Phytochemistry, Vol. 24, No. 11, pp. 2751–2752, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

INHIBITION OF LYCOPENE CYCLIZATION BY *CAPSICUM* CHROMOPLAST MEMBRANES BY 2-AZA-2,3-DIHYDROSQUALENE

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(*Received* 20 *March* 1985)

Key Word Index—*Capsicum annuum*; Solanaceae; chromoplast; lycopene cyclase; inhibitor.

Abstract—2-Aza-2,3-dihydrosqualene strongly inhibited lycopene cyclase from *Capsicum* chromoplast membranes.

INTRODUCTION

It has been demonstrated that 2-aza-2,3-dihydrosqualene is a potent inhibitor of 2,3-oxidosqualene cyclase from higher plants [1]. Unexpectedly, this compound induces an accumulation of lycopene in liquid suspension cultures of *Rubus fruticosus* [P. Benveniste, personal communication]. This phenomenon is also observed in liquid suspension cultures of *Nicotiana tabacum*, and, to a lesser extent, in green pericarp disks of pepper fruits pre-treated with tween 80 [unpublished observations].

It has been shown that lycopene is the substrate for the cyclase involved in β-carotene synthesis in *Lycopersicon esculentum* [2] and in *Capsicum annuum* chromoplasts [3]. Accordingly, we designed experiments to study the *in vitro* effect of 2-aza-2,3-dihydrosqualene on lycopene cyclization. In this paper, we show that 2-aza-2,3-dihydrosqualene inhibits lycopene cyclase from *Capsicum* chromoplast membranes.

RESULTS AND DISCUSSION

Capsicum chromoplast membranes were incubated with lycopene. At the end of the incubation procedure, a preliminary chromatography on silica gel separated the fraction containing bicyclic carotenes (α- and β-carotenes) from lycopene (system 1). Subsequently, this fraction was streaked on MgO-Kieselguhr [4] to separate α- and β-carotenes (system 2). The latter were rechromatographed on silica gel plates (system 3). At the completion of this procedure, α- and β-carotenes were radiochemically pure. HPLC [5] of the total carotene fraction is presented in Fig. 1. The radioactivity from lycopene was significantly incorporated into β-carotene. The conversion rate was about 150 000 dpm/hr/mg protein. No radioactivity was detected in α-carotene. This confirms the prevalence for the biosynthesis of carotenoids with a β ring during the ripening of *Capsicum* fruits [3]. Furthermore, the conversion was not performed by boiled chromoplast membranes.

2-Aza-2,3-dihydrosqualene emulsified with lycopene was investigated as an inhibitor of lycopene cyclase. The results obtained are shown in Table 1. They clearly demonstrate a marked inhibition of lycopene cyclase activity by 2-aza-2,3-dihydrosqualene. This data complements the results obtained *in vivo* with *Nicotiana tabacum* cell cultures grown for 4 weeks in the presence of 1.75 μg/ml of this compound. In the later case, lycopene was the major carotene detected (80%) followed by ζ- and γ-carotenes, while the xanthophyll and the chlorophyll fractions were qualitatively unaffected.

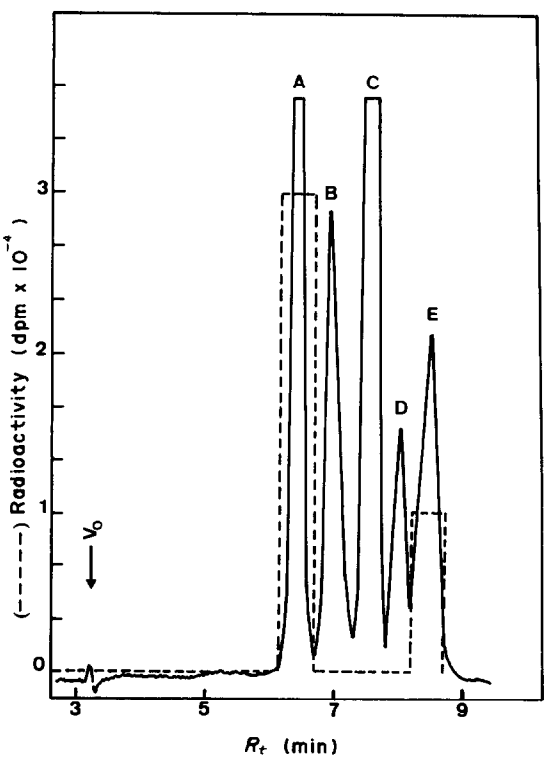

Fig. 1. Separation of labelled β-carotene from labelled lycopene by reverse phase HPLC. A, Lycopene; B, γ-carotene; C, ζ-carotene; D, α-carotene; E, β-carotene; Vo, void vol. After the addition of carrier amounts of authentic standards, the lipid extract was separated on a μC_{18} Bondapak column eluted with MeCN–EtOAc–$CHCl_3$ (7:2:1); flow rate: 1 ml/min; detection: 440 nm.

Table 1. Effect of 2-aza-2,3-dihydrosqualene on the cyclization of lycopene to β-carotene

2-Aza-2,3-dihydrosqualene (μM)	Radioactivity (dpm/mg protein)	
	β-carotene	Residual
0	150×10^3	—
1	26×10^3	17
2.5	21×10^3	14
5	6.8×10^3	5
10	3×10^3	2
20	1.5×10^3	1

*Residual activity $= \frac{\text{activity in the treated sample}}{\text{activity in the control}} \times 100$.

The exact mechanism involved in the inhibition of lycopene cyclase remains to be determined. Nevertheless, 2-aza-2,3-dihydrosqualene should be a useful tool for studies on carotenoid biosynthesis.

EXPERIMENTAL

Capsicum annuum L. chromoplast membranes, isolated as described previously [6], were incubated with labelled lycopene, generously given by Prof. Dr. Brubacher and Dr. Fricker from Hoffman LaRoche, in a medium containing (1 ml final vol.): 0.25 M sorbitol, 5 mM $MgCl_2$, 2 mM $MnCl_2$, 5 mM DTT, 2 μM FAD, 1 mM NADP, 5 mg Tween-80, 15,15'-[^{3}H]-lycopene (480 000 dpm, 160 Ci/mol), 50 mM Tris–maleate (pH 6.8) and chromoplast membranes equivalent to 0.5 mg protein. The incubation was performed at 25° for 1 hr. The reaction was terminated with 4 ml $CHCl_3$–MeOH (2:1). After the addition of 400 μg non-radioactive lycopene, ζ, γ, and α-carotenes, the lipid extract was subjected to TLC on silica gel G developed with petrol–Et_2O (99:1) (system 1). The bicyclic carotene fraction (α and β-carotenes) was streaked on MgO-Kieselguhr developed with C_6H_6–petrol (9:1) to resolve α- and β-carotenes (system 2). α and β-carotenes were further purified on silica gel developed with petrol (system 3). Additionally, the total carotene fraction was analysed by HPLC on μC_{18} Bondapak [5]. The incorporated radioactivity was determined in a liquid scintillation spectrometer. The proteins were determined according to the procedure of Bradford [7].

Acknowledgements—We thank Professor Cattel, Instituto di Chimica Farmaceutica Applicata, Turin, and Professor Benveniste, University of Strasbourg, for kind gift of 2-aza-2,3-dihydrosqualene and helpful advices. We are indebted to Mrs C. Agnès for liquid suspension cultures of *Nicotiana tabacum*.

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