Comparative Immunological and Chemical Analysis of Lipids and Carotenoids of the D1-Peptide and of the Light-Harvesting-Complex of Photosystem II of *Nicotiana tabacum*

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The light-harvesting-complex (LHCP) was isolated from photosystem II of *Nicotiana tabacum* var. John William's Broadleaf by means of the detergent acetyl-β-D-glucopyranoside and fractionating centrifugation. The D1-peptide of photosystem II was isolated as a dimer with the molecular mass of 66 kDa from the chlorophyll-deficient tobacco mutant *N. tabacum* Su/su by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis. Both preparations were characterized by means of the Western blot procedure using monospecific antisera to the proteins of photosystem II and monospecific antisera to lipids with which the lipids bound to peptides were determined. In parallel to this, lipids bound to the isolated LHCP-complex and to the isolated D1-peptide were determined by lipido-chemical methods.

The extraction of the isolated core peptide D1 with a mixture of boiling methanol and chloroform and subsequent HPLC-chromatography showed that in the D1-peptide isolated *via* SDS-polyacrylamide gel electrophoresis, monogalactolipids, phosphatidylglycerol and sulfolipid molecules are bound in the molar ratio 1:3:17. By means of the immunological procedure of Western blotting we were able to show that the 66 kDa band of the isolated dimeric D1 reacts positively only with the antisera to monogalactolipid, sulfolipid, β-carotene and violaxanthin. With the antiserum to digalactolipid and that to phosphatidylglycerol a positive reaction is only observed if the preparation used in the Western blot is not the isolated D1-peptide but a "total" photosystem II-preparation.

The lipid extraction of the LHCP-complex and the subsequent analysis by thin-layer chromatography led to the result that the isolated LHCP-complex contained in bound form 3 molecules monogalactolipid, 1 molecule of digalactolipid, 1 molecule of phosphatidylglycerol and 1 molecule of lutein. Less than 1 molecule of sulfolipid, β-carotene, neoxanthin and violaxanthin are found. In the Western blot analysis only the antiserum to monogalactolipid and phosphatidylglycerol and among the carotenoid antisera only the antisera to β-carotene, violaxanthin and to neoxanthin reacted. With the antisera to the digalactolipid, to the sulfolipid and the antisera to the xanthophylls, namely to lutein and neoxanthin, a positive reaction occurred only if the material used in the Western Blot was the "total" photosystem II-preparation.

By gas chromatography of the fatty acids of the isolated peptide fractions it was shown that, compared to the lipids of photosystem II and of the thylakoid membrane, in lipids of the isolated D1-peptide and of the LHCP-complex the saturation degree of fatty acids is strongly increased. Whereas palmitic acid in chloroplast lipids makes up for only 11% of the fatty acids, this saturated fatty acid increases in the lipids of the LHCP to 20% and makes up for 74% of total fatty acids in the lipids of the D1-peptide. Linoleic and linolenic acids are completely absent and oleic acid makes up for 14% of total fatty acids . In contrast to the lipids of the thylakoid membrane, the lipids bound to proteins/peptides are characterized by a strongly saturated character.

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