Evidence for the Existence of Two β-Carotene Pools and Two Biosynthetic β-Carotene Pathways in the Chloroplast

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Two β-Carotene pools were obtained in the chloroplast. It is concluded that one pool is very small like α-carotene and responsible exclusively for the biosynthesis of β-ionone-xanthophylls. The other β-carotene pool is proposed to be the one, that is located close to photosystem I and is involved in photosynthesis as a light protecting agent for chlorophylls against photo-oxidation. Furthermore evidence is given that both β-carotene pools are synthesized by independent biosynthetic pathways.

Introduction

The intraorganelle distribution of chlorophylls, carotenoids and quinones in the chloroplast is different. Chlorophylls are present exclusively in the thylakoids [1]. Besides chlorophylls, carotenoids and quinones are constituents of the photosynthetic membrane, but they are also present in the osmiophilic plastoglobuli [2, 3]. Furthermore investigations carried out by Douce reveal, that carotenoids (violaxanthin) are contained even in the chloroplast envelope [4, 5].

Fractionation studies of chloroplast lamellae have also obtained differences in the distribution of chlorophylls, carotenoids and quinones between photosystem I and II and the light-harvesting complex [6 – 10]. Whereas β-carotene and chlorophyll a are the major pigments of photosystem I-, chlorophyll a and lutein are present in large amounts in photosystem II-particles together with plastoquinone-9, α-tocopherol and lower concentrations of β-carotene, violaxanthin and neoxanthin. In contrast to photosystem I and II every carotenoid present in the chloroplast is contained in the light-harvesting complex together with chlorophyll a and b [10].

In this communication dual labelling experiments with [^{3}H]leucine/[^{14}C]acetate and [^{3}H]mevalonate/[^{14}C]acetate were achieved to obtain differences in the intraplastidic compartmentation of carotenoids in chloroplasts of green radish seedlings and *Chlorella pyrenoidosa.* The aim of these investigations was to indicate *in vivo* that β-carotene is contained in the chloroplast in two different pools which are probably synthesized by independent biosynthetic pathways.

Materials and Methods

Chlorella pyrenoidosa (211-8 l, algae collection Göttingen) was grown autotrophically for 7 – 10 days and then supplied with [4,5-^{3}H]leucine (500 µCi, specific activity 1.0 mCi/mmol) for a period of 18 h. During the final 40 min [2-^{14}C]acetate (200 µCi, specific activity 55 mCi/mmol) was also supplied. Thereafter the cells were washed free from exogenous labelled substrate and reincubated in the light in fresh nutrition medium. At different times after the beginning of the experiment *Chlorella* cells were harvested, carotenoids isolated, purified to a constant specific radioactivity and assayed for ^{14}C- and ^{3}H-radioactivity by liquid scintillation counting.

Radish seedlings grown for 6 days in light were incubated with [2-^{3}H]mevalonate (1 mCi, specific activity 100 – 500 mCi/mmol) together with [2-^{14}C]acetate (1 mCi, specific activity 40 – 60 mCi/mmol) for 24 h. Thereafter carotenoids were isolated, purified to a constant specific radioactivity and assayed for ^{14}C- and ^{3}H-radioactivity.

Results

Besides leucine, CO_2 , acetate and mevalonate are the most common precursors for carotenoid biosynthesis in algae and higher plants. The first specific reaction in terpenoid biosynthesis leading to the formation of carotenoids involves the biosynthesis of phytoene from geranylgeranylpyrophosphate [11].

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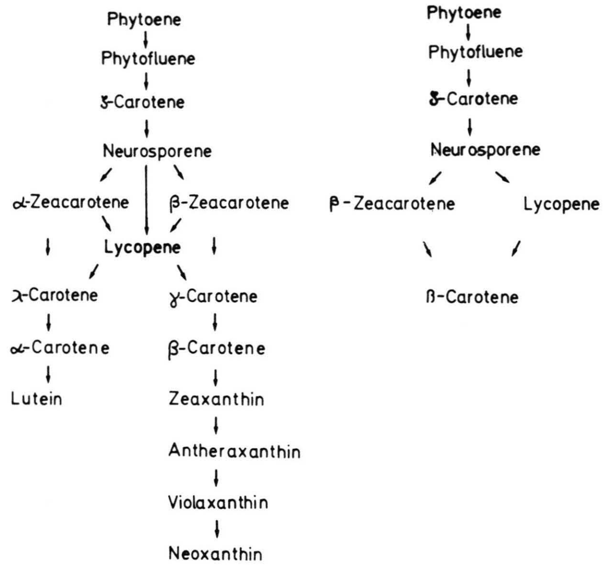

Fig. 1. General scheme for carotenoid biosynthesis [12] and the proposed independent β-carotene biosynthesis.

Phytoene then is desaturated *via* phytofluene, ζ-carotene and neurosporene to lycopene (Fig. 1). Lycopene finally cyclizes to λ- or γ-carotene. On the other hand the cyclization of neurosporene to β- and α-zeacarotene and finally to λ- or γ-carotene was proposed as an alternative reaction. λ-carotene cyclizes to α-carotene, whereas γ-carotene froms both α- and β-carotene. By introduction of oxygen α-carotene froms lutein, while β-carotene is the precursor of zeaxanthin.

It is generally believed that all carotenoids are synthesized in one biosynthetic pathway in the chloroplast stroma and thereafter attached to tne thylakoid membrane. If a carotenoid is synthesized via one biosynthetic pathway and is contained in the chloroplast in only one pool, then, in a pulse-chase-labelling experiment, the label in a carotenoid molecule that is derived from a terpenoid precursor like [^{3}H]leucine should follow the same kinetic as that of another *e. g.* [^{14}C]acetate resulting in a constant ^{3}H/^{14}C-ratio.

The ^{3}H- and ^{14}C-labelling kinetic of α- and β-carotene and their products lutein and zeaxanthin from *Chlorella pyrenoidosa* are shown in Fig. 2. After 18 h incubation time with [^{3}H]leucine all carotenoids were labelled by tritium. Mol specific radioactivity was higher in α-carotene than in lutein reflecting their precursor-product relationship. That lutein is synthesized directly from α-carotene can also be seen after the pulse chase when the ^{14}C- and ^{3}H-radioactivity is lost in α-carotene and at the same time increases in lutein. On the other hand zeaxanthin was labelled to a much higher extent by [^{3}H]leucine and [^{14}C]acetate than its precursor β-carotene. During the last 60 min of the experiment ^{14}C-radioactivity decreases in β-carotene and increases in zeaxanthin due to the transformation of [^{14}C-β]carotene into [^{14}C]zeaxanthin. ^{3}H-radioactivity, however, was not lost to the same extent but increased in β-carotene and zeaxanthin as well. The effect that the [^{14}C]label was lost in β-carotene to a greater extent than was ^{3}H-radioactivity, as well as the higher mol specific ^{3}H- and ^{14}C-radioactivity of zeaxanthin compared to β-carotene is in contrast to the previous assumption that a carotenoid is contained in the chloroplast in one pool. It can only be explained by the existence of two β-carotene pools. From this it is concluded, that one β-carotene pool is present in the chloroplast as a small biosynthetic pool like α-carotene. This pool has a higher mol specific radioactivity than zeaxanthin, whereas the other pool seems to be larger with a lower specific radioactivity.

Further evidence that β-carotene is contained in the chloroplast in two different pools was obtained by dual labelling experiments with [2-^{3}H]mevalonate and [2-^{14}C]acetate in chloroplasts of green radish seedlings. Besides this it was also investigated, whether these two β-carotene pools are synthesized in one or two independent biosynthetic pathways. It is known that carotenoid biosynthesis proceeds *via* geranylgeranylpyrophosphate and different acyclic carotene precursors like phytoene, phytofluene and lycopene that cyclizes to λ- and γ-carotene and finally to α- or β-carotene. If carotenoids are synthesized in one biosynthetic pathway, then [^{14}C]acetate and [^{3}H]mevalonate are incorporated in the acyclic carotenoid precursors and also in carotenes and xantho-

Table I. Mol-specific radioactivity of radish carotenoids after 24 hours incorporation with [2-^{14}C]acetate and [2-^{3}H]-mevalonic acid as precursors.

Carotenoid	[^{3}H]DPM/μmol	[^{14}C]DPM/μmol	^{3}H/^{14}C
lycopene	208×10^5	310×10^5	0.67
α-carotene	154×10^5	0	–
lutein	4×10^5	0	–
β-carotene	24×10^5	0.9×10^5	26.67
violaxanthin	6×10^5	0	–

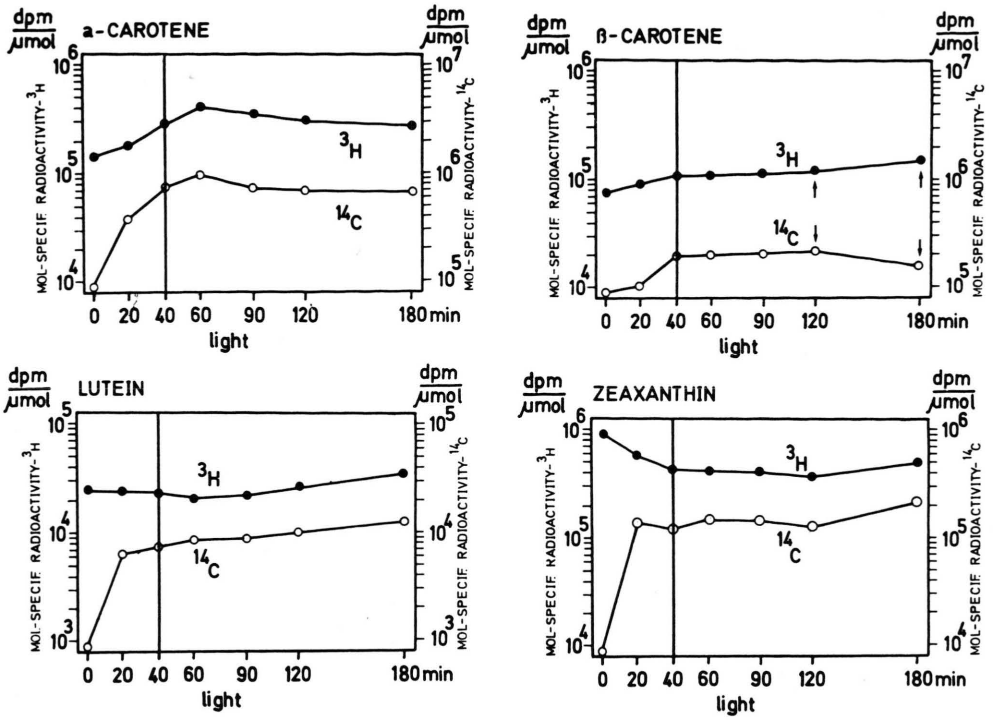

Fig. 2. Labelling kinetic of α- and β-carotene and their biosynthetic products lutein and zeaxanthin from *Chlorella pyrenoidosa* in a tracer kinetic experiment with [4,5-^{3}H]leucine and [2-^{14}C]acetate as precursors.

phylls after 24 h incubation time. As shown in Table I, [^{3}H]mevalonate and [^{14}C]acetate were incorporated in lycopene and β-carotene whereas α-carotene, lutein and violaxanthin were labeled only by [^{3}H]mevalonate. The highest mol specific radioactivity was obtained in lycopene and the lowest in lutein and violaxanthin. This is consistent with the first experiment.

However, the differences obtained in the label of lycopene and β-carotene compared to α-carotene, lutein and violaxanthin in this experiment are not conclusive with the fact that all carotenoids are synthesized in the chloroplast in one biosynthetic pathway. They rather support the existence of two β-carotene pools in the chloroplast and also the existence of two independent biosynthetic pathways for the formation of β-carotene. There seems to exist a small β-carotene pol that is responsible exclusively for the biosynthesis of β-ionone xanthophylls. Besides this pool a larger β-carotene pool is evident. This large β-carotene pool could be synthesized by an independent terpenoid pathway.

Discussion

Chlorophylls and carotenoids are involved in photosynthesis as light absorbing agents. Besides chlorophylls, β-carotene and lutein are the major chloroplast pigments [12]. Whereas the function of chlorophylls as light harvesting pigments in the reaction centers of photosystem I and II and the light-harvesting complex is known [10], only little information is present concerning the function of carotenoids. Carotenoids are involved in the activity of photosystem I and II [10, 12] but also in the xanthophyll cycle [13]. Particularly β-carotene is proposed to function as a light protecting agent for chlorophylls against photooxidation by deactivating chlorophyll triplets or singlet oxygen [14]. Furthermore it was pointed out, that a large β-carotene pool is associated with the antenna pigments of photosystem I [15].

The dual labelling experiments performed in this communication, concerning the analysis of the intraplastidic distribution of carotenoids are in agreement with previous investigations and reveal that β-carotene is contained in the chloroplast in different pools. It is concluded, that one β-carotene pool is as small as α-carotene. As a biosynthetic pool this β-carotene could be used for the biosynthesis of β-ionone-xanthophylls. Besides this biosynthetic pool another larger β-carotene pool is present in the chloroplast. This large pool could be the β-carotene pool that is located close to photosystem I and protects the chlorophylls against photo-oxidation [14, 15]. The total discrimination of [^{14}C]acetate compared to [^{3}H]mevalonate in all radish xanthophylls after 24 h of incubation implies that this large β-carotene pool of photosystem I is synthesized by an independent terpenoid pathway (Fig. 2). For these investigations represent only a preliminary result, further experiments concerning the intraplastidic distribution of carotenoids are in progress.

Acknowledgements

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[1] J. T. O. Kirk and R. A. E. Tilney-Bassett, The Plastids, 2nd ed., pp. 64 – 97, Elsevier, Biomedical Press, Oxford 1978.
[2] H. K. Lichtenthaler, Protoplasma **68,** 65 – 77 (1969).
[3] K. H. Grumbach and H. K. Lichtenthaler, Proc. 3rd Int. Congr. on Photosynth. (M. Avron, ed.), **Vol. I,** pp. 515 – 523, Elsevier, Amsterdam 1975.
[4] R. Douce, R. B. Holtz, and A. A. Benson, J. Biol. Chem. **248,** 7215 – 7222 (1975).
[5] R. Douce and J. Joyard, Advances in the Biochem. and Physiol. of Plant Lipids (L. A. Appelqvist and C. Liljenberg, eds.), pp. 79 – 89, Elsevier, Amsterdam 1978.
[6] L. P. Vernon, B. Ke, H. Mollenhauser, and E. R. Shaw, Progress in Photosynth. Res. (H. Metzner, ed.), **Vol. I,** pp. 137 – 148 IUBS, Tübingen 1969.
[7] H. K. Lichtenthaler and M. Tevini, Z. Pflanzenphysiol. **62,** 33 – 50 (1970).
[8] A. Radunz and G. H. Schmid, Z. Naturforsch. **28 c,** 36 – 44 (1973).
[9] L. H. Grimme, Ber. Deutsch. Bot. Ges. **87,** 509 – 514 (1974).
[10] J. P. Thornber, Ann. Rev. Plant. Physiol. **26,** 127 – 158 (1975).
[11] G. Britton, Chemistry and Biochemistry of Plant Pigments, 2nd (T. W. Goodwin, ed.), pp. 262 – 326, Academic Press, London 1976.
[12] G. F. W. Searle and J. S. C. Wessels, Biochim. Biophys. Acta **504,** 84 – 99 (1978).
[13] A. Hager, Ber. Deutsch. Bot. Ges. **88,** 27 – 44 (1975).
[14] H. T. Witt, Quart. Rev. Biophys. **4,** 365 – 477 (1971).
[15] W. Junge, H. Schaffernicht, and N. Nelson, Biochim. Biophys. Acta **462,** 73 – 85 (1977).