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Fractionation of the thylakoid membranes from tobacco. A tentative isolation of 'end membrane' and purified 'stroma lamellae' membranes

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Abstract

Thylakoids isolated from tobacco were fragmented by sonication and the vesicles so obtained were separated by partitioning in aqueous polymer two-phase systems. By this procedure, grana vesicles were separated from stroma exposed membrane vesicles. The latter vesicles could be further fractionated by countercurrent distribution, with dextran–polyethylene glycol phase systems, and divided into two main populations, tentatively named 'stroma lamellae' and 'end membrane'. Both these vesicle preparations have high chlorophyll *a*/*b* ratio, high photosystem (PS) I and low PS II content, suggesting their origin from stroma exposed regions of the thylakoid. The two vesicle populations have been compared with respect to biochemical composition and photosynthetic activity. The 'end membrane' has a higher chlorophyll *a*/*b* ratio (5.7 vs. 4.7), higher P700 content (4.7 vs. 3.3 mmol/mol of chlorophyll). The 'end membrane' has the lowest PS II content, the ratio PS I/PS II being more than 10, as shown by EPR measurements. The PS II in both fractions is of the β-type. The decay of fluorescence is different for the two populations, the 'stroma lamellae' showing a very slow decay even in the presence of $K_3Fe(CN)_6$ as an acceptor. The two vesicle populations have very different surface properties: the end membranes prefer the upper phase much more than the stroma lamellae, a fact which was utilized for their separation. Arguments are presented which support the suggestion that the two vesicle populations originate from the grana end membranes and the stroma lamellae, respectively.

Keywords: Thylakoid; End membrane; Stroma lamellae; Aqueous two-phase partitioning; Photosystem I; *(Nicotiana tabacum)*

1. Introduction

Fragmentation of the photosynthetic membrane followed by separation and characterization of the fragments has been a successful approach to increase our understanding of the structure and function of the photosynthetic apparatus. Previous studies from our and other laboratories have shown that a combination of press treatment or sonication together with partitioning in aqueous polymer two-phase system can be exploited to isolate subthylakoid membrane vesicles originating from the stroma lamellae, from the appressed grana core membrane and from the margins of the grana. These three vesicle populations have been characterized with respect to pigment and protein composition, photochemical activity, spectra and structure [1–6]. For a review of the procedure see [7].

Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; DQ, duroquinone; FNR, ferredoxin:$NADP^+$ oxidoreductase; MES, 2-[*N*-Morpholino]ethanesulfonic acid; $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidized form); PpBQ, phenyl-*p*-benzoquinone; PS I, photosystem I; PS II, photosystem II; P700, primary donor of PS I

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Based on these fractionation studies and electron microscopy, a model has been presented for the thylakoid membrane [8]. According to this model photosystem II (PS II) α is localized in the appressed grana core while PS I α is in the grana margin. These two photosystems are responsible for the linear electron transport while in the stroma lamellae, where PS I β and PS II β are localized, the cyclic electron transport around PS I takes place.

In this paper we describe experiments which demonstrate that the previously isolated stroma lamellae fraction is heterogeneous and can be separated into two populations. Our work suggests that one of these originates from the single paired stroma lamellae while the other originates from the grana end membrane, i.e., the stroma exposed membranes at the two ends of a grana stack (see Fig. 1 for the nomenclature of the different parts of the thylakoid).

2. Materials and methods

2.1. Cultivation of tobacco plants and preparation of thylakoid membranes

Tobacco plants (*Nicotiana tabacum*) were grown hydroponically for 4–5 weeks at 24°C with a 12/12 h light/dark period. A light intensity of 300 μE/m^2 s with cool fluorescent light was obtained by dysprosium lamps.

Thylakoids were prepared as described in [1]. Sonication of thylakoids was done according to [4] either with 1 or 2 mM $MgCl_2$ as indicated in the respective figure legends. The sonicate was fractionated either by countercurrent distribution or by a batch procedure as described earlier [1]. The polymer and ionic composition in the different experiments are given in the respective figure legends.

2.2. Partition for characterization of the surface properties

A series of tubes were set up, all containing the same amount of thylakoids or vesicles and the same ionic composition but different concentrations of polymers [9,10]. The ionic composition was 10 mM sodium phosphate (pH 7.4), 5 mM NaCl, 20 mM sucrose. The concentration of polymer is plotted as the abscissa (Fig. 4). The absorption at 680 nm of top phase diluted 2.5 times is plotted as the ordinate and it is expressed as per cent of absorption at 680 nm of a sample diluted similarly (Fig. 4).

2.3. Electron transport measurements

PS II activity was measured as oxygen evolution using a Clark-type O_2 electrode (Hansatech, Norfolk, UK). The buffer consisted of 100 mM sucrose, 50 mM Mes (pH 6.5), 10 mM NaCl, 5 mM $MgCl_2$. The sample concentration was 20 to 25 μg Chl/ml (1 ml final volume). Other assays comparing the reduction potential of PS II were done using either DQ or PpBQ at a concentration of 200 μM in both cases or $K_3Fe(CN)_6$ at a concentration of 2 mM. All measurements were made at 20°C.

The reduction of $NADP^+$ as PS I activity was followed spectrophotometrically at 340 nm according to Cleland and Bendall [11] in an Aminco DW-2 spectrophotometer except that only 2 μM ferredoxin was used. DCIP and ascorbic acid were included at 130 μM and 4.5 mM, respectively. The buffer consisted of 100 mM sucrose, 50 mM Tricine (pH 7.9), 5 mM $MgCl_2$, 5 mM KH_2PO_4, 6 mM glucose. Glucose oxidase and catalase were included in the assay mixture to maintain anaerobicity [11]. Sample concentration was 11 μg Chl/ml. The sample cuvette was illuminated from the side with saturating red light (RG630, heat filter, ∼1000 μE/m^2 s). A 340-nm interference filter and a 600-nm cut-off filter were placed in front of the photomultiplier. In some cases, 0.3 units/ml ferredoxin–NADP reductase was included. An absorbance coefficient of 5.1 mM^{-1} cm^{-1} for NADH at 340 nm was used [12]. The rate of $NADP^+$ reduction was calculated from the initial slope.

2.4. Measurements of cytochrome b_{559}, cytochrome f and P700 content

Cytochrome b_{559} content was estimated spectrophotometrically according to [13]. Cytochrome f was determined spectrophotometrically from the reduced minus oxidized absorbance change at 554 nm [14] using an absorption coefficient of 17.2 cm^2 mmol^{-1}. The reaction buffer contained 8 mM sodium phosphate buffer (pH 7.4), 4 mM NaCl,

80 mM sucrose, 1% Triton X-100. The concentration of Chl during all measurements was 100 μg/ml.

P700 content was obtained with an Aminco DW-2 spectrophotometer operated in a split beam mode. The amplitude of the light-minus-dark absorbance change at 700 nm was measured using an extinction coefficient of 64 $mM^{-1}\,cm^{-1}$ [15]. The reaction buffer contained 0.02% (w/w) sodium dodecylsulfate, 2 mM methylviologen, 2 mM sodium ascorbate, 15 mM Tricine (pH 7.8), 5 mM $MgCl_2$, 10 mM NaCl, 400 mM sucrose. Final concentrations of Chl during measurements was 20–30 μg/ml.

2.5. Fluorescence measurements and EPR spectroscopy

Flash-induced Chl *a* fluorescence was detected with a PAM 100 fluorimeter (Walz, Effeltrich, Germany). Single saturating actinic flashes were provided by a Walz XST-103 xenon flash lamp of 11 μs half peak width. A blue-green Scotch BG3 filter was used and the intensity at the sample level was 2×10^5 W/m^2. Data were collected and analysed using the Q_A-FIP program (version 4.3, Q_A-Data Oy, Turku, Finland).

X-Band EPR spectra at room temperature were recorded with a Bruker ESP380E spectrometer. For kinetic measurements, saturating laser flashes (6 ns, 300 mJ at 532 nm) from Nd-YAG laser were directed into the EPR cavity. Triggering of the laser and EPR recording were done by a home-made trigger device.

3. Results

3.1. Countercurrent distribution

Two different vesicle populations are obtained when stacked thylakoids are sonicated and then separated by countercurrent distribution using aqueous polymer two-phase systems [1]. One population originates from the grana (appressed grana core and grana margins) while the other originates from the stroma lamellae and probably also the grana end membranes [1,8]. The two populations have very different affinities for the two phases; the grana vesicles (inside-out) prefer the lower phase while the other vesicles (right side out) prefer the upper phase. Because of this a simple preparative batch procedure has been developed resulting in two fractions, B3 and T3, containing grana and stroma lamellae vesicles, respectively. In this work we used both countercurrent distribution and the batch procedure.

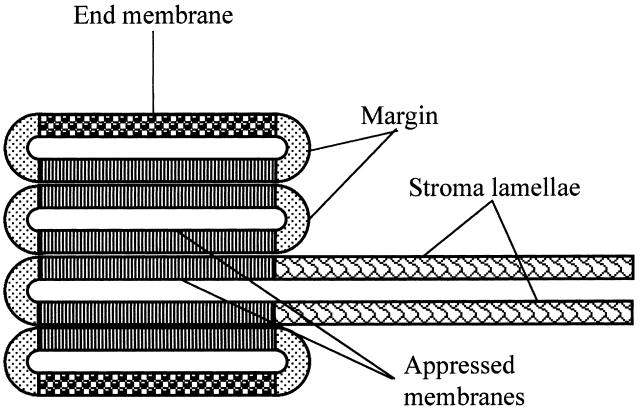

Fig. 1. Nomenclature of the different domains of the thylakoid from plant chloroplasts with stacked grana. The grana are composed of a central core with appressed circular membranes forming the grana stack and a periphery composed of the margins and the two flat, circular end membranes at the two ends of the grana stack. The stroma lamellae, which connect different grana stacks, are single paired. End membranes, margins and stroma lamellae are exposed to the surrounding chloroplast stroma.

Fig. 2A,B show such countercurrent distribution diagrams for tobacco thylakoids. In both cases, two well separated peaks are obtained. The left-hand peaks have low Chl *a*/*b* ratios and represent the grana vesicles while the right-hand peaks have high Chl *a*/*b* ratios and represent the stroma lamellae. Since the two peaks in Fig. 2A and B are so well separated we were able to use a batch procedure to prepare the stroma lamellae fraction in both cases. The only difference between the two experiments is the concentration of magnesium ions during sonication, being 1 mM in Fig. 2A and 2 mM in Fig. 2B. As seen, the yield of the right-hand peak is much less in the experiment of Fig. 2B, 23% instead of 39%. This led us to the conclusion that different regions of the thylakoid membrane were detached in the two cases, and we hypothesized that one would expect a heterogeneity of the vesicle population represented by the right-hand peak of Fig. 2A (stroma lamellae vesicles). To test this hypothesis we analysed, by countercurrent distribution, the stroma lamellae vesicles obtained by sonication in 1 and 2 mM

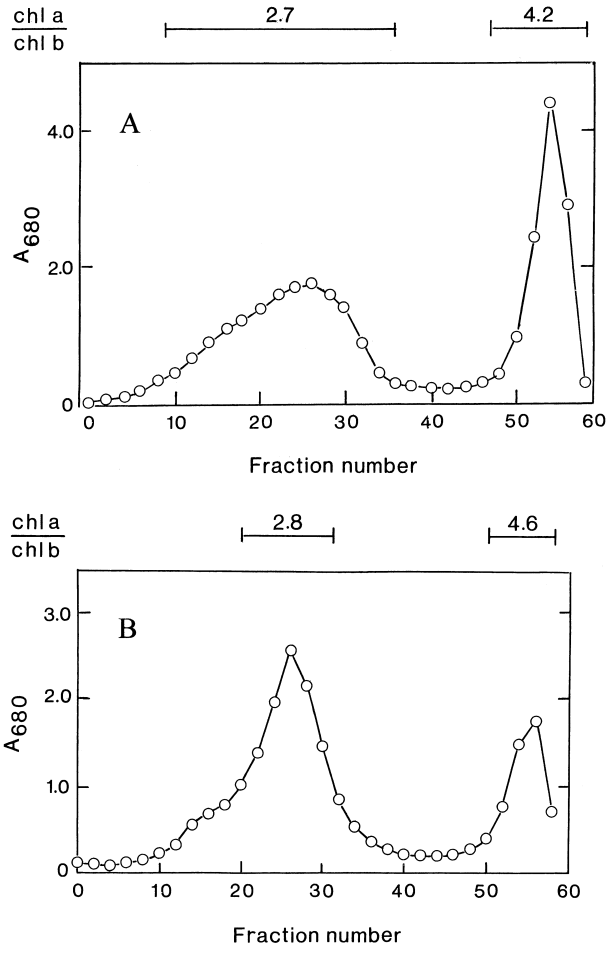

Fig. 2. Separation of grana vesicles (left) from stroma lamellae membrane vesicles (right), from tobacco. Stacked thylakoids were sonicated and then subjected to countercurrent distribution. Composition of phase system: 5.8% (w/w) dextran, 5.8% (w/w) polyethylene glycol, 10 mM sodium phosphate (pH 7.4), 5 mM NaCl, 20 mM sucrose. (A) Sonication in 1 mM $MgCl_2$. The right-hand peak comprises 39% of the total on a Chl basis. (B) Sonication in 2 mM $MgCl_2$. The right-hand peak comprises 23% of the total on a Chl basis.

$MgCl_2$, respectively. Fig. 3A,B show the countercurrent distribution of the two stroma lamellae vesicle populations, i.e., vesicles represented by the right-hand peaks of Fig. 2A,B. For these countercurrent distribution experiments we used a higher polymer concentration. Fig. 3A shows two major peaks, a broad peak to the left and a narrow one to the right; 34% of the material is in the latter peak. In the experiment of Fig. 3B, i.e., when sonication was carried out with 2 mM $MgCl_2$, one major peak is obtained with only 4% in the small peak to the right.

Apparently the vesicles represented by the right-hand peak of Fig. 3A have not been detached from the thylakoids during sonication with 2 mM $MgCl_2$ since they are almost absent in the experiment of Fig. 3B. The two vesicle populations of Fig. 3A both have high chlorophyll *a*/*b* ratios, indicative of the origin from stroma exposed membranes: they were still separated, showing that they have different surface properties. As a working hypothesis we suggested that the vesicles represented by the right-hand peak of Fig. 3A originate from the grana end membranes while those of the peak to the left originate from the single paired membranes of the stroma lamellae. These two fractions will hereafter be named 'end

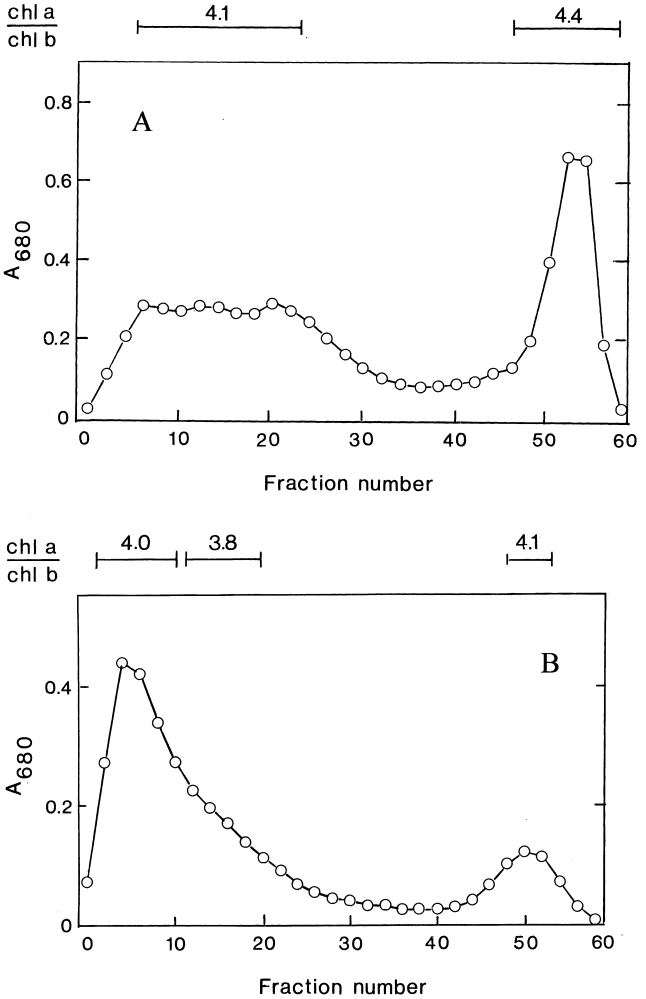

Fig. 3. (A) Countercurrent distribution of vesicles represented by the right-hand peak of Fig. 2A. (B) Countercurrent distribution of vesicle represented by the right-hand peak of Fig. 2B. Phase composition as in Fig. 2 except that the polymer concentration was 6.1% (w/w).

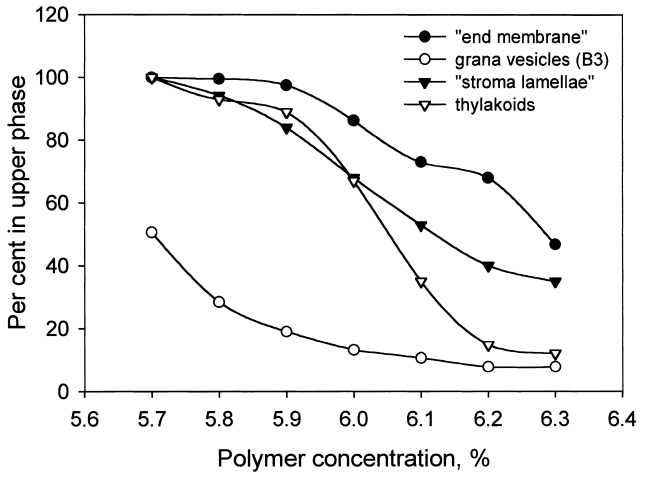

Fig. 4. Partition of different subchloroplast membrane vesicles in a phase system as function of the polymer concentration (per cent of each of dextran and polyethylene glycol).

membrane' and 'stroma lamellae', respectively. They can be isolated preparatively by the batch procedure [1,7] where they are recovered in the T3 fraction; in the case of 'end membrane' after sonication of thylakoids with 1 mM $MgCl_2$ and using a phase system with 6.1% (w/w) of each polymer, and in the case of 'stroma lamellae' after sonication of thylakoids with 2 mM $MgCl_2$ and using a phase system with 5.8% (w/w) of each polymer. These two vesicle populations have been characterized as follows.

3.2. Differences in partition behaviour

Partition of thylakoids and subthylakoid vesicles in a 'polymer series', i.e., in phase systems with increasing polymer concentration, is shown in Fig. 4. The inside-out grana vesicles have the lowest partition, i.e., they prefer the lower phase relatively more than the other particles. Thylakoids, 'stroma lamellae' and 'end membrane' are all mainly in the upper phase in the polymer concentration region 5.7–5.9. At higher concentration the curves for these three particles diverge, the 'end membranes' having the highest partition. This figure demonstrates differences in surface properties between the different vesicles. One can also learn from Fig. 4 that a polymer concentration of 5.8–5.9% is optimal for separation of grana vesicles from 'stroma lamellae' and 'end membrane' vesicles and that 6.1–6.2% is optimal for separation of the two latter vesicles.

3.3. Acceptor side properties of PS II

PS II centres are heterogeneous (PS II α and PS II β) with respect to antenna size and the ability to donate electrons to secondary electron acceptors like plastoquinone (see [16] for review). PS II β has a midpoint potential on the acceptor side (Q_A) of about +120 mV, while that of PS II α is −20 mV [17,18]. The redox potential of PS II can be assayed by measuring the rates of oxygen evolution in the presence of different types of quinones [19], which have similar lipophilicities but differ in midpoint potential. This allows a comparison of the redox properties of different PS II populations regardless of their location in the membrane. When PpBQ (E_m = +279 mV) [13] is used as an acceptor, PS II α and PS II β evolve oxygen. If PpBQ is substituted by DQ (E_m = +52 mV) only PS II α contributes to O_2 evolution. Table 1 shows the rates of oxygen evolution by the PS II centres in the 'end membrane' and 'stroma lamellae' fractions when measured in the presence of PpBQ, DQ or $K_3Fe(CN)_6$. Neither fraction contains PS II α as shown by the lack of oxygen evolution in the presence of DQ. 'End membrane' fractions contain only trace amounts of PS II β, shown by the very low activity in the presence of PpBQ and $K_3Fe(CN)_6$; this activity was about half that of 'stroma lamellae'.

Flash-induced fluorescence shows very small in-

Table 1
Acceptor side properties of PS II

Electron acceptor	E_m (mV)	Thylakoids	'Stroma lamellae'	'End membrane'
PpBQ	+279	95	24.5	11
DQ	+52	50.6	0	0
$K_3Fe(CN)_6$	+430	26.5	11.6	8.5

The rates of oxygen evolution (μmol O_2/mg of Chl h) by PS II in different fractions isolated from thylakoids when measured in the presence of different acceptors.

duction of variable fluorescence, F_v, which represents, although not entirely, the activity of PS II [20]. The F_v/F_m ratio (F_m represents maximal level of fluorescence after the saturating flash), measured in presence of DCMU, was 0.24 for the 'stroma lamellae' fraction, 0.21 for the 'end membrane', and 0.90 for thylakoids. Analysis of the decay of the fluorescence shows differences between the two fractions. In the 'stroma lamellae' fraction fluorescence shows a very slow decay (86 ms half time) even in the presence of acceptor $K_3Fe(CN)_6$ (56 ms half time) indicating impaired forward electron transport from primary quinone acceptor Q_A^-. In the presence of DCMU, decay half time was about 200 ms which reflects recombination between Q_A^- and the donor side of PS II. The fluorescence decay in the 'end membrane' fraction was different. Addition of $K_3Fe(CN)_6$ did not change fluorescence decay: half time was 12–14 ms. In the presence of DCMU, the decay half time was 54 ms. This is too fast for recombination with the functional donor side of PS II and probably reflects recombination between Q_A^- and Tyr_Z^{ox}, a secondary donor in PS II, indicating the presence of inactive PS II centres lacking Mn cluster. These results are in agreement with the conclusion that both fractions studied contain a small amount of PS II in the form of PS II β with, relative to PS II α, impaired activity on the acceptor side, and that the lower capacity for O_2 of the 'end membrane' fraction is due to some impairment on the donor side.

3.4. Reduction of $NADP^+$

The light-induced absorbance changes occurring at 340 nm were done to measure the reduction of $NADP^+$ by both fractions in the presence of donors like ascorbate and DCIP (Table 2). The activity of both fractions increased upon the addition FNR, 13 times for the 'end membrane' fraction and seven times for the 'stroma lamellae' fraction. This suggests that the 'stroma lamellae' fraction contains more native, membrane-bound FNR.

3.5. P700 content and light saturation curves

The 'stroma lamellae' fraction has a Chl *a/b* ratio of 4.7 and the 'end membrane' fraction 5.4–5.7 (Table 2). The estimation of P700 content shows that the 'end membrane' fraction is more enriched in PS I than the 'stroma lamellae'. For the 'end membrane' P700/Chl (mmol/mol) is about 4.7, while that for the 'stroma lamellae' is 3.3 and for thylakoids 1.7 (Table 2). Both fractions are saturated at approximately the same light intensity (data not shown), which does not suggest a significant difference in antenna size between the two fractions.

Analysis of PS I content by EPR measurements on the basis of signal I ($P700^+$, g value 2.0026, [21]) induced by continuous illumination, shows that the content of PS I in the 'end membrane' fraction was 1.23-times higher than in the 'stroma lamellae' fraction, Fig. 5A. Kinetics of $P700^+$ decay were similar in both preparations (2.2–2.4 s half time, Fig. 5B). The content of PS II was also estimated by EPR measurements of signal II_{slow} which originates from Tyr_D, another secondary donor in PS II with $g = 2.0046$ [21], and was found to be more than 10-times lower than PS I in both preparations (PS I/PS II = signal I/signal $II_{slow} > 10$).

3.6. Cytochrome b_{559} HP and cytochrome f content

We also measured the amount of cytochrome b_{559}.

Table 2
Chlorophyll *a/b* ratio, cytochrome b_{559} HP form (% of total cytochrome b_{559} content), P700 content and $NADP^+$ reduction in thylakoids, 'end membrane' and 'stroma lamellae' fractions

	Thylakoids	'Stroma lamellae'	'End membrane'
Chl *a/b* (mol/mol)	2.9	4.7	5.7
Cytochrome b_{559} HP (%)	75	24	12
P700/Chl (mmol/mol)	1.7	3.3	4.7
$NADP^+$ μmol $NADP^+$/mg Chl h	130	21	14
$NADP^+$[a] μmol $NADP^+$/mg Chl h	130	150	180

[a]With added soluble FNR, 0.25 units.

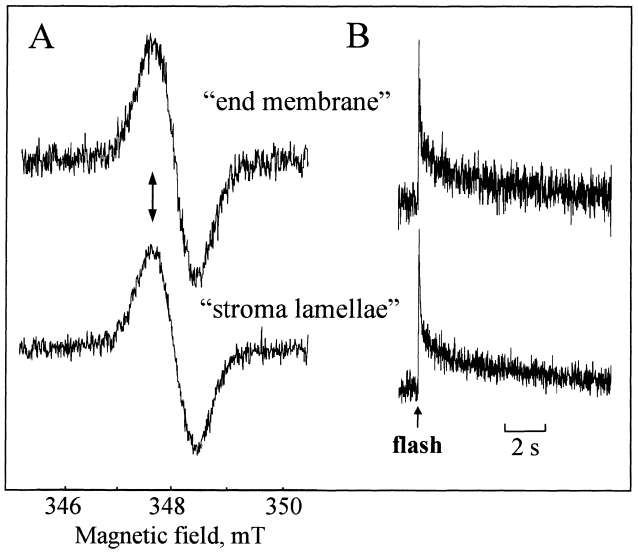

Fig. 5. (A) Induction of EPR signal I ($P700^+$) by continuous light (1000 W lamp, white light filtered through copper sulfate solution and directed into EPR cavity) in 'end membrane' and 'stroma lamellae' fractions. EPR conditions: microwave frequency 9.77 GHz; microwave power 114 μW; conversion time 81.92 ms; time constant 20.48 ms; modulation amplitude 0.5 mT; temperature 294 K. Arrow indicates field position for kinetic measurements. (B) Kinetic measurements of $P700^+$ decay in 'end membrane' and 'stroma lamellae' fractions. EPR conditions the same as in A except for conversion time 10.24 ms and time constant 2.56 ms. Both traces represent averaging of 100 transients. Chlorophyll concentration was 2–3 mg/ml for both fractions.

It is believed that different thermodynamic forms of cytochrome b_{559} are distributed asymmetrically between appressed (grana) and nonappressed (stroma exposed) regions of the thylakoid membrane. The low-potential form (LP) is associated with non-active PS II centres, isolated from stroma lamellae, while the high-potential form (HP) dominates in active centres, distributed in the granal part of membrane [22]. According to our measurements, if thylakoids contain 75% of cytochrome b_{559} in the HP form, then the 'stroma lamellae' contains 24.5% and 'end membrane' fraction only 12% (Table 2). This low content of cytochrome b_{559} HP in both fractions agrees with their origin from the stroma exposed part of the thylakoid. The cytochrome *f* concentration was the same in the 'end membrane' and the 'stroma lamellae' fractions (not shown).

4. Discussion

From electron micrographs of stacked thylakoids of higher plants, one can distinguish between different membrane domains (Fig. 1). So far, four different types of subthylakoid vesicles have been isolated by a combination of sonication and aqueous two-phase partitioning. They originate from the grana, stroma lamellae, grana margins and the appressed grana core membranes. They all differ in their biochemical composition and ability to carry out photosynthetic reactions [1–8].

The amount of end membrane in the thylakoid, as seen by electron microscopy, varies depending on species and growth conditions. Chloroplasts having grana with only a few paired membranes per granum have relatively more end membranes than chloroplasts with large grana stacks. In contrast, the amount of stroma lamellae is fairly constant, about 20% of the thylakoid, for many plant species (see Table 1 in [8]). Tobacco chloroplasts have small grana stacks and hence relatively many end membranes per chloroplast. They account for about the same percentage fraction of the thylakoid as the stroma lamellae [8].

The end membranes are single membranes with one surface, the lumen surface, adjacent to an appressed membrane pair of the granum and the other surface facing the stroma, while the stroma lamellae are single paired membranes surrounded by stroma on both sides. Thus, the end membranes are distinct structural domains which account for a substantial fraction of the thylakoid membrane and it is therefore of great interest to be able to isolate end membrane vesicles for biochemical and functional characterization.

The present work demonstrates that the so-called stroma lamellae fraction obtained either by countercurrent distribution or by a preparative batch procedure (T3) of sonicated thylakoids is heterogeneous and consists of two major populations of vesicles, here named 'end membrane' and 'stroma lamellae', respectively. Both have a high Chl *a*/*b* ratio, low PS II and high PS I content (PS I/PS II > 10). The PS II is of the β type. However, the two vesicle preparations differ significantly in their Chl *a*/*b* ratio, oxygen evolution, cytochrome b_{559} and P700 content, and also in the ability for $NADP^+$ reduction. The ki-

netics of the fluorescence decay differ also between the two populations. The 'end membrane' fraction is more enriched in PS I and more depleted in PS II than the 'stroma lamellae' fraction. This is indicated by the higher Chl *a*/*b* ratio and the higher P700 content of the 'end membrane' fraction. The 'end membrane' fraction also has higher FNR activity. Both fractions contain cytochrome *f* supporting the notion that the cytochrome *bf* complex is found all over the thylakoid membrane.

The largest difference between the two populations is in their surface properties; that is why they can be so well separated. One explanation for this difference could be difference in sideness. So far, however, we have not been able to demonstrate such a difference.

The question then arises from where in the thylakoid these two vesicle types originate. We suggest that one originates from the single paired stroma lamellae while the other comes from the grana end membranes for the following reasons.

(1) The yield of the right-hand peak of the countercurrent distribution (Fig. 2A) of sonicated thylakoids is in the range of 35–40%, which is much more than one would expect from the amount of single paired stroma lamellae found in electron micrographs of chloroplasts from sections of higher plant leaf cells. Measurements on electron micrographs from several plant species show that only $20 \pm 5\%$ of the thylakoid is in the form of single paired stroma lamellae [8]. Since the composition of the thylakoid is dominated by the light harvesting complexes, it is reasonable to assume that the length of thylakoid membrane is proportional to the Chl (*a*+*b*) content, i.e., approximately the absorption at 680 nm. The additional vesicles found in the fractions represented by the right-hand peak of Fig. 2A must therefore come from the grana region of the thylakoid, either the grana margins or the end membranes. The grana margin vesicles which have been isolated have a much lower Chl *a*/*b* ratio, about 3, and are therefore excluded. The remaining candidates are the grana end membranes. The possibility that these membranes are detached from the grana after press treatment was suggested already by Sane et al. [23] based on electron micrographs and fractionation studies.

(2) The yield of the right-hand peak is decreased when the thylakoids are sonicated in 2 mM instead of 1 mM $MgCl_2$; compare Fig. 2A,B. We interpret this as an effect of the stacking forces at the higher $MgCl_2$ concentrations. It has been shown that the grana stacks are more compressed at higher ionic strengths and particularly so at higher magnesium ion concentrations [24]. Also, the 'lumenal forces', i.e., the attractive forces between two inner sides of the thylakoid membrane, are stronger at higher $MgCl_2$ concentrations [25]. This means that the end membranes are more strongly bound to the grana stacks by the 'lumenal forces' and therefore are more resistant to sonication. The single paired stroma lamellae would not be resistant against fragmentation to the same extent because the single paired membranes are exposed about equally to the fragmentation forces both at 2 mM and 1 mM magnesium ions.

(3) The vesicles of the left-hand peak, named 'stroma lamellae', have a lower partition ratio, i.e., they prefer the upper phase less than the 'end membrane' vesicles, Fig. 4. This agrees with earlier reports on the partition of a stroma lamellae fraction obtained by press treatment and centrifugation (named Y100 or 100K) and of partition of whole thylakoids [10,26]. In all of these studies the partition ratio for the Y100 or the 100K fraction was lower than that of the thylakoids. Since the thylakoids expose both the end membrane surface and the stroma lamellae surface while the Y100 or 100K fractions only expose the stroma lamellae surface, these results are consistent with the notion that end membranes have a higher partition ratio than the stroma lamellae, i.e., the end membranes prefer the upper phase more.

(4) That the amount of vesicles is of the same order of magnitude in the two fractions represented by the two peaks in Fig. 3A is consistent with the approximately equal amounts of end membranes and stroma lamellae present in tobacco thylakoids as calculated from electron micrographs [8].

Together, the points above support the notion that the left-hand peak of Fig. 3A represents vesicles originating from the stroma lamellae and the right-hand peak vesicles originating from the grana end membranes. It remains to show, however, if the two membrane domains have different functions in the thylakoid.

The 'end membrane' fraction described here is a vesicle preparation with a high PS I content and the

lowest PS II content of vesicles described so far. Because of this and because of its high FNR activity, it seems that this preparation should be useful for studies on the function of PS I in a membrane which has not been treated with detergents.

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