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Heterogeneity in Photosystem II_α. Evidence from fluorescence and gel electrophoresis experiments

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The antenna size of different thylakoid membrane vesicles originating from grana partitions and enriched in PS II_α was determined by measuring the kinetics of the variable fluorescence and by gel electrophoresis of pigment-protein complexes. Inside-out thylakoid vesicles were fractionated by sonication and phase partition and separated into different subpopulations of PS II_α vesicles. The kinetics of the rise in the variable fluorescence of these subpopulations differed, demonstrating a heterogeneity in functional antenna size among the PS II_α. Analysis by gel electrophoresis showed that the fractions which have a more rapid rise in variable fluorescence, and hence a larger functional antenna size, also had a larger ratio of LHCII/PS II core polypeptides. The relative content of the 25 kDa polypeptide of LHCII increased with antenna size. There is thus a heterogeneity both with respect to the quantitative and the qualitative composition of the antenna of PS II_α. The results suggest that in vivo there are different domains in the partition region of the thylakoid membrane which have different average antenna size of PS II_α. The results lend further support to previous work in which heterogeneity of the antenna size of PS II_α of the same types of vesicles population was demonstrated by light saturation curves of oxygen evolution (Albertsson, P.-Å. and Yu S.-G. (1988) Biochim. Biophys. Acta 936, 215–221).

Two classes of photosystem II are present in the thylakoids of higher plants. One, PS II_α, which is located in the grana, has an antenna size which is 2–3-times larger than the other, PS II_β, which is located in the stroma membrane [1–6]. The two classes also differ in their redox properties [3,7,8]. For example, PS II_α can evolve oxygen with either phenylparabenzoquinone or ferricyanide as electron acceptors, while PS II_β works well with the quinone but poorly with ferricyanide [3]. For a general discussion of the heterogeneity of PS II, see the review by Black et al. [9].

In addition, there is also a heterogeneity within PS II_α. In a previous paper [10] we demonstrated that separate domains of the thylakoid membrane of spinach chloroplasts have different average antenna size of PS II_α. Sonication of inside-out thylakoid vesicles – which originate from the grana partitions, and harbour the PS II_α – followed by phase partition, separated vesicles into different populations having different average functional antenna size of PS II_α. The functional antenna size was determined from light saturation curves of the oxygen evolution with phenylparabenzoquinone as electron acceptor. The more hydrophilic acceptor, ferricyanide, can also be used to demonstrate this heterogeneity (unpublished data). We now describe further characterization of the different vesicle preparations and provide additional evidence for a heterogeneity in antenna size among PS II_α by two additional, independent methods: fluorescence spectroscopy and gel electrophoresis.

The isolation of chloroplasts and the preparation of inside-out thylakoid vesicles (B3) was done as described previously [10] except that a polymer concentration of dextran and poly(ethylene glycol) of 5.7% (w/w), respectively, was used to increase the yield. The sonication-phase partition procedure for the separation of fragments from the inside-out vesicles was done essentially as already described before [10–12]. A phase system containing the B3 fraction was subjected to repeated cycles of sonication-phase partition giving the fractions 180 s, 360 s, 540 s and BS according to the nomenclature described [10,12]. All these subfractions derive from the B3 fraction and have a high PS II

Abbreviations: PS II, Photosystem II; LHCII, light-harvesting chlorophyll *a*/*b* protein; SDS, sodium dodecylsulfate.

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activity in the form of PS II_α; their properties are described in more detail in a separate publication [12]. The BS fraction, which is obtained in a yield of about 30% of B3, has the lowest chlorophyll *a/b* ratio and consists of membrane vesicles with the highest enrichment of PS II as determined by oxygen evolution and content of PS II polypeptides [12].

The kinetics of the variable fluorescence of the different fractions show that the BS fraction needs the shortest time to reach half-maximal rise in variable fluorescence (Table I). A comparison of the kinetic constants of the increase of the area over the fluorescence induction curves was made by semilogarithmic plots according to the method of Melis and Homann [1,2] (Table I). This comparison shows also that the BS fraction has the most rapid rise in variable fluorescence. From the data of Table I one can conclude that the functional antenna size of all the three fractions originating from B3 differ. The order of the antenna size of the fractions, as determined by the fluorescence measurements, is the same as the order found be measuring the light saturation curves [10].

The content of chlorophyll-protein complex of PS II was determined by discontinuous sodium docecylsulfate-polyacrylamide gel electrophoresis [13]. The proportion of the light-harvesting pigment proteins of PS II and of the PS II core complex are given in Table II. All the membrane fractions have a higher ratio of LHCII per PS II core complex than the thylakoids, and the BS fraction has the highest of them all. Its ratio of LHCII per PS II core complex for the BS fraction is 11% larger than the same ratio for the B3 fraction.

LHCII is composed of several polypeptides, in the 25–30 kDa region. In order to compare the polypeptide composition of the LHCII of the different membrane fractions we analyzed the combined LHCII bands, from a gel electrophoresis similar to that described in Table II by a second, more denaturing gel electrophoresis, according to Larsson and Andersson [14]. This resolved the LHCII into two main bands, 25 and 27 kDa, respectively (Fig. 1). The ratio between these two polypeptides varies with the membrane fraction. The BS has the lowest 27/25 ratio. When the data of Fig. 1 are compared with those of Table II, one can see that the higher the ratio of LHCII to PS II core complex the more of the 25 kDa polypeptide is present in LHCII. There is both a quantitative and a qualitative difference between the antenna of the different subclasses of PS II_α.

In summary, we have demonstrated, by three independent methods, namely, the determination of the light saturation curve for O_2 evolution [10], the variable fluorescence and the polypeptide composition by gel electrophoresis, that the subclasses of PS II_α vesicles differ in the size of their antennae. The order of antenna size within the different fractions is the same when determined by all the three methods, BS having the largest antenna. Such PS II_α units can be built by adding to the antennae more of the LHCII polypeptides, whereby the functional antenna will be aug-

TABLE I

The half-rise time ($t_{1/2}$) and the velocity constant (K_α) of the area growth over the fluorescence induction curve of different thylakoid membrane fractions in the presence of DCMU

The value of K_α and the inverse value of $t_{1/2}$ are proportional to the functional antenna size. K_α is the constant for the fast component representing PS II_α. The fluorescence induction was measured at a room temperature of about 20 °C, with an modified Aminco Spectrophotometer. Actinic light of uniform field was provided in the blue-green region of the spectrum and admitted to the sample cuvette by an electric shutter (approx. opening time 20 ms). Fluorescence emission at 685 nm was detected at right angles to the direction of the actinic beam. A HBW-70 filter was placed in front of the photomultiplier tube in order to protect it from the actinic light and to separate the fluorescence beam. The signal was processed by the use of a Nicolet Signal Averager (Model 527) in combination with a computer system. The signal was displayed either on the storage in the averager or on the *X-Y* recorder (Model 2000). Integration of the area over the fluorescence induction curve and semilogarithmic plot were analysed, according to the method of Melis and Homann [1,2]. The velocity constant K_α was obtained from the initial slope of the plot ln(1 − area(t)) against time. The reaction mixture contained 50 mM Tricine (pH 7.8), 400 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and membrane fraction (5 μg chlorophyll/ml). Fluorescence was measured in the presence of 20 μM DCMU and after 5 min for dark adaptation.

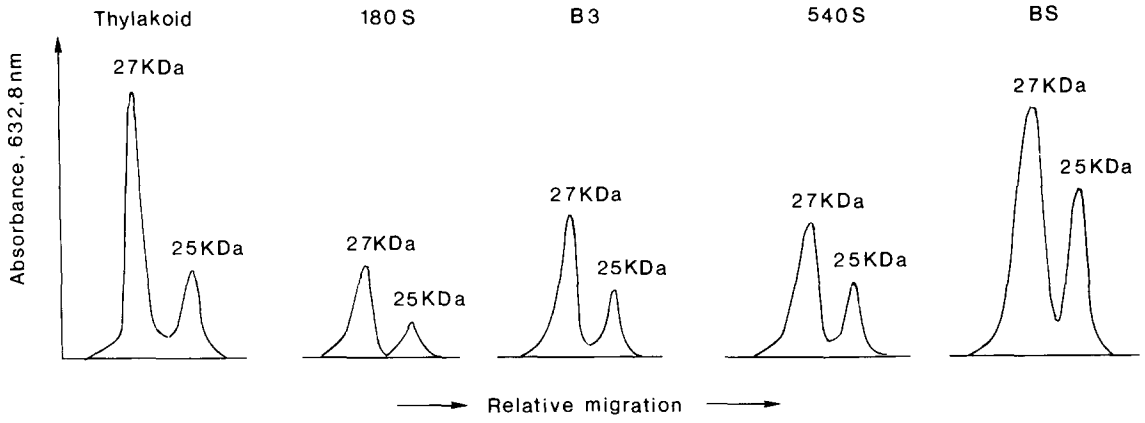
	Chl *a/b*	$t_{1/2}$ of F_v (ms)	K_α (s^{-1})
Thylakoid	2.7	62	10±1
180 S	3.3	57	10±1
540 S	2.3	51	12±1
B_3	2.3	51	14±1
BS	1.9	47	17±1

TABLE II

Relative proportion of light-harvesting pigment proteins of PS II and PS II-core complex proteins (including Cp43 and Cp47) in different thylakoid membrane fractions

Discontinuous tube SDS-PAGE was performed mainly as reported in Ref. 13. Solubilization of the material carried out at 4 °C with an SDS/chlorophyll ratio of 10. Gradient gels of 8–12% acrylamide were used. Electrophoresis was run at 4 °C for 3–4 h at a constant current density of 0.05 mA/mm². The gels were scanned using an LKB ultroscan laser densitometer. The chlorophyll-protein complexes were quantified from their peak areas. No significant amount of chlorophyll failed to enter the gel.

Fraction	LHCII (%)	CP_a (%)	$\frac{LHCII}{CP_a}$	Free Chl (%)
Thylakoid	43	19	2.3	3.7
180 s	53	19	2.7	4.7
B_3	56	20	2.8	3.6
720 s	62	21	3.0	4.6
BS	69	22	3.1	3.1



27KDa/25KDa	3.5	2.9	2.6	2.5	2.2
Chl a / Chl b	3.1	3.1	2.4	2.4	1.9

Fig. 1. The relative content of the 25 and 27 kDa polypeptides of LHCH of different thylakoid membrane fractions. Two-dimensional gel electrophoresis was used [14]. The first dimension electrophoresis, separating chlorophyll-protein complexes, was performed in the system of Camm and Green [15]. The band corresponding to the chlorophyll-protein complex of LHC II was cut out, incubated in the denaturating solubilizing buffer of Laemmli [16] and reelectrophoresed on a discontinuous gel according to Laemmli, using 12–22.5% gradient gels. The second-dimension gels were stained with Comassie brilliant blue and scanned using the laser densitometer. The relative amounts of LHCII polypeptides were quantified from the area under their peak. In both electrophoresis runs no significant amount of protein failed to enter the gels.

mented by discrete increments. In addition there is also a qualitative difference between the fractions, in that BS has a relatively larger content of the 25 kDa polypeptide. Recent studies have shown that LHCII consists of an inner part, which contains only the 27 kDa polypeptide, and a peripheral part which contains both the 25 kDa and the 27 kDa polypeptides [14,17]. All our PS II membrane vesicles contain both polypeptides, but those having a larger functional antenna are more enriched in 25 kDa. This shows that the increase in the antenna size is accomplished by increasing the peripheral part of the LHCII.

The inside-out vesicles (B3), which contain 75–85% of the PS II of the thylakoid in the form of $PSII_\alpha$, originate from the grana partitions [18]. Sonication of these vesicles produces new vesicles which are smaller in size than the B3 vesicles [19]. While the average diameter of the thin sections of the B3 vesicles is 0.3 μm, this diameter is reduced to 0.1–0.2 μm after sonication [19]. Moreover, counter-current distribution of sonicated inside-out vesicles results in peaks in the separation diagram, indicating that the inside-out vesicles contain discrete regions with different membrane properties [19–22]. Our present work has shown that the subclasses of PS II_α vesicles, obtained by sonication of inside-out vesicles, differ in their average PS II_α antenna size. Note that the material is in a vesicle form and has not been treated by detergents. We therefore conclude that the different populations of PS II_α are not intermixed but originate from separate domains in the native thylakoid membrane. For a discussion on the localization of these domains, see our previous paper [10].

Recently it has been shown that thylakoids from 'low-light' spinach have a larger LHCII/PS II core ratio and a smaller 27 kDa/25kDa ratio of the LHCII than thylakoids from medium or high-light spinach [23,24]. The authors conclude that the LHCII of the 'low-light' chloroplasts contains more of the peripheral, 25 kDa rich, pool of LHCH. Since the BS fraction contains the largest relative amount of the 25 kDa polypeptide, our results suggest that chloroplast from leaves grown under low-light conditions contain more of those grana domains from which the BS fraction originates. Our results also suggest that these "BS domains" may be more abundant in the interior or on the lower side of a leaf where the light intensity is lower, and where the chloroplasts may resemble the chloroplasts from plants grown under low-light conditions [25].

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