Intermolecular Interactions of Polypeptides and Lipids in the Thylakoid Membrane

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Intermolecular interactions between chloroplast lipids and a polypeptide fraction from thylakoids were investigated by far ultraviolet circular dichroism. The polypeptide fraction was isolated from dodecyl sulfate-containing buffers. It exhibits an average molecular weight of 24 000. The circular dichroism of this polypeptide fraction measured as mean residue ellipticity is greater in the presence of sodium dodecyl sulfate than in the absence of this detergent. This effect is reversible. Addition of sulfoquinovosyl diglyceride to the dodecyl sulfate-free solution of the polypeptide also causes an increase of the circular dichroism. This increase was only observed in the pH-range between 6.9 and 7.4. The effect of dodecyl sulfate or sulfolipid on the circular dichroism is interpreted to indicate an increase of α-helix content. Monogalactosyl diglyceride, digalactosyl diglyceride and phosphatidyl glycerol gave no reaction. The attempt to obtain a conformational analysis of the polypeptide in the different states did not yield an entirely satisfactory result. Antisera to sulfolipid inhibit photosynthetic electron transport of stroma-freed chloroplasts in the region of light reaction I. This inhibition is restricted to the same pH-range as the non-covalent binding of sulfolipid to the polypeptides. It appears that in the cell membrane-bound metabolic processes are regulated by this pH-dependence of the sulfolipid-polypeptide interactions.

From the literature it is known for some time that the amphiphilic chloroplast lipids form myelin figures in contact with water [1, 2]. These consist of liquid bimolecular lipid layers. Their stability is based on interactions of the lipid molecules among themselves and on interactions of the lipid molecules with water [3]. From results obtained by polarisation microscopy and small angle X-ray scattering it was assumed that bimolecular lipid films play a role in the structure of the thylakoid membrane [4–7]. More recent research shows that in aqueous media also proteins of the thylakoid membrane aggregate spontaneously and form membrane-like layers [8]. For the maintenance of the functioning membrane structure in addition to lipid-lipid and protein-protein interactions also interactions between lipid and protein molecules must exist. Only little concrete information is available on the nature of these interactions in biological membranes [3, 9–12]. In the following we report on evidence for lipid-protein interactions which are connected with conformational changes of the protein. The experiments were carried out with the mixture of all the polypeptides from thylakoids of *Antirrhinum majus* and with a distinct fraction from this mixture. This fraction exhibits a molecular weight of approximately 24000 and is the major polypeptide component [13, 14]. It appears that this polypeptide fraction is in fact composed of 4 different polypeptides with very similar molecular weights.

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Abbreviations: Sulfolipid, sulfoquinovosyl diglyceride; dodecyl suflate, sodium dodecyl sulfate; polypeptide 24000, polypeptide exhibiting an apparent molecular weight of 24000.

Materials and Methods

Isolation and solubilization of stroma-freed chloroplasts

Stroma-freed chloroplasts from *Antirrhinum majus* were isolated according to Kreutz and Menke [15]. Solubilization of the chloroplasts was described by Menke *et al.* [13]. The dodecyl sulfate concentration was 2.2%. Undissolved residues were removed from the solution by centrifugation (60 min at 31000 × *g*, 22 °C).

Preparation of thylakoid peptides. The supernatant was diluted with 9-times its volume of 0.01 M sodium phosphate buffer (pH 7.2) containing 0.1% mercaptoethanol. In order to remove the mercaptoethanol the solution was dialyzed for 48 hours against flowing 0.01 M sodium phosphate buffer

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(pH 7.2) containing 0.22% dodecyl sulfate. After filtration this solution was used for the determination of the circular dichroism spectrum.

In order to remove the lipids the undiluted solution was precipitated with 5-times its volume of cold acetone and then washed with the same solvent. The precipitate was dissolved in such an amount of 0.01 M sodium phosphate buffer (pH 7.2) containing 1% mercaptoethanol to give a final polypeptide concentration of 0.5%. During this dissolving procedure dodecyl sulfate was added to give a final concentration of 2.2%. This solution was diluted with 9-times its volume of 0.01 M sodium phosphate buffer (pH 7.2) containing 0.1% mercaptoethanol and then dialyzed and filtered as above. The lipid-containing and lipid-free solutions were freed from dodecyl sulfate according to the method of Weber and Kuter [16] using the anion exchanger Dowex AG 1-X2. The solvents in the dodecyl sulfate-free preparations were changed by dialysis or by diafiltration (Amicon, PM 10 membrane). The polypeptide mixture, used for spectroscopy was dissolved in 0.01 M sodium phosphate buffer.

Isolation of the polypeptide 24000. From the solution of stroma-freed chloroplasts in dodecyl sulfate-containing sodium phosphate buffer, the polypeptide fraction 24000 was isolated by repeated gel chromatography. The elution buffer contained 0.25% dodecyl sulfate and 0.1% mercaptoethanol in 0.01 M sodium phosphate buffer (pH 7.2). In the first step Sepharose 6B (Pharmacia) was used in four columns (10 × 96 cm) connected in series. Fractions, which exhibited in the dodecyl sulfate polyacrylamide gel electrophoresis only one band with the apparent molecular weight of 24000 were pooled. The gel electrophoreses were carried out according to Weber and Osborn [17, 18] and Menke and Schölzel [19]. The fractions from several runs were concentrated by ultrafiltration to approximately 1/20 the original volume (Amicon, HIDP10 hollow fiber). Further purification was obtained by gel filtration over Sephadex G-150 (Pharmacia) again in 4 in series connected columns (10 × 96 cm). The purest fractions were precipitated after concentration to 1/80 with 5-times the volume of cold acetone, in order to remove the lipids, and then washed as described above. In this case, only such an amount of buffer was added to the precipitation to give a final polypeptide concentration of 1%. Aggregated polypeptide was removed by another gel filtration on Sephadex G-150 (2.6 × 90 cm). For the optical tests of the dodecyl sulfate-containing solution the purified fraction was transferred by 48 h of dialysis into 0.05 M sodium chloride solution which contained 0.25% dodecyl sulfate. The pH of the sodium chloride solution was adjusted to 7.0 ± 0.2 with 0.1 N NaOH. In addition, preparations in 0.01 M sodium phosphate buffer (pH 7.2) containing 0.1% dodecyl sulfate were tested. The preparation of dodecyl sulfate-free preparations was carried out as described above. They contained less than 1 molecule dodecyl sulfate per molecule of polypeptide 24000 (Craubner *et al.* to be published).

Spectra were taken in 0.01 M sodium phosphate buffer (pH 6.8 to 7.5) or in 0.05 M sodium chloride, the pH of which was adjusted with 0.1 N NaOH to 7.0 ± 0.2. The protein concentration was determined by the Lowry procedure [20] with the purified polypeptide preparation from chloroplasts as a standard.

Isolation of the chloroplast lipids was described in earlier publications by Radunz [21–23].

Antisera. The preparation of the antiserum to the sulfolipid was described by Radunz and Berzborn [24]. Antisera to polypeptides were obtained as described by Menke *et al.* [13].

Circular dichroism was measured as described earlier [25, 26]. All solutions were cleared by filtration (Sartorius membrane filter, pore width 0.45 μm) or centrifugation. In order to investigate the effect of chloroplast lipids on the conformation of the polypeptides, 0.7 ml of a 0.1% solution of the respective lipid in acetone or benzene were filled into a 1 mm path length cuvet. After evaporation of the solvent with scrubbed nitrogen gas and in the vacuum, 0.7 ml of a 0.1% solution of the polypeptide in 0.05 M sodium chloride solution or in phosphate buffer were added. The solutions were shaken until the lipids were dispersed. Recording of the circular dichroism spectrum was begun after about 30 min. The data were calculated as mean residue ellipticity (Θ), using a mean residue molecular weight of 109. This value was calculated from the amino acid composition of the thylakoid membrane proteins [27]. After extraction of the lipids the protein content of the thylakoid membrane fragments was determined by the Kjeldahl method (N × 6.0). In all other cases the protein concentration was determined according to the Lowry procedure. Dodecyl sulfate does not interfere whereas lipids have to be removed prior to the assay.

For the conformational analysis the reference spectra by Chen *et al.* were used [28]. These had been determined from proteins, whose structure was known from X-ray analysis. It became evident that the spectra for the β-structure vary considerably with different proteins. A satisfactory conformational analysis with the averaged spectrum was obtained with fragments of the thylakoid membrane [26]. However, the analysis was not successful with the polypeptide fractions described in the present paper.

Considerable deviations of the calculated from the experimental curves were observed between 185 and 200 nm. In this case we obtain the described results using the variability width given by Chen *et al.* [28]. Nevertheless, the well known difficulties for polypeptides with low α-helix content remain. This is especially obvious from Fig. 9. Table I contains

Table I. Reference spectra of β-structure used for the conformational analysis.

Wavelength [nm]	Θ [deg·cm²·dmol⁻¹] β_1 [a]	β_2 [a]
191	−4000	+27800
193	+3200	+32500
195	+6800	+30000
197	+3200	+22000
199	−1500	+13000
201	− 500	+ 3600
203	−4000	− 2600
205	−7300	− 5100
207	−8500	− 5500
209	−9500	− 7000
211	−10200	− 9500
213	−9500	− 9000
215	−9200	− 7650
217	−8700	− 6000
219	−6700	− 3900
221	−3800	− 1400
223	− 600	+ 1500
225	+2000	+ 4000
227	+2900	+ 5000
229	+4000	+ 5000
231	+4000	+ 4200
233	+3100	+ 3100
235	+3000	+ 2100
237	+2800	+ 1800
239	+3100	+ 2400

[a] β_1 was used for the fragments of the thylakoid membrane. β_2 for the polypeptides.

the coordination values for the two β-structure spectra which have been used as reference spectra for the conformational analysis.

Infrared spectra. A suspension of stroma-freed chloroplasts or the protein solutions were dried onto 1 mm thick silicium plates. The spectra were recorded in absorbance mode with a Perkin-Elmer 325 Infrarot-Gitterspektrophotometer. An uncoated silicium plate was placed in the reference beam. In order to compensate for the higher reflexion losses at the uncoated plate the absorbance at 2000 cm^{-1} was compensated to zero. During the recording the preparations were cooled to 5 °C.

Partial reactions of photosynthetic electron transport and photophosphorylation reactions have been carried out according to Radunz *et al.* [29]. Chloroplast preparations from *Nicotiana tabacum* var. John William's Broadleaf were used when photosynthetic electron transport reactions were tested. The chloroplasts for these reactions were prepared according to Homann and Schmid [30].

Results and Discussion

Interaction between dodecyl sulfate and polypeptides

As the isolation of the polypeptide fraction was carried out by means of dodecyl sulfate, we report first on conformational changes which are caused by this detergent. Fig. 1 (dashed line) shows the far ultraviolet circular dichroism spectrum of the polypeptide mixture in the presence of 0.22% dodecyl sulfate. As the circular dichroism spectrum of the polypeptides in the thylakoid membrane is not known, the spectrum of fragments of the thylakoid membrane [26] was used for comparison (Fig. 1, solid line). This preparation was obtained by ultrasonication and fractionating centrifugation. From Fig. 1 (dashed line) it appears that dodecyl sulfate causes a decrease of the mean residue ellipticity. Removal of dodecyl sulfate by ion exchange chromatography

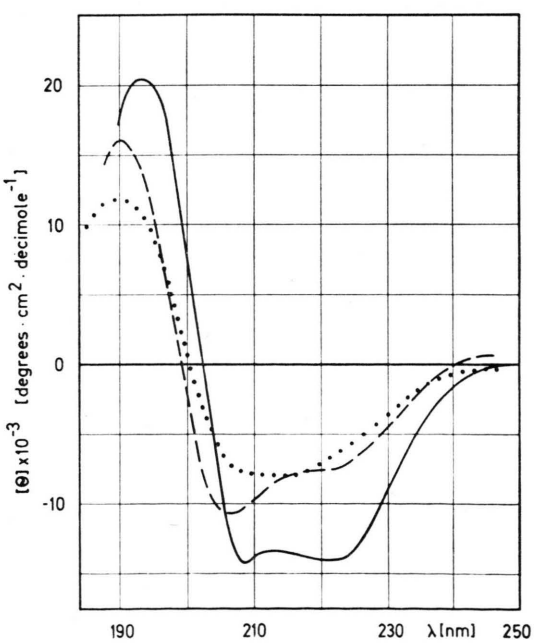

Fig. 1. Far ultraviolet circular dichroism spectra of fragments of the thylakoid membrane (solid line), of the polypeptide mixture obtained from the thylakoids dissolved in 0.22% dodecyl sulfate-containing phosphate buffer pH 7.2 (dashed line) and of the polypeptide mixture after removal of dodecyl sulfate (dotted line). Θ is the mean residue ellipticity and was determined as described in materials and methods.

causes a further decrease of the mean residue ellipticity (Fig. 1, dotted line). Addition of solid dodecyl sulfate into the cuvet, containing the detergent-free solution, results in a circular dichroism spectrum which does not differ from the spectrum of the original dodecyl sulfate-containing solution. Consequently, conformational changes of the polypeptide, induced by the removal of dodecyl sulfate, are reversible. If the extraction of the lipids from the dodecyl sulfate-containing solution is omitted, spectra are obtained which do almost not differ from that shown in Fig. 1. It appears that dodecyl sulfate separates the major part of the lipids from the original lipo-protein complex. This is also shown by gel chromatography and gel electrophoresis. The results obtained from our experiments are taken to mean that dissolution of thylakoids with dodecyl sulfate leads to a separation of lipids from the polypeptides, which in turn changes the conformation of the polypeptides. Removal of the dodecyl sulfate causes an additional conformational change. A renaturation as described by Weber and Kuter [16] for enzyme molecules was not observed.

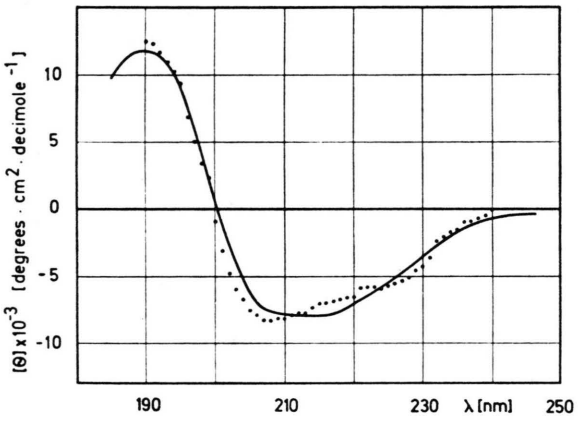

Fig. 3. Approximation of the circular dichroism spectrum of the polypeptide mixture after removal of the dodecyl sulfate. Solid line: experimental curve, dotted line: calculated curve.

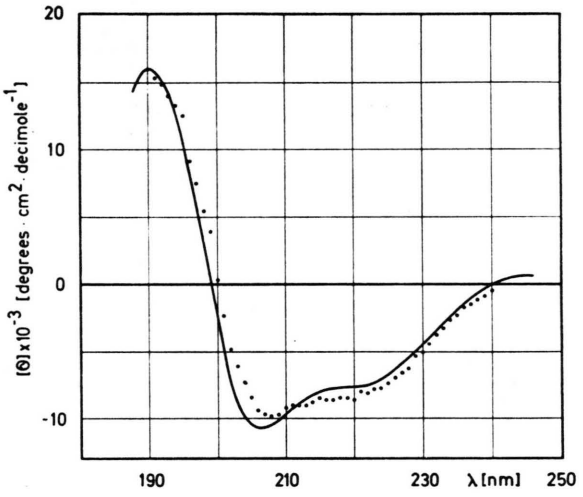

Fig. 2. Approximation of the circular dichroism spectrum of the polypeptide mixture dissolved in dodecyl sulfate-containing buffer, by linear superposition of the weighed reference spectra for α, β and random coil structure. Solid line: experimental curve, dotted line: calculated curve.

The result of our attempt to synthesize the spectra by linear superposition of the portions of α-helix, β-structure and random coil is summarized in Table II. From this table it is seen that dissolving of the thylakoid membrane in dodecyl sulfate results in a decrease of the α-helix content, and that removal of the detergent from the polypeptide mixture leads to a further decrease. Concomitant with this decrease an increase in β-structure and random coil is observed. The degree of approximation of the calculated spectrum to the experimental curve is shown in Figs 2 and 3. Recalling that this type of conformational analysis, even with better approximations than those shown in Figs 2 and 3 does frequently not yield fully satisfactory results, the

Table II. Results obtained by conformational analysis of thylakoid polypeptides.

	per cent			
	α-Helix	β-Structure	Random coil	Approximation in
Fragments of the thylakoid membrane	40	18	42	Menke and Hirtz 1973
Mixture of the thylakoid polypeytides dissolved in 0.22 per cent dodecyl sulfate	20	20	60	Fig. 2
Mixture of the thylakoid polypeptides after removal of the dodecyl sulfate	13	27	60	Fig. 3
Polypeptide fraction 24000 in 1 per cent dodecyl sulfate	11	14.5	74.5	Fig. 6
Polypeptide fraction 24000 after removal of the dodecyl sulfate	4	25	71	Fig. 7 (·····)
	4	48	48	Fig. 7 (+++)
Polypeptide fraction 24000 after addition of sulfolipid	7	25	48	Fig. 8

values of Table II should not be overestimated. However, the data clearly shows the direction in which the conformational changes occur.

The fact that denaturation is linked to an increase of β-structure is also shown by infrared spectroscopy. For stroma-freed chloroplasts the maximum of the amide I-band lies at 1657 cm^{-1} and that of the amide II-band at 1546 cm^{-1} (Fig. 4, upper curve). The spectrum as far as the position of the maximum and the shape of the absorption curve is concerned, does not differ much from the spectra of myoglobin (0% β-structure) or lysozyme (16% β-structure), recorded under equivalent conditions. From this it follows that in the thylakoid membrane the polypeptides are preponderantly present in the state of α-helix and random coil. Extraction of the preparations with 100% acetone reduces the ester carbonyl band at 1738 cm^{-1}, but leaves the structure of the amide bands almost unchanged (Fig. 4, middle curve). The dodecyl sulfate-freed mixture of all polypeptides of the thylakoid membrane yields an infrared spectrum which is characterized by a second maximum at 1627 cm^{-1} and a shoulder at approximately 1690 cm^{-1} (Fig. 4, lower curve).

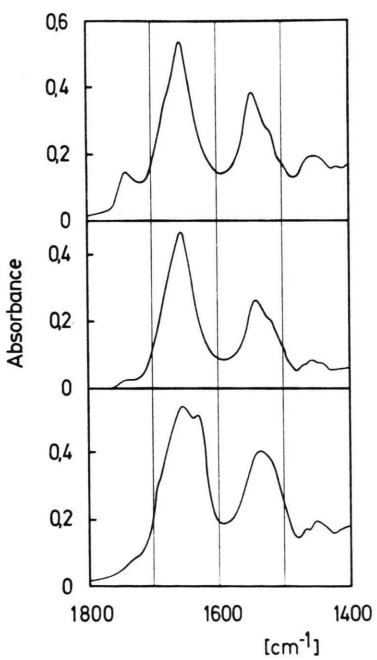

Fig. 4. Infrared spectra of solid films of chloroplast preparations on silicium plates. Upper curve: stroma-freed chloroplasts, middle curve: stroma-freed chloroplasts extracted with 100% acetone, lower curve: mixture of all polypeptides of the thylakoid membrane after removal of detergent by anion-exchange chromatography.

The amide II-band shows a shoulder at approximately 1520 cm^{-1}. According to the literature these bands are attributed to β-structure [31, 32]. Therefore, the circular dichroism data is confirmed as far as the observation is concerned that the dissolution of the thylakoids in dodecyl sulfate-containing solutions and the subsequent removal of the detergent leads to a considerable increase of β-structure. In this context it should be noted that the removal of the lipids, if carried out with acetone in the absence of water, does not induce this conformational change. In contrast to this, the extraction with 70% acetone leads to a transformation to β-structure. α-Helix-random coil transitions are not easily detected by infrared spectroscopy because the positions of the maxima differ by only 5 cm^{-1} [31, 32]. In earlier studies it was observed that dissolving of the thylakoids in dodecyl sulfate-containing water did not cause an appreciable alteration of the circular dichroism in the region of the peptide absorption [25]. We were not able to clarify, why the alteration described here did not take place at that time.

Corresponding experiments as with the polypeptide mixture were carried out with a polypeptide fraction exhibiting a molecular weight of 24000. In order to demonstrate the degree of purification, Fig. 5 shows the optical scans of dodecyl sulfate

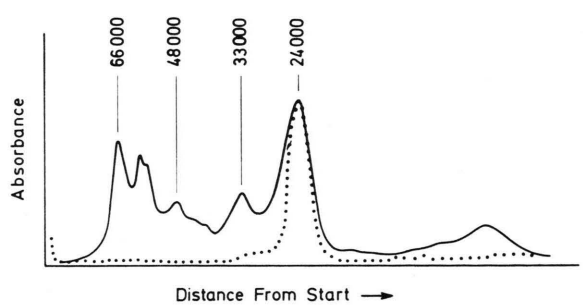

Fig. 5. Optical scans of dodecyl sulfate polyacrylamide gel electropherograms. Solid line: polypeptide mixture, dotted line: polypeptide fraction 24000. The gels are stained with Coomassie blue and scanned at $\lambda = 558$ nm. The numbers represent apparent molecular weights.

polyacrylamide gel electropherograms of the polypeptide 24000 and that of the mixture from which the polypeptide fraction originated. Fig. 6, dashed line, shows the far ultraviolet circular dichroism spectra of the polypeptide fraction 24000 in the presence of 1% dodecyl sulfate and in the absence of the detergent (solid line). Removal of dodecyl

sulfate causes as in the case of the polypeptide mixture a decrease of the mean residue ellipticity (Fig. 6). Also in this case the changes caused by the removal of the dodecyl sulfate are reversible. The approximation of the calculated to the experimental curves was less successful than for the polypeptide mixture (Figs 7, 9). However, the infrared spectrum of the dodecyl sulfate-freed polypeptide fraction 24000 differs hardly from the corresponding spectrum of the polypeptide mixture (Fig. 4, lower curve). Therefore, at any rate also in this case a decrease of the α-helix content and an increase of β-structure is the overall result (Table II).

The fact that proteins of the thylakoid membrane retain partially their secondary structure in dodecyl sulfate is of importance for the determination of apparent molecular weights by dodecyl sulfate polyacrylamide gel electrophoresis. This method, which is important for the determination of molecular weights of membrane proteins, leads only to right results, if the polypeptides dissolved in dodecyl sulfate-containing buffers are fully extended. In this extended state many soluble proteins bind per g 1.4 g dodecyl sulfate [33–35]. However, the polypeptides of the thylakoid membrane do not completely unfold in the presence of dodecyl sulfate even under reducing conditions. In line with this, the polypeptide fraction with the apparent molecular weight 24000 binds only 1.15 g dodecyl sulfate per g protein [14]. A molecular weight of 25000 was determined in the ultracentrifuge.

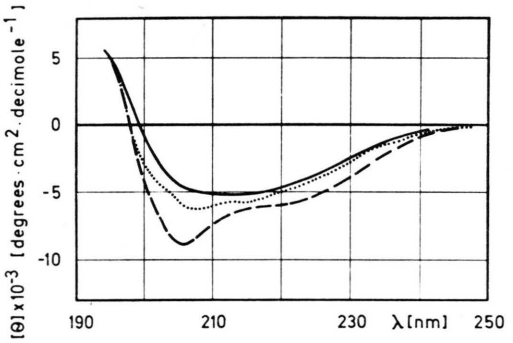

Fig. 6. Circular dichroism spectrum of the polypeptide fraction 24000 in the presence of 1% dodecyl sulfate (dashed line), after removal of dodecyl sulfate (solid line) and after addition of 0.1% sulfolipid to the detergent-free solution (dotted line). In this experiment all 3 measurements were carried out with the same polypeptide solution, to aliquots of which solid dodecyl sulfate or sulfolipid was added. The depicted dashed curve is identical to that obtained before the removal of dodecyl sulfate by ion exchange. The pH was 7.2.

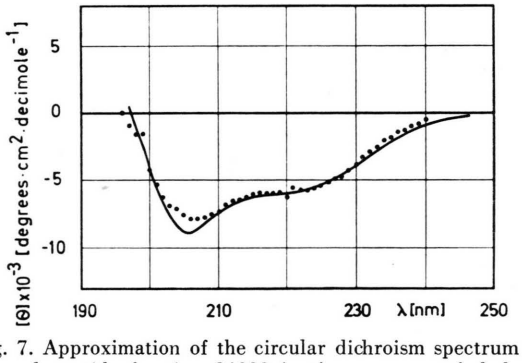

Fig. 7. Approximation of the circular dichroism spectrum of the polypeptide fraction 24000 in the presence of dodecyl sulfate. Solid line: experimental curve, dotted line: calculated curve.

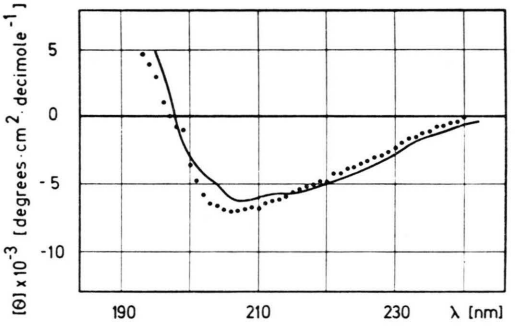

Fig. 8. Approximation of the circular dichroism spectrum of the polypeptide fraction 24000 in the presence of 0.1% sulfolipid. Solid line: experimental curve, dotted line: calculated curve.

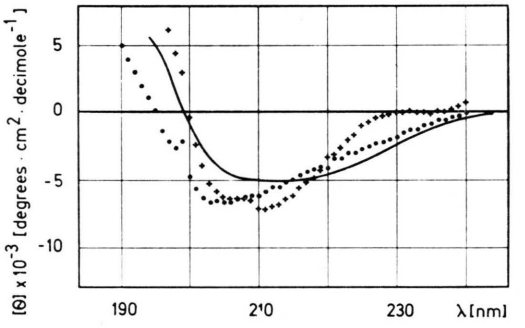

Fig. 9. Approximation of the circular dichroism spectrum of the polypeptide fraction 24000 after removal of dodecyl sulfate. Solid line: experimental curve, dotted line and crosses represent two different attempts of approximation.

Interactions between lipids and polypeptides

According to the results described above it appeared reasonable to look for interactions between

lipids and polypeptides both isolated from the thylakoid membrane. We tested the lipids monogalactosyl diglyceride, digalactosyl diplyceride, sulfoquinovosyl diglyceride and phosphatidyl glycerol. In addition, an extract containing all chloroplast lipids was used.

It became evident that only sulfolipid reacts with the polypeptides (Fig. 6, dotted line). The circular dichroism increases upon addition of sulfolipid although not as much as upon addition of dodecyl sulfate. Despite the fact, that the conformational analysis was not satisfactory (Fig. 8), we can say that this lipid causes apparently little increase in the α-helix content. Already with the unaided eye one can see that the sulfolipid behaves differently from the other lipids. All lipids tested dissolve from the cuvet walls forming temporarily a turbid suspension after one has filled in the polypeptide solution. Not reacting lipids sedimentate after a short time forming a layer at the bottom of the cuvet. The sediment consisted of birefringent sphero crystals as viewed under the polarisation microscope. The circular dichroism spectrum of the supernatant does not change. Therefore, the droplets did not take up any polypeptides. In contrast to this, the circular dichroism of the polypeptide changes in the presence of sulfolipid and no or almost no sediment is formed. After 1/2 hour the solution still appears slightly turbid but becomes clear in the course of several hours. The circular dichroism spectra of the turbid and the cleared solution are not different. Increase of the amount of added sulfolipid has no influence on the circular dichroism. Sulfolipid water suspensions exhibit no circular dichroism. The conformational change of the polypeptide induced by the sulfolipid depends on the pH of the suspension medium. A change in the circular dichroism in phosphate buffer was only observed in a pH-range between 6.9 and 7.4. The strongest change occurred at pH 7.2. However, the maximum is flat. This dependence of the reaction on the hydrogen ion concentration might be interpreted to indicate that in addition to hydrophobic also interionic interactions stabilize the binding between the polypeptide molecules and the sulfolipid. Addition of sulfolipid to the polypeptide mixture changes the circular dichroism to a just detectable degree. This rather low effect might be due to the fact that only certain polypeptide species react with the sulfolipid.

In investigations of membrane preparations and other particle suspensions spectral distortions are observed if the particle dimensions are in the order of magnitude of the wavelength of light. Causes for these optical artifacts are differential light scattering and absorption flattening [36–40]. The investigated fragments of the thylakoid membrane exhibit, according to earlier electron microscopic investigations, a mean diameter of 108 Å [26, 41]. Consequently, these fragments are smaller by one order of magnitude than the wavelength of the measuring light. The dodecyl sulfate polypeptide micelles exhibit the molecular weight of 54000. During the removal of the dodecyl sulfate the polypeptides aggregate in dependence on the pH. The molecular weight of the micelles becomes smaller with increasing pH and was found to be 340000 at pH 6.8 and 150000 at pH 7.5 [14]. All these preparations appear clear and light scattering effects play only a minor role. After the addition of sulfolipid scattering of the solution increases at least temporarily. In this case the stronger light scattering could have produced a flattening of the spectrum. In reality, however, the addition of sulfolipid leads to an increase of the ellipticity. Therefore, the conformational changes induced by sulfolipid are in reality rather stronger than our estimates. In addition, it must be borne in mind that scattering becomes lower with increasing pH, whereas the ellipticity change goes through a maximum in this range. Hence, it appears that we may exclude that our results are produced by optical artifacts.

Interactions between sulfolipid and polypeptides in the thylakoid membrane

The results presented show that interactions between polypeptide and sulfolipid molecules occur under *in vitro* conditions which does not mean, however, that such interactions also take place in the functioning thylakoid membrane. In earlier investigations we had found that monospecific antisera to sulfolipid did not influence electron transport in the thylakoid membrane, despite the fact that they were specifically adsorbed. However, a reinvestigation showed, that the electron transport is inhibited within a narrow pH-range. The inhibition is observed for a photosystem I-mediated electron transport reaction between the artificial electron donor 2,6-dichlorophenol indophenol/ascorbate and the artificial electron acceptor methylviologen. The effect was also observed when the concentration of

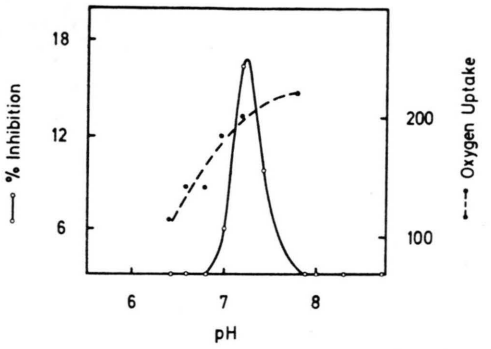

Fig. 10. Photosystem I mediated photoreduction of methylviologen with the electron donor couple 2,6-dichlorophenol indophenol/ascorbate in tobacco chloroplasts. Solid line: pH-dependence of the inhibition of the reaction by antibodies to sulfolipid, dashed line: pH-dependence of the reaction without additions.

Table III. Effect of the antiserum to sulfoquinovosyl diglyceride on cyclic and noncyclic photophosphorylation in tobacco chloroplasts.

Assay condition	Specific activity [a]
Phenazine methosulfate (PMS)	644
PMS + antiserum to sulfolipid	650
PMS + control serum	666
$K_3Fe(CN)_6$	61
$K_3Fe(CN)_6$ + antiserum to sulfolipid	57
$K_3Fe(CN)_6$ + control serum	49
$K_3Fe(CN)_6 + 10^{-5}$ M DCMU	0

[a] μmol [^{32}P]ATP formed $\times$ (mg Chlorophyll)$^{-1}$ h^{-1}; Temperature 14–15 °C; pH 7.2; light intensity 120 000 lx.

the artificial electron donor was high enough as to by-pass plastocyanin, which is the final electron donor to P_{700} according to Fujita and Murano [42]. Fig. 10, solid line, shows the dependence of this inhibition on the pH of the reaction medium. The maximal effect is at the pH 7.2. The pH-optimum for the electron transport reaction itself lies above pH 8 (Fig. 10, dashed curve). The maximal inhibition was 16%. Due to the limited accessibility of the thylakoid surface to antibodies the observed maximal inhibition can only be 30 – 40% [43]. Therefore, 16% inhibition is half the maximally possible value. The fact that a 100% inhibition is not observed is due to the grana structure of the chloroplasts. Photophosphorylation reactions such as the phenazine methosulfate- or ferricyanide-mediated photophosphorylation were not affected by the antiserum (Table III).

From the fact that the pH-maximum for the intermolecular interactions between sulfolipid and polypeptides *in vitro* coincides with the pH-optimum for the inhibition of electron transport by antibodies to sulfolipid, it may be concluded that also in the thylakoid membrane interactions between sulfolipid and protein molecules occur. It must be assumed that this interaction is not restricted to polypeptides with the molecular weight of 24000, because antibodies to this polypeptide fraction do not inhibit the electron transport [44]. As the sulfolipid does not participate in electron transport, the effect of the antibodies to the sulfolipid must be an indirect one. One could visualize this effect as being induced by the binding of an antibody molecule to a sulfolipid molecule which then causes a conformational change in the protein molecule to which the sulfolipid is attached. If this protein molecule was involved in photosynthetic electron transport its conformational change might induce a change in the electron transport rate. As the sulfolipid interacts only in a narrow pH-range with the protein molecule, it appears reasonable that antibodies to the sulfolipid influence electron transport also only in this pH-range. As an alternative it is thinkable, that binding of the antibodies changes the position of the protein molecule in relation to other molecules in the membrane, which also might lead to an inhibition of the electron transport. If one recalls that in the cell localized pH-changes take place, one can easily imagine that the pH-dependence of the interactions between the sulfolipid and proteins is a possible mechanism for the regulation of membrane-bound reactions. For the maintenance of the membrane structure the interactions between sulfolipid and proteins play apparently no important role, as the thylakoid membrane is also stable outside the pH-range in which these interactions occur. However, it cannot be excluded that interactions exist between sulfolipid and proteins outside this pH-range which are not linked with a conformational change of the protein. Moreover, the sulfolipid represents only 5% of the total lipid content [45]. Our results do not exclude that in the thylakoid membrane non-covalent bindings between proteins and other lipids exist. The stronger hydrophobic lipids can probably not easily enter into contact with polypeptides in aqueous media. Among the tested lipids the hydrophilic character is most pronounced with the sulfolipid so that Benson [2] calls it a natural surfactant. Under other conditions,

the prerequisite for the formation of non-polar bindings between these lipids and proteins might be more favorable.

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