Short Communication

Subfractionation of Inside-Out Thylakoid Vesicles*

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Thylakoid membranes can be fragmented by press treatment and separated by centrifugation and aqueous two-phase partition[1,2] into photosystem I- and photosystem II-rich vesicles.[3,4] From these studies it was concluded that the two photosystems are segregated into two separate domains in the native thylakoid membrane.[4]

The photosystem I vesicles originate from the non-appressed, stroma-exposed region of the thylakoid system, while the photosystem II vesicles originate from the grana partition.[5] The isolated photosystem II-rich vesicles have an inside-out conformation[6,7] and have been used as very powerful tools in studies of the transverse and lateral organization of the thylakoid membrane and of oxygen evolution.[8]

In this work we have further fragmented the inside-out, photosystem II-enriched vesicles by sonication. This yields a population of smaller vesicles of heterogeneous composition.[9,10] Using aqueous two-phase partition and counter-current distribution we have separated the vesicles into different populations. Their composition with regard to chlorophyll a and b content and their photosystem I activity (P 700) was determined.

Materials and methods

Inside-out vesicles from spinach were prepared as described in Ref. 11. The vesicles were included in a 7.5 g phase system comprising 5.6 % (*w/w*) Dextran 500, 5.6 % (*w/w*) PEG 4000, 10 mM sodium phosphate buffer (pH 7.4), 3 mM NaCl, 1 mM $MgCl_2$ and 20 mM sucrose. The sample was sonicated in a Branson Sonifier, Model B 30 (equipped with a 1/2-inch tip), for 2×30 s with a resting interval of 1 min and with continuous cooling. The ultrasonic exposure had an intensity output of 7, with 20 % duty pulses. The sonicate was then subjected to liquid interface countercurrent distribution[9,10] using a 60 cavity thin-layer apparatus. The phase system used was the same as that described above. Chlorophyll was determined according to Arnon.[12] The concentration of P 700 was measured directly from the magnitude of the light-minus-dark absorbance change at 700 nm.[13] A differential extinction coefficient of 64 mM^{-1} cm^{-1} was used.[14] The reaction mixture contained 0.02 % (*w/w*) sodium dodecylsulfate, 200 μM methyl viologen, 2 mM sodium ascorbate and chloroplast membranes yielding about 20 μM chlorophyll.

Plotting the data. If a membrane consists of two domains, one rich in a component A and the other in a component B, then there is a linear relation between the concentration of A (amount of A per membrane area) and the concentration of B (amount of B per membrane area) in fragments obtained by disintegrating the membrane

$$[A] = -\frac{[A_0]}{[B_0]}[B] + [A_0]$$

where $[A_0]$ is the concentration of A in a pure A fraction ($[B] = 0$) and $[B_0]$ is the concentration of B in a pure B fraction ($[A] = 0$).[10]

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Results and discussion

A counter-current distribution of sonicated inside-out (B3) vesicles is illustrated in Fig. 1. In agreement with previous results,[9,10] fractions with different chlorophyll a/b ratio and P 700 content were found. The fractions in the left part of the diagram had the lowest chlorophyll a/b ratio and the lowest P 700 content. Compared to previous results, we have now obtained fractions with lower chlorophyll a/b ratio (1.8) and greater depletion of P 700. We used the concentration of P 700 as representative of the photosystem I- and that of chlorophyll b as representative of the photosystem II-rich domain since the latter is dominated by the chlorophyll b-rich, light-harvesting protein II. Total chlorophyll concentration was chosen as representative of membrane area.

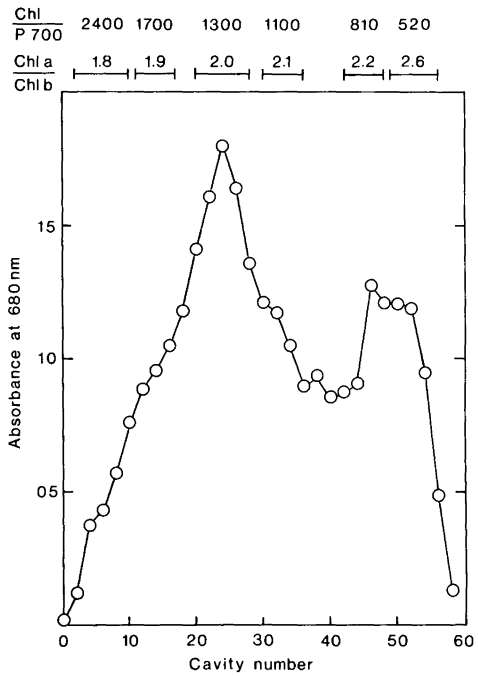

Fig. 1. Counter-current distribution of sonicated inside-out (B3) thylakoid vesicles. On top of the diagram are shown the chlorophyll a/b ratio (mol/mol) and chlorophyll/P 700 ratio (mol/mol) of pooled fractions. The chlorophyll a/b ratios of the Yeda press homogenate of the thylakoids and of the inside-out (B3) vesicles were 2.8 and 2.15, respectively. Corresponding values of the chlorophyll/P 700 ratio were 440 and 800, respectively.

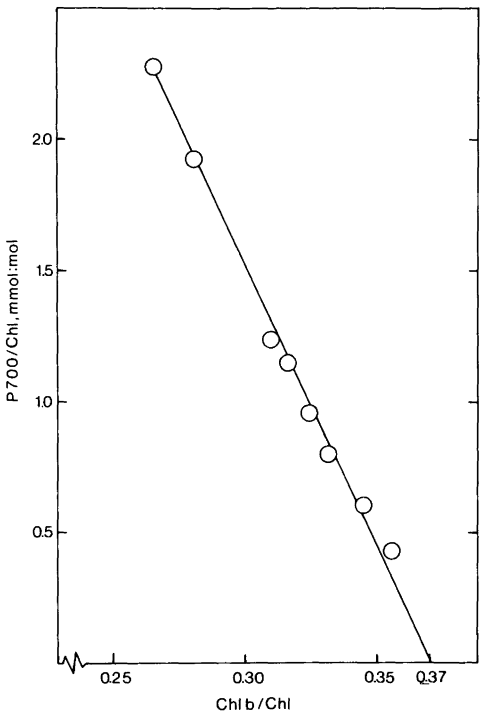

Fig. 2. Plot of P 700/chlorophyll ratio (mmol/mol) against chlorophyll b/total chlorophyll ratio (mol/mol) of different thylakoid vesicles. The points represent, from left to right: Yeda press homogenate of thylakoids, pooled fractions (Fig. 1) 49–56 and 42–28, inside-out vesicles (B3) and pooled fractions (Fig. 1) 30–36, 20–28, 11–17 and 2–10.

We plotted the concentration ratio P 700/total chlorophyll against chlorophyll b/total chlorophyll. As seen in Fig. 2, a straight line is obtained which can be extrapolated to a chlorophyll b/total chlorophyll ratio of 0.37 at zero P 700/total chlorophyll. This implies that if there is a region with zero P 700 concentration it should have a chlorophyll a/b (mol/mol) ratio of 1.7, which is in good agreement with previous extrapolation[10] and with what one should expect for a membrane preparation containing photosystem II reaction centres plus their antenna. In some of the sonicated fractions such low a/b ratios were indeed found; they also had extremely low P 700 concentrations, near the detection limit of the method for analysing for P 700. Thus, it can be concluded that sonication leads to fragmentation of inside-out thylakoid vesicles into smaller vesicles, some

of which lack P 700. Since these vesicles have a diameter of about 0.3 μm[9] it can be concluded that *in vivo* there are regions rich in PS II which do indeed lack P 700. These results therefore support the suggestion[3] that inside-out PS II vesicles (B3) originate from regions in the native thylakoid membrane containing only PS II, and they also support a model of the thylakoid membrane in which PS I is excluded from the partition region.[15,16] The PS I contaminant in inside-out vesicles obtained by press treatment is therefore not intermixed at the molecular level with PS II, as suggested by Atta-Asafo-Adjei and Dilley,[17] but is located in separate contaminating domains probably originating from stroma membranes.

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