Carotenoid Composition of Chlorophyll-Carotenoid-Proteins from Radish Chloroplasts

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The uneven distribution of carotenoids and chlorophylls between several chlorophyll-carotenoid-proteins isolated from radish chloroplasts by SDS-polyacrylamide-gel electrophoresis is described.

Lutein and *neoxanthin* are enriched in the light-harvesting chlorophyll a/b-protein $LHCP_3$, which exhibits low chlorophyll a/b ratios (1.1–1.3) and high values for the ratio chlorophyll a/β-carotene (a/c = 60–180).

β-carotene is bound not only to the chlorophyll a-protein CPI + CPIa of photosystem I, but also to the chlorophyll a-protein CPa. Both chlorophyll a-proteins are characterized by high values for the ratio a/b and low values for the ratio a/c.

The photosynthetic prenylpigments, chlorophylls and carotenoids, are bound within the chloroplasts to the photochemically active thylakoids [1–3]. The osmiophilic plastoglobuli of the chloroplast stroma, in turn, contain carotenoids only in trace amounts [4, 5]. Another chloroplast compartiment containing carotenoids is the chloroplast envelope with violaxanthin as main component [6]. We have estimated that in photosynthetically active chloroplasts, which possess a fully developed membrane system of stroma and grana thylakoids, more than 90% of the carotenoids are bound to the thylakoids.

Polyacrylamide-gel electrophoresis of sodium dodecylsulfate-digested chloroplasts or thylakoids reveals several chlorophyll-carotenoid-proteins which differ in their chlorophyll composition [7]. The main components are the light-harvesting chlorophyll a/b-protein $LHCP_3$ and the photochemically active P700 chlorophyll a-proteins of pigment system I, CPI and CPIa. Several other minor components have been found more recently, the additional light-harvesting chlorophyll proteins $LHCP_1$, $LHCP_2$, $LHCP_y$ and the chlorophyll a-protein CPa [8–11]. There is some indication that CPa may represent the reaction center of photosystem II [9, 12, 13].

Chlorophyll b was found to be associated together with chlorophyll a in the light-harvesting chlorophyll a/b-proteins (LHCP's). The latter exhibit low a/b ratios of 1 to 1.3 and possess lutein as main carotenoid [7, 8, 11]. The CPI, in turn, is characterized by high a/b ratios and β-carotene as main carotenoid [7, 11, 14]. Most of the pigment work with the chlorophyll-proteins was concerned with the partition of chlorophyll a and b between the different pigment proteins. A quantitative analysis of their carotenoid composition and the exact partition of β-carotene and lutein between the different chlorophyll-carotenoid-proteins was, however, not performed. Whether the other predominant thylakoid xanthophylls violaxanthin and neoxanthin are bound to the chlorophyll-carotenoid-proteins and in which way is not known either. In order to obtain more information on the binding and localization of carotenoids within the thylakoids, we determined the carotenoid composition of the major chlorophyll-carotenoid-proteins in green radish seedlings.

The pigment-proteins ($LHCP_3$, CPI + CPIa and CPa) were isolated from the chloroplasts of 7 d old radish seedlings (*Raphanus sativus* L.; 3 d dark growth + 4 d continuous white light; 3500 lux = 9 W × m^{-2}) by the SDS-PAGE method (Fig. 1) as described before [11]. After electrophoretic separation the corresponding pigment-proteins were cut off from the gel disks and eluted without further purifi-

Abbreviations: a/b, ratio chlorophyll a/b; CPI and CPIa, P700-containing chlorophyll a-β-carotene-proteins of photosystem I; CPa, chlorophyll a-β-carotene-protein; $LHCP_3$, main light-harvesting chlorophyll a/b-lutein-protein; PAGE = polyacrylamide-gel electrophoresis; SDS = sodium dodecylsulfate.

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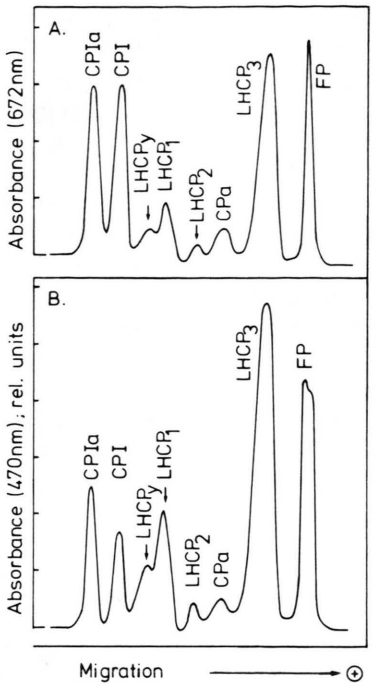

Fig. 1. Densitometer scans of the chlorophyll-carotenoid-proteins of radish chloroplasts separated by SDS-PAGE. A) Scan at 672 nm. B) Scan at 470 nm; the absorbance of the LHCP's and of the free pigment zone (FP) increases at 470 nm due to the enrichment of xanthophylls and chlorophyll b. The absorbance of CPI, CPIa and CPa, which contain only little chlorophyll b, correspondingly decreases at 470 nm.

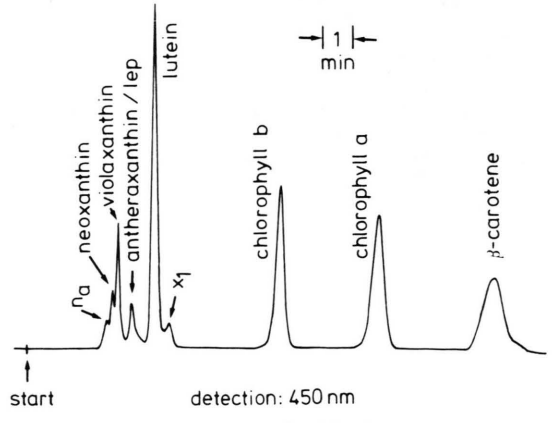

Fig. 2. High performance liquid chromatography of a prenylpigment extract from radish chloroplasts. X_1 = an unknown, minor xanthophyll, lep = luteinepoxid, n_a = neoxanthin a. (Nucleosil RP8, 5 µm; 9% water in methanol; 240 bar; flow rate: 1.3 ml/min.)

cation with a diluted buffer (0.01 M Tris-HCl, pH 8). The pigments were extracted with acetone and transferred to diethylether. Carotenoids and chlorophylls were determined by reversed-phase high performance liquid chromatography (HPLC) [15] as shown in Fig. 2.

As compared to the carotenoid composition of chloroplasts lutein and neoxanthin (consisting to about equal parts of neoxanthin and neoxanthin a) are enriched in the light-harvesting chlorophyll a/b-xanthophyll-protein $LHCP_3$. This also applies to the other LHCP's. β-carotene is present only in trace amounts, which do not seem to be a contamination, since these remain during further purification of the $LHCP_3$. On a molar basis there is about 1 xanthophyll per 2–3 chlorophylls in the $LHCP_3$.

The chlorophyll a-protein CPa, which is regarded as the possible reaction center of photosystem II, contains a very high percentage of β-carotene. Our purer CPa preparations exhibited rather high chlorophyll a/b ratios (6–8) and contained the higher percentage of β-carotene. Being present only in lower amounts it is often difficult to separate CPa fully from the major pigment-protein $LHCP_3$ with its low a/b ratio. From this one may conclude that the lutein content of the CPa protein could be lower than given in Table I. In any case, the identification of CPa can easily be performed via the chlorophyll a to β-carotene ratio (a/c) which shows low values for CPa (Table I), even when the fraction is contaminated by some $LHCP_3$. In fact, the a/c ratio proved to be a better parameter to define the

Table I. Percentage composition of carotenoids (weight %) and prenylpigment ratios of three chlorophyll-carotenoid-proteins isolated from radish choroplasts. Mean values and percentage ranges from 4 isolations. a + b/x + c = ratio chlorophylls to carotenoids; a/c = ratio chlorophyll a to β-carotene.

	Chloro-plasts	$LHCP_3$	CPa	CPI + CPIa
chlorophyll a/b	3.2	1.1–1.3	3.8	9–21
a + b/x + c	4	3–5	3–5	6–9
a/c	12	60–180	4–8	10
β-carotene	30	1.0–1.9	*60–75*	*56–64*
lutein	45	*56–65*	14–20	18–24
violaxanthin	11	4–5	4	7
neoxanthin	6	*21–29*	7	6
antheraxanthin/ luteinepoxid	5	~2	~2	3
xanthophyll x_1	~3	4	3	3

chlorophyll-carotenoid-proteins than the ratio chlorophyll a/b.

The P700-containing chlorophyll a-proteins of photosystem I, CPI and CPIa, – eluted here together – also show an enrichment of β-carotene as compared to the levels of whole chloroplasts. High a/b and low a/c ratios are typical for the CPI and CPIa as well as for the CPa.

The results of this paper were presented at the 6th International Carotenoid Symposium [16]. They show that β-carotene is not only a constituent of the photosystem I-pigment-proteins CPI + CPIa, but also a genuine component of the CPa which might represent the reaction center of photosystem II. Its function in the CPI, CPIa and CPa may thus be that of a photoprotective agent for chlorophyll a molecules by quenching excitation energy in the reaction center [17, 18]. In the CPa there is 1 molecule of β-carotene per 2–4 molecules of chlorophyll a and in the CPI + CPIa fraction 1 β-carotene per ca. 5 chlorophyll a molecules. The enrichment of β-carotene in CPa and CPI has now also been reported for spinach chloroplasts [19].

In contrast to lutein + neoxanthin and to β-carotene, which are preferentially bound either to the $LHCP_3$ or to CPI and CPa, there is no enrichment of violaxanthin and the other minor xanthophylls (antheraxanthin/luteinepoxid and x_1) in one of the chlorophyll-carotenoid-proteins (Table I). In the case of violaxanthin the level is lower in all pigment proteins than in the chloroplast fraction. This indicates that part of the violaxanthin is not bound to the chlorophyll-carotenoid-proteins and may be associated in the photosynthetic membrane with thylakoid lipids. This work is continued to further clarify the localization of violaxanthin and to quantify the partition of single carotenoids between all the chlorophyll-carotenoid-proteins of radish chloroplasts.

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