterization of Photosystem II in stroma thylakoid membranes. Photosynth Res 25: 107–117

Joliot P, Lavergne J and Beal D (1992) Plastoquinone compartmentation in chloroplasts. I. Evidence for domains with different rates of photo-reduction. Biochim Biophys Acta 1101: 1–12

Juhler RK, Andreasson E, Yu S-G and Albertsson P-Å (1993) Composition of photosynthetic pigments in thylakoid membrane fractions from spinach. Photosynth Res 35: 171–178

Juniper BE (1978) In: Kirk JTO and Tilney-Bassett RAE. The Plastids. Elsevier/North Holland Biomedical Press, Amsterdam/New York/Oxford

Laetsch WM (1972) In: Conn EE and Stumpf PK. Outlines of Biochemistry, 3rd ed. Wiley, New York

Ledbetter MC and Porter KR (1970) Introduction to the Fine Structure of Plant Cells. Springer, Berlin/Heidelberg/New York

Melis A, Spangfort M And Andersson B (1987) Light-absorption and electron-transport balance between Photosystem II and photosystem I in spinach chloroplasts. Photochem Photobiol 45: 129–136

Menke (1980) In: Strasburger's Textbook of Botany. Longman, London/New York

Miller K (1988) In: Stryer, L. Biochemistry, 3rd ed. Freeman, New York

Murakami S and Packer L (1970) Protonation and chloroplast membrane structure. J Cell Biol 47: 332–351

Ort DR (1986) Energy transduction in oxygenic photosynthesis: An overview of structure and mechanism. In: Staehelin LA and Arnzen CJ (eds) Encyclopedia of Plant Physiology, New Series, Vol 19, pp 143–196. Springer-Verlag, Berlin/Heidelberg/New York/Tokyo

Park RB (1965) In: Bonner J and Varner JE (eds) Plant Biochemistry. Academic Press, New York

Romanowska E and Albertsson P-A (1994) Isolation and characterization of the cytochrome *bf* complex from whole thylakoids, grana and stroma lamellae vesicles from spinach chloroplasts. Plant Cell Physiol 35: 557–568

Sane PV, Goodchild DJ and Park RB (1970) Characterization of chloroplast Photosystems I and 2 separated by a non-detergent method. Biochim Biophys Acta 216: 162–178

Sane PV (1977) The topography of the thylakoid membrane of the chloroplast. In: Trebst A and Avron M (eds) Encyclopedia of Plant Physiology New Series, Vol 5, pp 522–542. Springer Verlag, Berlin/Heidelberg/New York

Schmid GH, Jankowicz M and Menke W (1976) Cyclic photophosphorylation and chloroplast structure in the labellum of the orchid *Aceras anthropophorum*. J Microsc Biol Cell 26: 25–28

Shumway LK (1973) In: Edelstein SJ, Introductory Biochemistry. Holden-Day, Inc, San Francisco

Simpson D (1979) Freeze-fracture studies on Barley plastid membranes III. Location of the light-harvesting chlorophyll-protein. Carlsberg Res Commun 44: 305–336

Staehelin LA (1986) Chloroplast structure and supramolecular organization of photosynthetic membranes. In: Staehelin LA and Arnzen CJ (eds) Enzyclopedia of Plant Physiology, New Series, Vol 19, pp 1–84. Springer-Verlag, Berlin/Heidelberg/New York/Tokyo

Stefansson H, Wollenberger L and Albertsson P-Å (1994) Fragmentation and separation of the thylakoid membrane. Effect of light-induced protein phosphorylation on domain composition. J Chromatogr 668: 191–200

Svensson P and Albertsson P-Å (1989) Preparation of highly enriched Photosystem II membrane vesicles by a non-detergent method. Photosynth Res 20: 249–259

Svensson P, Andreasson E and Albertsson P-Å (1991) Heterogeneity among Photosystem I. Biochim Biophys Acta 1060: 45–50

Vallon O, Wollman FA and Olive J (1986) Lateral distribution of the main protein complexes of the photosynthetic apparatus in *Chlamydomonas reinhardtii* and in spinach: an immunocytochemical study using intact thylakoid membranes and a PS II enriched membrane preparation. Photobiochem Photobiophys 12: 203–220

Weibull C and Albertsson P-Å (1988) Ultrastructure of spinach thylakoids as seen in low-temperature and conventional embeddings. J Ultrastruct Mol Struct Res 100: 55–59

Whitmarsh I (1986) Mobile electron carriers in thylakoids. In: Staehlin LA and Arntzen CI (eds) Encyclopedia of Plant Physiology, New Series. Vol 19, pp 508–527. Springer-Verlag, Berlin/Heidelberg/New York/Tokyo

Wollenberger L, Stefansson H, Yu S-G and Albertsson P-Å (1994) Isolation and characterization of vesicles originating from the chloroplast grana margins. Biochim Biophys Acta 1184: 93–102

Wollenberger L, Weibull C and Albertsson P-Å (1995) Further characterization of the chloroplast grana margins: The non-detergent preparation of granal Photosystem I cannot reduce ferredoxin in the absence of $NADP^+$ reduction. Biochim Biophys Acta 1230: 10–22

Yu S-G, Björn G and Albertsson P-Å (1993) Characterization of a non-detergent PS II – cytochrome *b/f* preparation (BS) Photosynth Res 37: 227–236

Yu S-G, Stefansson H, Romanowska E and Albertsson P-Å (1994) Two dimensional electrophoresis of thylakoid membrane proteins and its application to microsequencing. Photosynth Res 41: 475–486