Phosphatidylglycerol and β-Carotene Bound onto the D 1-Core Peptide of Photosystem II in the Filamentous Cyanobacterium *Oscillatoria chalybea*

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D 1-Peptide, Phosphatidylglycerol, β-Carotene, Antibody, Lipid-Protein Binding, Cyanobacterium, Manganese Stabilizing Peptide

Photosystem II-particles from the cyanobacterium *Oscillatoria chalybea* were isolated by fractionating centrifugation. Purification of these particles was achieved by a 22 hours centrifugation over a linear sucrose density gradient at 217.500 × *g*. The obtained particle fraction exhibited an oxygen evolution activity which corresponded to three times the rate of intact cells and to five times the rate of intact thylakoids. The chlorophyll protein ratio was 1:10 and the ratio manganese/chlorophyll 1:34.

SDS-polyacrylamide gel electrophoresis showed that the photosystem II-fraction is composed of the core peptides D 1 and D 2, the chlorophyll-binding peptides CP 43 and CP 47, the extrinsic 33 kDa peptide (manganese stabilizing peptide, MSP) and phycobiliproteins with molecular masses between 16 to 20 kDa. Cyt b_{559} was not detected in our gel electrophoresis assay. Part of the peptides of the 30 kDa-region (D 1, D 2, MSP) occurred as aggregates with a molecular mass of 60 to 66 kDa.

The D 1-peptide was isolated from the PS II-preparation by SDS-gel electrophoresis. The intrinisic peptide reacts in the Western blot procedure with the antiserum to phosphatidylglycerol and with the antiserum to β-carotene. Incubation of the peptide with the antisera to monogalactosyldiglyceride, sulfoquinovosyldiglyceride and zeaxanthine resulted negatively. The binding of phosphatidylglycerol onto the D 1-peptide was confirmed by lipid analysis in HPLC and fatty acid analysis by gas chromatography. Only this lipid, respectively the typical fatty acid mixture of this lipid was detected. The lipid is characterized by the fact that the hexadecenoic acid does not exhibit *trans*-configuration, as is true for phosphatidylglycerol of higher plants and algae, but occurs in *cis*-configuration.

With the antibody being directed towards the glycerol-phosphate residue and not towards the fatty acids, it can be concluded from the reaction of the antibodies with the bound lipid that the lipid is bound to the peptide *via* the fatty acid. The negatively charged phosphatidylglycerol increases the hydrophobicity of the peptide and leads to a negatively charged surface favouring binding of cations like calcium and magnesium. The fact that incubation of this PS II-fraction with phospholipase inhibits photosynthetic activity by 25% which can be fully restored by addition of phosphatidylglycerol, shows that bound phosphatidylglycerol has a functional role.

Introduction

By comparative lipid analyses of the cyanobacterium *Oscillatoria chalybea* we were able to show that the amount of the two anionic lipids phosphatidylglycerol and sulfoquinovosyldiglyceride present depends on the composition of the culture medium [1]. Whereas in ammonia cultures the negatively charged phosphatidylglycerol is increased by 30%, it is the sulfoquinovosyldiglyceride which is increased by 30% when cyanobacteria are grown on nitrate as the sole nitrogen source. According to Tremolière *et al.* [2] and Remy *et al.* [3] the negatively charged phospholipid with the typical hexadecenoic acid in *trans*-configuration plays a functional role for the arrangement of the oligomeric structure of polypeptides of the light-harvesting complex in higher plants. According to investigations in our laboratory the light-harvesting complex of *Nicotiana tabacum* var. John William's Broadleaf contains in comparison to photosystem II and thylakoid preparations twice to three times the amount of phosphatidylglycerol [4, 5]. In cyanobacteria light absorption is carried out by phycobilisomes and we found no phosphatidylglycerol bound to proteins of phycobilisomes.

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It can be assumed that the function of the anionic lipid phosphatidylglycerol is not only restricted to the structural arrangement of the oligomers in the light-harvesting complex. By phospholipase treatment Siegenthaler *et al.* were able to show that in spinach chloroplasts not the phosphatidylglycerol molecules located on the outer surface play a role in photosynthetic electron transport but only the molecules located towards the inner thylakoid membrane surface [6]. Fragata *et al.* report on a stimulation of oxygen evolution in photosystem II preparations, if these preparations are integrated in phosphatidylglycerol vesicles [7]. In contrast to this, our own experiments show that antisera to the negatively charged lipids [8, 9] as well as antisera to the neutral galactolipids [10] inhibit photosystem I and photosystem II reactions on the donor sides of both photosystems. These experiments can only be explained in the sense that these lipids are bound to proteins involved in photosynthetic electron transport reactions. Furthermore, these experiments demonstrate that lipids on the outer surface of the membrane are bound to proteins of both photosystems. By means of the Western blot technique, we were able to show that the two galactolipids as well as sulfolipid are bound to the core peptides of photosystem II [11]. In this publication we report on the binding of phosphatidylglycerol and β-carotene on the D 1-core peptide of photosystem II of the cyanobacterium *Oscillatoria chalybea.* The binding of these lipids onto the D 1-peptide is demonstrated by monospecific polyclonal antisera with the Western blot technique and by lipid analysis by HPLC.

Material and Methods

Isolation of photosystem II-preparations

For the isolation a method was developed which was inspired by the method of Burnap *et al.* [12] and Bendall *et al.* [13] for the isolation of PS II-particles from thermophilic cyanobacteria. 13–16 days old nitrate-grown *Oscillatoria* filaments were harvested, washed and homogenized in 50 mM Hepes-buffer pH 7.5 containing 1 M sucrose, 10 mM NaCl and 10 mM $CaCl_2$. The homogenate was sedimented by 10 min of centrifugation at $5000 \times g$ and the sediment suspended in the above described Hepes-buffer which contained as protease inhibitor 1 mM PMSF (phenylmethylsulfonylfluoride) and 1 mM benzamidine. The intact cells were broken under cooling by 0.1–0.2 mm glass beads in a cell mill (Bead Beater, Biospec Products USA). In order to seperate thylakoids from the beads, the suspension was centrifuged for 1 minute at $500 \times g$. From the supernatant the thylakoids were sedimented by centrifugation during 30 min at $100{,}000 \times g$. In order to separate the major part of phycobilisomes the thylakoids were suspended in Hepes-buffer which contained 0.35% Na-cholate, incubated for 10 min and again centrifuged for 30 min at $100{,}000 \times g$. In order to obtain the PS II-particles the sedimented thylakoids were suspended in 50 mM Hepes-buffer containing instead of sucrose, 20% glycerol, 0.08% *n*-dodecyl-β-D-maltoside and 0.6% 1-*o*-octyl-β-D-glucopyranoside and incubated for 1 min. Thereafter the preparation was centrifuged at $160{,}000 \times g$ in a SW 60 Ti swinging buckett rotor in the ultracentrifuge (Beckmann). The supernatant contained purified PS II-particles. For further purification of this preparation the PS II-fractions were given on a linear sucrose density gradient of 5–25% sucrose in Hepes-buffer and centrifuged for 22 h at $215{,}000 \times g$ in a SW40Ti swinging buckett rotor (Beckmann). The gradient contained 0.03% dodecylmaltoside in order to avoid aggregation of the photosystem II-particles. The lower green band of the gradient contained the purified PS II-fractions (Fig. 1, d) which were immediately used for photosynthetic activity measurements and frozen before use for other investigations like SDS-gel electrophoresis and lipid determinations.

Chlorophyll and protein determinations

Chlorophyll was determined according to Schmid [14] and protein according to Lowry *et al.* [15].

Analysis of the polypeptide composition

Polypeptides of the PS II-fractions were analysed by polyacrylamide gel electrophoresis in a 12.5% or a 10% separation gel according to Laemmli [16]. The stacking gel was 3.8% and electrophoresis was carried out at 15 mA. The pre-treatment of the samples to be analysed was variable: thylakoids were heated in sample buffer 1:1 (v/v) to 100 °C for 1 min whereas PS II-particle preparations were incubated for 30 min with sample buffer at room temperature. The composition of the sample buffer was 1 ml 0.2 M Tris (pH 6.8) containing 5 mM EDTA, 1 M sucrose,

0.2 ml 20% SDS and 0.06 ml DTE (dithiotreitol 7.7 mg/ml).

Western blot procedure

Peptides separated and analysed by SDS-Polyacrylamide gel electrophoresis were transferred according to Rennart *et al.* [17] to nitrocellulose membranes (Schleicher u. Schüll Nr. B 85) or to methanol-stable Immobilon-P-membranes (Millipore) by diffusion during 16 h at room temperature. The specific antibodies were used in form of monospecific polyclonal antisera to the photosystem II peptides CP 47 from tobacco, D 1, D 2 and MSP (manganese stabilizing peptide) from oat, to L-aminoacid-oxidase (L-Aox) from *Synechococcus pcc 6301* [18], as well as to the α-subunit of the coupling factor of photophosphorylation CF_1 from spinach [19], to the lipids monogalactosyldiglyceride [10, 20], sulfoquinovosyldiglyceride [9], phosphatidylglycerol [8] and to the carotenoids β-carotene [21] and zeaxanthin [22]. The dilution factor of the sera was 1:100 to 1:200 (v/v). Binding of the primary antibody was detected with a peroxidase-marked anti-species antibody (Anti-IgG-antibody). The dilution was 1:200 (v/v). The peroxidase reaction was carried out with 4-chloro-1-naphthol/H_2O_2 as substrate. The composition of the buffer solutions, necessary for the different washing procedures, has been described earlier [11]. In order to determine the type of lipid binding, the peptides were transfered to the Immobilon-P-membranes and were treated before antibody incubation for 45 minutes with methanol.

Isolation of the D 1-peptide from photosystem II-fractions

After the electrophoretic analysis of the peptides, the gel was stained with 0.3 M $CuCl_2$. The D 1-containing region was cut out and destained with 0.25 M Tris-buffer pH 9, containing 1 mM EDTA. The D 1-peptide was eluted from the gel by electroelution at 200 V in an elution chamber from Schleicher u. Schüll. The elution buffer consisted of 20 mM Tris, 0.15 M glycine and 0.01% SDS (pH 9). A Bio-trap BT 1000 membrane from Schleicher u. Schüll was used as elution membrane. In order to extract all of the peptide out of the membranes, a 10 sec lasting pole reversal was applied. The purity test of the obtained D 1-peptide was carried out by SDS polyacrylamide electrophoresis and by Western blot procedure.

Measurement of photosystem II-activity

Oxygen evolution was measured with a Clark type oxygen electrode from Rank Brothers (England). Electron acceptors used for activity measurements of thylakoids and purified photosystem II-preparations were $K_3Fe(CN)_6$ and for activity measurements in whole cells *p*-benzoquinone. In order to test the influence of the phospholipid phosphatidylglycerol in *Oscillatoria chalybea,* photosystem II-particles were incubated with phospholipase A_2 (lipase concentration 1 mg/mg chlorophyll) with oxygen evolution measured after the treatment. In other experiments the photosystem II-particles were directly incubated with phosphatidylglycerol. In these experiments the phospholipid was emulsified by ultrasonication (20 min/30 W) in 50 mM Hepes-buffer pH 7.5. The ratio chlorophyll/phosphatidylglycerol was 1:20 w/w). Oxygen-evolution of the treated photosystem II-particles was measured during 90 min. Controls used were photosystem II-particles not treated with lipase or not incubated with phosphatidylglycerol.

Analysis of lipids and fatty acids

Photosystem II-particles were dialysed against water, precipitated with acetone and centrifuged at $5.000 \times g$ for 20 min. The sediment was extracted with boiling methanol and subsequently extracted with methanol/chloroform (1:2 v/v). For the HPLC-analysis a lipid solution in methanol was used. The analysis was carried out with a RP-18 column (Merck) in a HPLC-set-up from LKB (gradient pump 2249) with acetonitrile/water/phosphoric acid (85%) 60:39.6:0.4 (v/v). The column pressure was 5.5 bar and the flow-rate 0.5 ml/min. Detection of eluted lipids was obtained with a spectral-detector (LKB 2140) at 205 nm. The standard lipid used was phosphatidylglycerol with palmitic acid as the sole fatty acid (Sigma, Heidelberg).

For gas-chromatographic analysis of the fatty acids photosystem II-particles were saponified with 0.5 M methanolic NaOH under back-flow conditions during 1 h with the fatty acids extracted as described earlier [23]. Transformation to methylesters was obtained with 5% methanolic HCl. The analysis of the fatty acid methylesters was carried out with a Hewlett Packard Gas Chromatograph (Typ 5750). As stationary phase 10% ethylenglycol succinate on Chromosorb was used. The analysis was carried out

at a column temperature of 190 °C and a nitrogen pressure of 60 ml/min.

Results

1. Isolation and characterization of photosystem II-preparations

Photosystem II-particles from the filamentous cyanobacterium *Oscillatoria chalybea* were isolated by a procedure essentially corresponding to that of Burnap *et al.* [12] and Bendall *et al.* [13]. First thylakoids from cultures grown on nitrate as the sole nitrogen source were obtained by fractionating centrifugation. By sodium-cholate treatment the remainder of phycobiliproteins and CF_1 was removed. Thereafter, the thylakoid membrane was solubilized by incubation with the detergents *n*-dodecyl-β-D-maltoside and 1-O-octyl-β-glucopyranoside. Not solubilized membrane particles were sedimented in a 60 min centrifugation at 160,000 × *g*. The membrane-free supernatant was enriched with photosystem II-particles. The particles were further purified by 22 h centrifugation over a linear sucrose density gradient at 217,000 × *g*. The PS II-fraction was located in the lower green zone as shown in Fig. 1 A. Absorption spectra of this fraction (Fig. 1 B, d) show practically only the absorption band of chlorophyll *a*. This purification step eliminated carotenoids not belonging to the PS II-core complex (Fig. 1 B, a), the remaining phycobilisomes (Fig. 1 B, b) remainder of photosystem I-particles (Fig. 1 B, c) and the major portion of the CF_1-complex.

The thus obtained purified PS II-fractions did not contain any contamination by photosystem I-particles as shown by the complete absence of the P 700-absorption change, still present in the preparation before the sucrose density gradient centrifugation (Fig. 2). In comparison to intact cells or isolated thylakoids the obtained PS II-particles exhibited a 3 times, respectively 5 times higher activity of oxygen evolution (Table I). Further characteristics of the PS II-fraction such as chlorophyll, protein and manganese content are given in Table I.

The photosystem II-fraction obtained by sucrose density gradient centrifugation consists as shown by SDS-polyacrylamide gel electrophoresis (Fig. 3 d) of the core peptides D 1 and D 2, the chlorophyll-binding peptides with the molecular masses of 43

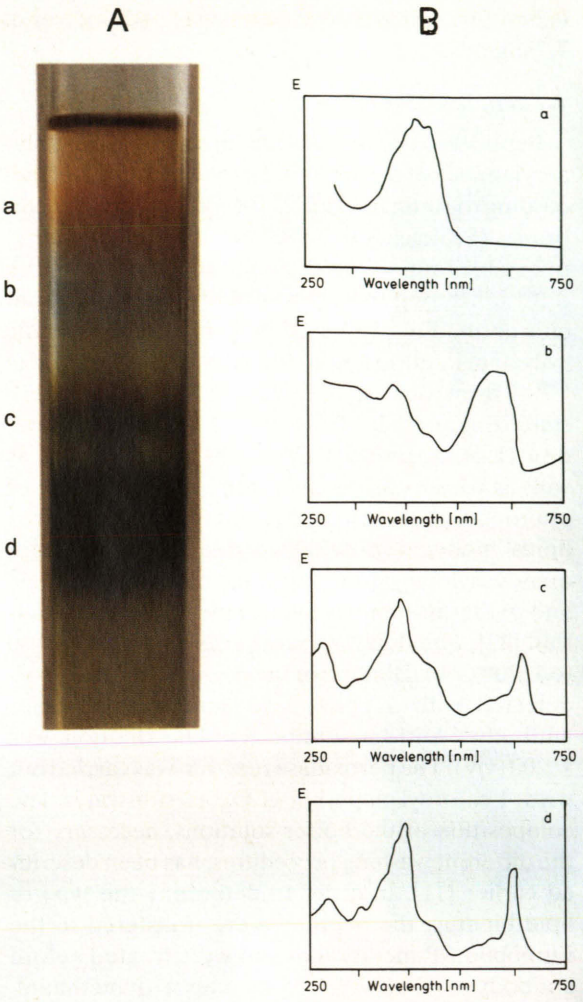

Fig. 1. A: Distribution of particle fractions from *Oscillatoria chalybea* thylakoids after centrifugation over a sucrose density gradient. The gradient contained 0.03% β-dodecylmaltoside to prevent aggregation of photosystem II-particles. B: Absorption spectra of the fractions: a) β-carotene; b) phycobiliproteins; c) photosystem I-fraction; d) photosystem II-fraction.

and 47 kDa, the extrinsic manganese stabilizing peptide (MSP) with the molecular mass of 34 kDa and a minor impurity of phycobiliproteins with molecular masses of 16–20 kDa. In the region of 60–66 kDa a broad peptide band is observed, which reacted with the D 1 antiserum. Hence this band appears to be aggregates of the PS II-peptides of the 30 kDa region, appearing under the electrophoresis conditions used. Peptides in the low molecular region below 10 kDa such as the $cyt_{b\,559}$-peptides

Table I. Characterization of the isolation steps for photosystem II-particles of *Oscillatoria chalybea*.

Cells, preparations	Chlorophyll/ protein	Mn/ chlorophyll	O_2-Evolution [μmol × mg Chl^{-1} × h^{-1}]
Cells	n.d.	n.d.	200
Thylakoids	1:14	n.d.	140
DM/ OGP-particles	1:12	1:17,5	635
PS II-particles (purified)	1:10	1:33,5	1010

Manganese determination has been made with ICP-mass spectroscopy; DM/OGP = Dodecyl-β-D-maltoside/Octyl-β-glucopyranoside.

with molecular masses of 6.5 and 9 kDa were not observed under our electrophoretic conditions.

The recognized peptides were characterized in the Western blot procedure with monospecific antisera (Fig. 4). In this analysis we have also included L-aminoacid oxidase which is supposed to play an important role in the water-splitting reaction [18].

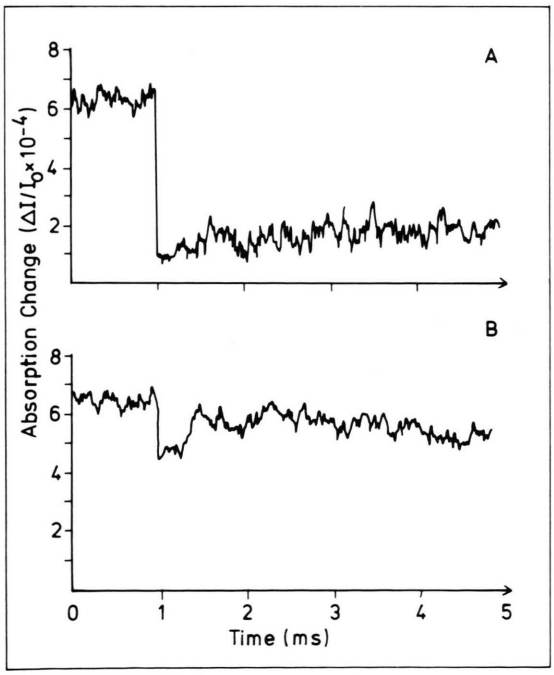

Fig. 2. Measurement of light-induced absorption change at 700 nm (P 700) in the photosystem II-particle preparation: A: before and B: after the gradient centrifugation.

An impurity of this photosystem II-preparation was the α-subunit of the coupling factor of photophosphorylation (CF_1). According to Luis *et al.* [24] and Avni *et al.* [25] the properties of a chaperone protein are attributed to this protein, which means that this protein determines the folding/conformation of other proteins such as that of the β-subunit in the CF_1 complex. It seems as if, due to this property, the α-subunit gets solidly adsorbed in the isolation procedure on certain peptides of the photosystem II-complex.

2. Lipid analysis

A qualitative lipid analysis by means of HPLC on a RP-18 column has led to the result that the purified PS II-fraction contained exclusively the negatively charged phospholipid phosphatidylglycerol (Fig. 5 a). The presence of this lipid was demonstrated also for the D 1/MSP-fraction obtained by electroelution (Fig. 5 c). Other lipids, as the galactolipids, occurring in large amounts in *Oscillatoria chalybea* and sulfoquinovosyldiglyceride were not identified in the lipid fraction of the PS II-preparation. A gas chromatographic analysis of the fatty acids showed that the fatty acid composition of the total lipid fraction from the PS II-preparation was identical to the fatty acid composition of phosphatidylglycerol isolated from *Oscillatoria chalybea* (Table II). This means that due to the fatty acid composition beside phosphatidylglycerol no other lipids are identified. A comparison of the fatty acids of phosphatidylglycerol from *Oscillatoria chalybea* with that of higher plants, *e.g.* from *Antirrhinum majus* (Table II) shows that *Oscillatoria chalybea* contains a hexadecenoic acid, which, however, in

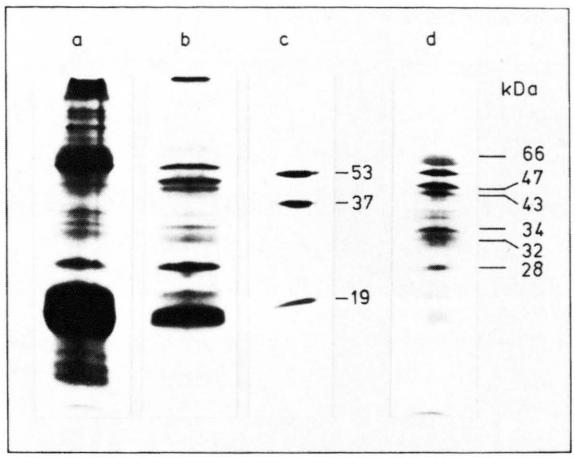

Fig. 3. Peptide composition of different particle preparation analyzed in the SDS-polyacrylamide gel electrophoresis. The acrylamide concentration was 3.5% for the stacking gel and 10% for the separation gel. a) Thylakoids; b) photosystem II-particles after treatment of the thylakoids with 0.08% β-dodecylmaltoside and 0.6% octyl-β-glucopyranoside; c) standards: 53 kDa glutamate dehydrogenase, 37 kDa D-aminoacidoxidase, 19 kDa myoglobine; d) photosystem II-particles after gradient centrifugation.

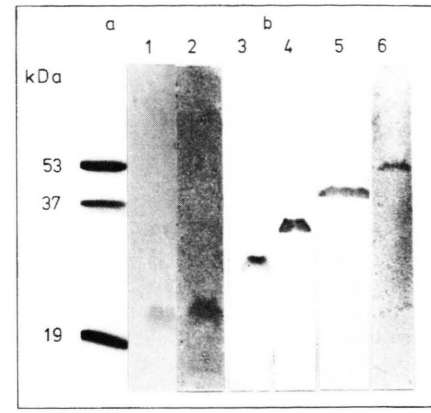

Fig. 4. Characterization of the peptides of the photosystem II-particle preparation by Western blot. a) SDS-polyacrylamide gel (10%) with protein standards (see Fig. 3); b) nitrocellulose membrane after reaction with antisera to 1) L-aminoacid oxidase; 2) D 2-peptide; 3) D 1-peptide; 4) MSP (manganese-stabilizing peptide); 5) CP43; 6) coupling factor of photophosphorylation.

contrast to that of higher plants occurs not in the *trans*- but only in the *cis*-configuration [26]. This *cis*-hexadecenoic acid in phosphatidylglycerol makes up for only 10% of the total fatty acids of this mixture in contrast to higher plants, where this fatty acids contributes to 40% of the fatty acid mixture of this phospholipid [1]. In addition, there are differences with respect to the degree of saturation of the fatty acid composition. Whereas the phosphatidylglycerol from *Antirrhinum majus* consists of only 20% saturated fatty acids, the contribution of saturated fatty acids is 56% of total fatty acids in *Oscillatoria chalybea.* In Table II, for comparison purpose, also the fatty acids of phosphatidylglycerol from the purple bacterium *Rhodopseudomonas sphaeroides* are included. This lipid also exhibits peculiarities in the sense that the contribution of unsaturated fatty acids is, as in phosphatidylglycerol of higher plants, very high (up to 90%) but does not contain as in cyanobacteria fatty acids in *trans*-configuration. The main component here is the so-called vaccenic acid, whose double bond lies between C_{11} and C_{12} and has *cis*-configuration [27]. Hence, the qualitative lipid analysis shows that phosphatidylglycerol occurring in the purified PS II-fraction is bound onto the D 1-peptide. Therefore, in the following the reaction of a monospecific antiserum to phosphatidylglycerol and the reaction of other lipid antisera, as well as that of the antisera to β-carotene and zeaxanthin are described. The photosystem II-fractions were first extracted with acetone, in order to remove loosely bound lipids, and subjected to a SDS-polyacrylamide gel electrophoresis in 12.5% polyacrylamide gel. Thereafter the peptides were transferred to methanol-stable *Immobilon P*-membranes and incubated with the respective monospecific polyclonal antisera. As seen in Fig. 6, 7 and 8, of all lipid antisera tested only the antiserum to phosphatidylglycerol marks the D 1-peptide. It was possible to dilute in this reaction the antisera by a factor of 100 to 200. The control sera did not react at all. This means that the negatively charged phosphatidylglycerol is bound onto the D 1-peptide. This binding is so strong that even washing of the *Immobilon P*-membrane after the transfer of the peptides with methanol does not prevent the reaction with the phosphatidylglycerol antiserum. For the structrual analysis it is important to note that the phosphatidylglycerol antiserum only reacts with the D 1-peptide, if present in the monomeric form. This is seen from the fact that the aggregated D 1/D 2/MSP-peptides, which form in the SDS-gel electrophoresis a broad band in the region of 60–66 kDa, do not react with the antiserum

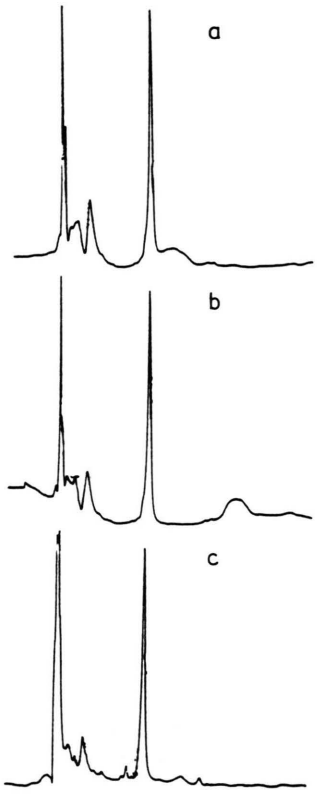

Fig. 5. Lipid analysis of the photosystem II-particles by HPLC. Column RP 18 (250 mm × 4 mm, Merck); isocratic elution (0.5 ml/min with acetonitrile/water/H_3PO_4 60%/39.6%/0.4%): a) lipids of PS II-particles; b) phosphatidylglycerol standard (Sigma); c) lipids of the D 1/MSP-peptide fraction purified by SDS-gel electrophoresis and electroelution.

(Fig. 7). Besides the antiserum to phosphatidylglycerol also the antiserum to β-carotene reacts with the D 1-peptide (Fig. 8 e). In this case the D 1-peptide is marked with high intensity when present in the monomeric form and with low intensity in the dimeric form *i.e.* the aggregated peptides.

Antisera to the second anionic lipid sulfoquinovosyldiglyceride (SQDG) occurring in *Oscillatoria chalybea* as well as those to the major lipids *i.e.* to the galactolipids do not react in the Western blot procedure with the peptides of the purified photosystem II-fraction (Fig. 8 c and d). This negative result does not mean, however, that these lipids do not occur on these peptides. As the D 1/D 2-peptides of the purified PS II-fraction strongly tend to aggregation in the SDS-gel electrophoresis, yielding

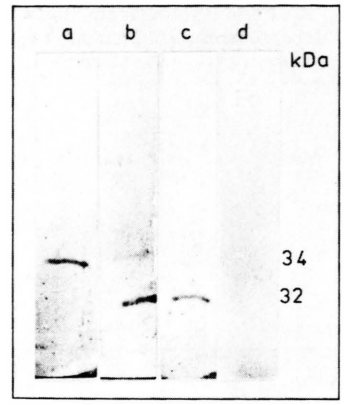

Fig. 6. Reaction of the PS II-particle preparation with different antisera in the Western blot. The picture shows the nitrocellulose membrane after reaction with the antisera to a) MSP; b) D 1; c) phosphatidylglycerol; d) control serum belonging to the phosphatidylglycerol immunization.

peptide bands in the region of 60 – 66 kDa (Fig. 6 c) in which lipid molecules might be inaccessible to antibodies due to conformational changes, the reactions with the SQDG- and MGDG-antisera were also carried out with unpurified PS II-fractions. Also in these cases the lipid antibodies did not react.

These results show that only the negatively charged phosphatidylglycerol and β-carotene are bound *via* the intrinsic D 1-peptide to the reaction

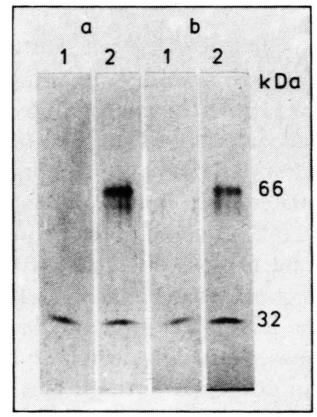

Fig. 7. Reaction of the polypeptides of the PS II-particle preparation with different antisera in the Western blot procedure before and after treatment of Immobilon-P-transfer membranes with methanol. a) Before methanol washing: 1) antiserum to phosphatidylglycerol; 2) antiserum to the D 1-peptide; b) after methanol washing: 1) antiserum to phosphatidylglycerol; 2) antiserum to the D 1-peptide.

Table II. Fatty acid composition of photosystem II-particles from *Oscillatoria chalybea* and of phosphatidylglycerol from *Oscillatoria chalybea, Antirrhinum majus* and *Rhodopseudomonas spheroides.*

Preparations	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Photosystem II-particles from *Oscillatoria chalybea*	–	56	10 *cis*	–	17	11	6
Phosphatidylglycerol from *Oscillatoria chalybea*	–	55	10 *cis*	–	16	11	9
Phosphatidylglycerol from *Antirrhinum majus*	1	19	41 *trans*	–	5	18	15
Phosphatidylglycerol from *Rhodopseudomonas spheroides*	–	4	1	8	87*	–	–

* Detected as vaccenic acid.

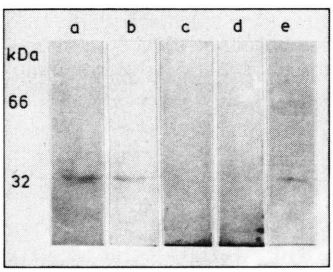

Fig. 8. Western blot analysis of the photosystem II-particles with antisera to lipids and to the D 1-peptide after treatment of the Immobilon-P-transfer membranes with methanol. a) Antiserum to D 1-peptide; b) antiserum to phosphatidylglycerol; c) antiserum to monogalactosyldiglyceride; d) antiserum to sulfoquinovosyldiglyceride; e) antiserum to β-carotene.

center of photosystem II. A comparative qualitative lipid analysis by the HPLC-chromatography permits the estimation that per reaction center 1 – 20 lipid molecules occur. Comparative experiments with photosystem II-particles from the cyanobacterium *Synechococcus* PCC 6301 show that also in this thermophilic cyanobacterium phosphatidylglycerol bound to the D 1-peptide can be identified.

In order to obtain further information on the functional influence of phosphatidylglycerol in the reaction center, O_2-evolution of the purified photosystem II-preparation was measured under the influence of the phosphatidylglycerol-decomposing enzyme phospholipase A_2. As can be seen from Fig. 9 a and b, a clear effect of the phospholipase A_2-treatment an oxygen evolution is observed. After an incubation of 30 min, O_2-evolution decreases substantially, being reduced by 25 per cent 2 h later in the treated preparation. A subsequent addition of phosphatidylglycerol as emulsion to this photosystem II-preparation stimulates photosynthetic O_2-evolution again, restoring after 20 min of incubation oxygen evolution to the original level of the untreated control preparation.

Discussion

With monospecific antisera to the glycolipids, phospholipids and carotenoids we were able to demonstrate in the Western blot procedure, that the D 1-peptide of photosystem II from the cyanobacterium *Oscillatoria chalybea* contains bound β-carotene and phosphatidylglycerol. Nanba and Satoh [28] have shown by HPLC analysis and absorption measurements that the core-complex of photosystem II from spinach contains, in addition to chlorophyll$_a$ and to pheophytine$_a$, β-carotene molecules. The same result was obtained by Barber *et al.* [29] for the reaction center complex of photosystem II from peas. The core-complex consists of the D 1- and D 2-peptides and the peptides of cytochrome b_{559}. In the present paper, by means of immunological methods using monospecific polyclonal antisera, it is shown for the first time that β-carotene is exclusively bound to the D 1-peptide. The result that the negatively charged phosphatidylglycerol is also bound to this peptide is also new. Quantitative lipid analysis by *High Pressure Liquid Chromatography* permitted to estimate that the molar ratio of phospholipid/D 1-peptide was maximally 20 : 1. Phosphatidylglycerol is the only phospholipid occurring in *Oscillatoria chalybea* [26]. This lipid from cyanobacteria differs in comparison to that of higher plants in the fatty acid com-

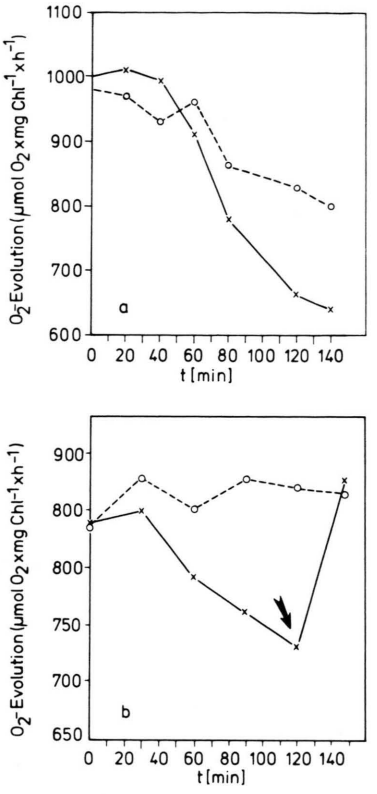

Fig. 9. Influence of phospholipase A_2 and phosphatidylglycerol on the ferricyanide Hill reaction of the photosystem II-particle fraction. (○) No addition; (x) phospholipase A_2 treatment. The arrow in b) indicates phosphatidylglycerol addition.

position and in the position of fatty acids on the glycerol, with the difference being on the one hand that the hexadecenoic monoenoic acid does not have *trans*-configuration but *cis*-configuration and that fatty acids with 16 carbon atoms are localized in position 1 and fatty acids with 18 carbon atoms in position 2 [26]. In contrast, in phosphatidylglycerol molecules from higher plants unsaturated fatty acids are bound in position 2 and saturated fatty acids in position 1 of the diglyceride. The configurational differences might have steric reasons, on the other hand it should be noted that fatty acids with *cis*-configuration have in comparison to fatty acids with *trans*-configuration of the same chain length a considerably lower melting point [30]. This means that this phosphatidylglycerol with its hexadecenoic acid in *cis*-configuration gives a higher fluidity to the membrane. It looks as if in phosphatidylglycerol from *Oscillatoria chalybea* the high amount of saturated fatty acids is compensated for by this acid in *cis*-configuration. Furthermore, this particular configuration might play a decisive role for binding of the lipid molecules on the D 1-peptide. Under the assumption that the phospholipid is covalently bound to the peptide, this binding should go *via* the fatty acid. This in turn means that the glycerol-phosphate residue should become accessible to antibodies. This concept is supported by the fact that the phosphatidylglycerol antibody is not directed towards the fatty acid region but towards the glycerol phosphate residue. Moreover, it is observed that treatment of the D 1-peptide with phospholipase A_2 partially inhibits oxygen evolution in photosystem II. Phospholipase A_2 splits fatty acids in position 2 of the diglyceride.

By the binding of the anionic phosphatidylglycerol the D 1-peptide has received a hydrophobic modification and a negatively charged surface. By means of this hydrophobic domain the D 1-peptide obtains the property to undergo not only intermolecular interactions with proteins, but also with lipids bound to other proteins and to lipids which build up the bimolecular lipid layer. Moreover, the negatively charged surface might permit ionic binding with cations like calcium and magnesium. In this sense the stimulatory effects of photosynthetic activity of photosystem II-reactions caused by magnesium ions might become understandable [31].

The type of binding in the fatty acid region of phosphatidylglycerol onto the D 1-peptide is not entirely clear and will be further studied. The literature describes a covalent binding of phosphatidylinositol onto cell wall glycoproteins in bacteria [32, 33]. Thus, phosphatidylinositol seems to be bound *via* the inositol residue to sugar residues in a glycane type of binding.

Furthermore covalent binding of saturated fatty acids like palmitic and myristic acid onto membrane-bound glycoproteins is described [35, 36]. Whereas binding of palmitic acid occurs either by ester binding with a serine residue or by thioester binding with a cystein residue, myristic acid is bound *via* an amide binding of an *n*-terminal glycine. In both cases, by the acylation as well as by the binding of the phospholipid the respective proteins obtain by the free methyl ends of the fatty acids hydrophobic poles by which they can be anchored in the lipid membrane. On the other hand the proteins

obtain by the acyl residues the capacity to permeate lipid membranes. By means of this hydrophobic polarization, proteins whose site of synthesis does not coincide with the functional site, can be translocated. Thus, Mattoo and Edelmann [37] have studied the acylation and translocation of the D1-precursor peptides in the Laminaceae water plant *Spirodela oligorrhiza.* They were able to show that biosynthesis and palmitoylation of the D1-precursor occurs membrane-bound on stroma-lamellae, before translocation of the palmitoylated D1 into the photosystem II-complex of the grana thylakoids takes place. For the bound (^{3}H)-labeled palmitic acid a half-life time of 3–6 min was observed. It is not clear whether palmitic acid is split off after translocation of the D1-peptide, of whether the D1-bound fatty acid has a high turnover. The fate of the palmitic acid is unresolved. We assume that in the cyanobacterium *Oscillatoria chalybea* biosynthesis of the D1-peptide might be differently regulated, as thylakoids occur single stranded in the cytoplasma.

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