THE ENZYMATIC CONVERSION OF PHYTOENE TO PHYTOFLUENE*

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Received June 6, 1962

In a previous publication Anderson and Porter (1962) reported the incorporation of C^{14} of terpenol pyrophosphates into phytoene, phytofluene, ζ-carotene, neurosporene, lycopene, γ-carotene and β-carotene by isolated tomato plastids. This result suggested that tomato plastids might be able to convert phytoene to phytofluene (Porter and Anderson, 1962). Experiments were therefore conducted, and results are herein reported which prove that tomato plastids can indeed effect the conversion of phytoene to phytofluene.

The preparation of C^{14}-labeled terpenol pyrophosphates from mevalonic acid by a rat liver enzyme system was reported previously (Anderson and Porter, 1962). The conversion of terpenol pyrophosphates to phytoene by tomato plastids was also reported. In the present experiments phytoene was synthesized from mevalonic acid of a specific activity of 2.2×10^6 counts per minute per μmole. The biosynthetic phytoene was then purified through chromatography on alumina (Anderson, Norgard and Porter, 1960)

* This investigation was supported by research grants, A-1383 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U.S. Public Health Service, and G-61-50 from the Life Insurance Medical Research Fund.

and the specific radioactivity of phytoene in each fraction eluted from the column was determined via measurement of light absorption and radioactivity. Eluate fractions which agreed closely in specific radioactivity were pooled and an aliquot of the composited sample was hydrogenated catalytically (Anderson and Porter, 1962). The resultant solution of lycopersane was washed thoroughly with water, evaporated to dryness, dissolved in petroleum ether and subjected to gas-liquid chromatography (GLC) on SE-30 at 270°C. Effluents from the chromatograph were trapped on glass wool and then eluted with petroleum ether prior to a determination of radioactivity. The lycopersane peak was found to be coincident with 95% or more of the radioactivity in the effluent gas. The phytoene used as substrate was therefore almost completely free of contaminating radioactivity.

Attempts to convert phytoene to phytofluene were made with the following system. Five mg. of Tween 20 in 0.5 ml. of 0.1 M phosphate buffer, pH 7.0, were placed in a 10 ml. Erlenmeyer flask. C^{14}-labeled phytoene (5300 counts per minute of a specific activity of 500,000 counts per minute per mg.) in petroleum ether was added and the solvent was removed under a stream of nitrogen. The contents of the flask were homogenized for 30 seconds under nitrogen with a Vortex stirrer. Tomato plastids prepared from 70-80 g. of tomatoes were introduced in 1.0 ml. of 0.1 M phosphate buffer, pH 7.0 and then an addition of two μmoles of TPN was made. The final incubation mixture, 1.7 ml., was incubated under air or nitrogen at 25°C. for 16-18 hours with gentle shaking.

At the end of the incubation period carrier phytofluene was added, the mixture saponified and the carotenes extracted. Separation of phytoene and phytofluene was effected through chromatography on alumina. At least 50 ml. of eluate (5 tubes) were collected between the last tube which contained spectro

photometrically-measurable amounts of phytoene and the first tube which contained phytofluene (Figure 1).

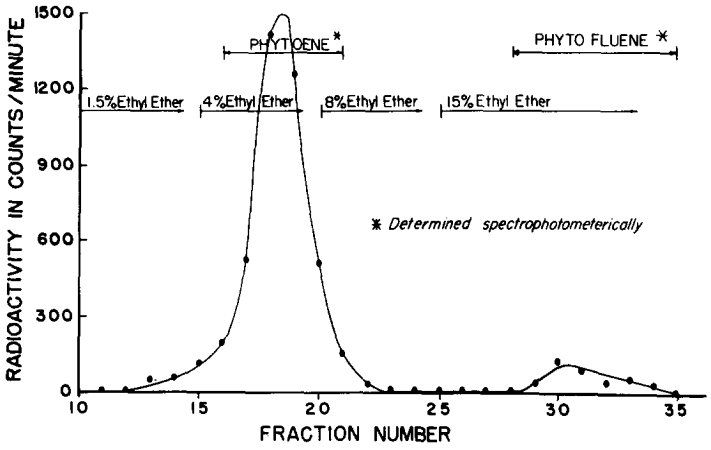

Figure 1. The Chromatographic Separation of Phytoene and Phytofluene.

The results of attempts to convert phytoene to phytofluene are summarized in Table 1. The experimental conditions are listed in column one, and the remaining columns report the quantity of radioactivity that was retained with each step of purification of the product. The values given are corrected for losses in aliquots which occurred in each purification step and for losses on GLC. The decline in values in the last column is primarily due to a slight lag in the collection of the effluent peak of lycopersane. Trapping of this trailing fraction followed by rechromatography on SE-30 has shown that this trailing material is lycopersane. Therefore, the actual values for C^{14} in lycopersane are somewhat higher than those reported in the last column.

The results of Table 1 provide proof of the conversion of phytoene to phytofluene by tomato plastids. Whether the phytoene is converted intact is as yet unproven, but the results of earlier studies (Anderson and Porter, 1962) suggest that such a conversion is probable. In addition, approximately 80% of the initial radioactivity was recovered in phytoene after incubation. The

TABLE I

INCORPORATION OF C^{14} OF PHYTOENE INTO PHYTOFLUENE

Incubation System[1]	C^{14} in Phytofluene		
	After Hydrogenation	After Chromatography[2]	After GLC
	cpm.	cpm.	cpm.
1. Complete	335	300	215
2. "	305	240	140
3. " , under N_2	330	230	130
4. " , under N_2	390	220	115
5. " , minus TPN	280	265	175
6. " , minus plastids	60	0	---

[1] The conditions of incubation are reported in the text.
[2] Lycopersane was eluted from a 1.8 x 7.0 cm column of alumina with 33 ml. of petroleum ether (30-60°C).

results of the present study might also suggest that oxygen is not required for the conversion of phytoene to phytofluene. However, this result should be viewed with caution, for the cofactor requirements have not been thoroughly studied. The failure of TPN to stimulate the formation of phytofluene may indicate either that adequate supplies of TPN are already present in the plastids, or that another hydrogen acceptor functions in the formation of phytofluene. Further studies are in progress on the mechanism and the requirements of this reaction.

Previous reports have provided some evidence for the interconversion of carotenes. Decker and Uehleke (1961) reported the conversion of labeled lycopene to β-carotene by green leaf plastids and the reverse reaction by tomato parenchymatous tissue. Evidence for the conversion of C^{14}-labeled

phytoene to δ-carotene by a cell-free extract of Staphyloccocus aureus was presented by Suzue (1961). These results when coupled with the results of this paper on the conversion of phytoene to phytofluene provide strong evidence for the sequential biosynthesis of carotenes (Porter and Anderson, 1962).

The technical assistance of Miss Marcia Morse is gratefully acknowledged.

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