Binding of Antibodies onto the Thylakoid Membrane. VI. Asymmetric Distribution of Lipids and Proteins in the Thylakoid Membrane

Alfons Radunz

Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), D-5000 Köln-Vogelsang

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Dedicated to Professor W. Menke *at the Occasion of His 70th Birthday*

Chloroplasts, Lipids, Proteins, Antibody Binding, Thylakoid Membrane Surface

The maximal binding of antibodies out of monospecific antisera to proteins and lipids onto three different chloroplast preparations is compared. In these preparations different parts of the thylakoid membrane surface are accessible to antibodies. Whereas stroma-freed chloroplasts bind antibodies only at the outer surface, also the inner membrane surface is exposed in the two chloroplast preparations which were obtained by ultrasonication and subsequent fractionating centrifugation. In the ultrasonic sediment the inner surface is prevailing. In the ultrasonic supernatant antibodies do not only react with the inner and outer surface but also with considerable parties of the interior of the thylakoid membrane.

It was found that the thylakoid membrane surface exposed to the stroma consists preponderantly of proteins whereas the surface directed towards the interior of the thylakoids consists mainly of lipids. All proteins involved in electron transport such as ferredoxin, ferredoxin-$NADP^+$-reductase, plastocyanin, cytochrome f and the coupling factor of photophosphorylation are detectable in the outer surface. The molecules of the coupling factor span the thylakoid membrane from the outside to the inside. They hinder the binding of antibodies to monogalactosyl diglyceride and to a polypeptide with the apparent molecular weight 24000. The polypeptide 24000 is a major component of the membrane proteins, and is detected on the outer and inner surface of the membrane. The major part of this polypeptide, however, is located in the interior of the thylakoid membrane.

The lipid mixture has a different composition in the outer surface than on the inside face of the membrane. The sulfoquinovosyl diglyceride and the phosphatides, phosphatidyl glycerol, phosphatidyl cholin and phosphatidyl inositol are stronger concentrated in the outer surface than in the inside face. The neutral monogalactosyl diglyceride and di- or trigalactosyl diglyceride, however, occur in the inner surface in higher concentrations than in the outer surface. The major part of the sulfoquinovosyl diglyceride and of the monogalactosyl diglyceride are located in the interior of the membrane.

Introduction

Fragments of the thylakoid membrane, which have been obtained from stroma-freed chloroplasts of *Antirrhinum majus* by ultrasonication and fractionating centrifugation, are able to bind maximally equal amounts of antibodies to lipids and proteins [1]. In contrast stroma-freed chloroplasts bind four times more antibodies to proteins than antibodies to lipids [2–5]. From this it is concluded that the major part of the thylakoid membrane surface directed towards the inside consists of lipids. This conclusion, however, is not entirely unequivocal; as stated already earlier the mentioned membrane fragments have an average diameter of 100 Å, whereas their thickness corresponds to that of the thylakoid membrane [6]. Therefore, ultrasonication not only exposes the inner surface of the thylakoid membrane to antibodies but in the fracture faces also considerable portions of the interior of the membrane. Therefore, it appeared interesting to study the antibody binding with a chloroplast preparation in which the portion of the new fracture faces is small in comparison to the portion of the exposed inner surface. In the present paper, therefore, the antibody binding is studied with the ultrasonic sediment, which is obtained by ultrasonication and centrifugation. The results are compared with earlier work.

Reprint requests to Dr. A. Radunz, Universität Bielefeld, Fakultät für Biologie, Zellphysiologie, Universitätsstr. 1.

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Methods

Quantitative lipid determination

The individual lipid components of the ultrasonic sediment were determined according to methods described earlier [1, 2, 7, 8] (Table I). The lipid

composition of stroma-freed chloroplasts and of the ultrasonic supernatant was taken from our earlier work [1, 2].

Preparation of the antisera

The antisera to lipids, pigments, proteins and chloroplasts listed in the tables are the same as those which are described in preceding articles [1–5, 9]. They were obtained by immunization of rabbits. The specificity of these antisera has also been characterised in earlier work [4, 9–21]. The antisera were heated for 20 min to 56 °C in order to inactivate residual complement.

Preparation of ultrasonic sediment

Stroma-freed chloroplasts prepared according to an earlier described procedure [1] from *Antirrhinum majus* were ultrasonicated according to Kannangara *et al.* [22] 8 times during 30 seconds and subsequently centrifuged for two hours at 33000 × *g*. This led to the sedimentation of about 60 per cent of the chloroplast material (= ultrasonic sediment). The remaining 40 per cent stayed as membrane fragments in the supernatant (= ultrasonic supernatant).

Quantitative binding of antibodies onto the ultrasonic sediment

The maximal binding of antibodies onto the ultrasonic sediment was determined as with stroma-freed chloroplasts in the region of excess of antibodies. Antigen samples of 30 µg each were supplemented with increasing amounts of serum in parallel series. The amount of serum was 0.04 to 5 ml (see

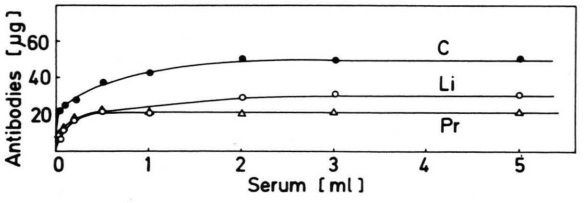

Fig. 1. Binding of antibodies onto 30 µg ultrasonic sediment from *Antirrhinum majus* chloroplasts plotted against serum volume. C, binding of antibodies to stroma-freed chloroplasts (antiserum contains antibodies to proteins and lipids). Li, binding of antibodies to all in Table I listed lipids and carotenoids with the exception of chlorophyll. Pr, binding of antibodies to proteins of the thylakoid membrane (proteins involved in electron transport and proteins insoluble in aqueous media with the apparent molecular weights 11000 to 66000). The values are averages of 12 individual determinations.

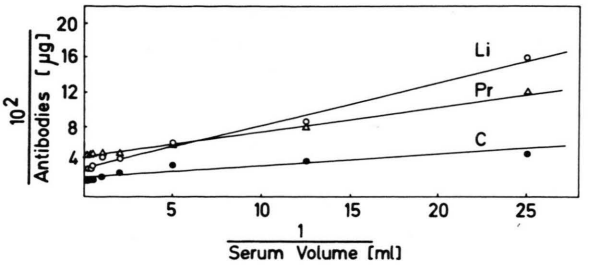

Fig. 2. Dependence of the amount of bound antibodies on the serum volume plotted in the reciprocal system (values from Fig. 1). C, antibodies to stroma-freed chloroplasts. Li, antibodies to membrane lipids. Pr, antibodies to proteins of the thylakoid membrane.

Fig. 1). The samples were permanently shaken for 6 h at room temperature and subsequently kept for 16 h at 5 °C. Thereafter the not bound gamma globulines and serum albumines were washed with physiological saline (0.8%) out of the preparation. The nitrogen determination and the subsequent calculation of the protein content were carried out according to the method described in part I [2]. Control experiments were carried out with sera, which were withdrawn from the animals before immunization. The obtained protein values of these controls were substracted from the protein values of the fragments treated with active sera, which yielded the amount of bound antibodies. These values were plotted in Fig. 1 as function of the added amount of serum. It was seen, that with the adsorption of antibodies to lipids and proteins onto the ultrasonic sediment, just as with the binding of antibodies onto stroma-freed chloroplasts a saturation value is reached. If the amount of bound antibodies is plotted in the reciprocal system as dependence on the added amount of antibodies (serum volume), as shown in Fig. 2, the values are located on a straight line. From the crossing point of this straight line with the *y*-axis the amount of antibodies, maximally bound, is calculated. These values coincide within the error limits of ± 2% with the saturation values obtained by the graphical plot. The results of the maximal binding are summarized in Tables II to V. For comparison purpose the data on antibody binding by stroma-freed chloroplasts and by the ultrasonic supernatant taken from earlier work are also listed in the tables.

As the lipid antisera also contained antibodies to methylated bovine serum albumine, the ultrasonic sediment was incubated in parallel assays with

antisera which contained antibodies to methylated bovine serum albumine. Out of these bovine serum albumin antisera no antibodies were adsorbed onto chloroplast fragments.

Double diffusion test in agarose

Immune precipitation as the test for monospecificity of the antisera to the coupling factor subunits was carried out in 0.8% agarose gel [23]. The 0.06 M barbiturate buffer had a pH of 8. The *Antirrhinum* chloroplasts used as antigen were prepared according to Homann and Schmid [24] suspended in a 0.06 M phosphate buffer containing 1% triton X 100 and sonicated twice for 30 sec [4]. The diffusion time was between 24 and 36 h. The plates were stained with a 0.2% amido black solution in acetic acid after being washed 1.7% sodium chloride solution.

Results and Discussion

Morphology and chemical properties of the chloroplast preparations

If stroma-freed chloroplasts of *Antirrhinum majus* are ultrasonicated in distilled water and fractionated by centrifugation two fractions are obtained [22]. The supernatant contains small fragments of the thylakoid membrane, which exhibit an average diameter of 100 Å [6]. These fragments suspended in the supernatant are designated as ultrasonic supernatant. The sediment consists mainly of small thylakoid stacks which originate from the grana of the chloroplasts. The outer thylakoids of these stacks which are in contact with the suspension medium are just as the thylakoids of the intergrana regions either disrupted or decomposed to small fragments, which stay in the supernatant [25]. In addition the preparation contains some disrupted thylakoids. Therefore in the fragments of the sediment considerable portions of the inner surface are exposed and accessible to antibodies. This fraction is called ultrasonic sediment. The relative portion of outer thylakoid surface is smaller in the ultrasonic sediment than in the fragments of the ultrasonic supernatant. Also the portion of membrane fracture sites is considerably smaller in the sediment. Moreover, in the ultrasonic sediment the surface accessible to antibodies is relatively small, since the thylakoids border in the stacks neighboring thylakoids.

Hence, in the three chloroplast preparations different sides of the membrane surface are accessible to antibodies. In stroma-freed chloroplasts antibodies are only bound to the outer surface of the thylakoids if the preparation does not contain disrupted thylakoids. The ultrasonic sediment, however, adsorbs antibodies onto the outer and inner membrane surface with preponderance of the adsorption onto the surface which is directed towards the inside. With the ultrasonic supernatant the outer and inner surface are equally accessible to antibodies. In addition considerable portions of the interior of the membrane are exposed. At the edges of the fragments dislocations of molecules probably

Table I. Chemical composition of stroma-freed chloroplasts, ultrasonic sediment and ultrasonic supernatant in per cent dry weight.

	Stroma-freed chloroplasts	Ultrasonic sediment	Ultrasonic supernatant
Proteins	50.00 ± 1	48.00 ± 1.00	51.00 ± 1.00
Lipids	43.40 ± 0.80	39.30 ± 0.70	37.90 ± 0.90
Monogalactosyl diglyceride	11.00 ± 0.30	7.60 ± 0.30	8.30 ± 0.30
Digalactosyl diglyceride	10.10 ± 0.30	4.80 ± 0.20	6.90 ± 0.30
Trigalactosyl diglyceride	0.90 ± 0.02	0.28 ± 0.03	1.10 ± 0.10
Sulfoquinovosyl diglyceride	2.00 ± 0.20	0.71 ± 0.06	1.50 ± 0.10
Phosphatidyl glycerol	1.85 ± 0.06	1.18 ± 0.15	1.60 ± 0.06
Phosphatidyl cholin	0.12 ± 0.01	0.16 ± 0.01	0.11 ± 0.07
Phosphatidyl inositol	0.14 ± 0.04	0.31 ± 0.01	0.28 ± 0.01
Chlorophyll a	9.30 ± 0.40	11.00 ± 0.40	10.20 ± 0.40
Chlorophyll b	3.30 ± 0.40	3.90 ± 0.30	3.00 ± 0.40
Lutein	0.88 ± 0.03	0.58 ± 0.02	0.45 ± 0.01
Neoxanthin	0.30 ± 0.01	0.19 ± 0.01	0.24 ± 0.01
Violaxanthin	0.29 ± 0.01	0.10 ± 0.01	0.22 ± 0.01
β-Carotene	0.62 ± 0.02	0.31 ± 0.01	0.38 ± 0.02

Chloroplasts were isolated from *Antirrhinum majus*.

occur. By comparison of the antibody binding by the three chloroplast preparations information on the nature of the antigens in the inner and outer membrane surface can be obtained and to a certain extend also information on the interior of the membrane.

A comparison of the antibody binding by these three chloroplast preparations, however, appears only meaningful, if the three preparations do not differ too much with respect to their chemical composition. As is seen in Table I the three fractions certain within the error limits the same amounts of proteins and lipids. Thus, the ultrasonic sediment, which mainly consists of grana stacks, does not contain additional amounts of lipids. The amount of chlorophyll shows no significant differences. On the other hand certain lipids occur in the three chloroplast preparations in different amounts.

Maximal binding of antibodies onto chloroplast preparations

The maximal binding of antibodies out of an antiserum to stroma-freed chloroplasts increases upon fragmentation of the lamellar system (Table II). This antiserum contains antibodies to proteins and lipids of the lamellar system. The increase of antibody binding referred to the weight unit, is to a certain extend a measure for the increase in antibody binding of the thylakoid membrane surface. As is seen from Table II, the maximal antibody binding out of an antiserum to stroma-freed chloroplasts is distinctly increased, but there is not so much increase as with the ultrasonic supernatant. This result is according to expectation, if the preparations exhibit the above described morphological structures with the ultrasonic supernatant the maxi-

Table II. Maximal binding of antibodies to lipids and proteins onto stroma-freed chloroplasts, ultrasonic sediment and ultrasonic supernatant.

Antiserum to	Stroma-freed chloroplasts	Ultrasonic sediment	Ultrasonic supernatant
Stroma-freed chloroplasts	1.05 ± 0.02	1.44 ± 0.07	2.54 ± 0.24
Total proteins [b]	1.02 ± 0.03	0.74 ± 0.02	1.56 ± 0.10
Total lipids [c]	0.24 ± 0.01	0.91 ± 0.05	1.50 ± 0.10
Monogalactosyl diglyceride	0.16 ± 0.02 (0.36) [a]	0.53 ± 0.04	1.16 ± 0.09
Tri- and digalactosyl diglyceride	0.17 ± 0.01	0.57 ± 0.03	0.98 ± 0.07
Sulfoquinovosyl diglyceride	0.12 ± 0.01	0.32 ± 0.01	1.16 ± 0.10
Phosphatides (Phosphatidyl glycerol, Phosphatidyl cholin, Phosphatidyl inositol, Cardiolipin)	0.20 ± 0.01	0.47 ± 0.05	1.20 ± 0.10
Carotenoids (Lutein, Neoxanthin, Violaxanthin, Zeaxanthin, β-Carotene)	0.09 ± 0.01	0.18 ± 0.01	
Polypeptide MW 24000	0.10 ± 0.01 (0.20) [a]	0.21 ± 0.01	0.72 ± 0.10
Coupling factor	0.40 ± 0.04	0.70 ± 0.03	0.76 ± 0.07
Subunits of the coupling factor			
α-component MW 66000	0.22 ± 0.01		
β-component MW 62000	0.31 ± 0.02		
γ-component MW 40000	0.26 ± 0.02		
δ-component MW 22000	0.29 ± 0.03		
ε-component MW 11000	0.42 ± 0.04		

The values are referred to 1 g dry weight of the chloroplast preparations. The binding data for stroma-freed chloroplasts and the ultrasonic supernatant are taken from earlier work. The values for the ultrasonic supernatant were multiplied with the factor 2, as the binding of antibodies was determined according to the quantitative precipitation reaction and because antibodies are bivalently bound onto membrane fragments of the ultrasonic supernatant. With stroma-freed chloroplasts and ultrasonic sediment the determination was carried out in the region of excess antibodies where antibodies consequently are monovalently bound.

[a] The values in brackets give the maximal binding of antibodies to monogalactosyl diglyceride and polypeptide MW 24000 after removal of the coupling factor.

[b] The antiserum to "Total proteins" contains antibodies to proteins of the thylakoid membrane.

[c] The antiserum to "Total lipids" contains antibodies to all in Table I listed lipids and carotenoids with the exception of chlorophyll.

mal binding is smaller than the increase of the geometrical surface. This is due to the hindering shape of the antibody molecules and to the fact that antibodies and membrane fragments have similar sizes. The size and shape of the antibodies is also the reason, why the sum of adsorbed antibodies out of a mixed lipid and protein antiserum is larger than the maximal adsorption of antibodies out of an antiserum to stroma-freed chloroplasts. As is shown in Table II 1 g ultrasonic sediment binds 1.2 × more lipid antibodies than antibodies to proteins. On the other hand stroma-freed chloroplasts bind 4 times more antibodies to protein than antibodies to lipids [2]. The ultrasonic supernatant binds approximately the same amount of antibodies to lipids and to proteins.

For the comparison of the antibody binding by the three chloroplast preparations the weight unit as reference is not suited since in these preparations different portions of the surface are accessible to antibodies. Therefore in Table III we refer to the amount of antibodies, which is maximally bound out of an antiserum to stroma-freed chloroplasts. This calculation shows that the ultrasonic sediment binds more antibodies to lipids than the ultrasonic supernatant despite the fact that with the membrane fragments of the ultrasonic supernatant not only the outer and inner surface of the thylakoid membrane are exposed but also portions of the interior of the membrane. This result is due to the fact that in the ultrasonic sediment more inside face of the membrane than outside face is accessible to antibodies. Concomitantly the ultrasonic sediment binds less protein antibodies than the ultrasonic supernatant and than stroma-freed chloroplasts. According to earlier studies the outside face of the thylakoid membrane consists by approx. 85% of protein molecules, the present study shows that the inner surface of the membrane consists mainly of lipid molecules.

Table III. Antibodies binding onto stroma-freed chloroplasts, ultrasonic sediment and ultrasonic supernatant.

Antiserum to	Stroma-freed chloroplasts	Ultrasonic sediment	Ultrasonic supernatant
Stroma-freed chloroplasts	1.00	1.00	1.00
Total Proteins [b]	0.97	0.51	0.61
Total Lipids [c]	0.23	0.63	0.59
Monogalactosyl diglyceride	0.15 (0.34) [a]	0.37	0.46
Tri- and digalactosyl diglyceride	0.16	0.40	0.39
Sulfoquinovosyl diglyceride	0.11	0.22	0.46
Phosphatides (Phosphatidyl glycerol, Phosphatidyl cholin, Phosphatidyl inositol, Cardiolipin)	0.19	0.33	0.47
Carotenoids (Lutein, Neoxanthin, Violaxanthin, Zeaxanthin, β-Carotene)	0.09	0.13	
Polypeptide MW 24000	0.10 (0.19) [a]	0.15	0.28
Coupling factor	0.38	0.49	0.30

The values are taken from Table II and referred to the amount of antibodies which chloroplasts can maximally bind out of an antiserum to stroma-freed chloroplasts. The explanations a to c are to be taken from Table II.

The adsorption of antibodies out of monospecific protein antisera led to the result, that the ultrasonic sediment binds 29 per cent more antibodies to the coupling factor than stroma-freed chloroplasts, if one refers the values to the maximal antibody binding (Table III). Since in the ultrasonic sediment proportionally more inner surface than outer surface of the thylakoid membrane is available for antibody binding, it follows that coupling factor antibody molecules are also adsorbed onto the inner membrane face and that also in the thylakoid membranes of the grana coupling factor occurs. This conclusion seems to be in contradiction with the observation, that ultrasonic supernatant binds somewhat less coupling factor antibodies than is bound by stroma-freed chloroplasts. The reason for this is that ultrasonic supernatant contains less coupling factor than stroma-freed chloroplasts and ultrasonic sediment. An earlier tested preparation of the ultrasonic supernatant contained no coupling factor [22]. Noteworthy is also the result which is obtained if one refers the binding of antibodies to coupling factor to the amount of antibodies which is maximally bound out of an antiserum to proteins of the thylakoid membrane (Table IV). Whereas the binding of antibodies to the coupling factor makes up for 39 per cent of the maximally bound antibodies with stroma-freed chloroplasts, this value increases to 94 per cent with the ultrasonic sediment. This means that the coupling factor on the inside face of the membrane exceeds the amount of all other protein molecules. It might mean that the thylakoids in the grana regions contain more coupling factor than those in the intergrana regions. These results

Table IV. Antibody binding onto stroma-freed chloroplasts, ultrasonic sediment and ultrasonic supernatant.

Antiserum to	Stroma-freed chloroplasts	Ultrasonic sediment	Ultrasonic supernatant
Total proteins	1.00	1.00	1.00
Polypeptide MW 24000	0.10	0.28	0.46
Coupling factor	0.39	0.94	0.49

The values are taken from Table II and referred to the amount of antibody, which the chloroplast preparations can maximally bind out of an antiserum to proteins to the thylakoid membrane.

are only understandable if the coupling factor molecules span the membrane.

In addition the binding of antibodies to the subunits of the coupling factor with the apparent molecular weights 66000, 62000, 39000, 22000 and 11000 onto stroma-freed chloroplasts was studied (Table II). These antisera not only precipitate an authentic coupling factor preparation but react also with stroma-freed chloroplasts (Table II). The antisera yield in the agarose double diffusion test against a chloroplast preparation of *Antirrhinum* solubilized in triton only one band (Fig. 3). The effect of these antisera on photophosphorylation, electron transport and proton transfer was described earlier [9]. Moreover, as is seen from Table II, 1 g stroma-freed chloroplasts binds 0.22 up to 0.42 g of antibodies out of the sera. The highest binding value was obtained for the ε-component with the apparent molecular weight 11000. Out of an antiserum to the complete coupling factor 0.4 g antibodies are adsorbed [3]. From this result it can be concluded that the portion of the coupling factor molecule, which is located in the outer thylakoid membrane surface, contains antigenic determinants of all five subunits.

Concerning the polypeptide with the molecular weight 24000, which has the amino terminal sequence Ala-Ala-Gly-Lys-Pro-Thr-Asp [4]*, it should be noted that the ultrasonic supernatant binds almost 3 times as much antibodies to this polypeptide than stroma-freed chloroplasts (Table III). After removal of the coupling factor by washing with an EDTA-containing solution [1] stroma-freed chloroplasts bind two times more antibodies to the polypeptide 24000. From this it is concluded that not all molecules of this antigen are located in the outer surface, and that a considerable portion lies inside the membrane. In addition the results listed in Table III and IV demonstrate that this polypeptide also occurs at the surface directed towards the inside.

Moreover, the question arises, whether the lipid composition at the outer and inner surface of the thylakoid membrane is the same. If this was the case chloroplast preparations in which only the outer surface is accessible to antibodies, should bind approximately the same relative amount of antibodies to the individual lipids as a chloroplast preparation which in addition can bind antibodies on the inside face of the membrane. In order to make an estimate, the amount of bound antibodies to a certain lipid must be referred to the amount of lipid antibodies which can be maximally bound by the chloroplast preparation (Table V). This is necessary, since during fragmentation the areas which are occupied by lipid molecules are changed. From Table V it is seen, that the lipid mixture on the inside and the outside face of the membrane has not the same composition. Sulfoquinovosyl diglyceride and phosphatides occur in higher amounts on the outside face than on the inside face. The big difference between the antibody binding out of the antiserum to sulfolipid by the ultrasonic sediment and the ultrasonic supernatant shows that this lipid is preponderantly located in the interior of the

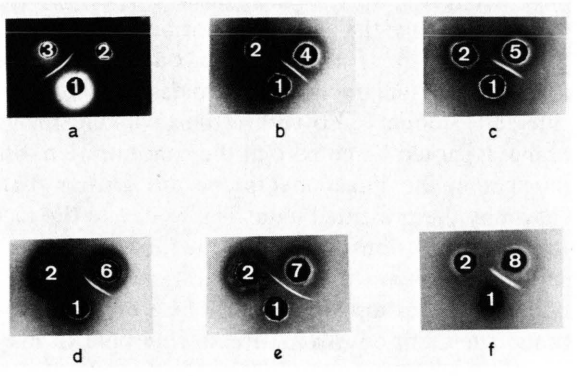

Fig. 3. Immune diffusion in agarose as test for monospecificity of the antisera to the coupling factor (a) and to the subunits with the molecular weights 66000 (b), 62000 (c), 39000 (d), 22000 (e) and 11000 (f). Hole 1: *Antirrhinum* chloroplasts with 1% triton X 100. Hole 2: control serum. (a) Hole 3: antiserum to coupling factor; (b) Hole 4: anti-α-CF_1; (c) Hole 5: anti-β-CF_1; (d) Hole 6: anti-γ-CF_1; (e) Hole 7: anti-δ-CF_1; (f) Hole 8: anti-ε-CF_1.

* The sequence analysis was carried out by Dr. Beyreuther, Genetic Institute of the University of Cologne.

Table V. Antibody binding onto stroma-freed chloroplasts, ultrasonic sediment and ultrasonic supernatant.

Antiserum to	Stroma-freed chloroplasts	Ultrasonic sediment	Ultrasonic supernatant
Total Lipids	1.00	1.00	1.00
Monogalactosyl diglyceride	0.67	0.58	0.77
Tri- and digalactosyl diglyceride	0.71	0.63	0.65
Sulfoquinovosyl diglyceride	0.50	0.35	0.77
Phosphatides	0.83	0.52	0.80
Carotenoids	0.38	0.20	

The values are taken from Table II and referred to the antibody amount that chloroplasts can maximally bind out of a lipid antiserum. The lipid antiserum contains antibodies to all in Table II listed membrane lipids and pigments with the exception of chlorophyll.

membrane, which means that the major part of sulfolipid molecules is located neither at the inside nor at the outside face of the thylakoid membrane. It should be noted that the sulfoquinovosyl diglyceride is the only colorless lipid, the antiserum of which inhibits electron transport of chloroplasts [26]. It seems as if intermolecular interactions exist between this lipid and proteins. The neutral galactolipids are stronger localised at the membrane surface directed towards the inside (Table III). As the membrane fragments of the ultrasonic supernatant bind 33 per cent more antibodies to monogalactosyl diglyceride than the ultrasonic sediment (Table V) and since, as shown earlier, stroma-freed chloroplasts bind twice the amount of antibodies to monogalactolipids after removal of the coupling factor, it is concluded that monogalactolipids occur in considerable amounts also within the thylakoid membrane. It should be noted that the concentration differences in the intact membrane are greater than shown by the presented data. This is due to the fact that our conclusions concerning the inner membrane surface are obtained with preparations which also bind antibodies also the outside face of the membrane. In addition stroma-freed chloroplasts may contain some disrupted thylakoids. But the result are qualitatively unequivocal.

Finally it should be noted, that these results describe the condition of not swellable thylakoid membranes in stroma-freed chloroplasts. This data will be probably modified if swellable chloroplasts with higher electron transport and photophosphorylation rates are studied. This is to be expected, because antibodies to the anionic lipids [5, 10, 11], pigments [14–21] as well as to proteins [27–33] which are involved in electron transport, are only monovalently bound stroma-freed chloroplasts, whereas these antibodies react bivalently with swellable chloroplasts.

Since a considerable amount of comparable data is available now, it is tempting, to calculate the quantitative composition of the surfaces of the thylakoid membrane. This, however, is principally not possible, as the number of bound antibody molecules does not correspond to the number of antigen molecules per surface unit. This is due to the fact that a bound antibody molecule may cover up several antigen molecules, if their local concentration is high enough. In addition, antibody molecules may also cover up alien antigens. This covering effect is the larger the smaller the antigen molecules are and the higher their concentration is. Due to this effect is the observation that the sum of the binding data of colorless lipids in Table II is twice to three times higher than the amount of antibodies which can be accommodated at the membrane surface. If one wants to get a picture of the composition of the lipids at the membrane surfaces one should refer the individual data of the lipids to the amount of antibodies to lipids which can be simultaneously accommodated at the membrane surface and not to the sum of antibodies that can be maximally bound.

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