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Carotenogenic enzymes from *Capsicum* chromoplasts

Bilal Camara

Université Paris VI, Laboratoire de Régulations Métaboliques et Différenciation des Plastes, Tour 53, 4 Place Jussieu, 75230 Paris Cedex 05

Abstract- The biosynthesis of carotenoids in Capsicum annuum chromoplasts has been studied. Isolated chromoplasts incorporated isopentenyl pyrophosphate into carotenes, while xanthophylls were poorly labelled. These reactions are compartmentalized: the soluble fraction (stroma) alone is able to incorporate isopentenyl pyrophosphate into phytoene, while the following steps occur only in the membrane fraction.

Phytoene synthetase has been characterized from this system after polyethylene glycol fractionation and gel filtration. The enzyme is resolved on the calibrated column, with an apparent molecular weight 190,000 daltons. Isopentenyl pyrophosphate is incorporated at 5 nmol/mg protein/h. The apparent K_m for isopentenyl pyrophosphate is 12 μM. The optimal pH is 7.6. When isopentenyl pyrophosphate is used as a substrate, the synthesis of phytoene is inhibited by allylic pyrophosphates and inorganic pyrophosphates at 50 μM and 1 mM concentration.

Lycopene cyclase has been solubilized from this system. The soluble preparation converts lycopene into ß-carotene. Neither α-carotene nor γ-carotene is detected. Characterization with respect to cofactor requirements shows that NADP and FAD are not required. The maximum conversion occurs in Tris-maleate buffer at pH 6.8. Lycopene cyclase activity was drastically inhibited (90%) by the two newly synthesized amine derivatives, undecyldiethylamine and tridecyldiethylamine at 2 μM.

Capsicum chromoplast membranes convert epoxy-xanthophylls to keto-xanthophylls. Capsanthin and capsorubin are synthesized from antheraxanthin and violaxanthin. The 5,6-epoxy group is strictly required for this conversion.

INTRODUCTION

Important data on carotenoid biosynthesis in higher plants have been obtained by Dr. Porter's group (1). To date, we have focused on the biogenesis of carotenoids in isolated Capsicum chromoplasts. We have shown that this system incorporated isopentenyl pyrophosphate into carotenes, while xanthophylls were poorly labelled, even after prolongated incubation times (2-4). These reactions are compartmentalized: the soluble fraction (stroma) alone is able to incorporate isopentenyl pyrophosphate into phytoene, while the later steps occur in the membrane fraction (5&6). Similar results have been reported from Narcissus chromoplasts (7). Here we present additional results on the following points: 1) isolation and characterization of phytoene synthetase from Capsicum chromoplasts, 2) solubilization of lycopene cyclase from Capsicum chromoplasts, 3) conversion of epoxy-xanthophylls to keto-xanthophylls by Capsicum chromoplast membranes.

ISOLATION AND CHARACTERIZATION OF PHYTOENE SYNTHETASE

Phytoene synthetase complex has been characterized from tomato fruit after ammonium sulfate precipitation (8). A similar procedure was used to precipitate this complex from Flavobacterium (9). Recently, we have observed that polyethylene glycol stimulates the synthesis of phytoene (10). Due to this effect and to its potential use as a precipitating agent (11) we started our purification procedure with PEG 6000.

A solution (w/w) of PEG 6000 in 50 mM Tris- HCl pH 7.6 containing 1 mM ß- mercaptoethanol was added to a crude stromal fraction (2.5 ml of PEG solution per 10 ml of stromal solution). Four fractions termed $C_{2.5}$, C_5, $C_{7.5}$, C_{10} and a supernatant were collected. The different fractions were tested for phytoene and labile terpene alcohols formation, using isopentenyl pyrophosphate as a substrate (more details will be given elsewhere). The profile obtained showed that each fraction contained isopentenyl pyrophosphate isomerase, prenyltransferase and phytoene synthetase activities. Furthermore the specific activity increased up to the C_{10} fraction. On the other hand the activity in the supernatant was negligible. When subjected to gel filtration on Sephacryl-S 300, all active fractions contained isopentenyl pyrophosphate isomerase, prenyltransferase and phytoene synthetase activities.

After calibration of the Sephacryl column with marker proteins, the estimated native molecular weight was 190,000 daltons. The specific activity was 5 nmol/mg protein/h.The apparent K_m for isopentenyl pyrophosphate was 12µM, which compares favourably to the value observed for the tomato enzyme (8). When isopentenyl pyrophosphate was used as a substrate, a significant inhibition was observed after addition of allylic pyrophosphates or inorganic pyrophosphates to the incubation medium. At 50µM concentration the residual activity was 77% 32%, 24%, 23% in the presence of dimethylallyl pyrophosphate, geranyl pyrophosphate, farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Higher concentrations of inorganic pyrophosphate were required for inhibition ; at 1 mM concentration the residual activity for phytoene synthesis was 13%.

SOLUBILIZATION OF LYCOPENE CYCLASE

In previous studies it was demonstrated that disrupted Capsicum chromoplasts converted labelled lycopene into ß-carotene (3).As a preliminary step in the solubilization procedure, we treated the chromoplast membranes with Tween-80 and Triton-X100. The cyclase activity was severely inhibited in the presence of Triton-X100, while Tween 80 stimulated this activity (12). Subsequently, we precipitated the chromoplast membrane proteins with acetone. The precipitate was washed with ether, and finally extracted with potassium phosphate buffer. After centrifugation for 4 h at 100,000g, the supernatant was saved for enzymatic determination. This preparation was incubated with labelled lycopene emulsified with Tween-80. As shown in Fig. 1 lycopene cyclase activity was maintained.

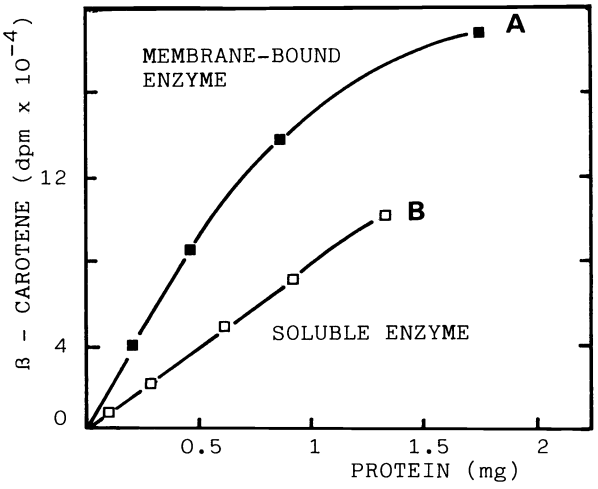


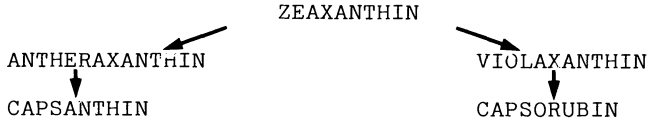
Fig. 1. Effect of undecyldiethylamine (A) and tridecyldiethylamine (B) on the cyclization of lycopene into ß-carotene.

Compared to similar results obtained with tomato enzymes (13), one could note that in Capsicum preparation, only ß-carotene is formed. This may be taken as evidence for the prevalence of the synthesis of carotenoids with a ß-ring actively synthesized during the ripening of Capsicum fruit. The maximal conversion of lycopene to ß-carotene occurred in Tris-maleate at pH 6.8. There was no strict requirement for NADP or FAD, as shown for tomato enzyme(1).

Due to the lipophilic nature of lycopene, we have synthesized several amphiphilic compounds and tested their effectiveness as in-vitro lycopene cyclase inhibitors. Two of them, undecyldiethylamine and tridecyldiethylamine, were incubated with the soluble lycopene cyclase described above. At 2µM concentration 90% inhibition was noted.

CONVERSION OF EPOXY-XANTHOPHYLLS INTO KETO-XANTHOPHYLLS

During the ripening of Capsicum fruits there is a massive accumulation of keto xanthopylls including capsanthin and capsorubin. Cholnoky et al.(14) proposed the first hypothesis for the biogenesis of these carotenoids as being in relation to the transport of photosynthetic oxygen by epoxy-xanthophylls. Later studies about the evolution pattern of the different carotenoids in different varieties of Capsicum fruits argued in favour of the key role played by epoxy-xanthophylls:antheraxanthin and violaxanthin (2, 15&16). Based on structural studies, a mechanism involving a pinacolic rearrangement of cyclohexenyl epoxides has been proposed (17). We have investigated these possibilities using Capsicum chromoplast membrane preparations (18-20). A summary of the results obtained are shown below.



Interestingly, when the 5,6-epoxy group is modified, the extent of conversion is drastically reduced.

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