Enzymic synthesis of bacterial phytoene from [2-^{14}C]mevalonic acid

A mutant of *Staphylococcus aureus*, which lacks the biosynthetic ability to produce bright-colored pigments, was separated and was found to accumulate the carotenoid phytoene[1]. The biosynthesis of phytoene by enzyme preparations from this mutant has been studied with [2-^{14}C]mevalonic acid.

The coccus was cultivated for 18 h at 34° in a nutrient broth containing 2% glycerol (pH 7.5) with continuous shaking. The resulting cells were collected by centrifugation at 4,000 rev./min for 10 min and were washed with 0.3% aq. NaCl.

After grinding the cells with alumina for 10 min under cooling, enzymes were extracted with 0.01 *M* Tris buffer (pH 8.0). The supernatant obtained after the removal of alumina and cell debris by centrifugation (5,000 rev./min for 10 min and 8,000 rev./min for 10 min) was used as the enzyme preparation for this study.

The incubation was carried out aerobically at 37° for 3 h. At the end of this period, 2 ml 10% ethanolic NaOH, 10 mg *p*-hydroquinone and 1.00 ml of a solution in petroleum ether of pure bacterial phytoene of absorbancy 8.500 at 286 mμ were added. The mixture was heated for 15 min in a water bath under N_2 with gentle reflux. After cooling, 2 ml water and 5 ml petroleum ether were added and after vigorous shaking the unsaponifiable matter was extracted into the petroleum ether layer. The petroleum ether extract was washed three times with water and dehydrated with anhyd. Na_2SO_4. The aliquot of the unsaponifiable fraction thus obtained was dried on alumina planchets and the radioactivity was counted as an infinitely thin sample in a gas-flow counter.

Separation of the carotenoid was carried out chromatographically on an activated-alumina column (150–200 mesh). The dry petroleum ether solution containing the unsaponifiable fraction was poured on the column of alumina (1 cm × 15 cm). After washing the column with petroleum ether, the carotenoid adsorbed was eluted with petroleum ether–ether (98:2). The ultraviolet-absorption spectrum of each 3 ml of the eluate was measured by a recording spectrophotometer. There was at least

TABLE I

EFFECT OF FLUORIDE, NICOTINAMIDE AND/OR EDTA UPON CONVERSION OF DL-[^{14}C]MEVALONIC ACID INTO UNSAPONIFIABLE FRACTION AND BACTERIAL PHYTOENE

Each system contained Tris buffer (pH 8.0), 40 μmoles; ATP, 30 μmoles; DPN, 2 mg; TPN, 0.5 mg; 6-phosphogluconate, 2 μmoles; enzyme solution, 0.3 ml (protein concentration, 52 mg/ml); DL-[2-^{14}C]mevalonic acid, 1.45 μg (10,000 counts/min); $MgCl_2$, 10 μmoles; $MnSO_4$, 4 μmoles, and (where indicated) EDTA, 7 μmoles, nicotinamide, 50 μmoles and/or KF, 30 μmoles, in a total volume of 0.6 ml. Incubation in air for 3 h at 37°.

Addition	*Unsaponifiable fraction (counts/min)*	*Bacterial phytoene (counts/min)*
None	2115	495
KF	800	435
KF, EDTA	125	115
KF, nicotinamide	1540	705
KF, nicotinamide, EDTA	125	30

Abbreviations: ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate.

90 % recovery of the phytoene. Samples (1.00 ml) of the two eluates containing the most phytoene were dried on alumina planchets and the radioactivity counted as before.

TABLE II

EFFECT OF COFACTORS ON THE BIOSYNTHESIS OF BACTERIAL PHYTOENE

Complete system contained Tris buffer (pH 8.0), 40 μmoles; ATP, 30 μmoles; DPN, 2 mg; TPN, 0.5 mg; 6-phosphogluconate, 2 μmoles; enzyme solution, 0.3 ml (protein concentration, 63 mg/ml); DL-[2-^{14}C]mevalonic acid, 1.45 μg (10,000 counts/min); $MgCl_2$, 10 μmoles; $MnSO_4$, 4 μmoles; nicotinamide, 50 μmoles; KF, 30 μmoles; and (where indicated) EDTA, 7 μmoles and bacterial nucleotide*, 10 mg in a total volume of 0.6 ml. Incubation in air at 37° for 3 h.

Cofactors	*(counts/min)*	
	Unsaponifiable fraction (counts/min)	*Bacterial phytoene (counts/min)*
Complete system	1433	1195
— ATP	262	149
— TPN	1895	1029
— TPN, 6-phosphogluconate	1098	935
— TPN, DPN	1044	711
— Mg, Mn + EDTA	454	83
+ Bacterial nucleotide	1892	1398

* Bacterial nucleotide was prepared from the boiled extract of cells by acetone precipitation and dried in a vacuum desiccator.

As shown in Table I the addition of nicotinamide favored the biosynthesis of bacterial phytoene. One reason may be the prevention of the breakdown of pyridine nucleotides[2]. The cofactor requirements are shown in Table II. ATP, TPNH and metals (Mg, Mn) are apparently essential as in the case of squalene[3–5]. There remains a possibility of the participation of other cofactor(s) in this system, judging from the stimulatory effect by the bacterial nucleotide.

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[1] G. SUZUE, *Arch. Biochem. Biophys.*, 88 (1960) 180.
[2] A. KORNBERG, AND O. LINDBERG, *J. Biol. Chem.*, 176 (1948) 665.
[3] B. H. AMDUR, H. RILLING, AND K. BLOCH, *J. Am. Chem. Soc.*, 79 (1957) 2646.
[4] F. LYNEN, *International Symposium on Enzyme Chemistry, Tokyo and Kyoto*, 1957, p. 57.
[5] G. POPJÁK, L. GOSSELIN, I. YOUHOTSKY-GORE, AND R. G. GOULD, *Biochem. J.*, 69 (1958) 238.

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