MULTIPLE FUNCTIONS OF ß-CAROTENE IN PHOTOSYSTEM I

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1. INTRODUCTION

It is well known, that carotenoids serve as light-harvesting pigments and protect the photosynthetic apparatus against photooxidative damage. Within the thylakoid membranes of higher plants carotenoids show a distinct pattern in their distribution among the antenna and reaction centre complexes (1). This specific association of the different carotenoids is not well understood with respect to their function in photosynthesis.

In the present study we analysed the ß-carotene content of the PS I core complex (RC I) and the PS I antenna (LHC I). Photobleaching experiments with RC I in the same way as structural investigations of LHC I lead to the conclusion that ß-carotene has several functions in photosystem I to maintain the structural and functional integrity during photosynthesis.

2. MATERIALS AND METHODS

PS I-200 from spinach was fractionated by sucrose density gradient centrifugation by the procedure of HAWORTH et al. (2). Photobleaching of RC I was induced by a slide projector (660W/m²). Pigment analysis by reverse-phase HPLC and mildly dissociating SDS-PAGE was performed according to KNOETZEL et al. (3). Low temperature fluorescence spectra were obtained from an Aminco SPF-500 spectrofluorimeter.

3. RESULTS AND DISCUSSION

In Tab. 1 the pigment composition of PS I-200, RC I and LHC I is shown. In RC I the dominant carotenoid is ß-carotene, whereas the

TABLE 1. Pigment content of photosystem I (PS I-200), a core preparation (RC I) and the PS I antenna (LHC I) (mol/mol P700)

	PS I-200	RC I	LHC I*
neoxanthin	1	0	1
violaxanthin	8	1	7
lutein	12	(1)	12
chl b	27	5	23
chl a	176	72	69
ß-carotene	30	15	8
P700	1	1	-

*derived from relative pigment composition assuming lutein being localized exclusively in LHC I

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xanthophylls of PS I are located in the peripheral antenna complex.

Illumination of RC I under photobleaching conditions induces a rapid decrease of pigment content (4). After prolonged photodestruction a complex with a stable pigment composition of approximately 20 molecules chlorophyll a (chl a), 2 - 3 ß-carotene and 1 chl a' per reaction centre is found, indicating a close spatial and functional relationship between these pigments (Tab. 2).

TABLE 2. Molar pigment/P700 ratio in RC I during illumination with strong white light (660 W/m²). Chl a' : C10-epimer of chl a. (N_2) : N_2-atmosphere during illumination.

pigment	pigment/P700 ratio[a] (mol/mol)							
	illumination time (h)							
	0	2	4	7	10	13	4 (N_2)	13 (N_2)
neoxanthin	0.01	-	-	-	-	-	-	-
violaxanthin	0.86	0.46	0.43	0.46	0.94	2.39	0.60	0.92
lutein	0.83	0.49	0.48	0.42	0.77	1.90	0.63	0.41
chl b	4.77	0.62	0.31	0.19	0.06	traces	1.53	0.27
chl a	72.5	25.87	22.11	18.00	19.53	16.71	35.78	20.32
chl a'	2.15	0.74	0.85	1.01	1.54	-[b]	0.91	0.91
ß-carotene	14.99	4.79	3.22	2.09	2.85	4.61	7.45	2.92

[a] photochemical assay [b] chl a and chl a' not separated

P700 is the most stable pigment in RC I, its photodestruction starts after 4 hours of illumination. At that time only 20 % of the initial ß-carotene is present. Photobleaching under nitrogen atmosphere gives comparable results (Tab. 2). From these data we conclude, that only 2 - 3 molecules ß-carotene in photosystem I are necessary to protect P700 very efficiently from damage due to excess of light. The other 12 molecules ß-carotene in RC I (see Tab. 1) are involved in the (less effective) protection of the chl a core antenna, as we concluded from the concomitant loss of both pigments (4).

During the preparation of RC I and LHC I from PS I-200 a considerable amount of chl a (31 molecules) and ß-carotene (7 molecules) is selectively lost as free pigments, as can be deduced from Tab. 1. This specific loss of the most lipophilic pigments of the thylakoid membranes leads to the suggestion, that these pigments are involved in the binding of the peripheral antenna to reaction centre polypeptides and allow an efficient energy transfer from the peripheral antenna to the core antenna.

Mildly dissociating SDS-PAGE of the LHC I fraction recovered from the sucrose density gradient reveals 3 pigmented bands (Fig. 1).

The 77 K fluorescence emission spectrum of the upper band of the LHC I fraction in Fig. 1 shows the long-wavelength emission at 730 nm of the PS I antenna (Fig. 2 B). This pigment-protein complex is designated LHC I (730) and corresponds probably to LHC I-730 from BASSI and SIMPSON (5). The fluorescence excitation spectrum demonstrates efficient energy transfer from chl b (474 nm) and carotenoids (shoulder at 486 nm) to chl a (Fig. 2 A).

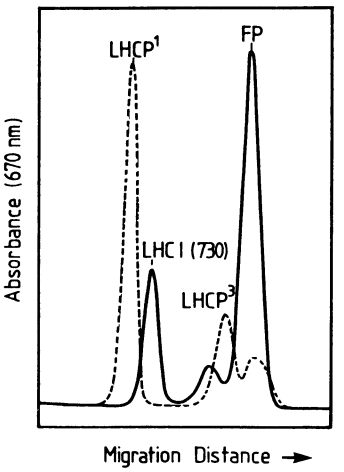

FIGURE 1. Mildly dissociating SDS-PAGE of the LHC I fraction (———) from the sucrose density gradient. For comparison, LHC II complexes (-----) are separated under identical conditions. FP = free pigments, $LHCP^1$ and $LHCP^3$ = oligomer and monomer of LHC II, respectively.

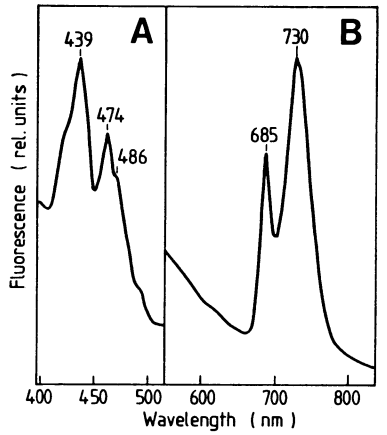

FIGURE 2. Fluorescence excitation (A) and emission spectrum (B) at 77 K of the upper band in Fig. 1 (LHC I (730)). Excitation at 439 nm, emission wavelength 730 nm.

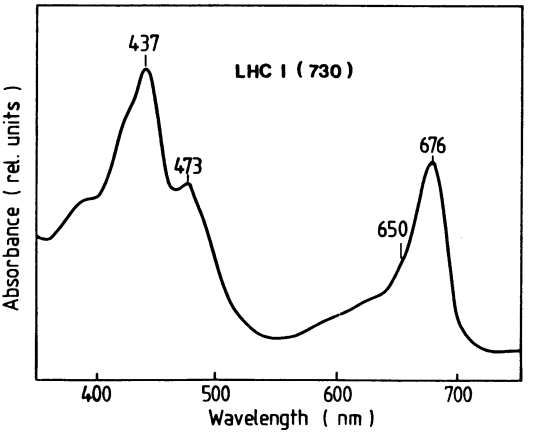

FIGURE 3. Room temperature absorbance spectrum of LHC I (730).

The absorbance spectrum of LHC I (730) is shown in Fig. 3. The maximun in the red is at 676 nm and the shoulder at 650 indicates the presence of chl b. The pigment analysis of LHC I (730) shows molar ratios of 0.5 for neoxanthin, 10.8 for violaxanthin, 17.3 for lutein, 40.5 for chl b and 2.4 for ß-carotene per 100 mol chl a. This pigment composition of LHC I (730) is different from the calculated pigment composition of LHC I in Tab. 1, probably due to the presence of a second pigment-

protein complex and the large amount of free pigments (40 - 60 %) after SDS-PAGE (Fig.1). In contrast to LHC II there is still some ß-carotene bound to LHC I (730), but most of this pigment is found in the free pigment zone, indicating the sensitivity of this pigment in the isolated PS I antenna against mild detergent treatment.

The intermediate band of the electrophoretic separation of LHC I in Fig. 1 is probably equivalent to LHC I-680 (5). This is confirmed by 77 K fluorescence emission at 680 nm and an absorbance maximum at 670 nm.

Taken together, ß-carotene displays three functions in LHC I. This carotenoid is associated with the long-wavelength chl a forms (6) which act as an energy sink in LHC I upon closure of the reaction centres (7). Recently we have shown that at least some of the ß-carotene molecules in the isolated LHC I are involved in photoprotection (4). Consequently we conclude that these ß-carotene molecules shield the energy valve system of the native PS I-200 complex from excessive excitation energy.

Additionally, beside the antenna function ß-carotene is an important structural element which enables the assembly of the PS I antenna system and connection to the core polypeptides.

Further investigations are necessary to answer the question whether all molecules of ß-carotene are involved in all three functions simultaneously, or whether each individual molecule fulfills a specific role.

4. REFERENCES

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