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Heterogeneity among Photosystem I

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Thylakoids from spinach were sonicated and separated by aqueous two-phase partitioning into two vesicle populations originating from grana (α vesicles) and stroma lamellae (β vesicles), respectively, according to a procedure described earlier (Andreasson et al. (1988) Biochim. Biophys. Acta 936, 339–350). The functional antenna sizes of Photosystem I for these two vesicle populations were determined by measuring the kinetics of the photooxidation of P700. Under green light illumination, the antenna size of Photosystem I of the α vesicles (PSI_α) was 40% larger (40% larger rate constant of photooxidation) than that of Photosystem I of the β vesicles (PSI_β). By using actinic light, which is preferentially absorbed by chlorophyll *b*, this difference was accentuated (60%) indicating that the antenna of PSIα contains relatively more chlorophyll *b*. The experiments did not support the possibility that this difference in functional antenna size was due to 'spill over' from Photosystem II. A model is presented which shows that PSIα is located in the periphery of the grana and PSI_β in the stroma lamellae.

Introduction

The number of functionally absorbing pigments in a photosystem, the antenna size, is an important parameter in photosynthesis [1,2]. It has been proposed that, at least for Photosystem II (PSII), the antenna size changes in response to different light conditions in higher plant chloroplasts [3–5]. In addition the PSII antenna within each chloroplast is heterogeneous in that a minor part of the PSII, designated $PSII_\beta$, has a significantly smaller antenna size than the major part, $PSII_\alpha$ [6,7]. There is also a heterogeneity within $PSII_\alpha$ [8,9].

The antenna size of Photosystem I (PSI) has previously been thought to be fairly homogeneous within each chloroplast. In a recent study [10], however, we presented evidence for a heterogeneity with respect to antenna size among PSI. Thylakoids were subjected to sonication and partitioning in an aqueous two-phase system. This yields grana membrane vesicles, the α fraction, enriched in PSII, but with a significant amount of PSI, and stroma membrane vesicles, the β fraction, enriched in PSI but also containing PSII. About 35% of the P700 content is in the α fraction and 65% in the β fraction. Light saturation curves of the PSI activity and kinetics of P700 photooxidation showed differences between the α and β vesicles indicating a larger antenna size of PSI in grana membranes than in stroma membranes.

Studies of the P700 photooxidation kinetics gives a direct estimation of the PSI antenna size. This method relies on the fact that the light absorption by PSI is proportional to the incident light intensity and the effective absorptive cross section of the PSI reaction center [11]. The effective absorptive cross section defines the antenna size. When thylakoid membranes are subjected to weak actinic illumination the rate of P700 photooxidation is limited by the rate of absorption by PSI, i.e. under light limiting conditions. The simplest kinetic pattern of P700 photooxidation occurs when all electron transport components between the photosystems are either oxidized or prevented from interacting with $P700^+$. This can be achieved by incubating the samples with KCN, with inhibits plastocyanin, thereby preventing electrons from reaching $P700^+$. The P700 photooxidation kinetics of our earlier study suggested that the antenna size of PSI in grana membranes is about 30% larger than that of PSI in stroma membranes. The PSI in the grana membranes with its larger

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; P700, reaction center of PSI; PSI and PSII, Photosystem I and II; HBW, half-band width.

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antenna size was named PSI_α and the PSI in stroma membranes was named PSI_β (compare with the nomenclature of PSII).

In the present work we further examine the photooxidation kinetics of PSI in the two thylakoid subpopulations, the α vesicles and the β vesicles, and in the starting material, sonicated thylakoids, under optimized conditions. We confirm the difference in antenna size between PSI_α and PSI_β. Our new data indicate that the difference is somewhat larger than previously observed. In addition we present evidence that the enlarged antenna of PSI_α contains a significant amount of chlorophyll *b*. Furthermore the difficulty in detecting heterogeneity in antenna size of whole thylakoids is discussed.

Materials and Methods

Chemicals

Dextran 500 was obtained from Pharmacia (Uppsala, Sweden). Poly(ethylene glycol) 4000 (Carbovax PEG 3350) was supplied by Union Carbide (New York, NY).

Vesicle isolation

Spinach (*Spinacia oleracia* L.) was grown at 20°C under coolwhite fluorescent light (incident intensity 400 μE m^2 s^{-1}) Chloroplast thylakoids were isolated as in [10]. The thylakoids were subjected to sonication followed by aqueous two-phase partitioning by a batch procedure in three steps according to [10]. The grana-derived membranes, the α fraction, and stroma lamellae membranes, the β fraction, were centrifuged 100 000 × *g* 90 min and resuspended in a small volume of 10 mM sodium phosphate buffer (pH 7.4), 5 mM NaCl and 100 mM sucrose. The prepared samples were immediately frozen in liquid nitrogen and stored there until use.

P700 kinetics

After 20 min of dark adaptation the samples were treated with potassium cyanide [12,13] for 2 h at 0°C. The incubation medium comprised 90 mM Tricine-KOH (pH 7.8), 150 mM KCN, 1 mM $MgCl_2$, 100 mM sucrose and 300 μM chlorophyll. After incubation the samples were diluted 10-times in a medium having the same composition as the incubation medium except for KCN. The P700 photooxidation kinetics were performed basically according to Melis [11] using an Aminco DW-2 spectrophotometer working in a dual wavelength mode. The measuring wavelength was set at 700 nm, while the reference beam was set at 730 nm. The optical pathlength of the cuvette for the measuring beam was 10 mm and for the actinic beam it was 4 mm. The measuring beams were transmitted by a RG 665 Schott filter. When green light was used, the actinic beam was transmitted by a broad band filter, 380–600 nm, and a 566.9 nm interference filter, HBW 80 nm, to give light between 500 nm and 600 nm. The so called chlorophyll *b*-light was obtained by transmittance of the actinic beam through a 480 nm interference filter HBW 8 nm. The actinic light intensities were 25 μE m^{-2} s^{-1} and 6 μE m^{-2} s^{-1} respectively. Signal averaging was performed with a Nicolet instrument corporation (NIC) model 527 instrument. The reaction mixture contained 100 mM sucrose, 90 mM Tricine (pH 7.8) 1 mM $MgCl_2$, 50 μM DCMU (omitted when indicated), 200 μM methylviologen and 30 μM chlorophyll.

Fluorescence induction measurements

Fluorescence induction measurements were performed at 20°C using an Aminco DW-2 spectrophotometer. The actinic illumination was provided in the green region of the spectrum by the same combination of filters as described above yielding exactly the same illumination conditions as when studying the P700 kinetics. The photomultiplier was shielded by an interference filter (RG 665). The signals were amplified by a differential amplifier (Tektronix AM 502). The reaction mixture contained 100 mM sucrose, 90 mM Tricine (pH 7.8), 1 mM $MgCl_2$, 25 μM DCMU (omitted when indicated) 100 μM methylviologen and thylakoid vesicles to yield a chlorophyll concentration of 6 μM. The thylakoid vesicle suspension was dark-adapted for at least 30 min before measurements.

Results

Grana-enriched membrane vesicles, α vesicles, and stroma enriched membrane vesicles, β vesicles, were obtained by aqueous two-phase partitioning of sonicated thylakoids [10]. Photooxidation kinetics of P700 were measured on these vesicle populations using weak green actinic illumination [11]. Typical traces (see Fig. 1) show that P700 in the α vesicles is photooxidized

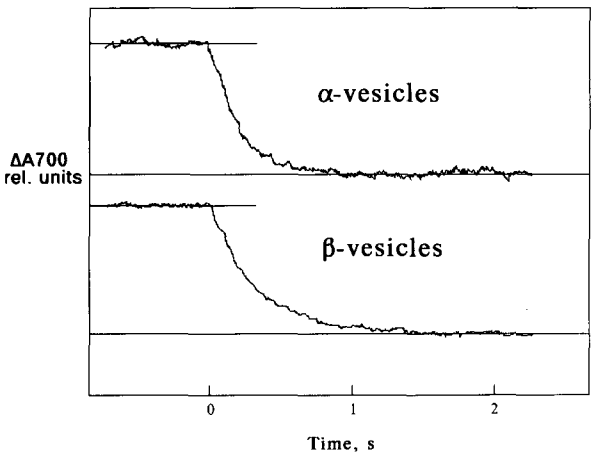


Fig. 1. Kinetic traces of the time-course of P700 photooxidation. The actinic, green light came on at 0 s. The traces of the α and β vesicles is the mean of 12 and 4 signals, respectively. Note the faster kinetics of the alpha vesicles.

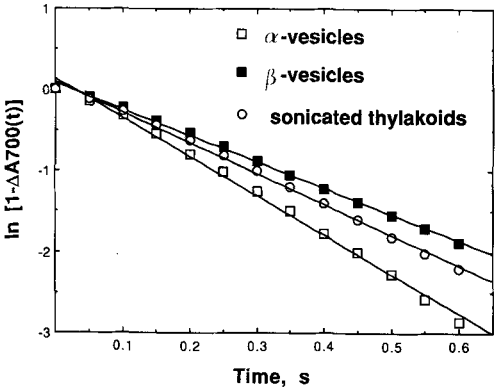


Fig. 2. First-order kinetic analysis of the traces in Fig. 1.

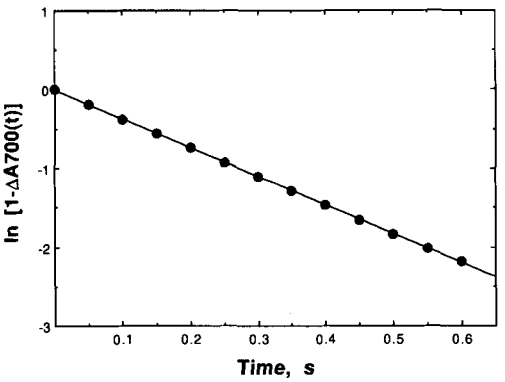


Fig. 3. Simulation of a first-order kinetic analysis of photooxidation of P700 in a mixture of two PSI populations, 35% having a 40% larger rate constant than the remaining 65%. The distortion from a straight line is not obvious.

significantly faster than P700 in the β vesicles. Semilogarithmic plots of these traces (see Fig. 2) give fairly straight lines with different slopes. The slopes are equivalent to the rate constants of P700 photooxidation [7,11] and are directly proportional to the antenna size of Photosystem I [11]. The rate constant of the α vesicles has a value that is about 40% higher than the rate constant of the β vesicles (Table I). This suggests that PSI_α has an antenna size that is about 40% larger than that of PSI_β. The rate constant for the starting material, sonicated thylakoids, shows an intermediate value.

In order to investigate why this heterogeneity is not obvious in the semilogarithmic plot of the P700 kinetics of whole thylakoids or sonicated thylakoids a simulation was performed. It was assumed that 35% of the PSI in thylakoids has a 40% larger antenna size than the remaining 65%. As shown in Fig. 3 there is no obvious bending of the curve, rather the points easily fit a straight line.

To test if there is also a qualitative difference between the antennae of PSI_α and PSI_β the kinetics of P700 photoxidation with an actinic light at 480 nm was studied. Using this light, which preferentially excites chlorophyll *b*, a larger difference in rate constants between PS_α and PSI_β, was obtained, PSI_α being 60% larger (Fig. 4, Table I). This suggests that a significant proportion of the enlarged antenna of PSI_α, is in the form of chlorophyll *b*.

In order to test whether 'spillover' from PSII might be the cause of the larger antenna of PSI_α a comparison was made between the kinetics of P700 photooxidation in α vesicles with and without closed PSII reaction centers. For this purpose dark adapted α vesicles were examined in the presence and absence of DCMU (closed and open PSII centers, respectively), as controls. In this experiment the KCN treatment was omitted since it was found to inhibit the PSII activity. The two kinetic traces for P700 photooxidation are almost identical in the two samples, with and without DCMU, Fig. 5a. The P700 is completely oxidized after 1.5 s. In a parallel experiment the kinetic traces of the variable fluorescence for the two samples were recorded. As can be seen in Fig. 5b the sample without DCMU shows a lag of about 2 s in the variable fluorescence indicative of quenching by the plastoquinone

TABLE I

Experimental values of the kinetic constant, K_{P700}, of P700 photooxidation for grana membranes (α vesicles), stroma lamellae membranes (β vesicles) and for the starting material, sonicated thylakoids (s.Thy), under different conditions

One experiment consists of a signal averaged output. The number of signals averaged varied depending on the type of vesicles measured; 12 for α vesicles, 3 for β vesicles and 6 for s.Thy.

	α vesicles	β vesicles	s.Thy
K_{P700} s^{-1} green light	4.6±0.3 (5)	3.2±0.1 (5)	3.7±0.3 (4)
K_{P700} s^{-1} Chl b-light	5.0±0.3 (4)	3.1±0.2 (4)	

Numbers in parantheses indicate the number of experiments.

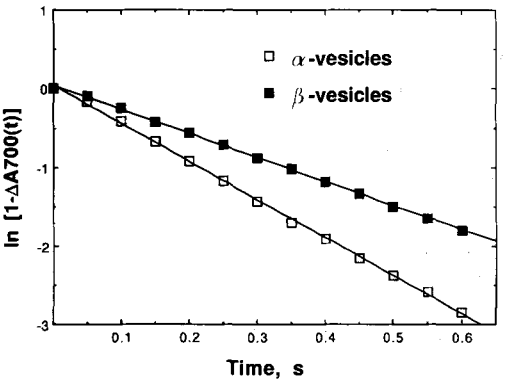
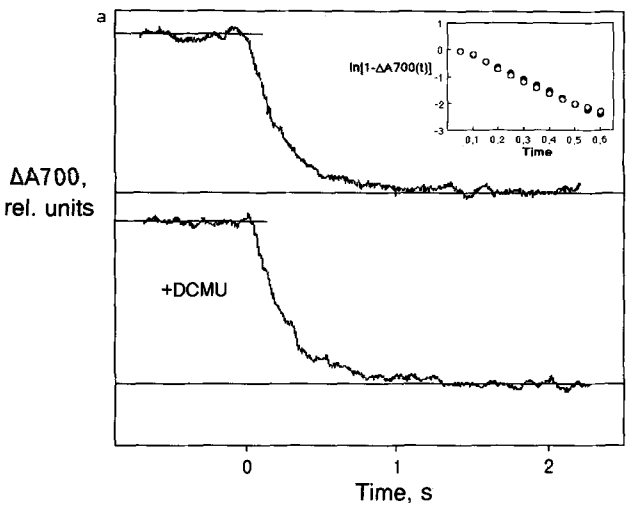


Fig. 4. First-order kinetic analysis of the traces of photooxidation of P700. In this case light that preferentially excites chlorophyll *b* was used (480 nm HBW 9). The difference in slope between α vesicles and β vesicles is more pronounced with this light than with the ordinary green light, (see Fig. 2 and Table 1).



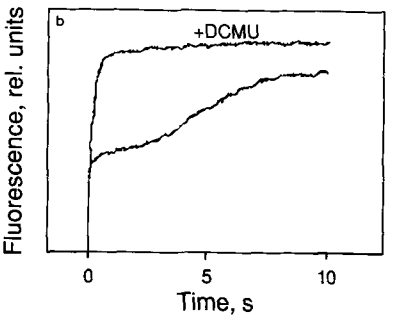


Fig. 5. (a) Kinetic traces of the time course of P700 photooxidation for α vesicles with and without DCMU added. The actinic light came on at 0 s. Inserted figure: First-order kinetic analysis of the traces (Hand-drawn best-fit curves were used for the analysis); ○, α vesicles; ●, α vesicles + DCMU. (b) Fluorescence yield at room temperature for α vesicles with and without DCMU added. The actinic light came on at 0 s. The light conditions were the same in the experiments of a and b.

pool, while the sample with DCMU shows an almost instantaneous rise in the variable fluorescence. This means that PSII in the sample without DCMU is active, and light absorbed by it during the first 2 s is used for electron transport to plastoquinone, while the PSII in the sample with DCMU is inactive. If there was any connection between the PSII and PSI units such that light absorbed by PSII could be utilized by PSI when the PSII centers were closed (spillover) then one would expect a faster photooxidation of P700 in the sample with DCMU. Since this is not the case (Fig. 5a, Table II) we conclude that the photooxidation of P700 is independent of the status of the PSII in the α vesicles. A similar study on the β vesicles could not be carried out since some of the plastocyanin may have been trapped in these vesicles during the preparation. The β vesicles have a right side out conformation, while the α vesicles are mainly inside out.

Discussion

These results demonstrate that PSI of spinach thylakoids can be separated into two populations, PSI_α and PSI_β, which differ both with regard to their antenna size and their pigment composition. PSI_α has an antenna size which is about 40% larger and also seems to contain more chlorophyll *b*.

Compared with our previous results, we now find a somewhat larger difference in rate constants between PSI_α and PSI_β (40% instead of 30%). We believe that this is a consequence of having in the present study, used a lower intensity of the actinic light. As pointed out by Melis [11] it is important to have such a low light intensity that light is rate limiting. Since PSI_α has a larger antenna, the size of this will be underestimated if the intensity of the actinic light increases over the range where it is strictly limiting.

The kinetic traces of P700 photooxidation of whole thylakoids do not reveal any heterogeneity among PSI. This is because the kinetic method can not resolve two components which differ by only 40% in their rate constants. This is shown by the simulation in Fig. 3. Our work stresses the importance of fragmentation and separation when one wants to study the heterogeneity among photosystems which differ with less than 100% in antenna size.

The molecular basis for the difference in antenna size is not known and we plan to investigate the difference in polypeptide composition between these two forms. This requires, however, access to pure preparations of the two forms of PSI. At the moment we can therefore only speculate. One alternative is that PSI_α is formed by the addition of a chlorophyll-protein complex to the PSI_β form. This additional antenna complex would have to contain relatively more chlorophyll *b* compared to the total antenna. It is of interest in this context that Bassi and Simpson [14] and Williams et al. [15] have, by detergent treatmant, isolated two types of PSI Complexes, one with and the other without a light-harvesting chlorophyll-protein complex, LHCII, which contains relatively much of chlorophyll *b*. It might be that PSI_α, but not PSI_β, contains this LHCII (or a LHCII like) complex and therefore is both

TABLE II

Experimental values of the kinetic constant, K_{P700}, of P700 photooxidation for grana membranes (α vesicles) with and without DCMU

Both experiments involve 12 individual measurements which were signal averaged

	α vesicles −DCMU	α vesicles +DCMU
K_{P700} s^{-1}	4.3	4.4

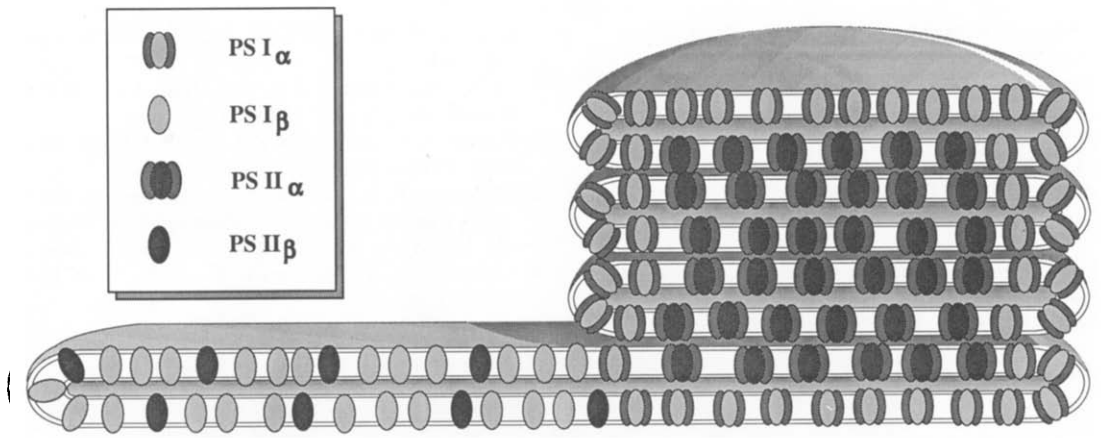


Fig. 6. Model of the thylakoid membrane with respect to the distribution of the photosystems. PSI_α and $PSII_\alpha$, which have the larger respective antennae are localized in the grana. $PSII_\alpha$ is in the central core of the grana partitions while the PSI_α is the periphery (margins, end membrane and a peripheral annulus of the grana disc). It is assumed that PSI_α and $PSII_\alpha$ are held in the grana by the stacking forces due to the presence of relatively more of LHCII in their antennae. PSI_β and $PSII_\beta$ are localized in the stroma lamellae. So far, there is no evidence for segregation between PSI_β and $PSII_\beta$.

larger and has more of chlorophyll b than PSI_β. It would also explain why PSI_α is located in the grana since the presence of LHCII, which promotes stacking, would keep the PSI_α in, or close to, the partition region.

Another alternative is that PSI_α has a larger functional antenna size because of spillover from PSII. Since PSI_α is found in α vesicles which contain relatively more PSII than the β vesicles one could argue that light absorbed by PSII, and not utilized for electron transport, could 'spill over' to PSI. Since we did not find any significant difference in the rate constants when DCMU was present or not (Fig. 5a, Table II) we conclude that 'spillover' is negligible under the conditions of our experiments.

It has been shown both by fractionation experiments [16–20] and by immunogold electron microscopy [21] that there is a considerable segregation between PSI and PSII in the grana region. PSII in the form of $PSII_\alpha$ is localized in the partition region [7] while PSI is localized in the periphery of the grana in addition to the stroma membranes [16–24]. Since we find that PSI_α is in the grana fraction (α vesicles) we concluded that it is the PSI_α which is localized in the periphery of the grana such as in the margins and the end membranes [10,23]. Since as much as about 40% of the chlorophyll of the α vesicles is associated with PSI [23,24] we have suggested that PSI_α is also localized in a peripheral annulus of the appressed grana disc [24], as shown in the model of Fig. 6. According to this model the PSI_α is held in the grana through association with LHCII (or LHCII like antenna complexes) by the same stacking forces which hold the $PSII_\alpha$ in the partition region.

According to our model, those photosystems which have the largest respective antennae, PSI_α and $PSII_\alpha$, are localized in the grana while the two β systems, PSI_β and $PSII_\beta$, which have the smaller antennae are in the stroma membrane. This must be of functional significance. We have elsewhere [23,24] suggested that the two α systems of the grana are responsible for the oxygenic electron transport and the reduction of NADP, while the stroma membranes with their two β systems carry out cyclic photophosphorylation. Since the β systems have smaller antennae these will be saturated at higher light intensities than the two α systems and their contribution to ATP synthesis might be relatively larger at high light intensities when relatively more ATP is needed for starch synthesis from glucose, for protein synthesis from amino acids and for repair processes.

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