Comparative Immunological Detection of Lipids and Carotenoids on Peptides of Photosystem I from Higher Plants and Cyanobacteria

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Dedicated to Professor Horst Senger on the occasion of his 65th birthday

Photosystem I, Western Blot, Lipid- and Pigment Composition, Lipid-Carotenoid Binding

Photosystem I preparations were obtained from wild-type tobacco *Nicotiana tabacum* var. JWB, three chlorophyll-deficient tobacco mutants: Su/su, Su/su var. Aurea and yellow- green leaf patches of the variegated mutant NC 95, *Spinacia oleracea* and furthermore from the mesophilic cyanobacterium *Synechococcus* PCC 7942 and the thermophilic cyanobacterium *Synechococcus* sp.. Peptides from these preparations were analyzed by SDS polyacrylamide gel electrophoresis and transferred for detection of bound lipids and carotenoids according to the Western blot procedure to nitrocellulose membranes. The PS I preparations from the *Nicotiana tabacum* species and spinach consist of the core complex **and** the LHCP I complex, the latter containing, however, traces of the LHCP II polypeptides. The core complex consists of the two core peptides with the apparent molecular mass of 66 kDa each and peptides with molecular masses of 22, 20, 19, 17, 16, 10 and 9 kDa. The LHCP I complex contains 4 subunits with molecular masses of 28, 26, 25 and 24 kDa. The PS I preparations of the two mutants Su/su and Su/su var. Aurea contain as impurities traces of the core peptides (D_1/D_2) and the two chlorophyll-binding peptides (CP43/CP47) of photosystem II. The PS I preparation from the mesophilic and thermophilic cyanobacterium consists of the two core peptides with the apparent molecular mass of 66 kDa and peptides with molecular masses of 16, 14 and 10 kDa. The peptides of the PS I preparations were characterized by specific PS I, CP I and LHCP I antisera. The antiserum to the PS I complex reacts in the Western blot with the homologous peptides of PS I from higher plants, but only with the CP I complex from the two cyanobacteria. In comparative studies with PS II from higher plants the PS I antiserum reacts with the LHCP II complex as expected. The antiserum to the CP I complex reacts only with the 66 kDa peptides of PS I from all objects. There is no cross reaction with the 66 kDa peptides (heterodimer of the D_1/D_2 peptides) of PS II. The antiserum to the LHCP I complex reacts only with the four LHCP I peptides of PS I from higher plants and as expected with the LHCP II of PS II: Because cyanobacteria do not have LHCP complexes, there is no reaction with the LHCP I antiserum. By means of polyclonal monospecific antisera to lipids it was shown by Western blot procedure that only two lipid species are bound to PS I peptides. The galactolipid monogalactosyldiglyceride is bound to the CP I complex of the *Nicotiana tabacum* species, spinach and the two cyanobacteria as well as to the LHCP I complex of the higher plants. The phospholipid phosphatidylglycerol is only associated with the CP I complex of the analyzed higher plants and cyanobacteria. With polyclonal monospecific antisera to carotenoids it was demonstrated that β-carotene, lutein, neoxanthin and zeaxanthin are associated with the CP I complex of the higher plants and the cyanobacteria analyzed. Violaxanthin is also bound to the CP I complex of the two cyanobacteria, whereas it is bound together with neoxanthin to the LHCP I complex of the higher plants.

Introduction

The special arrangement of certain lipids and carotenoids with proteins in the thylakoid membrane seems to be very important for photosynthetic electron transport reactions. While bound lipids probably stabilize conformation and activity of proteins and protein complexes, like the PS I and PS II complex (Sandermann, 1978; Webb and

Green, 1991), bound carotenoids seem to serve mainly as light harvesting pigments and protect the photosynthetic apparatus against photooxydative damage (Cogdell, 1985, 1988; Damm *et al.*, 1987, 1990; Krinsky, 1979; Siefermann-Harms, 1985). The lipids and carotenoids are not only differently distributed between the two photosystems, but also between the functional complexes within the respective photosystem, namely the reaction center core complex and light harvesting complex of PS I and PS II (Böger and Sandmann, 1990, 1993; Braumann *et al.*, 1982; Damm *et al.*, 1987, 1990; Li *et al.*, 1989; Murata *et al.*, 1990; Siefermann-Harms, 1980, 1985; Trémolières *et al.*, 1981). This different structural arrangment of lipids and carotenoids underlines their special functions in photosynthesis. Our own studies have shown that antisera to lipids and carotenoids inhibit PS I and PS II reactions on the donor side of both photosystems (Lehmann-Kirk *et al.*, 1979a, b; Menke *et al.*, 1976; Radunz, 1984; Radunz and Bader, 1982; Radunz and Schmid, 1973, 1975; Radunz *et al.*, 1984a, b). These inhibitory effects can be explained by assuming that the binding of antibodies induces a conformational change in certain electron transport peptides, thus leading to an inhibition of electron flow. The experiments demonstrate that lipids and carotenoids are antigenic determinants bound to these components. By means of the Western blot procedure we were able to show that the galactolipid monogalactosyldiglyceride, the phospholipid phosphatidylglycerol and the carotenoids β-carotene, lutein, neoxanthin and violaxanthin are bound on various polypeptides of the PS I complex (Makewicz *et al.*, 1994, 1995a, b). It should be emphasized that in contrast to the reactions of the monogalactolipid and of the phosphatidylglycerol, the antiserum to the digalactolipid and to the anionic lipid sulfoquinovosyldiglyceride also inhibits photosynthetic electron transport in the region of photosystem I and photosystem II (Radunz *et al.*, 1984a). By means of the Western blot procedure, however, these lipids were only detectable on peptides of photosystem II (Voß *et al.*, 1992, Makewicz *et al.*, 1995a). In the present publication we compare the presence of lipids and carotenoids bound onto peptides of the PS I complex from the wild-type tobacco *Nicotiana tabacum* var. JWB and three chlorophyll mutants, namely the yellow-green mutant Su/su, the

yellow mutant Su/su var. Aurea and the variegated mutant NC 95, furthermore from *Spinacia oleracea*, from the mesophilic cyanobacterium *Synechococcus* PCC 7942 and the thermophilic cyanobacterium *Synechococcus* sp.. The tobacco mutants differ from the wild-type in the morphology of their lamellar system and in their photosynthetic activity (Burk and Menser, 1964; Homann and Schmid, 1967; Schmid, 1967a, b; Schmid and Gaffron, 1967a, b; Schmid *et al.*, 1966; Okabe *et al.*, 1977). Whereas the lamellar system of chloroplasts from the wild-type tobacco JWB is composed of grana and intergrana regions, the lamellar system of chloroplasts of the mutants Su/su is mainly composed of extended intergrana regions and lower stacked grana regions. The lamellar system of the mutant Su/su var. Aurea consists just of membrane doublings and very extended intergrana regions. The mutant NC 95 has green leaf areas with chloroplasts of normal morphological structure of the lamellar system like the wild-type JWB and yellow-green leaf areas with chloroplasts which consist only of intergrana thylakoids which exhibit only photosystem I reactions (Homann and Schmid, 1967).

Material and Methods

Organisms, growth conditions and photosystem I isolation

PS I complexes were isolated from wild-type tobacco *Nicotiana tabacum* var. JWB and from three chlorophyll-deficient tobacco mutants: the yellow-green mutant Su/su, the yellow mutant Su/su var. Aurea and yellow-green leaf patches of the variegated mutant NC 95 (Burk and Menser, 1964; Homann and Schmid, 1967; Schmid, 1967a, b; Schmid and Gaffron, 1967a, b; Schmid *et al.*, 1966; Okabe *et al.*, 1977). PS I complexes were also isolated from *Spinacia oleracea* and from the mesophilic cyanobacterium *Synechococcus* PCC 7942 and the thermophilic cyanobacterium *Synechococcus* sp..

The *Nicotiana tabacum* species were grown in a climatized growth chamber with a light/dark cycle of 14 h/10 h, a day temperature of 27 °C and a relative humidity at 60%. Leaves were harvested after 2–3 months. Spinach was purchased from the local market. The mesophilic cyanobacterium *Synechococcus* PCC 7942 was grown in BG 11 medium (Stanier *et al.*, 1971), pH 7.0, with 17.65 mM

$NaNO_3$ in 250 ml bottles with constant illumination (2000 lux), supplied with 2% CO_2 enriched air at 38 °C. Cells were harvested after 2 days.

The thermophilic cyanobacterium *Synechococcus* sp. was grown in medium D (Castenholz, 1969), pH 7.5, with 5.6 mM $NaHCO_3$, 1.0 mM KNO_3 and 8.1 mM $NaNO_3$ in 1 l bottles with constant illumination (2000 lux), supplied with 5% CO_2 enriched air at 50 °C. Cells were harvested after 3 days.

PS I preparations from higher plants were prepared according to Wynn and Malkin (1988). Solubilized PS I and LHCP complexes were loaded onto a 0.4–1 M sucrose gradient containing 0.02% Triton X-100 with a 2 M sucrose cushion and centrifuged at 100.000 x g for 18 h at 4 °C. The dark green band at the top of the 2 M cushion contained the native PS I complexes (Makewicz *et al.*, 1994).

To prepare thylakoid membranes from the mesophilic cyanobacterium *Synechococcus* PCC 7942 the cells were centrifuged for 20 min at 2200 x g, washed with buffer I (0.05 M Hepes-NaOH, pH 6.5, 0.05 M $CaCl_2$, 0.4 M sucrose), pelleted again (20 min at 2200 x g, 4 °C) and resuspended again in buffer I. After addition of benzonase (Boehringer, Mannheim) (0.8 µ/ml cell suspension), the cell suspension was passed through a French press at 20000 psi and centrifuged for 5 min at 1100 x g at 4 °C to remove whole cells. The supernatant was then centrifuged (30 min at 27000 x g, 4 °C) and the resulting pellet containing the thylakoids was suspended in buffer II (0.05 M Hepes-NaOH, pH 6.5, 0.4 M sucrose). After centrifugations (30 min at 27100 x g, 4 °C) the membranes were resuspended in buffer II to give a final concentration of 1 mg chlorophyll/ml.

Thylakoid membranes from the thermophilic cyanobacterium *Synechococcus* sp. were obtained according to the procedure of Satoh *et al.* (1985) with slight modifications. Cells were harvested by centrifugation (20 min at 3000 x g), washed with 20 mM Hepes-NaOH, pH 7.5, containing 10 mM NaCl, centrifuged again and the resulting pellet was resuspended in 100 ml 50 mM Hepes-NaOH, pH 7.5, containing 0.5 M manitol. The cell suspension was treated with lysozyme (E. Merck, Darmstadt) (1 mg/ml cell suspension) for 1 h at 35 °C in the dark. After centrifugation (12 min at 16000 x g) the pellet was resuspended in 75 ml 50 mM Mes-

NaOH, pH 6.5, containing 10 mM NaCl and passed twice through a French press at 6000 psi. After addition of DNase (0.02 mg/ml cell suspension) and 5 mM $MgCl_2$ the suspension was centrifuged for 5 min at 12000 x g to remove whole cells. The supernatant was then centrifuged for 45 min at 250000 x g. The resulting pellet, containing the thylakoid membranes, was suspended in 20 mM Mes-NaOH, pH 6.5, containing 0.5 M manitol and 10 mM NaCl to give a final concentration of 1 mg Chl/ml.

Extraction and purification of PS I complexes from thylakoid membranes from both types of cyanobacteria were done according to Witt *et al.* (1987) with slight modifications. PS II complexes were extracted by addition of 0.3% sulfobetaine 12 (SB 12) and incubated for 20 min at room temperature in the dark. After centrifugation (40 min at 140000 x g, 4 °C) the resulting supernatant contained the extracted PS II complexes. The pellet was resuspended in 20 mM Mes-NaOH, pH 6.5, containing 0.5 M manitol and 10 mM NaCl and PS I complexes were extracted by addition of 1% SB 12 and incubated for 20 min at room temperature in the dark. After centrifugation (40 min at 140000 x g, 4 °C) the resulting supernatant contained the extracted PS I complexes. This extract was loaded onto a linear sucrose gradient (10–40% sucrose in 20 mM Mes-NaOH, pH 6.5, containing 10 mM $MgCl_2$ and 20 mM $CaCl_2$) and was centrifuged for 16 h at 100000 x g at 4 °C. The aggregated PS I complexes (upper part of the sucrose gradient) were dialyzed against 0.1 M Tris-HCl, pH 8.3, containing 10 mM $MgCl_2$, 20 mM $CaCl_2$ and 0.5 M manitol and subsequently concentrated.

SDS PAGE and Western blotting

PS I polypeptides were modified in the SDS polyacrylamide gel electrophoresis according to the methods of Weber and Osborn (1969) and Laemmli (1970). A 1.5 mm gel with a 10–20% gradient separation gel and a 3% collection gel was used. Prior to electrophoresis, samples of PS I (10 µg protein) were solubilized with 100 mM DTT, 2% SDS, 10% glycerol, 0.01% BPB and 10 mM Tris-HCl buffer, pH 8.4, at 50 °C for 30 min. Electrophoresis was carried out at a constant current of 25 mA for 4 h at room temperature. Following electrophoresis the gel was stained with Coomassie Brilliant Blue.

Western blotting was performed as described by Renart *et al.* (1979). The separated PS I polypeptides were transferred by pressure from SDS gels to nitrocellulose membranes for 20 h at room temperature. The membranes were blocked with 2.5% fish gelatine. The dilution of the first antibody depends on the antiserum used. The second antibody, peroxidase-conjugated pig immunglobulins to rabbit immunglobulins (anti-rabbit IgG, DAKO) was diluted 500-fold. Specifically bound antibodies were stained by the reaction of peroxidase with H_2O_2 and 4-chloro-1-naphtol (Sigma).

Antisera

The used antisera to PS I, CP I, LHCP I, lipids and to the carotenoids were obtained by immunization of rabbits and are characterized in earlier publications (Makewicz *et al.*, 1994 and 1995a, b; Radunz, 1970, 1971, 1976; Radunz and Bader, 1982; Radunz and Berzborn, 1970; Radunz and Schmid, 1973, 1975, 1979; Radunz *et al.*, 1984a, b; Schmid *et al.*, 1993).

Chlorophyll and protein determination

Chlorophyll was determined according to Schmid (1971) in methanol/water 90/10 (v/v) and

protein determinations were done according to Smith *et al.* (1985).

Results

PS I complexes

PS I complexes from wild-type tobacco, the three chlorophyll-deficient tobacco mutants Su/su, Su/su var. Aurea, yellow-green leaf patches of NC 95 and from spinach were prepared according to Wynn and Malkin (1988). These preparations were further purified by 18 h centrifugations at 100000 x *g* over a linear 0.4–1 M sucrose density gradient. PS I complexes from the mesophilic and thermophilic cyanobacteria were prepared according to Witt *et al.* (1987) These preparations were further purified by 16 h centrifugations at 10000 x *g* over a linear 10–40% sucrose denisty gradient. Fig. 1 shows the polypeptide composition of the PS I complexes from the higher plants and the two cyanobacteria by SDS polyacrylamide gel electrophoresis. The PS I preparations from the higher plants consist of the core complex and the LHCP I complex which contains also traces of LHCP II polypeptides. The core complex consists of the two core peptides with the apparent molecular mass of 66 kDa and 7 peptides with molecular masses of
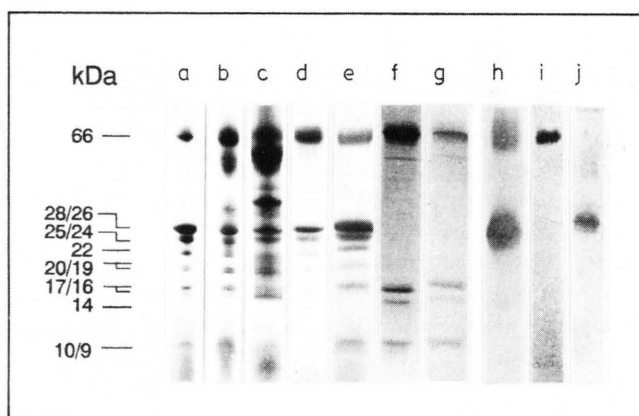

Fig. 1. Polypeptide composition of the PS I complexes from different plants demonstrated by SDS-PAGE and detection of polypeptides of PS I by means of the Western blot procedure.
a) – g) polypeptide composition of the PS I complex by SDS-PAGE; a) *Nicotiana tabacum* var. JWB (wild tpye); b) *Nicotiana tabacum* Su/su; c) *Nicotiana tabacum* Su/su var. Aurea; d) *Nicotiana tabacum* NC 95, yellow-green leaf patches; e) *Spinacia oleracea*; f) mesophilic cyanobacterium *Synechcococcus* PCC 7942; g) thermophilic cyanobacterium *Synechococcus* sp.; h) – i) nitrocellulose membranes with PS I peptides from *Nicotiana tabacum* var. JWB after reaction with h) an antiserum to PS I; i) an antiserum to CP I; j) an antiserum to the LHCP complex.
Subunits of the light harvesting complex (LHCP) consist of the molecular masses 24, 25, 26 and 28 kDa. Peptides with masses of 1–2 kDa difference do not separate on such a gel. Thus, the LHCP gives two bands, one at 24/25 kDa and the other at 26/28 kDa.

22, 20, 19, 17, 16, 10 and 9 kDa. The two core peptides, called CP I, appear as a single protein band. The LHCP I complex contains 4 subunits with molecular masses of 28, 26, 25 and 24 kDa. The PS I preparations of the two mutants Su/su and Su/su var. Aurea contain even after purification over the sucrose density gradient as impurities traces of the core peptides (D_1/D_2) and the two chlorophyll binding peptides (CP 43/CP47) of PS II. The PS I preparations from the mesophilic and thermophilic cyanobacterium consist of the two core peptides with the apparent molecular mass of 66 kDa and peptides with molecular masses of 16, 14 and 10 kDa. There is no LHCP complex in cyanobacteria. A polyclonal monospecific antiserum to the PS I complex reacts in the Western blot with the homologous peptides of PS I from the higher plants, but only with the CP I complex from the two cyanobacteria (Fig. 1). In comparative studies with PS II from higher plants the PS I antiserum reacts with the LHCP II complex as expected, because LHCP II peptides are able to migrate to PS I under certain circumstances (Jansson, 1994). Other PS II peptides were not labelled. The antiserum to the CP I complex reacts exclusively with the 66 kDa peptides of PS I from all objects. There is no cross reaction with the 66 kDa peptides (Heterodimer of the D_1/D_2 peptides) of PS II. The antiserum to the LHCP I complex reacts with the four LHCP I peptides of PS I from the higher plants tested and also as expected with the LHCP II of PS II. Because cyanobacteria do not have LHCP complexes there is no reaction with the LHCP I antiserum.

Detection of lipids bound onto PS I peptides

For the detection of lipids bound onto peptides of PS I the separated peptides were transferred from the SDS gel to nitrocellulose membranes and immunostained with polyclonal monospecific lipid antisera. Although different lipid antisera have been tested there is only a positive reaction with the antiserum to the galactolipid monogalactosyldiglyceride and to the phospholipid phosphatidylglycerol. No positive reaction could be obtained with antisera to other lipids like digalactosyldiglyceride and sulfoquinovosyldiglyceride. In a previous publication these results obtained with immunological methods were corroborated by

HPLC-analyses of the lipids (Makewicz *et al.*, 1995). The antiserum to phosphatidylglycerol marks only the CP I complex of all higher plants and the two cyanobacteria, whereas the antiserum to monogalactosyldiglyceride labels the CP I complex of all analyzed objects and also the four peptides of the LHCP I complex of the higher plants tested (Fig. 2). The corresponding control sera show absolutely no reaction with any PS I peptide. Positive immunoreactions after washing the PS I peptides with solvents like methanol or acetone before SDS polyacrylamide gel electrophoresis confirm a strong lipid binding to PS I peptides, a binding which can, however, be destroyed with phospholipase A_2 (Makewicz *et al.*, 1995a).

In order to analyze the importance of the lipids monogalactosyldiglyceride and phosphatidylglycerol for the function of the PS I complex, the influence of phospholipase A_2 on electron transport reactions in the region of PS I was tested. For this test PS I particles from *Nicotiana tabacum* var. JWB were incubated with the enzyme at 30 °C. The rate of electron transport was measured as O_2-uptake in a PS I-mediated Mehler-reaction using methylviologen as the acceptor and the electron donor couple 2,6-dichlorophenolindophenol/ascorbate in the presence of DCMU (Fig. 3). Whereas the rate of the control preparations remains essentially constant, the enzyme treated sample shows decreasing reaction rates. The inhibition reached 40%. If after 120 minutes an emulsion of monogalactosyldiglyceride and phosphatidylglycerol (ratio 1:1 v/v) was given to the enzyme-treated sample, a reconstitution of the preparations was obtained a few minutes later (30 min). This reaction is absolutely specific for the lipids tested. If an emulsion of phosphatidylserine which is a phospholipid that does not occur in chloroplasts, is added, no reactivation is observed. This demonstrates that the lipids monogalactosyldiglyceride and phosphatidylglycerol play a functional role in PS I.

Detection of carotenoids bound to PS I peptides

For the detection of carotenoids bound onto peptides of PS I the separated peptides were transferred from the SDS gel to nitrocellulose membranes and incubated with polyclonal monospecific antisera. The labelling experiments show that
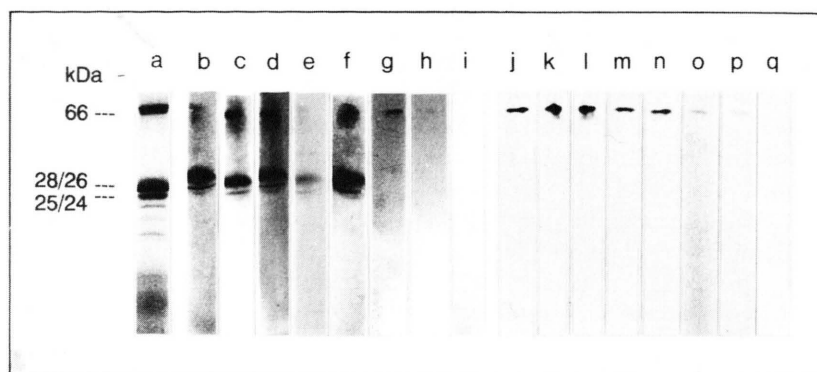
Fig. 2. Immunological evidence of bound monogalactosyldiglyceride and phosphatidylglycerol on peptides of the PS I complex from different organisms by means of the Western blot procedure.
a) SDS-PAGE with PS I peptides from JWB (10 µg chlorophyll); b) – h) nitrocellulose membranes with PS I peptides after reaction with the monogalactosyldiglyceride antiserum; j) – p) after reaction with the phosphatidylglycerol antiserum.
PS I peptides from b) and j), *Nicotiana tabacum* var. JWB (10 µg chlorophyll); c) and k), the tobacco mutant Su/su (10 µg chlorophyll); d) and l), the tobacco mutant Su/su var. Aurea (10 µg chlorophyll); e) and m), the mutant *NC 95*, yellow-green leaf patches (10 µg chlorophyll); f) and n), spinach *Spinacia oleracea* (10 µg chlorophyll); g) and o), the mesophilic cyanobacterium *Synechococcus* PCC 7942 (3 µg chlorophyll); h) and p), the thermophilic cyanobacterium *Synechococcus* sp. (3 µg chlorophyll); i) and q), *Nicotiana tabacum* var. JWB after reaction with the corresponding control sera.
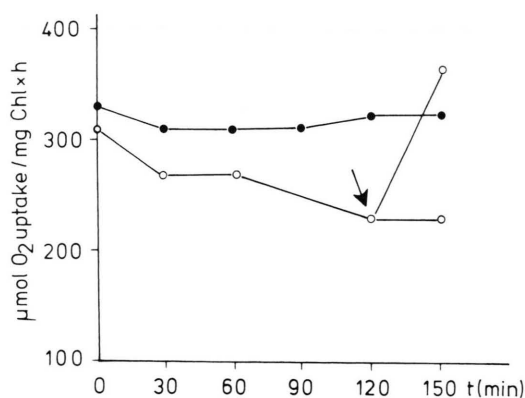The dilution of the MGDG antiserum was 1:400 and the dilution of the PG antiserum was 1:150.

Fig. 3. Influence of phospholipase A_2 on photosystem I-mediated photosynthetic electron transport of *N. tabacum* var. JWB as well as reactivation of electron transport by addition of a lipid emulsion of monogalactosyldiglyceride (MGDG) and phosphatidylglycerol (PG).
The PS I preparation was treated for 30 min with phospholipase A_2. 1 mg phospholipase was used for an amount of PS I preparation corresponding to 1 mg chlorophyll. The measurement of photosynthetic electron transport was carried out as PS I mediated O_2-uptake in a Mehler reaction, in 30 min intervals over 150 min. The lipid emulsion consisted of equal parts of (150 µg) monogalactosyldiglyceride and (150 µg) phosphatidylglycerol per reaction assay (corresponding to 30 µg chlorophyll). The measuring points have a mean error of 5%.
●—● untreated PS I preparation; ○—○ phospholipase A_2-treated PS I preparation; ╱ addition of the MGDG and PG emulsion.

β-carotene, lutein, neoxanthin and zeaxanthin are bound to the CP I complex of the analyzed higher plants and cyanobacteria (Figs 4 and 5). Neoxanthin is also bound to the two large subunits of the LHCP I complex of the higher plants with the molecular masses of 28 and 26 kDa (Fig. 4). The antiserum to violaxanthin also labels the large subunits of the LHCP I complex of the higher plants and in contrast to this, the CP I complex of both cyanobacteria (Fig. 4). The corresponding control sera show absolutely no reaction with any PS I peptide. Although the major portion of the carotenoids occurs after SDS gel electrophoresis as soluble pigments, there are carotenoids which are bound very strongly to the PS I peptides. Peptides washed with acetone or strongly acid buffer with the aim to remove or destroy carotenoids still show a positive labelling (Makewicz *et al.*, 1994). This underlines the tight binding of carotenoids to the respective peptides and the good protection of carotenoids between the proteins.

Discussion

With polyclonal monospecific antisera to glycolipids, phospholipids and carotenoids we are able to demonstrate in the Western blot procedure that the two core peptides (CP I) of PS I from four
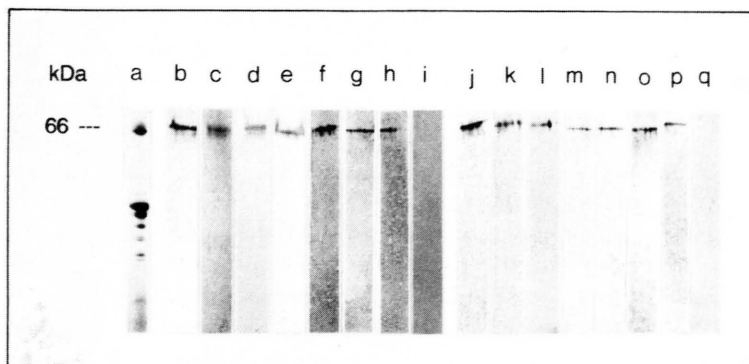
Fig. 4. Immunological evidence for the binding of lutein and zeaxanthin to peptides of the PS I complex from different organisms by means of the Western blot procedure.
a) SDS-PAGE with PS I peptides from JWB (10 μg chlorophyll); b) – h) nitrocellulose membranes with PS I peptides after reaction with the lutein antiserum; j) – p) after reaction with the zeaxanthin antiserum.
PS I peptides from b) and j), *Nicotiana tabacum* var. JWB (10 μg chlorophyll); c) and k), the tobacco mutant Su/su (10 μg chlorophyll); d) and l), the tobacco mutant Su/su var. Aurea (10 μg chlorophyll); e) and m), the tobacco mutant *NC 95*, yellow-green leaf patches (10 μg chlorophyll); f) and n), spinach *Spinacia oleracea* (10 μg chlorophyll); g) and o), the mesophilic cyanobacterium *Synechococcus* PCC 7942 (3 μg chlorophyll); h) and p), the thermophilic cyanobacterium *Synechococcus* sp. (3 μg chlorophyll); i) and q), *Nicotiana tabacum* var. JWB after reaction with the corresponding control sera. The dilution of the lutein and the zeaxanthin antiserum was 1:150.
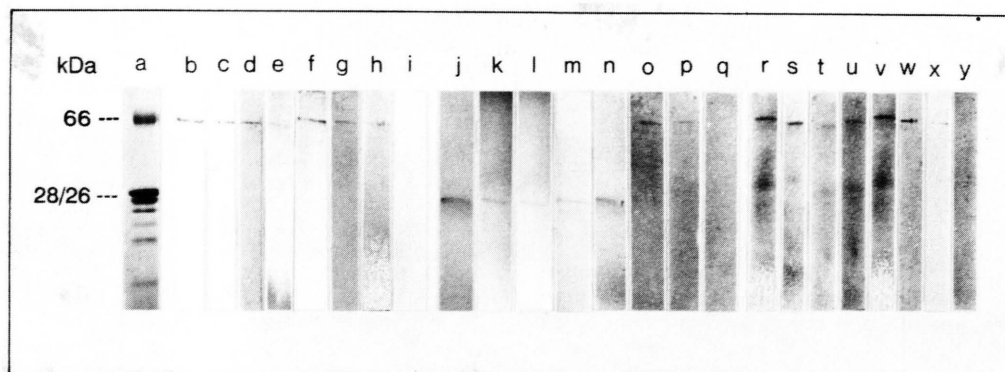

Fig. 5. Immunological evidence for the binding of β-carotene, violaxanthin and neoxanthin to peptides of the PS I complex from different organisms by means of the Western blot procedure.
a) SDS-PAGE with PS I peptides from JWB (10 μg chlorophyll); b) – h) nitrocellulose membranes with PS I peptides after reaction with the β-carotene antiserum (dilution 1:150); j) – p) after reaction with the violaxanthin antiserum (dilution 1:150); r) – x) after reaction with the neoxanthin antiserum (dilution 1:250).
PS I peptides from b), j) and r) *Nicotiana tabacum* var. JWB (10 μg chlorophyll); c), k) and s), the tobacco mutant Su/su (10 μg chlorophyll); d), l) and t), the tobacco mutant Su/su var. Aurea (10 μg chlorophyll); e), m) and u), the mutant *NC 95*, yellow-green leaf patches (10 μg chlorophyll); f), n) and v), spinach *Spinacia oleracea* (10 μg chlorophyll); g), o) and w), from the mesophilic cyanobacterium *Synechococcus* PCC 7942 (3 μg chlorophyll); h), p) and x), from the thermophilic cyanobacterium *Synechococcus* sp. (3 μg chlorophyll); i), q) and y), *Nicotiana tabacum* var. JWB after reaction with the corresponding control sera.

tobacco species, from spinach and a mesophilic and a thermophilic cyanobacterium contain bound monogalactosyldiglyceride, phosphatidylglycerol, β-carotene, lutein, neoxanthin and zeaxanthin. The CP I complex from the two cyanobacteria contains in addition violaxanthin. Monogalactosyldiglyceride, neoxanthin and violaxanthin can be found in the LHCP I complex of the analyzed higher plants. The results concerning these lipids fit into those of the literature. Trémolières *et al.*

(1981), Li *et al.* (1989) and Murata *et al.* (1990) have found that monogalactosyldiglyceride and phosphatidylglycerol occur in a high amount in the region of PS I and PS II. Both lipids seem to be necessary for building up a hydrophobic environment in which the PS I complex is embedded. By the binding of the anionic phosphatidylglycerol the CP I complex receives a hydrophobic modification and a negatively charged surface. From the reaction of the antibodies with the bound lipids it can be concluded that the lipids are bound to the peptides via their fatty acids and that the polar head groups serve as antigenic determinants. Until now, it is known that β-carotene is bound to the core peptides of PS I (Damm *et al.*, 1987, 1990) and PS II (Namba and Satoh, 1987; Barber *et al.*, 1987; Kruse *et al.*, 1993) and furthermore, that the xanthophylls are bound to the LHCP complex of both photosystems (Siefermann-Harms, 1987). However, it is new that lutein in substantial amounts but also neoxanthin, zeaxanthin and in the cyanobacteria violaxanthin are also bound to the CP I complex. In an earlier publication we have shown that unspecific adsorption or translocation of carotenoids can be excluded (Makewicz *et al.*, 1994). The results confirm that carotenoids are probably noncovalently bound as chromatophores onto PS I peptides and that the ionon rings serve as antigenic determinants. The function of the bound carotenoids seems to be clear (light absorption, energy transfer and protective functions), but it is not really known if there are differences in the binding mode or in the accessibility of carotenoids for antibodies in the different functional complexes, such as the CP I and LHCP I complex.

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