

Membrane and Vesicle Formation from Fragments and Proteins of Thylakoids

WILHELM MENKE, ALFONS RADUNZ, and FRIEDERIKE KOENIG

Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), Köln-Vogelsang

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Fragments of the thylakoid membrane, obtained by ultrasonication of stroma-freed chloroplasts and subsequent fractioning centrifugation, aggregate in buffer solutions of suitable ionic strength in the pH-range of 6.0—8.5. This aggregation leads to the formation of vesicles and membranes, which may reach a size of up to 0.5 μ m. A suspension of the fragments, having an average diameter of 100 \AA , is stable in water.

A protein preparation, obtained by treatment of stroma-freed chloroplasts with sodium deoxycholate and subsequent gel filtration, contains 4 % chlorophyll but no detectable amounts of colourless chloroplast lipids, nor deoxycholate. This preparation aggregates in the pH-range of 6.0—7.0 equally to membranes and vesicle-like structures. The solution of this protein, exhibiting a molecular weight of 600 000, is stable in water.

The proteins of both preparations are not denatured and both preparations exhibit photochemical reactions.

In recent years it has been frequently reported that supermolecular structural units of the cell may be separated into their components, and may be subsequently reconstituted from these components^{1,2}. To our knowledge, a reaggregation of fragments of the lamellar system of chloroplasts has only been reported by ARNTZEN *et al.*³. These authors separated the lamellar system of spinach chloroplasts by french press treatment and subsequent fractioning centrifugation into two fractions. From the sediment they extracted, by means of digitonin, a fraction which exhibited photosystem I activity. When the ionic strength of the medium was increased, this fraction aggregated into membranes. TAKACS and HOLT⁴ and LOACH *et al.*⁵ reported on the dissolution and reaggregation of bacterial thylakoids. However, none of the preparations obtained was detergent-free. As it is not known whether detergents might possibly play a role in membrane formation, it cannot be deduced from these experiments that the particles themselves are able to aggregate into membranes. Furthermore, some of these preparations contained at least as much lipid as is required for a film.

In the following, we report on the formation of vesicles and membranes from detergent-free fragments

of the thylakoid membrane. It is further demonstrated that protein preparations, which do not contain enough lipids for the formation of a film and which are detergent-free, are able to form membranes.

Materials and Methods

The preparation of fragments of the thylakoid membrane (ultrasonic supernatant) from stroma-freed chloroplasts of *Antirrhinum majus* was carried out as described earlier^{6,7}. The preparation of the protein fraction was described recently⁸. In order to determine the influence of pH and ionic strength on the aggregation, equal volumes of a suspension of fragments of the thylakoid membrane were added to the same amount of buffer solutions. Phosphate buffer according to Sørensen (0.012–0.6 M, pH 5.0–8.0) and tris/HCl buffer Tris(hydroxymethyl)aminomethane 0.05–0.5 M, pH 7.2–9.0) were used. In order to investigate the influence of bivalent cations on the aggregation, EDTA (Ethylene diamine tetraacetate) up to a final concentration of 2–7 mmol/l was added to these buffers.

The proteins (Fraction 1)⁸, dissolved in distilled water, were dialyzed against the buffer solutions. The buffers used were: citric acid-phosphate buffer according to McIlloine (0.1 M citric acid and 0.2 M Na_2HPO_4 , pH 2.2 and 4.0), glycine buffer according to Sørensen (7.505 g glycine and 5.85 g NaCl/l , adjusted with 0.1 N HCl to pH 3.0), ammonium formate (0.05 M pH 3.8), phosphate buffer according to Søren-

Requests for reprints should be sent to Prof. Dr. W. MENKE, Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), D-5000 Köln 30.



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sen (0.01–0.1 M, pH 5.0–8.0) and tris/HCl buffer (0.2 M, pH 8.5).

In order to check the reversibility of the membrane formation, we dialyzed against flowing distilled water.

The particle sizes were measured on electron micrographs, obtained from negatively-stained preparations, at a magnification of 100 000 with an Elmiskop 101 (Siemens) equipped with a sample cooling device. Staining of the preparations was achieved with uranyl acetate. Over 3000 particles from each preparation were measured.

Membrane and Vesicle Formation from Fragments of the Thylakoid Membrane

Suspensions of membrane fragments, obtained by ultrasonication of stroma-freed chloroplasts of *Antirrhinum majus* and subsequent centrifugation were stable in distilled water⁶. The particles exhibited an average diameter of 100 ± 1 Å. The most frequent value was equally 100 Å. The fragments reduced methylviologen or anthraquinone-2-sulfonate in the light with dichlorophenolindophenol/ascorbate as the electron donor⁷. Their lipid content was 38 % and approximately the same as that of the thylakoid membrane⁷. When the preparation was suspended in buffers in the pH range of 6.0–8.5, extended membranes and vesicle-like structures were observed in the undisturbed clear water (Figs 1*–4). These structures reached a size of up to 0.5 mm. We were able to show that the membrane formation occurred in tris as well as in phosphate buffers. At a pH-value of 7.2–7.5 membrane formation occurred in phosphate and tris buffers in a concentration range of 0.006–0.1 moles/l, whereas at higher buffer concentrations, precipitation was observed. At pH 8.5 (tris buffer), membranes and vesicles are formed only at higher buffer concentrations. At pH 5.0 and below, the preparation was precipitated. Addition of EDTA did not prevent the membrane formation, however at pH 8.5 it appears that membrane formation occurred to a smaller degree in the presence of EDTA. Membrane formation was observed over a temperature range of 0–37 °C. Removal of the ions by dialysis against water did not lead to the dissolution of the membranes.

Reaggregation of Proteins of the Thylakoid Membrane into Membranes

Recently, we described the preparation of a protein fraction from stroma-freed chloroplasts of *Antirrhinum*

*majus*⁸. For the dissolution of the lamellar system, sodium deoxycholate in alkaline solution was used. Lipids and detergent were removed by gel filtration. The protein particles, referred to as Fraction 1, exhibited a molecular weight of 600 000 and had an average diameter of 99 ± 1 Å. The particles contained approximately 4 % chlorophyll. Colourless chloroplast lipids and deoxycholate were not detectable. The circular dichroism of this protein in the region of peptide absorption was, within the limits of error, the same as that of fragments of the thylakoid membrane. The preparation reduced methylviologen and anthraquinone-2-sulfonate in the light with the dichlorophenolindophenol/ascorbate couple as the electron donor. The protein was soluble in water. It precipitated during dialysis against buffer in a pH range between 3 and 6. Between pH 6 and 7 the preparations appeared clear, despite the fact that the proteins were retained by paper filters. Upon examination under the light microscope we observed that the proteins were aggregated into membranes. These membranes were of different shapes and sizes, according to the conditions during formation. In general, we observed a few disc-shaped particles aside to extended membranes, which may achieve a size of up to 0.5 mm in the same preparation. The large membranes tended to roll up (Fig. 5). Vesicle-like formations were less frequently observed (Fig. 6). Occasionally, membranes with branchings (Fig. 7) or even giant thylakoid-like structures were found (Fig. 8). A detailed description of the conditions, which are necessary for the different formation of the membranes, cannot be made. However, we observed that near the precipitation zone, for example at pH 6, smaller membrane pieces and amorphous appearing precipitations were prevalent. Membranes were only occasionally observed on the acid side of the precipitation zone at pH 3. It is remarkable that no aggregation took place in water. This was demonstrated by the fact that the preparation exhibited the same molecular weight both in water and in carbonate solution (pH \approx 11.5)⁸. The membranes were not soluble in pure water.

Discussion

To our knowledge no report exists describing that membrane protein particles containing no detectable amounts of detergent and which in addition contain only a small fraction of the original lipids, can aggregate into membranes. The 4 % chlorophyll and the

* Figs 1–8 see Table page 64a.

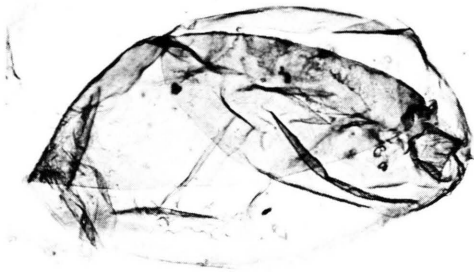


Fig. 1.

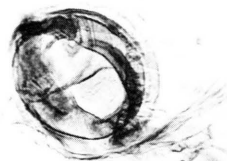


Fig. 2.



Fig. 3.



Fig. 4.

Figs. 1—4. Membranes and vesicles formed from fragments of the thylakoid membrane. Mag. 170 : 1.

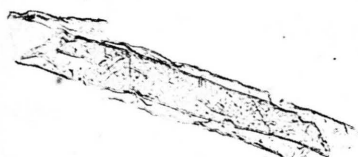


Fig. 5.

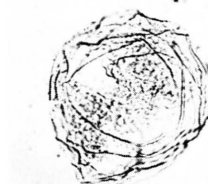


Fig. 6.



Fig. 7.



Fig. 8.

Figs. 5—8. Membranes formed out of proteins of the thylakoid membrane. Fig. 8. Phase-interference-contrast picture Mag. 170 : 1.

2–3 % ether-soluble components present in our preparation cannot suffice for the formation of a lipid film. However, they may play a role in the stabilization of the conformation of the polypeptides. Our experiments show that the probably disc-shaped and uniformly-sized protein particles derived from the thylakoid membrane may order themselves in a preferentially sidewise manner thus forming extended membranes of different thickness without the presence of a lipid film. From the dependence of the membrane formation on the pH value and the ionic strength, it becomes clear that interionic actions are participating in this process. Bivalent cations, like Mg^{++} and Ca^{++} , however, do not play a role, because the membrane formation also occurs without the addition of these cations. This appears worth mentioning because frequently, according to the literature, reaggregations are only observed in the presence of Mg^{++} ^{9–15, 4}.

Less surprising is the observation that fragments of the thylakoid membrane reaggregated into membranes, since they contained the original amounts of lipids. According to our observations, lipids appear to favor the formation of closed vesicles and vesicle-like structures. As EDTA did not impede membrane formation, it appears that in this case too, divalent cations are

not necessary. However, in pure water, that is, in the absence of ions, no aggregation was observed.

The fact that the membranes, once formed, did not dissolve after removal of ions from the medium, suggests the participation of an entropy effect in membrane formation. As detergents cause the dissolution of membranes, it is assumed that mutual hydrophobic action plays a role in the stabilization of the membrane structure¹⁶.

The fact that proteins as well as lipids are capable of forming membranes, may serve as an argument in favor of a layer model rather than of a mosaic model of the thylakoid membrane.

In context with discussion on the chemiosmotic theory of photophosphorylation, it should be noted that our protein preparation, in which coupling factor is detectable, is able to form ATP at a low rate in the light⁸. Whether this is related to the presence of vesicles and whether the membranes are semipermeable, despite their low lipid content, is to be investigated. that our protein preparation, in which coupling factor

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