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# LATERAL HETEROGENEITY IN THE DISTRIBUTION OF CHLOROPHYLL-PROTEIN COMPLEXES OF THE THYLAKOID MEMBRANES OF SPINACH CHLOROPLASTS

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## Summary

The lateral distribution of the main chlorophyll-protein complexes between appressed and non-appressed thylakoid membranes has been studied. The reaction centre complexes of Photosystems I and II and the light-harvesting complex have been resolved by an SDS-polyacrylamide gel electrophoretic method which permits most of the chlorophyll to remain protein-bound.

The analyses were applied to subchloroplast fractions shown to be derived from different thylakoid regions. Stroma thylakoids were separated from grana stacks by centrifugation following chloroplast disruption by press treatment or digitonin. Vesicles derived from the grana partitions were isolated by aqueous polymer two-phase partition. A substantial depletion in the amount of Photosystem I chlorophyll-protein complex and an enrichment in the Photosystem II reaction centre complex and the light-harvesting complex occurred in the appressed grana partition region. The high enrichment in this fraction compared to grana stack fractions derived from press or digitonin treatments, suggests that the grana Photosystem I is restricted mainly to the non-appressed grana end membranes and margins, and that the grana partitions possess mainly Photosystem II reaction centre complex and the light-harvesting complex.

In contrast, stroma thylakoids are highly enriched in the Photosystem I reaction centre complex. They possess also some 10—20% of the total Photosystem II reaction centre complex and the light-harvesting complex.

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Abbreviations: CP, chlorophyll *a*-protein complex; Chl, chlorophyll; $CF_1$, chloroplast ATPase; LHCP, light-harvesting chlorophyll *a*/*b*-protein complex.

The ratio of light-harvesting complex to Photosystem II reaction centre complex is rather constant in all subchloroplast fractions suggesting a close association between these complexes. This was not so for the ratio of light-harvesting complex and the Photosystem I reaction centre complex.

The lateral heterogeneity in the distribution of the photosystems between appressed and non-appressed membranes must have a profound impact on current understanding of both the distribution of excitation energy and photosynthetic electron transport between the photosystems.

## Introduction

Twenty years after Hill and Bendall [1] formulated the Z-scheme for the interaction of the two photosystems of green plant photosynthesis, knowledge of the molecular organization of the photosystems in the thylakoid membrane is limited. Despite many structural and functional studies on thylakoids and membrane fragments [2—5] even the localization of the two photosystems both along and across the chloroplast membrane is not resolved. Structurally, the thylakoid membrane of most mature higher plant chloroplasts consists of a network of single unstacked stroma thylakoids connected to regions of stacked grana thylakoids. There are two different outer membrane surfaces, appressed and non-appressed. Non-appressed membranes are those exposed to the stroma which comprise the stroma thylakoids, the end granal membranes and the margins of the grana, and the appressed membranes are the grana partitions. Freeze-fracture electron microscopy reveals a marked difference in particle size and distribution between appressed and non-appressed thylakoids [4,6].

While the structural heterogeneity along the chloroplast membrane is apparent, more and more evidence suggests a functional heterogeneity. Antibody labelling and freeze-etching studies demonstrated that the chloroplast ATPase ($CF_1$) was located only on the outer surfaces of non-appressed thylakoid membranes [7]. Early immunological studies [8] suggested, and later proteolytic experiments [9] confirmed, that ferredoxin-$NADP^+$ reductase was also excluded from the partition regions. Thus, both ATP synthesis and $NADP^+$ reduction occur only at the outer surfaces of non-appressed thylakoids. In contrast, Photosystem II seems to be restricted mainly to the grana partitions as demonstrated by lactoperoxidase iodination [10] and proteolysis [9] of chloroplasts. Membrane fractionation studies have also pointed to a differentiation of function. Based on electron microscopy, Sane et al. [11] proposed that the light membrane fragments isolated after passage of spinach chloroplasts through the French press were fragments of stroma thylakoids, while the heavy fraction were grana thylakoids which were not disrupted by press treatment. Since the light fraction had Photosystem I properties mainly, and the heavy fraction had both Photosystem II and Photosystem I properties, Park and Sane [12] proposed a model for the distribution of the photosystems, which had Photosystem I located in both grana and stroma thylakoids, whereas Photosystem II was restricted to grana membranes only. More recent evidence suggests that about 20% of Photosystem II is located in the stroma thylakoids [10]. Digitonin also releases a Photosystem II-enriched granal fraction, and

Photosystem I-enriched fragments in higher yield than the French press light fraction [13].

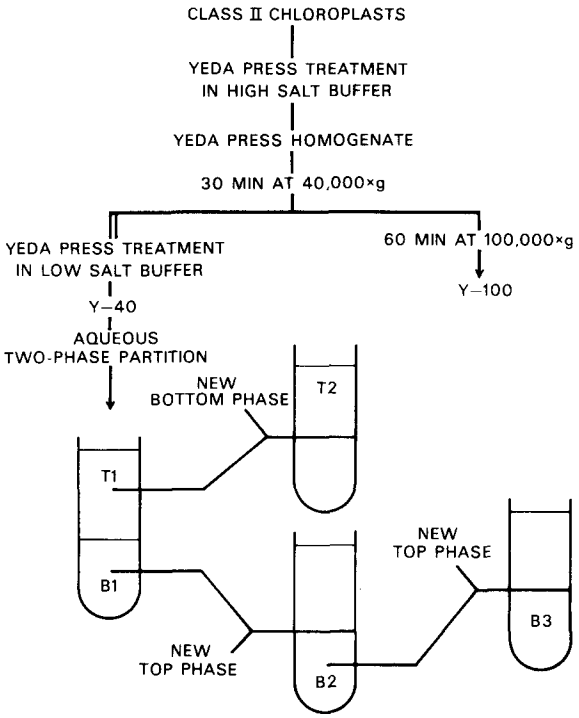
Membrane fractionation studies using centrifugation following mechanical or detergent fragmentation do not allow the localization of the photosystems with respect to the appressed partition regions. In an alternative approach, Yeda press-disrupted grana have been fractionated by aqueous polymer two-phase partition [14,15], a method which separates membranes according to differences in membrane surface properties [16,17]. In this way, inside-out thylakoid vesicles have been separated from normal right-side out vesicles [18]. By studying the mechanism by which the inverted vesicles were formed [19] it was shown that during Yeda press treatment, some grana rupture but remain tightly appressed. The resealing of these appressed membrane pairs from adjacent thylakoids results in inside-out vesicles. As a consequence of such a mechanism it is evident that the inside-out vesicles orginate from the partition regions of grana. This conclusion is supported by their freeze-fracture pattern [20]. These membrane vesicles also have high Photosystem II activity without the addition of artificial donors [15] indicating preservation of their native structure. These vesicles thus differ from the heavy French press grana fraction in being largely depleted in grana end membranes and margins. A comparison of appressed and non-appressed thylakoid membranes is therefore possible by studying the light stromal fraction (Y-100) and the inside-out vesicles (B3) obtained by differential centrifugation and phase partition following Yeda press treatment (Scheme I).

Earlier attempts to study the functional difference between grana and stroma thylakoid-enriched fractions have relied mainly on measurements of Photosystems I and II photochemical activities. Quantitation of the photosystems based on such an approach is hampered by inactivations of photochemical activities due to fragmentation, and by the restricted accessibility of donors and acceptors to the membrane catalytic sites.

This study compares subchloroplast fractions derived from appressed and non-appressed thylakoids with respect to their content of the three main chlorophyll-protein complexes of the photosynthetic unit, the reaction centre complexes of Photosystem I and Photosystem II and the light-harvesting chlorophyll *a*/*b*-protein complex. The analysis involved a recent sodium dodecyl sulphate polyacrylamide gel electrophoretic method which allows most of the chlorophyll to remain attached to protein [21], thereby permitting the relative amounts of chlorophyll associated with the photosystems to be determined. The results reveal a lateral heterogeneity in the distribution of the chlorophyll-protein complexes along the thylakoid membrane system. The partition region is very depleted in the Photosystem I reaction centre complex and enriched in the Photosystem II reaction centre complex and the light-harvesting complex. In contrast, the non-appressed membranes show a substantial increase in the Photosystem I reaction centre complex.

## Methods

*Spinacea oleracea* L. was grown in water-culture in a glasshouse. Class II chloroplasts were isolated [14] and resuspended in 50 mM sodium phosphate




**Scheme I. Yeda press disruption of chloroplast thylakoids followed by fractionation using differential centrifugation and phase partition.**

buffer, (pH 7.4), 150 mM NaCl (high salt buffer) and disrupted by two passes through a Yeda press at a nitrogen pressure of 10 MPa (Scheme I). The supernatant after centrifugation at 40 000 × *g* for 30 min was recentrifuged at 100 000 × *g* for 1 h (Y-100) and stored until use. The pellet (Y-40) sedimented after centrifugation at 40 000 × *g* for 30 min was resuspended in 10 mM sodium phosphate buffer (pH 7.4), 5 mM NaCl, 100 mM sucrose (low salt buffer) and passed twice through the Yeda press. After removal of starch grains by centrifugation at 1000 × *g* for 15 min, the grana vesicles in low salt buffer were fractionated by phase partition [15,18] (Scheme I). Grana vesicles (5.0 ml at 0.8 mg Chl/ml) were added to a polymer mixture to yield a 25-g phase system of the following final composition: 5.7% (w/w) dextran T500, 5.7% (w/w) polyethylene glycol 4000, 10 mmol sodium phosphate buffer (pH 7.4), 5 mmol NaCl and 20 mmol sucrose (the mmol values refer to 1 kg of phase system). The phase system was carefully mixed and centrifuged in a swing-out bucket rotor at 1500 × *g* for 3 min to facilitate phase settling. The top phase (T1) and bottom phase (B1) were collected and repartitioned with pure bottom and top phase, respectively, to give fractions T2 and B3. The B2 fraction was further partitioned to yield fraction B3. Fractions T2 and B3 were diluted with low salt buffer and centrifuged at 100 000 × *g* for 120 min to remove polymers. All fractions were resuspended in 50 mM Tris buffer (pH 8.0) at a high chlorophyll concentration (0.5—3 mg Chl/ml) and stored overnight in liquid $N_2$. Chlorophyll concentrations and the chlorophyll *a*/*b* ratios were determined in 80%

acetone [22] or by fluorescence for fractions with low chlorophyll *b* contents [23].

Spinach chloroplasts isolated in 50 mM phosphate buffer (pH 7.2), 300 mM sucrose, 100 mM KCl were also fragmented by passage through the French press [11] or by digitonin [13] as described previously. The membrane fractions were isolated by differential centrifugation: the French press fractions, F-10 and F-100, represent the 10 000 × *g* and 100 000 × *g* pellet fractions and the digitonin fractions, D-10 and D-144, represent the 10 000 × *g* and 144 000 × *g* fractions, respectively.

Chloroplasts and subchloroplast membrane fractions were solubilized prior to use at 4°C with 0.3 M Tris-HCl (pH 8.8), 10% glycerol, 0.375% SDS and an SDS/Chl ratio of 7.5. Discontinuous polyacrylamide gel electrophoresis was carried out at 4°C in tube gels as described before [24] except that gradient gels (8—11% polyacrylamide) were used instead of 8% polyacrylamide. The relative distribution of chlorophyll in the chlorophyll-protein complexes in the gels was determined as in Ref. 24.

## Results

The relative distributions of the chlorophyll-protein complexes in spinach thylakoids and membrane fractions derived from appressed and non-appressed regions were determined following their resolution by sodium dodecyl sulphate discontinuous polyacrylamide gel electrophoresis at 4°C by a method [21,24] which allows most of the chlorophyll to remain bound to protein. Spinach thylakoids give rise to six chlorophyll-protein complexes (Fig. 1a) which have been characterized by their absorption and fluorescence properties, *P*-700 content and polypeptide composition [21,24]. There are two chlorophyll *a*-protein complexes (CP1a and CP1) which both possess photoreactive *P*-700 and antennae chlorophyll *a* molecules with fluorescence properties of Photosystem I only; these belong to the Photosystem I reaction centre complex [24].

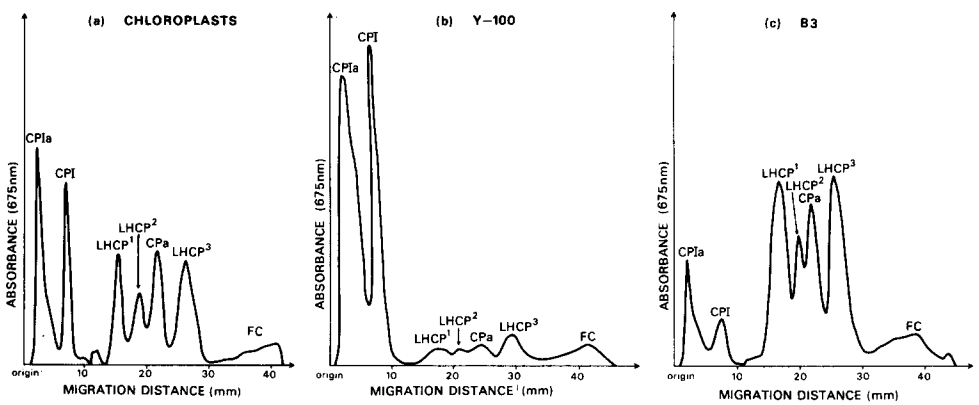



**Fig. 1. The relative distribution of chlorophyll amongst the chlorophyll-protein complexes resolved by SDS discontinuous polyacrylamide gel electrophoresis at 4°C. Gels of SDS-solubilized (a) spinach chloroplasts, (b) the Yeda press fraction sedimenting at 100 000 × *g* (Y-100), and (c) the phase-partition fraction, B3, were scanned at 675 nm.**

TABLE I

RELATIVE CHLOROPHYLL DISTRIBUTION OF CHLOROPHYLL-PROTEIN COMPLEXES FROM YEDA PRESS SUBCHLOROPLAST FRACTIONS AND SPINACH CHLOROPLASTS

**Designation of fractions according to Fig. 1. The chlorophyll-protein complexes were resolved by SDS-polyacrylamide gel electrophoresis and scanned to determine the relative amount of chlorophyll. The numbers presented are average values from six chloroplast preparations. Chlorophyll *a*/*b* ratios were determined by absorption [15] or by fluorescence (Y-100) [31].**

| Fraction | Average percentage of total chlorophyll | | | | Chl *a*/ Chl *b* | Total Chl (%) |
|---|---|---|---|---|---|---|
| | Photosystem I reaction centre complex CP1 + CP1a | Photosystem II reaction centre complex CPa | Light-harvesting complex $LHCP^{1-3}$ | Free chlorophyll | | |
| Chloroplasts | 27 | 13 | 52 | 8 | 2.8 | 100 |
| Y-40 | 25 | 13 | 52 | 10 | 2.7 | 93 |
| T2 | 27 | 12 | 51 | 10 | 2.8 | 76 |
| B3 | 10 | 14 | 65 | 11 | 2.0 | 7 |
| Y-100 | 69 | 5 | 17 | 9 | 9.5 | 7 |

Although no *P*-680 has been detected in the third chlorophyll-*a*-protein complex (CPa), it is considered from indirect evidence to be the reaction centre complex of Photosystem II [21,26—28]. First, chloroplasts with Photosystem II activity but no LHCP isolated from Chl *b*-less mutant barley [27] and developing plastids greened with periodic light [28] have much more chlorophyll associated with CPa. Second, the fluorescence properties of CPa from mutant barley [27] are similar to those of a spinach Photosystem II reaction centre complex [29]. The three chlorophyll *a*/*b*-protein complexes resolved ($LHCP^1$, $LHCP^2$ and $LHCP^3$) are all associated with the light-harvesting complex since they have similar spectral properties, pigment and polypeptide composition [21,24,30,31].

Table I summarizes the relative distribution of chlorophyll between the main chlorophyll-protein complexes in thylakoids and subchloroplast fractions obtained from Yeda press disruption. Due to the evidence presented above the amounts of chlorophyll of the three LHCP bands have been added together to give the chlorophyll content of the total light-harvesting chlorophyll-protein complex. In the same way the amounts of chlorophyll associated with CP1a and CP1 have been combined to give the total chlorophyll content of the reaction centre complex of Photosystem I. As can be seen in Table I for thylakoids, some 27% of the total chlorophyll is associated with the reaction centre complex of Photosystem I (CP1 and CP1a), 52% with the light-harvesting complex and 13% with the reaction centre complex of Photosystem II. Some chlorophyll (8%) has been dissociated from proteins and moves at the gel front as detergent-chlorophyll micelles.

The relative proportions of chlorophyll present in the chlorophyll-protein complexes associated with membrane fractions derived from appressed and non-appressed thylakoid regions are very different to those found in the complete thylakoid membrane system (Fig. 1a—c and Table I). The Y-100 fraction, containing small stroma thylakoid vesicles, showed a pronounced enrichment

TABLE II

RATIOS BETWEEN DIFFERENT CHLOROPHYLL-PROTEIN COMPLEXES IN CHLOROPLASTS AND THE Y-100 AND B3 SUBCHLOROPLAST FRACTIONS

| Fraction | Ratio | | |
|---|---|---|---|
| | LHCP / CP1 | LHCP / CPa | CP1 / CPa |
| Chloroplasts | 1.9 | 4.1 | 2.2 |
| Y-100 | 0.24 | 3.6 | 15 |
| B3 | 6.5 | 4.7 | 0.72 |

in CP1 and CP1a (69% of the total chlorophyll) and a concomitant depletion in the amount of chlorophyll associated with both CPa and LHCP (5% and 17%, respectively) (Fig. 1b). Thus the relative amount of chlorophyll associated with the CP1 complexes in this fraction is almost 3 times more than is found in unfractionated thylakoids and there is a corresponding decrease in both LHCP and CPa. This gives a CP1/CPa ratio of 15 compared to 2.2 in chloroplasts (Table II). The LHCP/CP1 ratio is 0.24 which is considerably lower than in chloroplasts (1.9).

The chlorophyll distribution pattern for the B3 fraction (Fig. 1c and Table I), isolated by phase-partition (Scheme I) is very different to that of the chloroplast and stroma thylakoid vesicles. Fraction B3 which consists of inside-out vesicles derived from the grana partitions is largely devoid of grana end membranes and margins [15,19,20]. These vesicles are considerably enhanced in Photosystem II activity, and their native membrane structure has been preserved since artificial donors are not required for oxygen evolution [15]. Most of the chlorophyll of fraction B3 was found in LHCP (65%) and CPa (14%), whereas CP1a and CP1 together only accounted for 10% of the total chlorophyll. The amount of chlorophyll associated with the reaction centre complex of Photosystem I was about three times less than that of chloroplasts. This latter value is likely to correspond to the actual proportion of chlorophyll associated with the Photosystem I reaction centre complex in the partition region vesicles, since virtually no free chlorophyll is derived from CP1a and CP1 during electrophoresis [24]. The LHCP/CP1 ratio is increased to 6.5 from 1.9 in the starting material (Table II). The most striking differences are observed by comparing the B3 and Y-100 fractions. The CP1/CPa ratio of 15 in the Y-100 fraction corresponds to a ratio of 0.72 in the B3 fraction (Table II). The LHCP/CP1 ratios are 0.24 and 6.5 for the Y-100 and B3 fractions, respectively. Although the LHCP/CP1 and CP1/CPa ratios both vary considerably between chloroplast and subchloroplast fractions derived from the appressed and non-appressed membrane regions, the LHCP/CPa ratio is fairly constant (3.6—4.7). The actual LHCP/CPa ratio in vivo is likely to be less than 4, since CPa is the most labile complex in the electrophoretic procedure used here and most of the free chlorophyll originates from CPa [21].

In the light of previous studies [15] which showed that the chlorophyll *a*/*b* ratio in the Y-100 fraction was high and that in the B3 fraction was low, the differences obtained in the amounts of LHCP are to be expected. It is known

TABLE III

RELATIVE CHLOROPHYLL DISTRIBUTION OF CHLOROPHYLL-PROTEIN COMPLEXES OF FRENCH PRESS AND DIGITONIN SUBCHLOROPLAST FRACTIONS OF SPINACH CHLOROPLASTS

| Fraction | Average percentage of total chlorophyll | | | | |
|---|---|---|---|---|---|
| | Photosystem I reaction centre complex CP1 + CP1a | Photosystem II reaction centre complex CPa | Light-harvesting complex $LHCP^{1-3}$ | Free chlorophyll | Chl *a*/ Chl *b* |
| Chloroplasts | 30 | 11 | 49 | 10 | 2.6 |
| F-10 | 20 | 13 | 60 | 7 | 2.3 |
| F-144 | 60 | 6 | 23 | 11 | 5.2 |
| D-10 | 15 | 11 | 65 | 9 | 2.1 |
| D-144 | 67 | 4 | 19 | 10 | 5.4 |

that all of the chlorophyll *b* is associated with LHCP [32]. The relative distribution of the chlorophyll-protein complexes in the bulk fractions, Y-40 and T2, is shown also in Table I. The Y-40 fraction, comprising 93% of the total chlorophyll of unfractionated chloroplast thylakoids, had virtually the same distribution pattern as the unfractionated chloroplasts. The T2 fraction from the phase partition fractionation (Scheme I), which accounts for 82% of the chlorophyll of the Y-40 fraction, showed only a slight enrichment in the amount of chlorophyll associated with CP1 and CP1a. Since the amount of free chlorophyll in each fraction formed during electrophoresis is of the same order (8—11%) (Table I), a direct comparison between the various fractions is feasible. If the chlorophyll contents of the individual chlorophyll-protein complexes in each fraction are added, taking into account the relative amounts of chlorophyll in the fractions, the combined calculated value corresponds to the composition of the starting chloroplast material. This observation shows that there is no loss or alteration of chlorophyll-protein complexes during the fractionation of the chloroplasts.

In order to compare the above analyses with other membrane fractions we analysed the relative chlorophyll distribution between the chlorophyll-protein complexes of the subchloroplast fractions isolated by differential centrifugation following French press [11] or digitonin [13] treatment of chloroplasts (Table III). The chlorophyll distribution of the French press light fraction (F-100) derived from stroma thylakoids showed an enrichment in the amount of chlorophyll associated with CP1a and CP1, and a depletion in that located in CPa and LHCP. The heavy fraction (F-10) derived from grana stacks was depleted in CP1a and CP1 (20%) and enriched in LHCP (60%) and CPa (13%). A relative enhancement of LHCP in F-10, and of CP1 in F-100, was noted before [33] but the high amounts of free chlorophyll gave lower yields, and CPa was not detected. The more extreme distribution pattern in the B3 fraction compared to that of the F-10 fraction, supports other observations [15,19, 20] that this fraction differs from the F-10 fraction in having lost most of its grana margins and end membranes. For the digitonin fractionation procedure [13] the same principal differences in chlorophyll distribution differences were

found between the light (D-144) and heavy centrifugal fractions (D-10). Fraction D-144 was considerably enriched in CP1 (67%) and depleted in LHCP (19%) and CPa (4%). The chlorophyll of the D-10 fraction was distributed between LHCP (65%), CPa (11%) and CP1 (15%). The values are in agreement with a recent electrophoretic study of the D-10 and D-144 fractions from barley [28] although the amount of chlorophyll associated with CPa (4%) in the barley D-10 fraction was lower [28]. The structural origins of the digitonin fractions cannot be judged directly.

As was the case for the Yeda press fractions (Table II) the French press and digitonin fractions show that the LHCP/CPa ratios are not very different (F-10 and F-144, 4.6 and 3.8; D-10 and D-144, 5.9 and 4.7). In contrast, the LHCP/CP1a + CP1 ratios are widely different (F-10 and F-144, 3.0 and 0.38; D-10 and D-144, 4.3 and 0.28). However, extensive analyses of French press and digitonin subchloroplast fractions were not undertaken making these ratios less reliable compared to those of Yeda press fractions.

Might the enrichment of CPa and LHCP and depletion of CP1 be even greater in the partitions in vivo than indicated by the experimental values in the B3 fraction? Since fraction B3 contains about 75% inside-out vesicles originating from grana partitions [19,20] the right-side out material might contain some grana end membranes and margins, as some residual coupling factor was detected. (Berzborn, R.J. and Andersson, B., unpublished observation). The depletion of CP1a and CP1 in the B3 fraction (Table I) should therefore only be considered as a minimum for native grana partitions which might have very little, if any, Photosystem I chlorophyll-protein complex.

Such a possibility can be verified by calculating the amount of CP1a and CP1 in the partition regions. This value was calculated by subtracting the experimental amount of CP1a and CP1 found in exposed membranes (69%; Y-100 fraction) from that found in unfractionated chloroplasts (27%) (Table I). Table IV shows the results of such calculations assuming different amounts of chlorophyll to be associated with appressed and non-appressed membranes. Assuming 30% of the total chlorophyll is located in non-appressed membranes,

TABLE IV

CALCULATED PROPORTION OF THE PHOTOSYSTEM I REACTION CENTRE COMPLEX IN THE GRANA PARTITIONS

Varying total chlorophyll distributions were assumed for the appressed and non-appressed thylakoid regions. The proportion of Photosystem I reaction centre in the partition region was then calculated by subtracting the amount of chlorophyll found in the non-appressed region (i.e. 69% of total chlorophyll of Y-100 was in CP1a and CP1) from that found in untreated chloroplasts (27% of total chlorophyll in CP1a and CP1).

| Assumed proportion of total chlorophyll in non-appressed thylakoids (%) | Amount of Photosystem I centre complex in grana partitions (%) |
|---|---|
| 20 | 17 |
| 25 | 14 |
| 30 | 9 |
| 35 | 4 |
| 40 | 0 |

the calculated value of CP1a and CP1 corresponds with that found experimentally in the B3 fraction (Table I). As estimated by electron microscopy with spinach grown under normal light conditions, the stroma thylakoids and grana end membranes contribute up to 30% of the length of thylakoid membranes [34]. With the contribution of grana margins, the proportion is likely to be between 30 and 40% of the total length of the thylakoid. Taking these figures as a measure of the amount of chlorophyll in the non-appressed thylakoids, the content of the Photosystem I reaction centre complex in the partition region is very low (Table IV). While these calculations include some assumptions they point to the partition regions being very depleted in Photosystem I, as is suggested by the experimental value for the B3 fraction.

## Discussion

In addition to the asymmetric distribution of lipids and proteins across membrane bilayers there is also increasing evidence demonstrating a non-random distribution of components in the lateral plane of the membrane. This has been shown to be the case for artifical bilayers [35,36] and several native membranes [37]. Especially in the case when membrane-membrane interactions occur as in grana stacking, biophysical evidence [35] suggests that components which are highly charged at the outer membrane surface would migrate out and the less charged components would be drawn into the appressed region, thereby resulting in a lateral redistribution of membrane components. Indeed for chloroplast thylakoids, lateral heterogeneity in the distribution of components has been demonstrated for the chloroplast coupling factor [7] and ferredoxin-$NADP^+$ reductase [9] which are both located in non-appressed regions only. A marked difference in the lateral distribution of the supramolecular complexes of thylakoids is demonstrated by the varying densities and sizes of the freeze-fracture particles in appressed and non-appressed regions [4,6].

The present study approaches the lateral heterogeneity of the photosystems along thylakoid membranes by comparing the relative content of the three main chlorophyll-protein complexes in various subchloroplast fractions. The relative proportions of chlorophyll associated with the three chlorophyll-protein complexes in membrane fractions derived from appressed and non-appressed thylakoids are strikingly different from one another, and from intact thylakoids. The enrichments in the amount of chlorophyll associated with CP1a and CP1 in the Y-100, F-100 and D-144 fractions, and of CPa and LHCP in the B3, F-10 and D-10 fractions, are in agreement with the known enhanced Photosystem I and Photosystem II activities, respectively. Analysis by the electrophoretic method is more reliable than attempted quantitation of the photosystems in subchloroplast fractions based on photochemical activities, since activity measurements may be distorted by inactivations due to membrane fragmentation, or by the restricted accessibility of electron donors, acceptors or effectors at the catalytic site.

The stroma thylakoid fractions (Y-100, F-100, D-144) have a substantially higher Photosystem I reaction centre complex content and much less Photosystem II reaction centre complex and light-harvesting chlorophyll-protein complex. This confirms earlier observations, that stroma thylakoids are highly

enriched in Photosystem I. Nevertheless, a minor proportion (calculated to be 10—20%) of the Photosystem II reaction centre complex and light-harvesting complex is present in stroma thylakoids (Table I, Fig. 1c). This is in accord with observations by Armond and Arntzen [10] that some 20% of Photosystem II activity is located in stroma thylakoids.

The most striking observation, however, is that the partition region shows such a pronounced depletion in Photosystem I reaction centre chlorophylls. There is also an increase in the light-harvesting complex and Photosystem II chlorophyll-protein complex. This indicates that the grana partitions are mainly Photosystem II regions, and that grana Photosystem I is restricted mainly to the non-appressed grana end membranes and margins. Previous studies where Photosystem I particles were isolated by detergent treatment of grana-enriched fractions [38,39] have been taken as evidence that Photosystems I and II were both located in the grana stacks. However, these results do not exclude a compartmentation of the photosystems within the grana stacks as suggested by the present study. We suggest that the Photosystem II reaction centre complex and the light-harvesting complex are located mainly in partition regions, and that the Photosystem I reaction centre complex is located mainly in the non-appressed membrane regions together with ferredoxin-$NADP^+$ reductase and the chloroplast coupling factor (Fig. 2).

A lateral heterogeneity in the distribution of the charged groups along the outer membrane surface of chloroplast thylakoids has been demonstrated by cross partition [40]. The outer surface of stroma thylakoids had an overall isoelectric point of 4.7 compared to 4.2 for the partition region. Although many different surface-exposed components may contribute to these overall isoelectric points, they are strikingly similar to those of isolated chlorophyll-protein complex 1 (4.8) and isolated light-harvesting complex (4.2—4.3) [41,42]. These observations are in agreement with the proposed lateral heterogeneity in the distribution of the chlorophyll-protein complexes suggested by the present study.

Another important observation is that the light-harvesting complex seems to be associated structurally with the reaction centre complex of Photosystem II, since rather constant LHCP/CPa ratios are found in the subchloroplast fractions, particularly with the Yeda press fractions (Table II). In contrast, no close association between the light-harvesting complex and the reaction centre com-

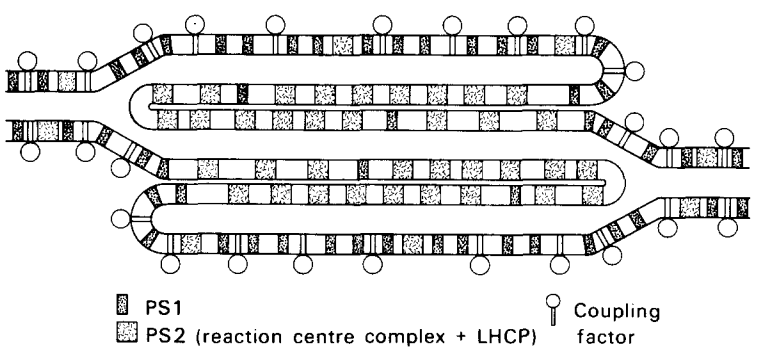



**Fig. 2. A schematic model showing the distribution of Photosystem I and Photosystem II between appressed and non-appressed thylakoid membranes of higher plant chloroplasts which contain grana.**

plex of Photosystem I is likely, since very different ratios between these complexes are found in the various fractions (Table II). While a close functional and structural association between the light-harvesting complex and the Photosystem II reaction centre complex has been suggested from fluorescence, isolation and developmental studies, and cation effects (see Ref. 4), this is the first direct evidence for rather similar amounts of the two complexes in grana and stroma thylakoids. This data suggests that the light-harvesting complex present in the non-appressed thylakoids is mainly associated with the residual Photosystem II reaction centre complex rather than the dominant Photosystem I reaction centre complex.

The consequences of the demonstrated localization of the chlorophyll-protein complexes in different membrane regions (Fig. 2) must have a profound impact on our current understanding of the transfer of light energy from the antennae pigments to the traps of Photosystems I and II, and also on the electron transport between the two photosystems. Two models have been suggested for the arrangement of the many antennae chlorophylls that are associated with the reaction centre of each photosystem. In the separate package model, Photosystems I and II coexist as separate entities in isolation from each other, whereas they are in close contact in the continuous array model which is favoured by most researchers (cf. Refs. 43, 44—47). The constant LHCP/CPa ratios in the present study point to a separate package model as suggested by Boardman et al. [5]. In addition, the low LHCP content in the stroma thylakoid fraction and its likely association with CPa, point to an even more extreme separate package model, where chlorophyll-protein complex 1 is mainly independent of LHCP, which is associated with the Photosystem II reaction centre complex. If indeed most of Photosystem I is located in a different membrane compartment to that of the Photosystem II reaction centre complex and the light-harvesting complex, the continuous array model is no longer appropriate for the bulk of the chlorophyll of grana-containing chloroplasts. Re-interpretation is needed of the effects of cations on the functional aspects of destacked and stacked chloroplast thylakoids in vitro. For example, the lack of 'spillover' in grana-containing chloroplasts [4], may be due in part to the scarcity of Photosystem I in the partition region.

Further, if most of Photosystem I and Photosystem II are spatially separated, then a lateral shuttle of reducing equivalents between the two physically separated photosystems is obligatory. The most likely candidate would be the lipid-soluble molecules of the plastoquinone pool as suggested earlier [48]. Such small amphipathic molecules are known to have fast diffusion rates in the lateral plane of membranes [49]. Further, they are present in at least 20-fold excess compared to *P*-680 and *P*-700, thereby partly compensating for the distances between Photosystems II and I. Therefore, it seems that plastoquinone is not only involved in the transport of protons across the membrane from its outer surface to the inner surface [50], but also acts as a lateral shuttle of reducing equivalents along the membrane, thereby providing the vital link between the spatially separated photosystems. The compartmentation of Photosystems I and II between non-appressed and appressed membrane regions may be a means for the regulation of photosynthetic function in different light environments.

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Since the acceptance of this paper, a freeze-fracture study of a Photosystem I mutant [51] suggests an exclusion of photosystem I from grana partitions, in accordance with our present results.

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