

Structural Modifications of the Photosynthetic Apparatus in the Region of Photosystem I in *Nicotiana tabacum* as a Consequence of an Increased CO₂-Content of the Atmosphere

A. Makewicz, A. Radunz and G. H. Schmid

Lehrstuhl für Zellphysiologie, Fakultät für Biologie, Universität Bielefeld, Postfach 100131, D-33501 Bielefeld, Bundesrepublik Deutschland

Dedicated to Professor Wilhelm Menke on the occasion of his 85th birthday

Z. Naturforsch. **50c**, 511–520 (1995); received July 11, 1995

N. tabacum, Western Blot, Lipid- and Carotenoid-Antisera, 700 ppm CO₂ in Air, Photosystem I

Nicotiana tabacum was grown with a CO₂-content in the atmosphere of 350 ppm and 700 ppm. After three weeks of growth in the respective atmosphere we were able to show that chloroplasts from plants grown under 700 ppm CO₂ exhibited an 18% higher photosystem I-mediated electron transport activity. Furthermore, we were able to show that in plants grown under the higher CO₂-concentration the peptide composition of photosystem I had been quantitatively changed. It appeared that the CPI-peptides were increased, whereas the LHCPI peptides were decreased. Corresponding to this change in the peptide composition the ratio chlorophyll/carotenoids/protein changes from 1:0.16:3.6 in the control plants to 1:0.23:5 in the "CO₂-plants". The chlorophyll a/b ratio changes from 1.9 in the control plants to 2.3 in the "CO₂ plants". Furthermore, the carotenoid composition appears to be changed. β -carotene increases by 60% as expected from the increased CPI-content. The xanthophylls lutein, violaxanthin, neoxanthin and zeaxanthin increase also but to a lesser extent. Using specific antisera to lipids, we were able to show that photosystem I contains only the membrane lipids monogalactosyldiglyceride and phosphatidylglycerol. Both lipids are specifically bound in a restricted number onto peptides of the photosystem I complex. Both lipids differ with respect to their binding strength on the peptides of photosystem I. The changes in the peptide and lipid composition were established by chemical analyses and by immunological techniques (Western blot) using monospecific antisera to the photosystem I peptides, to the chloroplast lipids as well as to the chloroplast carotenoids. From the quantitative and qualitative analyses of the peptides, carotenoids and lipids, the molecular distribution of the carotenoids and lipids in the CPI and LHCPI complex of photosystem I was established.

Introduction

Caused by anthropogenic activities, the CO₂-content of the atmosphere increases constantly. It is assumed that the CO₂-content of air might actually double from its actual value 350 ppm to 700 ppm in the next 50 years (Houghton *et al.*, 1990). In context with this expected change the question arises which influence this increased CO₂-content might have on plant growth. In this context in the past years the effect of increased

CO₂-concentrations on photosynthetic rates and the properties of the bifunctional enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco) and its gene expression was analyzed (Mullet *et al.*, 1980; Sage *et al.*, 1989; Bruce and Malkin, 1988; Stitt and Schulze, 1994; Van Oosten *et al.*, 1994; Makino, 1994). These experiments have shown that the influence of an increased CO₂-concentration depends on the organism studied, as well as on factors such as light intensity and temperature. It appears that an increased CO₂-concentration stimulates CO₂-fixation in C₃-plants by an increase in the Rubisco activity, which in turn is reflected by higher rates of photosynthesis. The long-term effect generally reported is that this stimulated photosynthetic activity is not maintained. It appears that limitations for example in the region of

Abbreviations: PSI, Photosystem I; CPI, core complex I; LHCPI, light harvesting complex I; LHCPII, light harvesting complex II.

Reprint requests to Prof. Dr. Georg H. Schmid.

0939–5075/95/0700–0511 \$ 06.00 © 1995 Verlag der Zeitschrift für Naturforschung. All rights reserved.

N



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

the transport systems might lead to an inhibition of photosynthesis.

In the present paper we investigated the effect of an increased CO₂-content of air of 700 ppm on the peptide and pigment composition of photosystem I. We analyze the molecular modifications of the lipid-pigment-peptide complex of photosystem I of *Nicotiana tabacum* (Bednarz *et al.*, 1988; Makewicz *et al.*, 1994 and 1995) grown from the cotyledon stage onward in air containing 700 ppm CO₂. The analysis was carried out with biochemical and immunological methods.

Materials and Methods

Growth of Nicotiana tabacum var. John William's Broadleaf

Plants were grown in a fully climatized growth chamber. The light/dark cycle was 14 h/10 h. Day temperature was 27 and night temperature 21 °C. Humidity was kept during the light cycle at ≈60% and during the dark period at ≈70%. The light intensity was 120 μ Einstein · m⁻² · sec⁻¹. The CO₂-content of the atmosphere was regulated by the following principle: The growth chamber contains glass compartments which were maintained by regulated gassing with an atmosphere of air containing 700 ppm. The not compartemented space was gassed with normal air. The CO₂-content of the gass phases was regularly verified by mass spectrometry as described earlier by Ishii and Schmid (1982) or by Schmid *et al.* (1981).

Isolation of photosystem I-particles

The PSI-preparations were prepared according to Wynn and Malkin (1988). Solubilized PSI and LHCP complexes were loaded onto a 0.4–1 M sucrose gradient that contained 0.02% Triton X-100 with a 2 M sucrose cushion and centrifuged at 100,000×g for 18 h at 4 °C. The dark green band at the top of the 2 M sucrose cushion contained the native PSI-particles (Makewicz *et al.*, 1994).

Peptide analysis and Western blotting

The polypeptides of PSI 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 were solubilized with 100 mM DTT, 2% SDS, 10% glycerol, 0.01% BPB (Brome Phenol Blue) and 10 mM Tris-HCl buffer (pH 8.3) at 50 °C for 30 min. Electrophoresis was carried out with a constant current of 25 mA for 4 h at room temperature. Following electrophoresis the gel was stained with Coomassie Brilliant Blue (Makewicz *et al.*, 1994).

Western blotting was performed as described by Renart *et al.* (1979). The proteins 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 depended on the anti-serum used. The second antibody, peroxidase-conjugated pig immunoglobulins to rabbit immunoglobulins (anti-rabbit IgG, DAKO), was diluted 500-fold. Specifically bound antibodies were stained by the reaction of peroxidase with H₂O₂ and 4-chloro-1-naphthol (Sigma). For densitometric analyses of the SDS-gels, the protein bands were stained with Coomassie Brilliant Blue and analyzed with a flat bed scanner – Scanjet IIp – from Hewlett Packard.

Antisera

The used antisera to photosystem I, core peptide I (CPI), photosystem II, D1-peptide, the peptides of the light harvesting complex of photosystem I (LHCPI) and to the light harvesting complex of photosystem II (LHCPII), to the glycolipids, to phosphatidylglycerol and to the carotenoids were obtained by immunization of rabbits and are characterized in earlier publications (Makewicz *et al.*, 1994 and 1995; Schmid *et al.*, 1993; Radunz, 1970, 1971, 1976; Radunz and Bader, 1982; Radunz and Berzborn, 1970; Radunz and Schmid, 1973, 1975 and 1979; Radunz *et al.*, 1984 a and b; Radunz, in preparation).

Quantitative determinations of chlorophyll, the carotenoids and proteins

Chlorophyll was determined according to Schmid (1971) in methanol/water (9:1). Carotenoids were determined using a standard curve by measuring the extinction in 90% methanol and 10% water at 430 nm. The contribution of chlorophylls to the measured extinction value at this

wavelength was subtracted. With material of plants grown in air 13 μg of chlorophyll per ml test solution give an extinction value of 0.5 at this wavelength. The protein determination was carried out according to Smith *et al.*, (1985).

Analysis of the lipids and carotenoids of photosystem I

For the analysis of carotenoids the PSI-particles were dialyzed against water, precipitated with acetone for 50 min at 4 °C, centrifuged at 5000 $\times g$ for 20 min and washed two times with water. For the carotenoid analysis the acetone supernatant was used. For the HPLC analysis of lipids the obtained sediment was extracted with boiling methanol and subsequently extracted with methanol/chloroform (1:2, v/v). A lipid solution in methanol was used for the HPLC. The analysis was carried out with a RP-18 column with acetonitrile/water/phosphoric acid (85%) 60:39.9:0.1 (v/v/v) with a flow-rate of 0.5 ml/min. Detection of eluted lipids was obtained with a spectral-detector at 205 nm. For the HPLC analysis of carotenoids the acetone extract from the acetone precipitation was concentrated and a carotenoid solution in diethylether was used. The analysis was carried out with a RP-18 column with a linear gradient of 75% methanol/acetonitrile (25:75, v/v) and 25% water for 20 min and 100% methanol/acetonitrile (25:75, v/v) for another 20 min with a flow-rate of 1.5 ml/min. Detection of eluted carotenoids was obtained at 445 nm with a spectral-detector.

Measurement of photosynthetic activities

Photosystem I activity was measured in a Clark-type electrode from Rank Brothers, Botisham, England using the electron donor couple 2,6-dichlorophenol indophenol/ascorbate and methylviologen as an acceptor, essentially as described earlier (Schmid *et al.*, 1981; Makewicz *et al.*, 1994; Radunz and Bader, 1982). Concentrations of the electron donors and acceptors used are given in Table I.

Results and Discussion

Tobacco plants grown for 3 weeks in an atmosphere containing 700 ppm CO₂ in air are shown in Fig. 1 of an earlier publication (He *et al.*, 1995).

The comparison with plants grown under 350 ppm CO₂ but otherwise identical conditions (light, temperature, nutrients) shows that the “700 ppm plants” have grown much better. Correspondingly, the dry matter of the plants, soluble leaf proteins and carbohydrates as well as the chlorophyll content are increased (data not shown). The “700 ppm plants” exhibit an 18% higher photosynthetic activity in the region of photosystem I (Table I). Leaves of the plants grown under the higher CO₂-concentration have a not so flat leaf surface as is usual for tobacco plants, but exhibit rolled up or curled leaf edges. It looks as if the leaf areas around the midrib grow faster than the leaf edge region. An interesting observation is that these plants have a higher mildew resistance than the control plants which were grown at 350 ppm CO₂ in air.

From these plants grown for 3 weeks under 700 ppm CO₂ or control conditions (350 ppm CO₂) photosystem I complexes were isolated according to Wynn and Malkin (1988) and purified by centrifugation at 100,000 $\times g$ over a sucrose density gradient. In Fig. 1 the peptide compositions of the photosystem I complex of “700 ppm plants” and the control plants are shown. As seen from the figure the qualitative composition of the peptides shows no difference. However, the quantita-

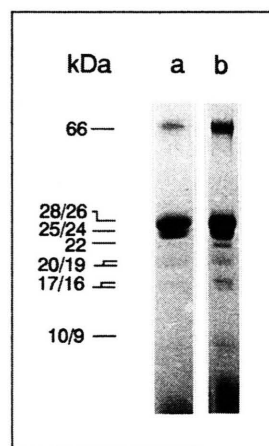


Fig. 1. Peptide composition of Photosystem I complexes from *Nicotiana tabacum* var. JWB in a SDS-polyacrylamide gel electrophoresis in dependence on the CO₂ content in air. a) PSI preparation of *N. tabacum* 3 weeks after growth in normal air (350 ppm CO₂) (control preparation); b) PSI preparation of *N. tabacum* 3 weeks after growth under 700 ppm CO₂ in air (CO₂ preparation).

tive difference is impressing. The 66 kDa band corresponding to the core-peptides of photosystem I (CPI) is substantially increased. The LHCPI, that is the bands in the molecular mass region of 28–24 kDa is comparable in both plants, whereas smaller peptides are only seen in the 700 ppm sample which means that they are increased in comparison to the 350 ppm sample. The result means that in comparison to the concentration of reaction centers the LHCPI is decreased in the “700 ppm plants”. The plant reacts to the increase of the CO₂-concentration to 700 ppm in the manner of plants which during their growth conditions are exposed for a prolonged time period to high light intensities. By means of specific antisera to the CPI peptide and to the LHCPI (and for a control to LHCPII) the Western blot analysis of the peptide pattern confirms the qualitative assessment of the gel electrophoresis (Fig. 2). In the 700 ppm plants the CPI-peptide band is somewhat stronger marked (Fig. 2).

Measurement of the chlorophylls, carotenoid and peptide content of the photosystem I complex shows that the ratio of these components depends on the growth conditions (Table I). Whereas in the control plants grown under the normal CO₂-condition of 350 ppm CO₂ in air the ratio chlorophyll/carotenoids/peptides is 1/0.16/3.6, the ratio shifts in the 700 ppm plants to 1/0.23/5. On a chlorophyll basis this corresponds to an increase in the carotenoid and an increase in the peptide content, implicating that in photosystem I a shift of the molar ratio of these components has occurred. Moreover, the ratio chlorophyll *a/b* does not remain constant and shifts from 1.9 in the control plants

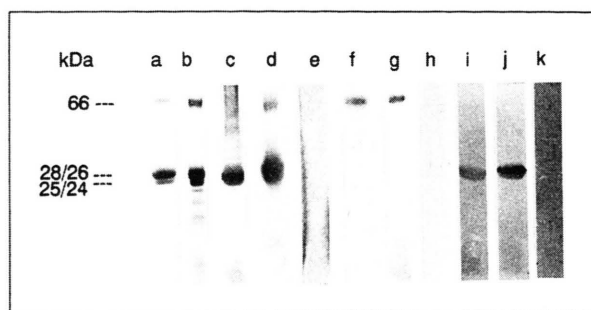


Fig. 2. Immunological test for the PSI-, CPI- and LHCPI-protein complexes in PSI preparations of *Nicotiana tabacum* var. JWB by the Western blot procedure in dependence on the CO₂ content of air during the growth period of 3 weeks. a) SDS-page with PSI-particles (corresponding to 10 µg chlorophyll) from plants of *Nicotiana tabacum* var. JWB grown under 350 ppm CO₂ in air (control preparation); b) SDS-page with PSI-particles (10 µg chlorophyll) from plants of *Nicotiana tabacum* var. JWB grown under 700 ppm CO₂ in air (CO₂-preparation); c)–k) Nitrocellulose membranes with the PSI-preparations to be compared after reaction with the protein antisera; c) control preparation and d) CO₂-preparation after reaction with the PSI-antiserum (dilution: 1:200); f) control preparation and g) CO₂-preparation after reaction with the CPI-antiserum (dilution 1:150); i) control preparation and j) CO₂-preparation after reaction with the LHCPI-antiserum (dilution 1:2000); e), h) and k) CO₂-preparation after reaction with the corresponding control sera.

to 2.3 in the “700 ppm plants” corresponding to a decrease in the chlorophyll *b* content which in turn would correspond to a decrease of the LHCPI-portion of the photosystem I complex. By means of monospecific antisera to lipids and carotenoids the binding of these components onto the peptides of photosystem I was analyzed in the Western blot

Table I. Chlorophyll-, carotenoid- and pigment content as well as photosynthetic activity of Photosystem I preparations from plants of *Nicotiana tabacum* var. JWB that have been grown under a CO₂-content of 350 ppm or 700 ppm.

PSI-preparation from	Age of plants (week)	Ratio chlorophyll <i>a/b</i>	Ratio chlorophyll to protein	Ratio carotenoid to chlorophyll	Ratio carotenoid to protein	PSI-activity* µmol O ₂ mg Chl ⁻¹ × h ⁻¹
Control Plants	3	1.9 ± 0.13	1:3.6	1:6.4	1:23.0	230 ± 14
“700 ppm Plants”	3	2.3 ± 0.18	1:5.0	1:4.3	1:21.5	280 ± 12

* Photosystem I activity was measured as reduction of methylviologen with the electron donor couple 2,6-dichlorophenol indophenol (DCPiP)/ascorbate with a Clark type electrode. Methylviologen concentration 10⁻⁴ M, DCPiP concentration 1.7 × 10⁻⁴ M, ascorbate 0.3 mM. Control plants were cultivated for 3 weeks in 350 ppm CO₂ in air, whereas “700 ppm plants” were cultivated in air with an increased CO₂-content of 700 ppm. Deviation of the other data was between 2 and 3 percent.

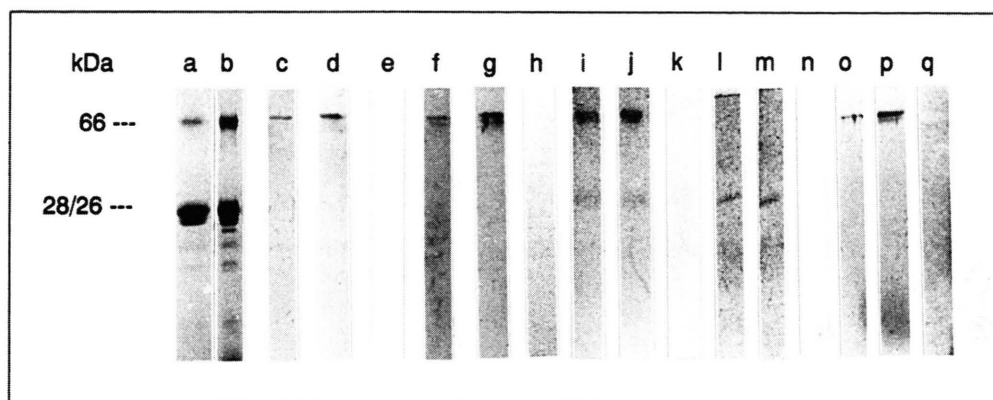


Fig. 3. Immunological detection of the carotenoids β -carotene, lutein, neoxanthin, violaxanthin, neoxanthin and zeaxanthin on peptides of the PSI complex of *Nicotiana tabacum* var. JWB by means of Western blotting in dependence on the CO₂-content of air under which the plants have been grown for 3 weeks. a) SDS-page with PSI-particles (10 μ g chlorophyll) from *N. tabacum* plants grown under 350 ppm CO₂ in air (control preparation); b) SDS-page with PSI-particles (10 μ g chlorophyll) from *N. tabacum* var. JWB plants grown under 700 ppm CO₂ in air (CO₂-preparation); c)–q) nitrocellulose membranes with PSI-preparations to be compared after reaction with the carotenoid antisera; c) control preparation and d) CO₂-preparation after the reaction with the antiserum to β -carotene (dilution 1:150); f) control preparation and g) CO₂-preparation after reaction with the antiserum to lutein (dilution 1:150); i) control preparation and j) CO₂-preparation after reaction with the antiserum to neoxanthin (dilution 1:250); l) control preparation and m) CO₂-preparation after reaction with the antiserum to violaxanthin (dilution 1:150); o) control preparation and p) CO₂-preparation after reaction with the zeaxanthin antiserum (dilution 1:150); e), h), k), n) and q) CO₂-preparations after reaction with the respective control sera.

procedure (Figs. 3 and 4). By means of this method it was shown that the CPI-band was specifically stronger marked by specific antibodies to β -carotene, to lutein (Fig. 3) and phosphatidylglycerol (Fig. 4), if the plants were grown under 700 ppm CO₂. Neoxanthin and zeaxanthin are present in the CPI to a very minor extent. It is interesting to note that lutein and β -carotene in both plants are only detected in the CPI-band. Violaxanthin occurs only in the LHCPI-band which contains in addition some neoxanthin (Fig. 3). The intensity of the marking of the LHCP-bands with the antiserum to violaxanthin appears stronger in the “700 ppm plants” than in the control plants. The antiserum to monogalactosyldiglyceride marks somewhat the CPI-band and intensively the peptides of the LHCPI complex (24–28 kDa region). It appears that this antiserum marks the peptides of the “700 ppm plants” stronger than the corresponding bands of the control plants (Fig. 4). It is clearly seen that all the different peptides of the LHCPI complex are marked which implies that all peptides of this complex carry monogalactosyldiglyceride molecules. Careful scrutiny of the

Western blot picture reveals a slight shift of the marked regions in comparison to the peptide positions of the peptides of the complex. This might be an indication that monogalactolipids are the binding elements between the peptides of the LHCPI complex. In contrast to this, the antiserum to phosphatidylglycerol reacts *only* with the CPI-band and here stronger with the “700 ppm plants” than with the control plants (Fig. 4). The distinction whether both peptides of the reaction core react with the antiserum or only one band cannot be made by the presented Western blot experiment. Furthermore, the experiments show that the peptides of the photosystem I complex do not react with the antisera to digalactosyldiglyceride or to sulfoquinovosyldiglyceride, hence these lipids do not occur on these peptides.

As the quantitative determination of carotenoids and chlorophylls has led to the result that the ratio of these two components has changed in dependence on the CO₂-content of air, the composition of the photosystem I preparation with respect to carotenoids and lipids was analyzed by HPLC. Lipids as well as carotenoids are adsorbed

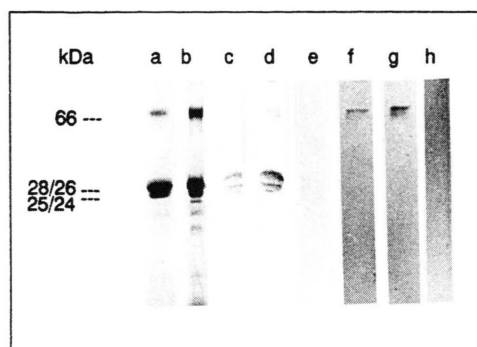


Fig. 4. Immunological detection of the lipids monogalactosyldiglyceride and phosphatidylglycerol on peptides of the PSI complex of *Nicotiana tabacum* var. JWB by means of Western blotting in dependence on the CO₂-content of air under which the plants have been grown for 3 weeks. a) SDS-page with PSI-particles (10 µg chlorophyll) from *N. tabacum* var. JWB plants grown under 350 ppm CO₂ in air (control preparation); b) SDS-page with PSI-particles (10 µg chlorophyll) from *N. tabacum* var. JWB plants grown under 700 ppm CO₂ in air (CO₂-preparation); c)–h) nitrocellulose membranes with the PSI-preparations to be compared after reaction with the antisera to lipids; c) control preparation and d) CO₂-preparation after the reaction with the antiserum to monogalactolipid (dilution 1:400); f) control preparation and g) CO₂-preparation after reaction with the antiserum to phosphatidylglycerol (dilution 1:150); e) and h) CO₂-preparations after reaction with the respective control serum.

with different strength to proteins as the simple extraction experiments with solvents of different polarities carried out by Costes and Bazier (1972)

and Costes *et al.* (1978) have shown. Moreover, the photosystem I particles contain unspecifically adsorbed quantities of lipids. Therefore, in order to obtain reproduceable results, the unspecifically adsorbed components had to be removed from the preparation. Table II shows that carotenoids are extracted by acetone to different degrees from the photosystem I preparation in dependence on the extraction time. β -Carotene and lutein are in comparison to the other xanthophylls stronger bound. Only a 50 min lasting acetone extraction removes all xanthophylls and β -carotene (Table II). By means of the Western blot procedure, we were able to show that even after an extraction of the PSI-preparation with boiling methanol/chloroform, a few carotenoid molecules still remain bound to the PSI-peptides and can by use of monospecific carotenoid antisera still be detected in certain peptides of the PSI complex in the Western blot procedure. Thus, the core-peptides and to a certain extent also the peptides of LHCPI still react positively with the carotenoid antisera. It should be emphasized again that this strong binding concerns only a few remaining molecules which do not contribute to the quantitative HPLC analysis. But from these experiments it is seen that β -carotene and lutein are firmly bound to the core peptides (Table II). From all the membrane lipids as shown by HPLC-analysis only monogalactosyldiglyceride and phosphatidylglycerol are found to

Table II. Extractability of carotenoids and lipids from photosystem I-preparations of *N. tabacum* var. JWB in dependence on the extraction time and the polarity of the solvent.

Preparation	β -carotene	Lutein	Neoxanthin	Violaxanthin	Zeaxanthin	Monogalactolipid MGDG	Phosphatidylglycerol PG
PSI, 20 min, acetone extraction	–	73	7	11	9		
PSI, 50 min, acetone extraction	55	36	2	4	3		
PSI, 50 min, acetone precipitation and extraction of the sediment with boiling methanol/chloroform	–	100	–	–	–		
CPI, 50 min, acetone extraction	27	73	–	–	–		
LHCPI, 50 min, acetone extraction	–	–	–	–	–		
PSI, 30 min, acetone precipitation and extraction of the sediment with boiling methanol/chloroform						27	73
CPI, 30 min, acetone precipitation and extraction of the sediment with boiling methanol/chloroform						49	52
CPI, 50 min, acetone precipitation and extraction of the sediment with boiling methanol/chloroform						–	100

The analysis of the carotenoids and lipids was carried out by HPLC. The values are per cent of total carotenoids or lipids.

Table III. Composition of carotenoids and lipids of the photosystem I-complex of *N. tabacum* var. JWB in dependence on the CO₂-content of the atmosphere under which the plants have been grown for 3 weeks.

	Carotenoid fraction in % total carotenoids					Lipid fraction in % total lipids	
	β-Carotene	Lutein	Neoxanthin	Violaxanthin	Zeaxanthin	Monogalactosyldiglyceride MGDG	Phosphatidylglycerol PG
PSI-control preparation	31	52	4	7	6	76	24
PSI-CO ₂ -preparation	50	42	2	3	3	–	100

Values are per cent total carotenoid or lipid. The carotenoid fractions were extracted by a 50 minutes acetone treatment. For the lipid fraction the PSI preparation was precipitated during 50 minutes with acetone and only the sediment was extracted with boiling methanol/chloroform. The analysis was carried out by HPLC (see Fig. 5).

be bound onto peptides of the PSI-preparation. Digalactolipid and sulfolipid do not occur, as already seen and demonstrated by immunological methods. If the preparation was precipitated by acetone (duration of the pre-extraction procedure 30 minutes!) and then extracted with boiling methanol/chloroform a mixture of monogalactosyldiglyceride and phosphatidylglycerol was obtained consisting by 3/4 of phosphatidylglycerol and by 1/4 of monogalactosyldiglyceride. Both lipids differ with respect to their binding strength to the peptides of photosystem I as seen from the fact that a 50 minutes extraction with acetone removes all the monogalactosyldiglyceride from CPI, whereas phosphatidylglycerol is only removed by the boiling polar solvent mixture methanol/chloroform.

In order to characterize lipids as antigenic determinants in the Western blot procedure and in order to characterize the type of the lipid-protein binding, enzymic decomposition experiments were carried out. After the transfer of the PSI-peptides to the nitrocellulose membranes, the membranes were incubated with phospholipase A₂ and with

lipase from hog rumen. After this treatment the reaction with the lipid antisera in the Western blot analysis came out negatively, meaning that the antigenic determinants are no more available. As the antibodies in the case of the monogalactolipid react with the galactose-glycerol-region and in the case of phosphatidylglycerol with the glycerol-phosphate-region and as the lipases used split off fatty acids, it must be assumed that these lipids are bound *via* their fatty acids onto the protein. This binding onto the protein could go *via* intermolecular interactions in a non-covalent manner.

In order to study the influence of the increased CO₂-content of air onto the composition of carotenoids and lipids, the carotenoids extractable with acetone and the lipids extractable with methanol/chloroform were analyzed by HPLC. As shown in Table III, β-carotene and lutein make up for 80% of the total carotenoid content of the PSI-preparation. The remainder is distributed between neoxanthin/violaxanthin and zeaxanthin. The lipid fraction of such a preparation consists to 3/4 of monogalactosyldiglyceride and to 1/4 of phosphatidylglycerol. The composition of the lipids of

Table IV. Number of bound molecules of chlorophylls, carotenoids and of the lipids monogalactosyldiglyceride and phosphatidylglycerol on the core and light-harvesting complex of photosystem I in plants of *Nicotiana tabacum* var. JWB grown under an atmosphere of 350 ppm CO₂ (a) or 700 ppm CO₂ (b) for 3 weeks.

Complex/molecules	Photosystem I [PSI]		Core-protein-complex I [CPI]		Light-harvesting-complex I [LHCPI]	
	a)	b)	a)	b)	a)	b)
Chlorophylls	189	132	57	40	132	92
Total carotenoids	54	57	49	54	50	3
β-Carotene	16	28	16	28	0	0
Lutein	28	23	28	23	0	0
Neoxanthin	3	2	2	1	1	1
Violaxanthin	4	2	0	0	4	2
Zeaxanthin	3	2	3	2	0	0
Monogalactosyldiglyceride	85	0	27	0	58	0
Phosphatidylglycerol	42	7	42	7	0	0

The values result from the quantitative determinations in Table I and from the HPLC analyses of Tables II and III.

photosystem I apparently depends on outer factors as it is seen that the composition of the carotenoids *and* lipids does not remain constant if the CO₂-content in air is increased to 700 ppm. Whereas β -carotene increases substantially, lutein decreases by $\approx 18\%$ and the other xanthophylls neoxanthin, violaxanthin and zeaxanthin decrease by $\approx 50\%$ (Table III). The observation fits into results of the literature in the sense that β -carotene which is practically exclusively bound to the core peptides (Siefermann-Harms, 1985; Kruse *et al.*,

1994), increases due to the increase in reaction core peptides when the plants are grown with 700 ppm CO₂ in air (Fig. 2). With the exception of lutein the decrease of the xanthophylls is explained by the decrease of LHCPI-peptides under the increased CO₂-concentration. As already mentioned above, the lipids monogalactosyldiglyceride and phosphatidylglycerol differ with respect to their binding strength to the peptides of photosystem I. Table III clearly shown, that growth under 700 ppm CO₂ leads to a substantial alteration of the structure of photosystem I, in which only phosphatidylglycerol remains bound to PSI, whereas monogalactosyldiglyceride changes its binding status in comparison to the control preparation (Table III).

As seen from these results, growth under the increased CO₂ content of air leads to a structural modification of the photosynthetic apparatus in the region of photosystem I. Thus, the content in core peptides (CPI) increases and that in light antenna peptides decreases which changes the chlorophyll/carotenoid and lipid content of the complex and/or the ratios of these components to each other.

From the quantitative determinations of the chlorophylls, carotenoids and lipids on the one hand and from the qualitative analysis of the carotenoid and lipid composition by HPLC (Fig. 5) on the other hand, the molar distribution of carotenoids and lipids on the CPI and LHCPI complexes was determined (Table IV). According to the statements of Mullet *et al.* (1980), Bruce and Malkin (1988) and Scheller and Møller (1990) 30% of the chlorophylls are located in CPI and 70% in the LHCPI which is a ratio of approx. 1:2. Our analysis *via* quantitative chlorophyll and protein determination (see Table I) leads to the same result. Corresponding to this ratio CPI/LHCPI we found for the distribution of monogalactosyldiglyceride also a ratio of 1:2. It appears, however, that growth under the increased CO₂ content of 700 ppm leads to changes in the binding status of this lipid. On the other hand phosphatidylglycerol is by principle only bound to the core peptides and not to the LHCPI and the growth condition of 700 ppm CO₂ in air reduces the number of these lipid molecules, bound as effector molecules to these peptides substantially in number (from 42 to 7). At this point it should be mentioned that the

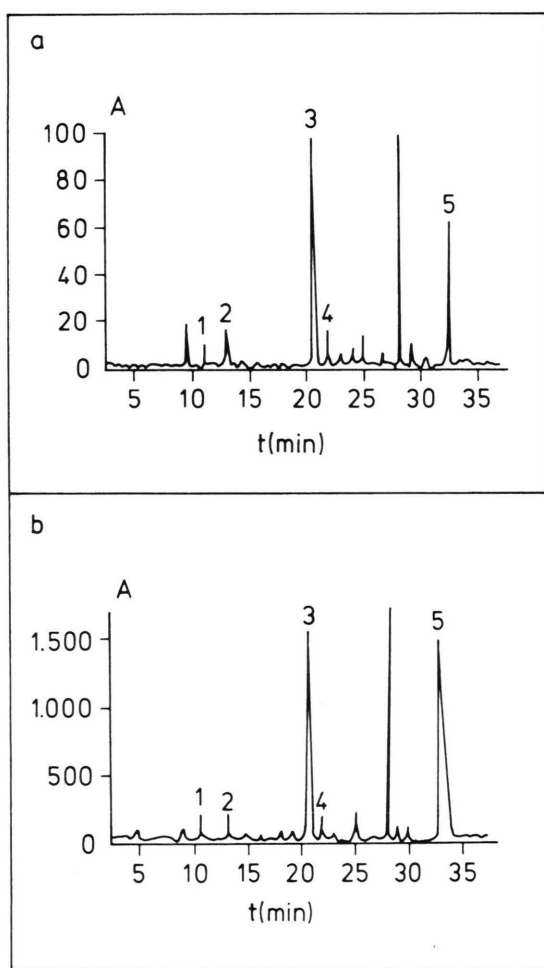


Fig. 5. HPLC-analysis of the carotenoids of the PSI complex of *N. tabacum* var. JWB in dependence on the CO₂-content in air under which the plants have been grown for 3 weeks. The carotenoids had been extracted by a 50 min acetone treatment. a) control preparation; b) CO₂-preparation. The numbers at the peaks represent: 1 neoxanthin, 2 violaxanthin, 3 lutein, 4 zeaxanthin and 5 β -carotene.

Western blot method permits detection of phosphatidylglycerol on LHCPII (Radunz, in preparation). Basis for the calculation of the carotenoid distribution is that CPI is composed of two 82 kDa peptides and that LHCPI is composed according to Jansson (1994) of 2 times 4 monomers of LHCPI and 2 trimers of LHCPII. This together with the Western blot analysis (Fig. 3) and the

HPLC analysis (Fig. 5) leads to the results summarized in Table IV. The result is that β -carotene and lutein, the two major carotenoid compounds, are in essence only detectable on the CPI complex. Violaxanthin occurs exclusively on the LHCPI. The astonishing outcome of this analysis is that the carotenoid content of the light antenna is so small and that of the CPI complex so high.

- Bednarz J., Radunz A. and Schmid G. H. (1988), Lipid composition of photosystem I and II in the tobacco mutant *Nicotiana tabacum* NC 95. *Z. Naturforsch.* **43c**, 423–430.
- Bruce B. D. and Malkin R. (1988), Subunit stoichiometry of the chloroplasts photosystem I complex. *J. Biol. Chem.* **263**, 7302–7308.
- Costes C. and Bazier R. (1972), Heterogeneity and structural role of lipids in chloroplasts. In: *IInd Intern. Congress on Photosynthesis* (G. Forti, M. Avron and A. Melandri, eds.), Dr. W. Junk N. V., pp. 1635–1640.
- Costes C., Bazier R., Baltscheffsky H. and Hallberg C. (1978), Mild extraction of lipids and pigments from *Rhodospirillum rubrum* chromatophores. *Plant Sci. Lett.* **12**, 241–249.
- He P., Bader K. P., Radunz A. and Schmid G. H., Consequences of high CO₂-concentration in air on growth and gas-exchange rates in tobacco mutants. *Z. Naturforsch.*, submitted.
- Houghton J. T., Jenkins G. J., Ephraums J. J. and Woodwell G. M. (1990), *Climate change: The IPCC scientific assessment*. Cambridge University Press, Cambridge, UK.
- Ishii R. and Schmid G. H. (1982), Studies on ¹⁸O₂-uptake in the light by entire plants of different tobacco mutants. *Z. Naturforsch.* **37c**, 93–101.
- Jansson S. (1994), The light-harvesting chlorophyll *a/b*-binding proteins. *Biochim. Biophys. Acta* **1184**, 1–19.
- Kruse O., Radunz A. and Schmid G. H. (1994), Phosphatidylglycerol and β -carotene bound onto the D1 core peptide of photosystem II in the filamentous cyanobacterium *Oscillatoria chalybea*. *Z. Naturforsch.* **49c**, 115–124.
- Laemmli U. K. (1970), Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**, 680–685.
- Lehmann-Kirk U., Bader K. P., Schmid G. H. and Radunz A. (1979a), Inhibition of photosynthetic electron transport in tobacco chloroplasts and thylakoids of the blue-green alga *Oscillatoria chalybea* by an antiserum to synthetic zeaxanthin. *Z. Naturforsch.* **34c**, 1218–1221.
- Lehmann-Kirk U., Schmid G. H. and Radunz A. (1979b), The effect of antibodies to violaxanthin on photosynthetic electron transport. *Z. Naturforsch.* **34c**, 427–430.
- Makewicz A., Radunz A. and Schmid G. H. (1994), Immunological evidence for the binding of β -carotene and xanthophylls onto peptides of photosystem I from *Nicotiana tabacum*. *Z. Naturforsch.* **49c**, 427–438.
- Makewicz A., Radunz A. and Schmid G. H. (1995), Detection of phosphatidylglycerol and monogalactosyldiglyceride on peptides of photosystem I in *Nicotiana tabacum* species. In: *Plant Lipid Metabolism* (J. C. Kader and P. Mazliak, eds.), Kluwer Academic Publishers, Dordrecht, Boston, London, pp. 156–160.
- Makino A. (1994), Biochemistry of C₃-photosynthesis in high CO₂. *J. Plant Res.* **107**, 79–84.
- Mullet J. E., Burke J. J. and Arntzen C. J. (1980), Chlorophyll proteins of photosystem I. *Plant Physiol.* **65**, 814–822.
- Radunz A. (1970), Serological localization of monogalactosyldiglyceride in thylakoid membranes. *Z. Naturforsch.* **27b**, 822–826.
- Radunz A. (1971), Phosphatidylglycerin-Antiserum und seine Reaktionen mit Chloroplasten. *Z. Naturforsch.* **26b**, 916–919.
- Radunz A. (1976), Localization of the tri- and digalactosyldiglyceride in the thylakoid membrane with serological methods. *Z. Naturforsch.* **31c**, 589–593.
- Radunz A. (1995), in preparation.
- Radunz A. and Bader K. P. (1982), Influence of β -carotene antibodies on the photosynthetic electron transport in chloroplasts of higher plants and in thylakoids of the blue-green alga *Oscillatoria chalybea*. In: *Biochemistry and Metabolism of Plant Lipids* (J. F. G. M. Wintermans and P. J. C. Kuiper, eds.), Elsevier Biomedical Press, Amsterdam, New York, Oxford, pp. 377–383.
- Radunz A., Bader K. P. and Schmid G. H. (1984a), Influence of antisera to sulfoquinovosyldiglyceride and to β -sitosterol on the photosynthetic electron transport in chloroplasts from higher plants. In: *Struct. Function & Metabol. Plant Lipid 9* (A. Siegenthaler and W. Eichenberger, eds.), Elsevier Science Publishers B. V., pp. 479–484.
- Radunz A., Bader K. P. and Schmid G. H. (1984b), Serological investigations of the function of galactolipids in the thylakoid membrane. *Z. Pflanzenphysiol.* **114**, 227–231.
- Radunz A. and Berzborn R. (1970), Antibodies against sulphoquinovosyl-diacyl glycerol and their reaction with chloroplasts. *Z. Naturforsch.* **25b**, 412–419.
- Radunz A. and Schmid G. H. (1973), Reactions of antisera to lutein and plastoquinone with chloroplast preparations and their effects on photosynthetic electron transport. *Z. Naturforsch.* **28c**, 36–44.
- Radunz A. and Schmid G. H. (1975), The effect of antibodies to neoxanthin on electron transport on the oxygen-evolving side of photosystem II and the reac-

- tions of this antiserum with various chloroplast preparations. *Z. Naturforsch.* **30c**, 622–627.
- Radunz A. and Schmid G. H. (1979), On the localization and function of the xanthophylls in the thylakoid membrane. *Ber. Dtsch. Bot. Ges.* **92**, 437–443.
- Renart J., Reiser J. and Stark G. R. (1979), Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: A method for studying antibody specificity and antigen structure. *Proc. Natl. Acad. Sci. USA* **76**, 3116–3120.
- Robertson E. J. R. and Leech R. M. (1995), Significant changes of cell and chloroplast development in young wheat leaves (*Triticum aestivum* cv Hereward) grown in elevated CO₂. *Plant Physiol.* **107**, 63–71.
- Sage R. F., Sharkey T. D. and Seemann J. R. (1989), Acclimation of photosynthesis to elevated CO₂ in five C3 species. *Plant Physiol.* **89**, 590–596.
- Schmid G. H. (1971), Origin and properties of mutant plants: Yellow tobacco. In: *Methods in Enzymology*, Vol. 23 (San Pietro, A., ed.), Academic Press, New York, London, pp. 171–194.
- Schmid G. H., Price J. M. and Gaffron H. (1966), Lamellar structure in chlorophyll-deficient but normally active chloroplasts. *J. de Microscopie* **5**, 205–212.
- Schmid G. H., Bader K. P., Gerster R., Triantaphylides C. and André M. (1981), Dependence of photorespiration and photosynthetic unit sizes on two interdependent nuclear gene factors in tobacco. *Z. Naturforsch.* **36c**, 662–671.
- Schmid G. H., Radunz A. and Gröschel-Stewart U. (1993), *Immunologie und ihre Anwendung in der Biologie*. Georg Thieme Verlag, Stuttgart, New York.
- Scheller H. V. and Möller B. L. (1990), Photosystem I polypeptides. *Plant Physiol.* **78**, 484–494.
- Siefermann-Harms D. (1985), Carotenoids in photosynthesis. I. Location in photosynthetic membranes and light-harvesting function. *Biochim. Biophys. Acta* **811**, 325–355.
- Smith P. K., Krohn R. I., Hermanson G. T., Mallia A. K., Gartner F. H., Provenzano M. D., Fujimoto E. K., Goecke N. M., Olson B. J. and Klenk D. C. (1985), Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76–85.
- Stitt M. and Schulze D. (1994), Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. *Plant, Cell and Environment* **17**, 465–487.
- Van Oosten J.-J., Wilkins D. and Besford R. T. (1994), Regulation of the expression of photosynthetic nuclear genes by CO₂ is mimicked by regulation by carbohydrates: A mechanism for the acclimation of photosynthesis to high CO₂? *Plant, Cell and Environment* **17**, 913–923.
- Weber K. and Osborn M. (1969), The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamid gel electrophoresis. *J. Biol. Chem.* **244**, 4406–4412.
- Wynn R. M. and Malkin R. (1988), Characterization of an isolated chloroplast membrane Fe-S protein and its identification as the photosystem I Fe-SA/Fe-SB. *FEBS Lett.* **229**, 293–297.