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SHORT COMMUNICATION

β-IONONE AND ITS EFFECT ON THE INCORPORATION OF ^{14}C MEVALONATE INTO CAROTENES AND HIGH COUNTING FRACTIONS IN CARROT ROOT

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Abstract—β-Ionone vapours stimulate the incorporation of [2-^{14}C]mevalonic acid into carotenes and high counting non-saponifiable fractions in carrot root. Radioactive α- and β-carotene purified by thin-layer chromatography on MgO:Hiflo Super-Cel (1:2 by weight) with 10% $CaSO_4$ binder is contaminated with high counting impurities. Recrystallization to constant specific activity or preliminary thin-layer chromatography on Silica Gel G is necessary to obtain α- and β-carotenes free of these high counting impurities.

INTRODUCTION

REYES, Chichester and Nakayama[1] have reported that β-ionone stimulates general isoprenoid biosynthesis in *Phycomyces blakesleeanus*. We have found that carrot root slices incubated with [2-^{14}C]mevalonic acid in the presence of β-ionone vapours produce about 2½ times more carotenes and high counting fractions than the control without β-ionone. The existence of high counting fractions which appear with carotenes when tomato homogenates are incubated with [2-^{14}C]mevalonic acid has been reported.[2,3] These high counting fractions are non-saponifiable and non-carotene in nature. Mercer and Goodwin[4] have identified the high counting fractions from maize as lanosterol, β-amyrin and a Δ^7 stenol.

RESULTS AND DISCUSSION

Table 1 shows the effect of purified carotene fractions on specific radioactivity data. Carrot slices were incubated in the presence of [2-^{14}C]mevalonic acid, ATP, and phosphate buffer. After extraction, saponification and chromatography on MgO:Hiflo Super-Cel thin-layers, the specific activities of the individual bands were determined using scintillation counting techniques. α- and β-carotene samples were then crystallized to constant specific activity. It can be seen that after the first crystallization, a considerable amount of radioactivity was lost and that after two crystallizations, little activity was associated with the crystalline pigments. Specific activities of the carotenes fell sharply on crystallization; thus what initially appeared to be high specific activities actually turned out to be about 20–30 per cent of the initial values.

[1] P. REYES, C. O. CHICHESTER and T. O. M. NAKAYAMA, *Biochim. Biophys. Acta* **90**, 578 (1964).
[2] D. G. ANDERSON, D. W. NORGARD and J. W. PORTER, *Biochem. Biophys. Res. Commun.* **1**, 83 (1960).
[3] D. G. ANDERSON, D. W. NORGARD and J. W. PORTER, *Arch. Biochem. Biophys.* **88**, 68 (1960).
[4] E. I. MERCER and T. W. GOODWIN, *Biochem. J.* **88**, 46p (1963).

TABLE 1. INCORPORATION OF [2-^{14}C]MEVALONIC ACID INTO CAROTENES

Sample	Total activity (disintegrations/min)			Specific activity (disintegrations/min/mg pigment)		
	Initial	First crystals	Second crystals	Initial	First crystals	Second crystals
Phytofluene	290	—	—	48,136	—	—
α-Carotene	2,060	248	165	124,844	25,817	22,111
β-Carotene	2,877	724	486	59,710	21,759	19,544
ζ-Carotene	703	—	—	42,443	—	—

Phytofluene and ζ-carotene were not purified further.

Earlier work in our laboratory had shown that specific radioactivities of non-crystallized carotenes were meaningless. When radioactive phytoene was incubated with lyophilized carrot slices in the presence of ATP and phosphate buffer, the α- and β-carotene bands became highly labelled. However, upon recrystallization of the pigments, the specific activities fell to zero.[5]

The effect of β-ionone on carotenes and high counting fractions is shown in the following experiment. Two sets of carrot slices were incubated as above, one as a control, the other in the presence of β-ionone vapours. The work-up of the samples was identical to the above, mentioned experiment up to the chromatography step. This time the non-saponifiable fraction was first chromatographed on a Silica gel G thin-layer in an attempt to separate the carotenes from the high counting fractions. Table 2 shows the results of this chromatogram. The carotenes which ran at the solvent front contained the majority of the radioactivity. Table 2 also shows that β-ionone vapours increase the incorporation of radioactivity into all fractions by a factor of $2\frac{1}{2}$.

TABLE 2. THE DISTRIBUTION OF RADIOACTIVITY FOUND IN CAROTENE AND NON-CAROTENE BANDS AFTER CHROMATOGRAPHY ON SILICA GEL G

R_f values	Total activity (disintegrations/min)	
	Control	β-Ionone
1·0 (carotenes)*	25,983	111,042
0·80	2,016	4,990
0·60	1,372	2,929
0·45	5,078	11,169
0·30	5,921	10,768

The large number of counts found in the "0·80 fraction" contains some trailing carotenes.

* The radioactivity of the carotene band was determined by taking the sum of all the radioactivity found in the individual carotenes. See Table 3.

[5] H. YOKOYAMA and C. O. CHICHESTER, Unpublished observations.

The carotene band was then chromatographed on a Mgo:Hiflo Super-Cel thin-layer and the total radioactivity and specific activity of the carotene bands determined. The results are shown in Table 3. The fraction running ahead of the phytofluene band, designated as "front", contained the majority of the radioactivity. Again the presence of β-ionone vapours increased the incorporation of radioactivity into all fractions. Upon crystallization of α- and β-carotene, the specific activities drop but not to the same degree as in the first experiment. The preliminary silica gel G thin-layer chromatography then removed a substantial amount of the high counting impurities present in the non-saponifiable fraction of carrot root.

TABLE 3. TOTAL ACTIVITY AND SPECIFIC ACTIVITY OF CAROTENE FRACTIONS

	Total activity (disintegrations/min)				Specific activity (disintegrations/min/mg pigment)			
	Control		+ β-Ionone		Control		+ β-Ionone	
Sample	Initial	First crystals	Initial	First crystals	Initial	First crystals	Initial	First crystals
"Front"	24,546	—	97,930	—	—	—	—	—
Phytofluene*	115	—	571	—	11,853	—	29,453	—
α-Carotene	248	207	819	639	16,453	15,134	22,401	19,467
β-Carotene	1,010	707	2,749	1,780	21,180	16,045	24,993	17,453
ζ-Carotene*	57	—	107	—	6,225	—	7,915	—

* Phytofluene and ζ-carotene were not purified further.
"Front" was not separated into individual polyenes so no specific activities were calculated.

An alternative method for the purification of α- and β-carotene has been described by Goodwin and Williams.[6] The method utilizes two thin-layer systems, one with Kieselguhr, the other with alumina. This method is also effective in the removal of high counting non-saponifiable impurities.

From the above experiments, it is concluded that (1) β-ionone stimulates incorporation of [2-^{14}C]mevalonic acid into carotenes and high counting, non-saponifiable fractions in carrot root, and (2) one must use caution when interpreting radioactivity data from carotene biosynthetic studies.

EXPERIMENTAL

Incubation of carrot slices. Thin slices (1–2 mm thick) from the lower third of young Imperator variety carrot roots were incubated in a petri dish in the presence of [2-^{14}C] mevalonic acid, 6 μmoles containing $3{\cdot}0 \times 10^6$ disintegrations/min (sp. act. 0·31 μc/μmole); ATP, 1 μmole; and potassium phosphate buffer, pH 6·8, 0·5 m-mole in a total volume of 10 ml. After 24-hr incubation at room temperature the pigments were extracted.

In experiments where β-ionone vapours were used, the experimental petri dish contained a 1-cm square of filter paper saturated with β-ionone (Novoviol Beta, Fritzsche Brothers Inc., New York). The filter paper was placed on the under side of the lid of the petri dish.

Extraction and chromatography of pigments. The pigments were extracted with acetone in a blender and the acetone fraction transferred to light petroleum (b.p. 30–60°) with the aid

[6] T. W. GOODWIN and R. J. H. WILLIAMS, *Biochem. J.* 97, 28c (1965).

of water. After saponification for 15 min on the steam bath with 20% (w/v) methanolic KOH, the non-saponifiable fraction was washed free of alkali with water. The non-saponifiable fraction was then chromatographed on a 1-mm thin-layer of MgO:Hiflo Super-Cel (1:2 by weight) containing 10% $CaSO_4$. The thin-layer was activated at 130° for 2 hr immediately prior to use. Development was with 2% acetone in light petroleum in the saturation chamber described by Davies.[7]

Silica gel G chromatography. The non-saponifiable fraction was chromatographed on a 0·75 mm layer of silica gel G which had been previously activated at 120° for 45 min. Development was with toluene:ethyl acetate (9:1 v/v) in the saturation chamber. The orange carotene band ran at the solvent front and was removed. The remainder of the layer was partially covered with a glass plate and a small portion sprayed with chlorosulphonic acid spray to locate the colourless bands.[8] The unsprayed portion of the bands were removed and counted in the scintillation counter.

Determination of radioactivity and specific activity. Bands were scraped from the thin-layers and eluted with acetone. The eluates were then transferred to light petroleum and quantitated using extinction coefficients.[9] α- and β-carotene samples were crystallized to constant specific activity after addition of non-radioactive carrier. Synthetic α- and β-carotene were gifts from Dr. O. Isler, F. Hoffman-La Roche Ltd., Basle, Switzerland. The samples were then evaporated to dryness and counted in a Tri-Carb Liquid scintillation spectrometer according to Shneour *et al.*[10] Specific activities were expressed as disintegrations/min/mg pigment.

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[7] B. H. DAVIES, *J. Chromatog.* **10**, 518 (1963).
[8] E. STAHL and H. JORK, In *Thin-Layer Chromatography* (Edited by E. STAHL), p. 201. Academic Press, New York (1965).
[9] B. H. DAVIES, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), p. 529. Academic Press, New York (1965).
[10] E. A. SHNEOUR, S. ARONOFF and R. M. KIRK, *Intern. J. Appl. Radiation Isotopes* **13**, 623 (1962).