PHOTOINDUCED PREPHYTOENE PYROPHOSPHATE SYNTHESIS IN A MYCOBACTERIUM SP[1]

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Summary: The formation of prephytoene pyrophosphate and the enzyme necessary for its synthesis are absent in a dark grown Mycobacterium sp. Photoinduction of carotene synthesis in this microorganism results in de novo synthesis of this carotene precursor and its synthetase. This enzymatic reaction is the first to be fully photoinduced in this organism.

Some non-photosynthetic microorganisms synthesize carotenes and carotenogenic enzymes in response to light (1). One of these photochromogenic bacteria, a Mycobacterium sp, synthesizes phytoene as the first detectable response to light (2). In a previous communication that described the isolation and characterization of the carotene precursor, prephytoene pyrophosphate[3], cell-free extracts of photoinduced Mycobacterium sp were used to synthesize this intermediate (3). These experiments established that the photoinduced bacteria could synthesize prephytoene pyrophosphate; however, uninduced cells were not tested for this capability at that time. In this communication we report that the synthesis of this carotene precursor and the enzyme necessary for its synthesis are absent in uninduced cells and that this synthetic capacity appears in response to light stimulation.

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[3] The same intermediate has been detected in the biosynthetic pathway to phytoene in extracts of tomato fruit (4,9). Qureshi, Barnes, and Porter found lycopersene to lie on the pathway between this precursor and phytoene; so they termed this precursor prelycopersene pyrophosphate (4). Since we have been unable to detect lycopersene as an intermediate in carotene synthesis in this bacteria (see Results), we feel constrained to retain the more general name, prephytoene pyrophosphate.

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METHODS AND MATERIALS

A Mycobacterium sp used previously was grown and harvested as before (2). Cells were washed with 0.05 M potassium phosphate buffer (pH 7.4) containing 1 mM $MgCl_2$ and were either used immediately or stored frozen for a short period of time.

Two grams of bacteria were suspended in 100 ml of the magnesium-containing phosphate buffer and treated in one of three ways. Photoinduced bacteria were prepared by exposing the bacterial suspension to 500 foot-candles of fluorescent light at 0° for 15 min. Induced-inhibited bacteria were treated in the same manner, but, after photoinduction, 10 mg of chloramphenicol was added to the suspension to inhibit protein synthesis. Uninduced bacteria were kept in the dark at all times. For preincubation, glucose (1% w/v) and ammonium sulfate (.5% w/v) were added to the suspensions which were then shaken for 2 hours at 30° in the dark.

The bacteria were collected by centrifugation, washed with 0.05 M potassium phosphate buffer (pH 7.4) containing 1 mM $MgCl_2$ and then suspended in equal volume of this buffer. Cells were broken in a French pressure cell at 15 to 20,000 psi. A small crystal of D Nase was added to the homogenate which was then incubated at 30° for 10 min. For experiments shown in Fig. 1, a 25,000 x g supernatant was used. Whole homogenates were used for the incubations for the synthesis of phytoene (Fig. 2) from geranylgeranyl pyrophosphate.

All-trans geranylgeranyl pyrophosphate, all-trans [^{3}H] geranylgeranyl pyrophosphate, and prephytoene pyrophosphate were prepared as before (3).

RESULTS

Extracts obtained from uninduced, induced, and induced-inhibited bacteria were incubated with [^{3}H] geranylgeranyl pyrophosphate. The incubation mixtures were extracted several times with n-butanol, which was then removed under a stream of nitrogen. The residue thus obtained was transferred to thin-layer plates of buffered silica gel H with a mixture of n-butanol,

benzene, and methanol, 1:1:1 (3). Chemically prepared geranylgeranyl pyrophosphate, geranylgeranyl monophosphate, and prephytoene pyrophosphate were used as non-radioactive carriers. After developing in a chloroform: methanol:water (50:50:10 v/v) system, the plates were scanned for radioactivity and the markers visualized by iodine staining. The thin-layer absorbant was divided into appropriate zones which were counted in a liquid scintillation spectrometer. This procedure was necessary since an enormous differential quenching was found on radioscanning of tritium-containing chromatograms. The data shown in Fig. 1 indicate that extracts of induced, uninhibited cells are capable of synthesizing prephytoene pyrophosphate, while extracts of uninduced or induced-inhibited (not shown) did not synthesize this compound.

The identity of prephytoene pyrophosphate was confirmed by elution of this zone and subsequent treatment of the eluate with $LiAlH_4$. The alcohol thus obtained cochromatographed on thin layer with authentic prephytoene alcohol (3).

In a similar set of experiments, using whole homogenates of bacteria, the incubation mixtures were extracted with butanol which was removed under a stream of nitrogen. Trans phytoene isolated from this Mycobacterium sp[4] and cis phytoene from a mutant (C_5) of Phycomyces blakesleeanus that accumulates phytoene (6) were added as carriers, and the mixture was chromatographed on a 1 x 13 cm column of 1% deactivated alumina. The cis and trans phytoenes were located by their absorbancy at 286 nM and the radioactivity was monitored by liquid scintillation spectrometry.

The results shown in Fig. 2 indicate that only induced bacteria synthesize phytoene and that in these bacteria the predominant isomer is trans. The peaks of radioactivity that eluted before the phytoene region were analyzed by gas-liquid chromatography using lycopersene as a standard. In all

[4] The trans nature of this phytoene will be the subject of another communication. Trans phytoene has been isolated from other bacteria (5).

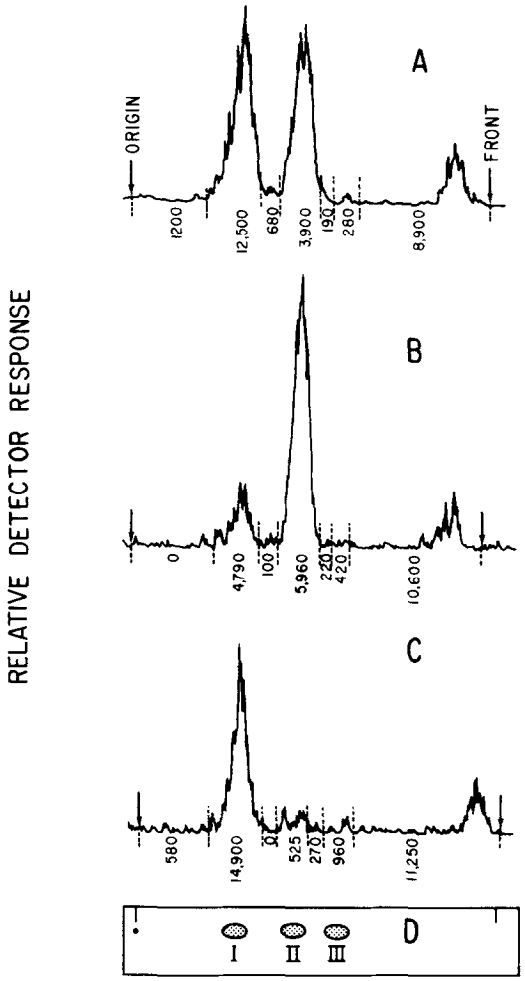

Fig. 1 Radiochromatogram scans of thin-layer chromatographs showing prephytoene pyrophosphate synthesis by photoinduced bacteria. Bacterial enzymes from either photoinduced or uninduced cells, $MgCl_2$ (1 mM), phosphate buffer (pH 7.4, 5 mM), and [^{3}H] geranylgeranyl pyrophosphate (1.3 nmoles 75 mc/mmole) were incubated in a final volume of 0.1 ml for 30 min. at 30°. Butanol extracts of the incubation mixtures were then chromatographed on buffered silica gel H plates.

A. Photoinduced, .8 mg protein.
B. Photoinduced, 2 mg protein.
C. Uninduced, 2.5 mg protein.
D. Reference compounds I, geranylgeranyl pyrophosphate; II, prephytoene pyrophosphate; III, geranylgeranyl monophosphate.

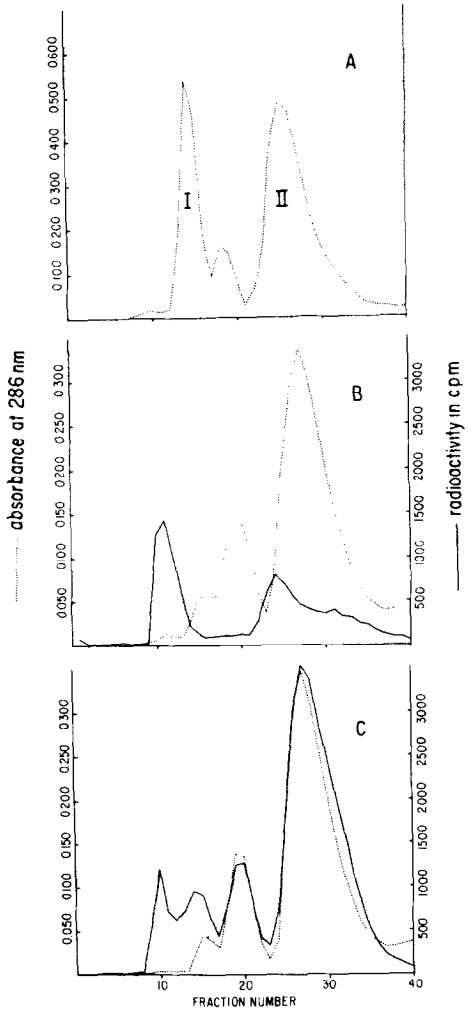

Fig. 2

A. Separation of cis(I) and trans(II) phytoene on a 1% deactivated alumina. A linear gradient of Skellysolve B to 9% diethyl ether in Skellysolve B was used for column elution. Five-milliliter fractions were collected.

B. Bacterial whole homogenates from uninduced cells (1.6 ml) were incubated with [^{3}H] geranylgeranyl pyrophosphate (16 nmoles) for 1-1/2 hr at 30° C. The incubation mixture was extracted three times with 2 ml of n-butanol. The n-butanol was evaporated under a stream of nitrogen. The residue was combined with trans phytoene previously isolated from photoinduced Mycobacterium sp and chromatographed on an alumina column.

C. The same procedure as described in B was followed except whole homogenates from photoinduced cells were used in the incubation.

cases the radioactivity emerged from the column much earlier than did lycopersene. It is probable that these compounds are elimination products of the substrate, geranylgeranyl pyrophosphate.

DISCUSSION

The data presented in this paper establish that the synthesis of the enzymes necessary for the production of both phytoene and prephytoene pyrophosphate by a Mycobacterium sp is photoinduced. In other experiments, we have shown that the enzyme necessary for the synthesis of geranylgeranyl pyrophosphate from farnesyl pyrophosphate and isopentenyl pyrophosphate is constitutive in this organism but that its concentration is enhanced by photoinduction (7). Thus, it is apparent that prephytoene pyrophosphate synthesis is the first fully photoinduced step in carotenogenesis in this bacteria. The location of the photoinduced step is not general for other photosensitive microorganisms since dark-grown Neurospora crassa synthesize phytoene and, after photoinduction, convert it to other carotenes (8).

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REFERENCES

1. Batra, P. P., in Photophysiology (A. C. Giese, ed.), Academic Press, New York (1971), Vol. 6, p. 47.
2. Rilling, H. C., Biochim. Biophys. Acta, 79, 464 (1964).
3. Altman, L. J., Ash, L., Kowerski, R. C., Epstein, W. W., Larsen, B. R., Rilling, H. C., Muscio, F., and Gregonis, D. E., J. Am. Chem. Soc., 94, 3257 (1972).
4. Qureshi, A. A., Barnes, F. J., and Porter, J. W., J. Biol. Chem. 247, 6730 (1972).
5. Weeks, O. B., in Aspects of Terpenoid Chemistry and Biochemistry (T.W. Goodwin, ed.), Academic Press, New York (1971), p. 301.
6. Meissner, G., and Delbrück, M., Plant Physiol., 43, 1279 (1968).
7. Johnson, J., and Rilling, H. C., Unpublished data.
8. Zalokar, M., Arch. Biochem. Biophys., 50, 71 (1954).
9. Barnes, F. J., Qureshi, A. A., Semmler, E. J., and Porter, J. W., J. Biol. Chem. 248, 2768 (1973).