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The domain organization of the plant thylakoid membrane

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A model of the photosynthetic membrane from higher plants is presented. The different photosystems, PSIα, PSIβ, PSIIα and PSIIβ, are located in separate domains. The photosystems with the largest antenna systems, the alpha systems, are in the grana and the other in the stroma lamellae. In each grana disc PSIα is located in a flat annulus surrounding a circular PSIIα domain. In this the PSIIα units with the largest antennae are found in the center. The model is consistent with results from recent membrane fractionation experiments.

Photosynthesis; Thylakoid structure; Membrane domain; Photosystem I; Photosystem II; Cytochrome *f*

The structure of the photosynthetic membrane (the thylakoid) from plants is very complex and it has to be since it carries out diverse functions under varying conditions of light and temperature. Its main function is to capture light quanta and to convert light energy into useful chemical energy. To this end the light energy is used to drive a series of redox reactions whereby water is oxidized to protons and oxygen while ferredoxin is reduced concomitantly with the production of ATP.

The thylakoid membrane consists of essentially two compartments: the grana and the stroma lamellae interconnecting the grana. One can also distinguish between appressed and stroma-exposed membranes or between different membrane domains such as the partitions (appressed), the margins of both grana and stroma lamellae, the grana end membranes (stroma exposed) and the zone at the border between the grana and the stroma lamellae (Fig. 1).

It was shown long ago that, following mechanical press treatment of thylakoid membranes, centrifugation could separate a small vesicle fraction containing PSI from a heavier grana-rich fraction containing both PSI and PSII. It was concluded that the small vesicle fraction originated from the stroma lamellae, that these contain PSI but very little PSII, and that the grana of the native thylakoid contains both PSI and PSII [1].

Separation of press-treated thylakoids by aqueous two-phase partition resulted in the isolation of inside-out vesicles of the size of about 0.5 μm, which were highly enriched in PSII [2-6]. The vesicles were assumed to originate from PSII domains of about the same size in the grana [2], more precisely the appressed partitions [7-9]. A segregation of PSI and PSII has also been demonstrated by electron microscopy [10-12] and there has emerged a picture of an almost complete lateral segregation of PSI and PSII with PSI localized in the stroma exposed domains and PSII in the appressed partition region [7-9].

The thylakoid membrane, however, is much more complex in its structure. The photosystems are heterogenous and there are different types of both PSI and PSII. We here present a model for the structure and function of the thylakoid membrane which is based on recent fractionation experiments and which takes into account the heterogeneity of the photosystems. We assume the presence of two main types of PSI (α and β) and two main types of PSII (α and β). We also discuss the model with respect to both the Z-scheme of electron transport where the two photosystems cooperate in series and the alternative scheme of Arnon in which they function independently in parallel.

Photosystem II is heterogenous, both with regard to its antenna size and its redox properties. For a review see Black et al. [13]. One can distinguish between at least two types. One type, PSIIα, has an antenna size which is about two times larger than the other, PSIIβ. PSIIα is localized in the grana partitions and PSIIβ in the stroma lamellae [14]. Both types can evolve oxygen but the redox properties on the acceptor site are different. Both work well with PPBQ as electron acceptor but PSIIα is more efficient in reducing ferricyanide. Recently it has also been shown that PSIIα, but not PSIIβ, can reduce duroquinone [15], giving additional support to the notion that these two photosystems are indeed functionally different in the electron transport. The main role of PSIIα is in oxygenic electron transport. The function of PSIIβ is not known but

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Abbreviations: PSI, photosystem I; PSII, photosystem II; Chl, chlorophyll; P700, reaction center of PSI; PPBQ, phenyl-*p*-benzoquinone

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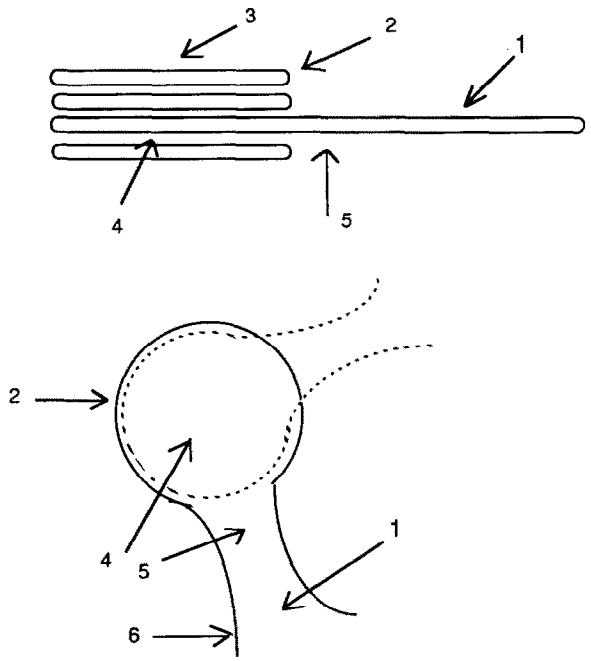

Fig. 1. Domains of the thylakoid membrane. Upper, cross-section; lower, top view. (1) flat stroma lamellae; (2) margin of grana; (3) end membrane; (4) partition region; (5) zone between grana and stroma lamellae; (6) margin of stroma lamellae.

several alternatives have been suggested; that PSIIβ supplies the necessary redox conditions to allow cyclic electron transport around PSI in the stroma membrane region [14]; that PSIIβ is a precursor to PSII [16]; that PSIIβ is a stage in a repair cycle of damaged PSII [16] and that conversion of PSIIα into PSIIβ is a way of regulating PSII activity and preventing photoinhibition [17].

Photosystem IIα in turn is heterogenous. Sonication of inside-out vesicles which harbour PSIIα, yields smaller vesicles, which can be separated into at least three subclasses (α_1, α_2 and α_3) that have different antenna sizes [18–19]. These originate from separate domains in the grana that have different average antenna sizes. It has been suggested that the PSIIα units with the largest antenna sizes are localized in the central core of the partition regions [18]. Alternatively the different PSIIα subclasses may arise from chloroplasts occurring at different layers in the leaf [18].

Photosystem I is also heterogenous. It has been demonstrated that there are at least two types of PSI with respect to the antenna size [20]. PSIα which is located in the grana has an antenna which is about 30% larger than that of PSIβ which is located in the stroma membranes. So far no difference in redox properties between these two systems has been demonstrated. However, the PSI activity at light saturation per P700 is different for PSIα and PSIβ [20], so one might expect differences in the redox properties between these photosystems also.

The cytochrome *b/f* complex is distributed all over the thylakoid membrane (see [6,11,12] for references) but not evenly. It is enriched together with PSIIα [20–22]. The content per chlorophyll is about 1.5–2 times higher in the most enriched PSIIα vesicles compared to the most PSI-enriched vesicles [21]. It is generally assumed that it is the same cytochrome *b/f* complex which is distributed over the thylakoid membrane, but this does not necessarily have to be the case. We propose that, just as in the case of PSI and PSII, there are at least two pools of the cytochrome *b/f* complex; one, α, associated with PSIIα in the grana while the other, β, is localized in the stroma lamellae and that they have different functions. The cytochrome $(b/f)\alpha$ is functioning in oxygenic electron transport while the $(b/f)\beta$ complex has its role in the cyclic electron transport around PSIβ in the stroma lamellae.

The ATP synthase is localized in unstacked membranes [12]. Upon fractionation of thylakoid membrane vesicles the ATP synthase complex seems to be enriched together with PSI; there are two pools of ATP synthase associated with PSIα-rich and PSIβ-rich vesicles, respectively [20].

Quantitative separation of grana and stroma membranes

We have recently devised a method whereby grana and stroma lamellae are quantitatively separated by one procedure using a non-detergent medium [20]. Stacked thylakoids are sonicated and the vesicles obtained are separated by counter-current distribution with an aqueous polymer two phase system. Two well-separated peaks are obtained in the separation diagram (Fig. 2) with negligible amounts of material between the two fractions. The left peak (alpha) originates from the grana; it is enriched in PSII in the form of PSIIα but also contains a considerable amount of PSI in the form PSIα. The peak to the right (beta) originates from the stroma lamellae and contains mainly PSI in the form of PSIβ and also PSIIβ (Table I). From the data in Table I we can calculate that there are about twice as many PSIIα reaction centers compared to PSIα in the alpha vesicles and 4–5 times more PSIβ reaction centers than PSIIβ centers in the beta vesicles.

Thus, the two photosystems with the larger respective antennae (PSIα and PSIIα) are located in the grana while the two photosystems with the smaller antennae, PSIβ and PSIIβ, are located in the stroma lamellae.

Since the separation of the two types of vesicles is so clear cut one can calculate the amount of chlorophyll associated with the four photosystems based on the chlorophyll distribution between the two peaks, the P700 content and the relative antenna sizes [23]. This calculation (Table II) shows that a substantial amount of chlorophyll is associated with PSIα located in the alpha-vesicles; it accounts for about 40% of the total PSI chlorophyll. About 25% and 38% of the total

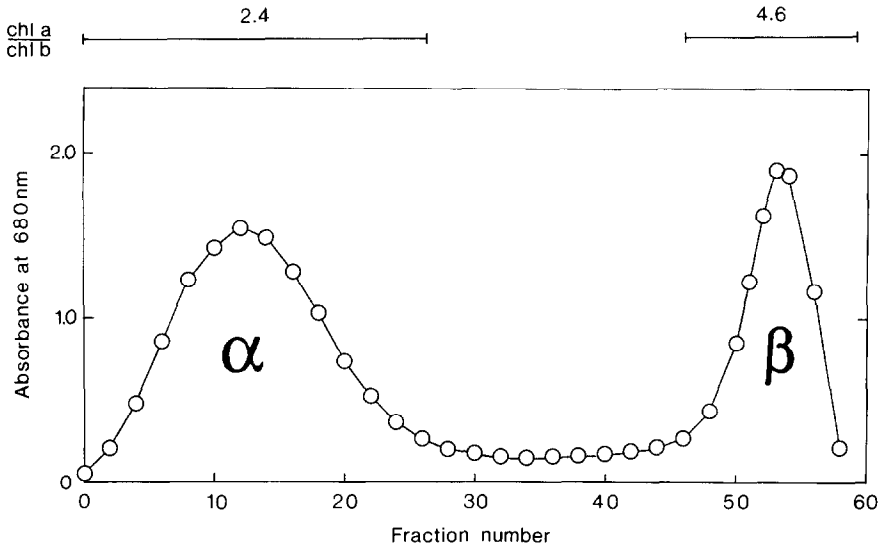

Fig. 2. Separation of sonicated, stacked thylakoids from spinach by counter-current distribution. The alpha peak originates from the grana and the beta peak from the stroma membranes [20].

chlorophyll is associated with PSIα and PSIIα respectively, i.e. as much as 40% of the grana chlorophyll is associated with PSIα.

The appressed grana and non-appressed stroma lamellae are different in all physical and chemical properties studied so far. They differ in resistance to mechanical stress by press treatment or sonication, resistance towards detergent solubilization [24], and with respect to polypeptide, pigment and lipid composition, surface electrical properties, etc [6]. (They are indeed so different that they can be considered to be two different membranes rather than different regions of the same membrane.) We propose that the grana and stroma lamellae have separate autonomous functions, i.e. they do not have to cooperate as is often assumed in the literature. If we accept the Z-scheme, we propose that the main function of the grana lamellae, with its two alpha systems, is to carry out oxygenic electron transport and reduce ferredoxin concommitantly with ATP production while the function of the stroma lamellae is to carry out cyclic photophosphorylation as it was originally suggested by Sane et al. [1].

Table I

Distribution of components between the two vesicle populations of Fig. 1 [20]. The thylakoids have 385 Chl/P700 and 400 Chl/atrazin binding sites [23]

	Chl *a/b*	Chl (%)	P700 (%)	PSII activity	Cyt. *f* (%)
α	2.4	63	36	85	57
β	4.6	37	64	15	43

Table II

Distribution of chlorophyll, in %, among the 4 photosystems [23]

PSIα	PSIIα	PSIβ	PSIIβ	PSI	PSII
25	38	34	3	59	41

However, one should also consider the possibility that PSIIα reduces ferredoxin directly without the involvement of PSIα according to the alternative scheme proposed by Arnon et al. [25]. It has been demonstrated for inside-out vesicles, i.e. PSIIα vesicles, [26,27] whole cells [28] and for isolated reaction centers [29] that PSII alone can reduce ferredoxin. If we accept the alternative scheme of Arnon, PSIIα would reduce ferredoxin directly without the involvement of PSIα whose function would be to carry out cyclic photophosphorylation together with PSIβ. It is of interest in this context that immunogold electron microscopy indicates that a significant amount of ferredoxin-NADP reductase is present in grana [12].

In both alternatives the grana are entirely responsible for the reduction of ferredoxin and NADP. We propose that the alpha systems in the grana are saturated earlier than the beta systems of the stroma lamellae when light increases. Hence, the stroma lamellae contribute relatively more ATP at high light intensities.

Where are PSIα and PSIIα localized in the grana?

Although PSIα and PSIIα are localized in the same vesicle population, the alpha vesicles, they are not randomly mixed. They can be separated by further sonication and phase partition of the inside-out (alpha) vesicles [22,30,31]. One subfraction contains almost only PSIIα and another is enriched in PSI [30,31]. These vesicles have a diameter of about 0.1–0.3 μm [22] and they must originate from domains of about the same size in the grana region. Since there is strong evidence for the localization of PSIIα in the partition regions both from fractionation experiments and electron microscopy studies it was suggested that PSIα is located in the remaining domains of the grana, i.e. the margins and the end membranes [20,23]. Selective solubilization of the margins with a detergent [32,33] and immuno-

electron microscopy [12] has indicated the presence of PSI in these domains. However, one should not exclude the presence of PSIα in separate domains of the appressed partitions. If the PSIα antennae contain LHCII polypeptides these may anchor the PSIα in the partition region and take part in the stacking process. A model which includes appressed PSI-rich membranes in grana has been described elsewhere [30].

In the model of Fig. 3 we place the PSIα not only at the curved edge of the margins but in a flat annulus that covers 40% of the circular grana membrane in order to accomodate the 40% of the grana chlorophyll which is associated with PSIα. The annulus of PSIα surrounds the circular area in the center which harbours PSIIα and which accounts for 60% of the area of the grana membrane. Hence, the ratio between the areas of the PSIα and PSIIα domains is 2 to 3 which is about the ratio between the chlorophyll associated with the two photosystems (Table II). This means that for a grana membrane which is 0.5 μm in diameter the width of the PSIα annulus is 0.06 μm. This model of the grana membrane is consistent with both the results of our fractionation experiments [20,22,23,30,31] and with immuno electron microscopy, see Fig. 6h in ref. [12].

The PSIIα domain of the grana membrane in turn consists of subdomains containing the subclasses of PSIIα. PSIIα_1 which has the largest antenna is located in a circular disc in the center, PSIIα_2 and PSIIα_3 in concentric rings between the central core of PSIIα_1 and the peripheral PSIα annulus as shown in Fig. 3. It makes sense to place the PSIIα_1 domain which has the largest antennae in the center of the granum where the light intensity will be less due to attenuation by the surrounding rings of membrane. (The PSIIα_1 domain is the origin of the BS fraction which has been characterized elsewhere [31] and which accounts for 20–30% of the alpha vesicles.) The relative area of the different PSIIα domains may vary with the light intensity during growth, the area of PSIIα_1 being larger in chloroplasts which dwell in low light. In support of this is the finding that the yield of the BS fraction is larger when isolated from inside-out vesicles from plants grown under low light conditions [34].

The stroma lamellae contain the two beta systems. (So far there is no evidence of a segregation between PSIβ and PSIIβ in the stroma lamellae.) The granum with its two alpha systems is the main machinery of the thylakoid for oxygenic electron transport and ferredoxin reduction both at low and high light intensities. The two beta systems are responsible for cyclic photophosphorylation, both at low and high light intensities, but contribute relatively more to ATP synthesis at high light intensities when relatively more ATP is needed by the chloroplast for the synthesis of proteins from amino acids, starch from glucose, repair of the photosynthetic apparatus and other ATP-requiring processes in the chloroplast. If the PSIIβ is only a stage

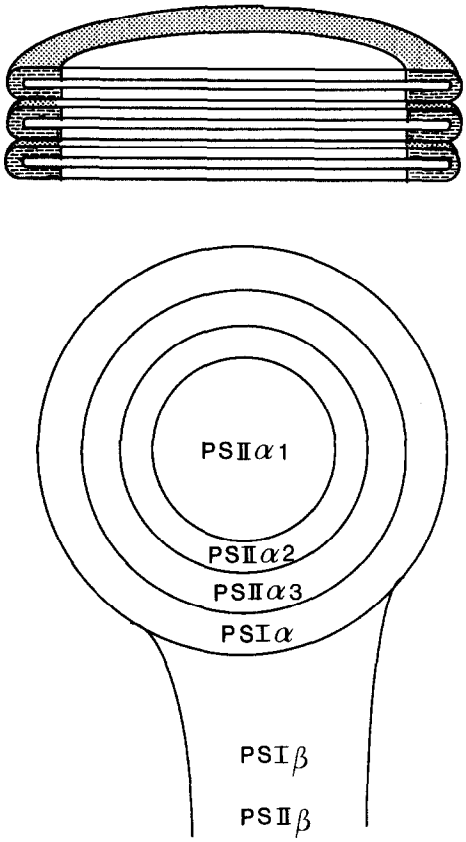

Fig. 3. Model of the thylakoid membrane from higher plants. Upper: section through the interior of a granum. The grey, flat annulus is the PSIα domain which encloses the central (white) domain of PSIIα. The connections to the stroma lamellae have been omitted. Lower: top view of a single thyalkoid; the PSIIα_1, PSIIα_2 and PSIIα_3 stand for domains with PSIIα units having different antennae sizes ($\alpha_1 > \alpha_2 > \alpha_3$). PSIα stands for the grey annular zone of the upper figure.

in a repair cycle [16] and not active in electron transport then PSIβ alone would be responsible for cyclic electron transport in the stroma membranes.

In each granum there is more chlorophyll associated with PSIIα than with PSIα (ratio about 3 to 2) and the question then arises, what about the balance in electron transport between the two photosystems according to the Z-scheme. This may not be a problem, however, since the capacity for light absorption by PSIIα is reduced relative to PSIα for the following reasons.

(1) The PSIIα domain is localized in the interior of the granum. It is surrounded by a layer of PSIα with a lateral thickness of about 0.6 μm. Light has to pass through this layer before reaching PSIIα which will be exposed to lower light intensity compared to PSIα. It is difficult to quantify this attenuation of light intensity but if we assume that the concentration of chlorophyll in the thylakoid membrane is 90 mM [35] and that the absorptivity is, on average, 70 $mM^{-1} \cdot cm^{-1}$ at 678 nm, then light illuminating the granum parallel to the membranes will be attenuated by about 10% after passing through a 0.06 μm thick PSIα layer (For simplicity we

assume Beers law to hold). Calculation of the absorption of light at 678 nm parallel to the grana membrane, by the entire grana disc, shows that the absorption of quanta per chlorophyll, on average, is 20–30% less for the PSIIα domain compared to the PSIα annulus.

(2) PSIIα antennae contain more chlorophyll *b* than PSIα. As discussed by Melis et al. [36] the chlorophyll *b*-rich antennae might be less effective in absorption of light particularly in the red region of the spectrum where the integrated absorbance of light by chlorophyll *a* in the 550–750 nm region is approximately 1.5-fold greater than chlorophyll *b* in 80% aceton. PSI-rich lamellae contain more β-carotene and this pigment transfers excitation energy specificially to the reaction center of PSI [36]. Hence, it appears that PSI because of its lower content of chlorophyll *b* and higher content of β-carotene, has some advantage over PSII in absorbing light expressed per total chlorophyll.

The discussion above is relevant when light is limiting. When light is saturating it is the turnover rate of the redox components which determine the balance between the two photosystems. Since the oxidation of the plastoquinone pool is the rate-limiting step in linear electron transport PSIα will not be rate limiting even if the number of its reaction centers is only half that of PSII.

In summary, the attenuation of the light by the PSIα layer around the PSIIα layer together with the different pigment composition of the two photosystems suggests that PSIα and PSIIα may well cooperate in a balanced electron transport according to the Z-scheme both at low and high light intensities.

The shielding of the inner PSIIα core of the granum by the annuli of the PSIα domain can also protect the PSIIα against photoinhibition as suggested by Critchley [37].

Our model emphasizes the autonomy of the grana and the stroma lamellae and is only a static model. In our opinion, studies on dynamic phenomena of the photosynthetic apparatus such as state 1 and state 2 transitions and phosphorylation-induced movements of light harvesting complexes should (if they occur) not only be studied with respect to movement from the grana to stroma lamellae, as has been the case hitherto, but also with respect to movements within the grana membrane. One should also be aware that there are at least two pools of cytochrome *b/f* and two pools of ATP synthase, when one interprets data from experiments with whole thylakoids, intact chloroplasts, or whole cells.

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