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BIOSYNTHESIS OF PLASTOQUINONE AND β-CAROTENE IN ISOLATED CHLOROPLASTS

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Abstract—Intact, isolated spinach chloroplasts incorporated ^{14}C from $^{14}CO_2$ into plastoquinone and β-carotene under photosynthetic conditions. Addition of unlabelled L-tyrosine, *p*-hydroxyphenylpyruvate, or homogentisate increased the incorporation of ^{14}C into plastoquinone, but decreased that into β-carotene.

INTRODUCTION

Using the method developed by Goodwin [1], it was elucidated that carotene [1] and the polyprenyl moiety of plastoquinone [2] are synthesized in the chloroplasts of higher plants, although carotene is also synthesized in chromoplasts [3–6]. From the work of Porter's laboratory it would be inferred that synthesis of α- and β-carotene via Δ^3-isopentenyl pyrophosphate, prephytoene pyrophosphate [3], (probably lycopersene [3]), 15-*cis* phytoene [4], 15-*cis* phytofluene, all-*trans* phytofluene [5], neurosporene, and lycopene [4] to δ- and γ-carotene [6] is performed by soluble enzymes of the plastids.

During their studies on the synthesis of the prenylquinones, plastoquinone and tocopherol, Whistance and Threlfall [7] found, that homogentisate is a precursor of the phenolic moiety. However, in the case of the polyprenyl moiety, a mixture of polyprenyls, formed by *Micrococcus lysodeicticus*, had to be added to effect its synthesis in chloroplast particles [8]. Isotopic studies, presented here, indicated that isolated intact chloroplasts are capable of complete synthesis of β-carotene and plastoquinone under photosynthetic conditions with $^{14}CO_2$. The rate of synthesis of plastoquinone was enhanced by addition of L-tyrosine, *p*-hydroxyphenylpyruvate, or homogentisate.

RESULTS

$^{14}CO_2$ was used as substrate for comparing the polyprenyl synthesis of plastoquinone and β-carotene but the phenolic substrate was not labelled. In spite of the long pathway starting from CO_2, a significant incorporation into both polyprenyls was detected in intact chloroplasts after treating them for only 30 min.

β-Carotene

After carrying out TLC of the petrol-soluble portion with system 1 [9], the carotene zone was saponified in order to eliminate contaminating lipids, especially triglycerides. Chromatography of the non-saponifiable portion was performed with TLC system 5 [10]. Carotenes (or precursors) other than β-carotene (light petroleum; λ_{max} 452; 476 nm) could not be identified in noticeable amounts by treatment with I_2 vapour, $SbCl_5$ or by observation in UV. The radioscans of the TLC showed only one maximum which can be correlated with the zone of β-carotene.

The results of the experiments, showing the incorporation of ^{14}C from $^{14}CO_2$ into polyprenyls of intact chloroplasts, are listed in Table 1. In order to synthesize polyprenyls, chloroplasts must be completely intact. In contrast, only negligible incorporation could be detected in experiments containing only a portion of *ca* 20% of intact chloroplasts. This may have been caused by dilution of the coenzymes and enzymes of photosynthetic carboxylation [11] and carotene synthesis [3–6] by the buffer solution when the chloroplasts were disrupted.

No detectable incorporation of ^{14}C from $^{14}CO_2$ into β-carotene could be obtained if the experiments were performed in darkness (Table 1). The effectiveness of illumination with low doses only (0.15×10^6 erg cm^{-2} sec^{-1} = 15% of the full dose) decreased it to 40% of the (light + homogentisate) value. The problem is to find out whether the synthesis of carotene is caused by a light-intensity-dependent production of substrate formed by photosynthesis, or by a photoinduction of carotene synthesis [4,5,12]. It might be due to photoinduction because low doses were sufficient for restoration of the reaction.

Maximum rate of incorporation of ^{14}C from $^{14}CO_2$ into β-carotene was obtained without adding phenolic substrate. An addition of *p*-hydroxyphenylpyruvate reduced the rate by half, whereas an addition of homogentisate of L-tyrosine decreased the rate by one third to one quarter.

Plastoquinone and plastoquinol

As plastoquinone forms a link in the electron transport carrier system of photosynthesis in chloroplasts, it is subjected to a current change between the reduced and the oxidized states [13]. The corresponding ratio of plastoquinone/plastoquinol is dependent on light conditions [13], substrate concentration etc. A ratio of plasto-

Table 1. Incorporation of ^{14}C from $^{14}CO_2$ into plastoquinone + plastoquinol and β-carotene of isolated spinach chloroplasts

Conditions	Plastoquinone + Plastoquinol† dpm/mg chlorophyll*	rel.		β-Carotene‡ dpm/mg chlorophyll*	rel.	
Dark + homogentisate	86	0,28	0,15	55	0,03	0,08
Light (low intensity) + homogentisate	318	1,02	0,55	291	0,14	0,40
Light + homogentisate	581	1,87	1,00	724	0,35	1,00
Light + L-tyrosine	364	1,17		423	0,21	
Light + *p*-hydroxyphenylpyruvate	1585	5,11		1232	0,59	
Light without phenolic substrate	310	1,00		2058	1,00	

* Chlorophyll (mg/expt). mean 2,9; min. 2,1; max. 3,77;

† plastoquinone + plastoquinol (nmol/mg chlorophyll). 11,5 7,1 17,9

‡ β-carotene (nmol/mg chlorophyll). 51.0 38.0 64.0

The values are averages of several determinations; each was standardized against a (light + homogentisate)-experiment, running at the same time.

quinone/plastoquinol of 1:1 was found under present conditions (white light; 1×10^6 erg cm^{-2} sec^{-1}; stopping the reaction by freezing (−20°) within 3 min). Plastoquinone predominated in experiments with low intensity of light.

The specific radioactivity of plastoquinone and plastoquinol was in the same range (plastoquinone 68.6 dpm/nmol; plastoquinol 46 dpm/nmol). This could be explained by the rapid change between the reduced and the oxidized states [13]. Probably the incorporation took place statistically over the total molecule; however, at the present time only incorporation into the prenyl moiety has been investigated by [14].

On comparing the ^{14}C-incorporation into plastoquinone and plastoquinol with that into β-carotene, it could be inferred that both have similar light dependencies. On adding homogentisate, the ratio of incorporation *without light:with low light intensities:with full light intensity* into plastoquinone + plastoquinol was 0.15:0.55:1.0, and that into β-carotene was 0.08:0.40:1.0. Thus in this case, light appears to have the same effect.

In isolated intact chloroplasts, the synthesis of plastoquinone was strongly dependent on availability of phenolic substrate. When chloroplasts were exposed to $^{14}CO_2$ under photosynthetic conditions, the ratio of incorporation of ^{14}C *without phenolic substrate:with* L-*tyrosine:with* p-*hydroxyphenylpyruvate:with homogentisate* into plastoquinone + plastoquinol was 1.0:1.2:5.1:1.9. According to [7,14], homogentisate is the immediate precursor of plastoquinone synthesis. On the other hand, in experiments presented here, a considerably higher rate of incorporation of ^{14}C could be found after the addition of *p*-hydroxyphenylpyruvate. Therefore, it is probable that a selective mechanism of transport of phenolic compounds across the chloroplast membrane exists (compare [15]).

α-Tocopherol

On the basis of the results of the preceding paper [9] it could be assumed that a non-plastidic synthesis not only of δ-, γ-, and β-tocopherol may occur, as proposed by [16], but also of α-tocopherol. Likewise in intact, isolated chloroplasts no incorporation of ^{14}C from $^{14}CO_2$ could be observed into α-tocopherol as well as into α-tocopherolquinone and α-tocopherolquinol. Because α-tocopherol is localized in chloroplasts [16] this implies the possibility that α-tocopherol compounds may be transported from the extraplastidic to the intraplastidic site.

DISCUSSION

It can be concluded that isolated chloroplasts are capable of total synthesis of β-carotene and the prenyl moiety of plastoquinone. This is in agreement with the former studies on total plants [1,2]. From these results it can be suggested that chloroplasts possess systems of acetate synthesis in addition to the enzyme systems of mevalonate and polyprenyl synthesis as demonstrated in the case of fatty acid synthesis [17,18]. It is difficult to speculate about the mechanism of acetate synthesis under photosynthetic conditions (compare [19]). The cooperation of chloroplasts and mitochondria in acetate synthesis, postulated by [20], and acetate synthesis via the glycollate pathway [21] may function as additional pathways in the intact cell.

The phenolic substrate added as L-tyrosine, *p*-hydroxyphenylpyruvate or homogentisate represented a critical factor in the synthesis of plastoquinone in isolated intact chloroplasts. It is unknown whether chloroplasts are capable of forming tyrosine or intermediate products to a sufficient extent, or if they are dependent on supply of tyrosine from the extraplastidic site. Probably, there are differences in the capacity of the shikimate pathway that are dependent on the stage of plant growth [22].

In sonicated chloroplast particles, Thomas and Threlfall [8] found predominately octa- and nonaprenyltoluquinol after addition of homogentisate, whereas plastoquinone and plastoquinol were the main products in intact chloroplasts of the experiment presented here. This must be attributed to the action of an enzyme system, which should have methylated the prenyltoluquinols in chloroplasts (compare [23]).

EXPERIMENTAL

Isolation of intact chloroplasts. Leaves of farm-grown spinach (*ca* 6 weeks old) were used for the isolation of chloroplasts. The preparation (0°) was performed as described in [11]. The percentage of intact chloroplasts was determined with the Hill-reaction [24] and amounted to 85–90%. The hexokinase test for adhering cytoplasm was negative.

Administration of $NaH^{14}CO_3$ and phenolic substrate. The chloroplast suspension (*ca* 3 mg chlorophyll in 1 ml medium B pH 6,7 [11] was pipetted into a cuvette ($d = 0.5$ cm; vol = 10 ml; $T = 20°$) with a mixture of 8.15 ml medium C pH 7.6 [11], 0.4 ml 200 mM $NaHCO_3$, 0.05 ml $NaH^{14}CO_3$ (= 100 μCi), 0.4 ml 25 mM *L*-tyrosine, *p*-hydroxyphenylpyruvate, or homogentisate (the latter dissolved in 0.5 mg ascorbic acid/ml [7]). The suspension was illuminated for 30 min with white light of an intensity of 1 × 10^6 erg cm^{-2} sec^{-1} using a Zeutschel monochromator system.

Extraction and purification of polyprenyl compounds. Extraction, TLC, and determination of plastoquinone, plastoquinol, α-tocopherol, α-tocopherolquinone and α-tocopherolquinol was performed as described in [9]. After oxidizing the plastoquinol zone, it was rechromatographed on Si gel with petrol–Et_2O 1:1 (system 1 [9]). The carotene zone developed with the same system was eluted with EtOH and saponified as described in [25]. The purified carotene was chromatographed with system 5 (Si gel petrol (40–60°) + 5% C_6H_6 [10] and determined by the spectral properties in petrol soln [26].

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REFERENCES

1. Goodwin, T. W. (1958) *Biochem. J.* **68**, 26P.
2. Griffiths, W. T., Threlfall, D. R. and Goodwin, T. W. (1964) *Biochem. J.* **90**, 40P.
3. Barnes, F. J., Qureshi, A. A., Semmler, E. J. and Porter, J. W. (1973) *J. Biol. Chem.* **248**, 2768.
4. Subbarayan, C., Kushwaha, S. C., Suzue, G. and Porter, J. W. (1970) *Arch. Biochem. Biophys.* **137**, 547.
5. Qureshi, A. A., Kim, M., Qureshi, N. and Porter, J. W. (1974) *Arch. Biochem. Biophys.* **162**, 108.
6. Kushwaha, S. C., Subbarayan, C., Beeler, D. A. and Porter, J. W. (1969) *J. Biol. Chem.* **244**, 3635.
7. Whistance, G. R. and Threlfall, D. R. (1970) *Biochem. J.* **117**, 593.
8. Thomas, G. and Threlfall, D. R. (1974) *Biochem. J.* **142**, 437.
9. Schultz, G., Huchzermeyer, Y., Reupke, B. and Bickel, H. (1976) *Phytochemistry* **15**, (in press).
10. Buggy, M. J., Britton, G. and Goodwin, T. W. (1969) *Biochem. J.* **114**, 641.
11. Jensen, R. G. and Bassham, J. A. (1966) *Proc. Natl. Acad. Sci. U.S.* **56**, 1095.
12. Rau, W. (1975) *Ber. Deut. Bot. Ges.* **88**, 45.
13. Witt, H. T. (1971) *Quart. Rev. Biophys.* **4**, 365.
14. Threlfall, D. R., Griffiths W. T. and Goodwin, T. W. (1967) *Biochem. J.* **103**, 831.
15. Walker, D. A. (1974) In: *Plant Biochemistry* (Northcote, D. H., ed.), *Intern. Rev. Sci., Biochemistry Series I*, Vol. 11, p. 1. Butterworths University Press, Kent.
16. Newton, R. P. and Pennock, J. F. (1971) *Phytochemistry* **10**, 2323.
17. Everson, R. G. and Gibbs, M. (1967) *Plant Physiol.* **72**, 1153.
18. Stumpf, P. K. and Boardman, N. K. (1970) *J. Biol. Chem.* **245**, 2579.
19. Bassham, J. A. (1965) In: *Plant Biochemistry* (Bonner, J. and Varner, J. E., eds.), p. 875. Academic Press, New York.
20. Kirk, J. T. O. (1970) *Ann. Rev. Plant Physiol.* **21**, 11.
21. Rogers, L. J., Shah, S. P. J. and Goodwin, T. W. (1968) *Photosynthetica* **2**, 184.
22. Dittrich, P. and Kandler, O. (1971) *Ber. Deut. Bot. Ges.* **84**, 465.
23. Whistance, G. R. and Threlfall, D. R. (1968) *Biochem. J.* **109**, 577.
24. Heber, U. and Santarius, K. A. (1970) *Z. Naturforsch.* **256**, 718.
25. Harborne, J. B. (1973) *Phytochemical Methods*, p. 122, Chapman & Hall, London.
26. Davies, B. H. (1965) In: *The Chemistry and Biochemistry of Plant Pigments* (Goodwin, T. W., ed.), p. 489, Academic Press, New York.