Distribution of *cis-trans*-β-Carotene in Pigment-Protein Fractions of Photosystem I and II

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The distribution of isomers of β-carotene is reported in the CP-47 pigment-protein complex of photosystem II and photosystem I (PS I) from barley. The distribution of carotenoids from light harvester complex II is reported. CP-47 lacks 9,9′-*cis*-carotene, but contains 9,13′-*cis*-carotene; PS I lacks 9,13′-*cis* but contains 9,9′-*cis*. More 9-*cis*, 9,13-*cis*, 9,15-*cis*, and epoxide is observed in CP-47 than in PS I.

Introduction

Photosystem II (PS II) and photosystem I (PS I) are composed of several pigment-protein complexes. PS II contains six polypeptide subunits: CP-47 and CP-43 kDa, two polypeptides of cytochrome b_{559}, and the polypeptides D-1 and D-2 [1, 2]. The presence and number of β-carotene molecules in PS I and PS II of photosynthesis has been well established (for a review see [3]). The lipophilic carotenes are organized in pigment-protein complexes, in PS I and PS II. Based on the abundance of carotenoids in photosynthetic membranes it is suggested that they serve some role in photosynthetic reactions or in stabilizing the photosynthetic apparatus [3]. The thylakoid membranes of spinach and a blue-green alga contain about 80% *all-trans*-β-carotene and about 20% of various *cis* isomers. It was shown that there are a few highly oriented carotenoids associated with the PS-II complex [4]. The presence and identity of the isomers in PS I and PS II of spinach and a thermophilic blue-green alga was determined by Ashikawa *et al.* [5]. Illumination of the thylakoid results in isomerization of *cis* forms of carotene into the *all-trans* forms. The thylakoid membranes contain, in addition to the *cis-trans* isomers of β-carotene, *all-trans*-β-carotene-5-6-epoxide [6].

In the present work the distribution of carotene isomers in some pigment-protein complexes of PS II and PS I of barley were analyzed using high performance liquid chromatography (HPLC). LHCP-II is composed of several component polypeptides of molecular weight 25 kDa, each of which contains 8 chlorophyll *a*, 6 chlorophyll *b*, and 2 xanthophyll molecules [7].

The subcomplex of PS II examined (CP47*), contains D-1/D-2, cyt b_{559} and CP-47. CP-47 contains 15–20 chlorophyll *a* and around 6 β-carotene molecules [8]. The LHC-II, which is assumed to contain five 25 kDa polypeptides, has a total of about 65 molecules of chlorophyll *a* and *b*, plus 10 molecules of xanthophyll. The complex of PS I, contains CP-1 and LHC-1 [9].

Materials and Methods

For HPLC a lime column was used on a Waters Associates chromatograph, model 6000 Wisp, equipped with a UV-vis detector which was set at 430 nm. The stainless steel column was 4.6 mm ID × 250 mm. It was packed as described by Tsukida *et al.* [10], by Dr. Walther Batsberg Pedersen (of RISO, Denmark). The calcium hydroxide was guaranteed analytical reagent grade, with particle size 1–10 μm from Kishida Chem. Co., Osaka (actually a gift from Dr. Niels Henrick Jensen of Radiometer, Denmark). The solvent used to elute the column was 0.3% acetone in *n*-hexane. Flow rate was 1 ml/min.

Carotenoids were extracted from the reaction centers and membrane preparations with 100% acetone. The pigment extracts were transferred into hexane, by addition of water. Acetone was removed from the hexane by repeated washing with water. Water was separated from hexane using a separatory funnel. The hexane fraction was applied to the column and the absorption of the eluant was monitored at

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436 nm. The absorption spectra of the eluants were compared with those shown by Koyama *et al.* [11] for the *cis-trans* isomers of β-carotene in hexane. Absorption spectra were measured with a Varian, Cary model 219 spectrophotometer.

A mixture of *cis* isomers of β-carotene was prepared by melting *all-trans*-β-carotene, for a few minutes, in an argon enriched atmosphere (oxygen was not completely removed). The β-carotene was a gift from Hoffmann-La Roche, Basel, Switzerland, and used without further purification. The data published by Tsukida *et al.* [10] were used as a guide to identify the relative positions of the *cis* forms of β-carotene in the eluants from HPLC. The type of *cis*-β-carotene in the pigment-protein complexes was determined by comparing the position of each band on the chromatogram with bands from the standard mixture of *cis-trans*-β-carotene.

The conditions for growing barley seedlings (*Hordeum vulgare* cv. Svalof's Bonus) was described by Hinz [4]. Grana membranes were prepared from barley using a modification of the method of Berthold *et al.* [12] as described by Møller and Høj [13]. Pigment-protein complexes were prepared as described by Bassi *et al.* [14] and Hinz and Welinder [7]. The pigment-protein samples were frozen (at −30 °C) for several months before the carotenoids were extracted.

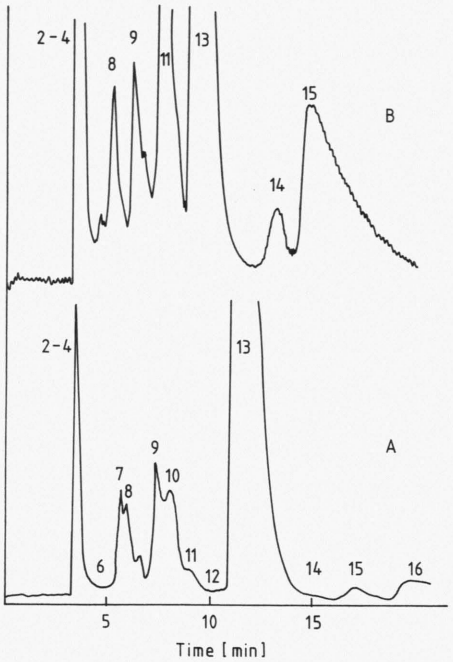

Fig. 1. HPLC elution pattern of thermally isomerized *all-trans*-β-carotene is shown by curve A. The elution pattern of carotenes extracted from PS-I particles is shown by curve B. The isomer assigned to each peak number is given in Table I. Experimental details are given in the Materials and Methods.

Results and Discussion

The mixture of thermally isomerized *all-trans*-β-carotene was analyzed by HPLC as described above in the Methods section. The result is shown in Fig. 1A. Each peak is numbered and identified in Table I. *All-trans*-β-carotene eluted at 10.6±0.3 min. The amount of each *cis* isomer present in the mixture depends for one thing, upon the duration of time *all-trans* was heated above the melting temperature. Differences in elution time are ascribed to variation in temperature and trace amounts of water and/or

Table I. HPLC of carotene.

Peak #	*cis* carotene	Ratio of *cis*/*all-trans* peaks; Fig. and curve #	
		1B	2A
2–4	15 or 13,15	0.118	0.089
6	9,13′		0.021
8	9,15	0.019	0.037
9	9,13	0.025	0.053
11	epoxide	0.18	0.41
13	*all-trans*	1.	1.
14	9,9′	0.017	
15	9	0.092	0.37

1B is PS I; 2A is CP47*.

acetone present in the solvents. The peak numbered "11", which was not identified by Tsukida *et al.* [10], is probably the 5,6-epoxide of *all-trans*-β-carotene as identified by Ashikawa *et al.* [6]. With the procedure used to analyze the isomers of carotene, the 15-*cis* and 13,15-*cis* was not resolved. Consequently, the peak corresponding to these isomers is given the double identification 15; 13,15-*cis*. This mixture was used as a standard to identify peaks in the HPLC of the extracts of the pigment-protein fractions.

PS-I particles, composed of CP-I and LHC-I, were extracted as described above. The result of HPLC analysis is shown in Fig. 1B. Identification of the numbered peaks is given in Table I. The ratio of absorbance of the *cis/trans* peaks is also given in Table I. This ratio may be taken as an estimate of the relative concentration of *cis*- to *all-trans*-carotene. It was difficult to distinguish the difference between 13-*cis*- and 9,15-*cis*-carotene, since these two isomers are relatively close to one another in the HPLC pattern. Furthermore, the small samples did not permit accurate measurement of the absorption spectrum of all fractions.

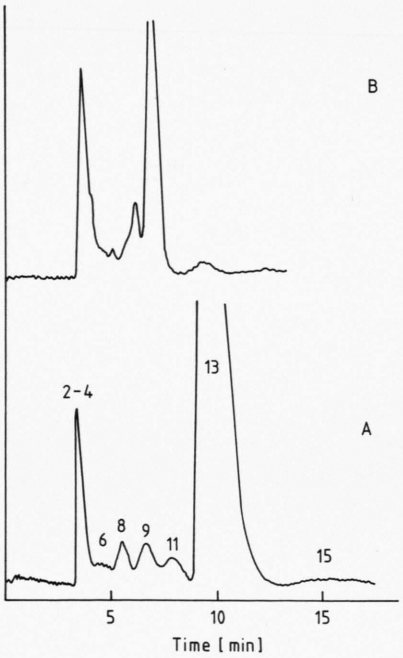

Fig. 2. HPLC elution patterns of carotenes and xanthophylls extracted from A_2 and LHC-II, are shown by curves A and B. The isomer assigned to each peak is given in Table I.

The HPLC analysis of fraction CP47* from reaction center II [15], composed of CP-47 and D-1/D-2 (free of CP-43 and LHC-II), is shown in Fig. 2A. The identity and relative concentration of *cis* carotenes are given in Table I.

The PS-I reaction center and light harvesting complex (LHC-I) was obtained from the supernatant of a BBY preparation. The HPLC analysis of the carotenes is shown in Fig. 2B and the ratio of *cis*- to *all-trans* peaks is shown in Table I. Our preliminary analysis of PS I and PS II show a larger amount of *cis* forms in the former, in agreement with the results of Ashikawa *et al.* [5]. However, we find this is not the case when comparing the amount of *cis* forms in PS I and CP47* (Table I).

When comparing the results of the HPLC it is readily seen that there are significant differences in the isomers of carotene in PS I and CP47* of PS II (refer to Table I). CP47* appears to lack 9,9′-*cis*-carotene, which is observed in the PS-I particles. On the other hand 9,13′-*cis* is observed in CP47* while none is detected in PS I. There is about 4 times more 9-*cis* in CP47* than PS I. In addition, about twice as much epoxide, 9,13-*cis* and 9,15-*cis* are observed in CP47* compared to PS I. When comparing PS I and CP47* there appears to be a greater proportion of *cis* forms in PS I than the PS- II core complex CP47* (columns 1B, 2A).

The LHC-II composed of CP-25 and CP-27 was prepared from BBY particles. The HPLC analysis of the LHC-II shows the presence of four bands, see Fig. 2B. The elution pattern of LHC-II is quite different from those obtained from PS-I or PS-II complexes. LHC contains only xanthophylls and no carotenoids. It was reported that most xanthophylls and other pigments remain trapped on the column and do not elute during the HPLC run [6]. Apparently, there are at least four different types of xanthophylls present in the LHC-II, that are not retained on the lime column. Consequently, the elution pattern shown for PS II could contain contributions from the xanthophylls from LHC. This observation can introduce some uncertainty as to the relative amounts of the various *cis*-β-carotene in reaction center particles.

While there is a preponderance of certain *cis* isomers in specific groups of pigment-protein complexes, it is not yet possible to identify specific *cis* carotenes uniquely associated with a complex.

[1] K. Satoh, Photochem. Photobiol. **42,** 845–853 (1985).
[2] W. R. Widger, W. A. Cramer, M. Hermodson, and R. G. Herrmann, FEBS Lett. **191,** 186–190 (1985).
[3] D. Siefermann-Harms, Biochim. Biophys. Acta **811,** 325–355 (1985).
[4] U. G. Hinz, Carlsberg Res. Commun. **50,** 285–298 (1985).
[5] I. Ashikawa, A. Miyata, H. Koike, Y. Inoue, and Y. Koyama, Biochemistry **25,** 6154–6160 (1986).
[6] I. Ashikawa, M. Kito, K. Satoh, H. Koike, Y. Inoue, K. Saiki, K. Tsukida, and Y. Koyama, Photochem. Photobiol. **46,** 269–275 (1987).
[7] U. Hinz and K. G. Welinder, Carlsberg Res. Commun. **52,** 39–54 (1987).
[8] J. Breton and K. Satoh, Biochim. Biophys. Acta **892,** 99–107 (1987).
[9] R. Bassi and D. Simpson, I. Eur. J. Biochem. **163,** 221–230 (1987).
[10] K. Tsukida, K. Saiki, T. Takii, and Y. Koyama, J. Chromatography **245,** 359–364 (1982).
[11] Y. Koyama, T. Takii, K. Saiki, and K. Tsukida, Photobiochem. Photobiophys. **5,** 139–150 (1983).
[12] D. A. Berthold, G. T. Babcock, and C. F. Yocum, FEBS Lett. **134,** 231–234 (1981).
[13] B. L. Møller and P. B. Høj, Carlsberg Res. Commun. **48,** 161–185 (1983).
[14] R. Bassi, U. Hinz, and R. Barbato, Carlsberg Res. Commun. **50,** 347–367 (1985).
[15] R. Bassi *et al.*, J. Biol. Chem. **262,** 13021–13029 (1987).