Glutaric Acid, a New Precursor of Biotin Biosynthesis

Sir:

Many workers^{1~6)} have reported that pimelic acid participates in the biosynthesis of biotin as an important precursor since the nutritive requirement of *Corynebacterium diphtheriae* for pimelic acid was found by du Vigneaud *et al.*⁷⁾ However, the study on the biosynthesis of biotin not *via* pimelic acid has not yet been reported.

In the course of investigation on the biosynthesis of biotin by microorganisms, we have found that the resting cells of some strains belonging to the genus *Agrobacterium* synthesized biotin-vitamins, mainly desthiobiotin, from glutaric acid, but not from pimelic acid. This communication briefly describes the facts that glutaric acid may be thought to be an essential precursor in the biosynthesis of biotin with *Agrobacterium*.

Bacteria used in this study were *Agrobacterium tumefaciens* IAM 1525, *A. radiobacter* IAM 1526 and *A. radiobacter* IAM 1527. These bacteria were cultivated at 28°C for 24 hr on a reciprocal shaker with the same medium as described previously.⁶⁾ The harvested cells were suspended in 0.85% sodium

chloride solution and then shaken at 28°C for 1 hr. After the starvation, the cells were harvested, washed twice and resuspended in the saline. The suspension was used in the experiment as resting cells preparation. The reaction was arrested by heating the mixture in a boiling water bath for 2 min. Biotin-vitamins synthesized in the reaction mixture were quantitatively determined by microbiological assays with *Saccharomyces cerevisiae*⁸⁾ and *Lactobacillus arabinosus*.⁹⁾ The bioautographic technique was also used to determine the sort of the vitamins.

TABLE I. EFFECT OF DICARBOXYLIC ACIDS ON THE BIOSYNTHESIS OF BIOTIN-VITAMINS BY *Agrobacterium* sp.

Addition	Biotin-vitamins synthesized (μ g/ml) by		
	<i>A. tumefaciens</i> IAM 1525	<i>A. radiobacter</i> IAM 1526	<i>A. radiobacter</i> IAM 1527
Oxalic acid	trace	trace	trace
Malonic acid	"	"	"
Glutaric acid	2.70	0.86	0.48
Adipic acid	trace	trace	trace
Pimelic acid	■	"	"
Suberic acid	"	■	"
Azelaic acid	"	"	"
Sebacic acid	"	"	"

Reaction mixture, containing about 80 mg (as dry matter) of resting cells, 2 mg of indicated compound and 200 μ moles of Tris-maleate buffer, pH 7.0, in a total volume of 1 ml, was aerobically incubated for 5 hr.

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2) K. Ogata, T. Tochikura, S. Iwahara, S. Takasawa, K. Ikushima, A. Nishimura and M. Kikuchi, *Agr. Biol. Chem.*, **29**, 889 (1965).

3) H. L. Elford and L. D. Wright, *Biochem. Biophys. Res. Commun.*, **10**, 373 (1963).

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7) V. du Vigneaud, K. Dittmer, E. Hague and B. Long, *Science*, **96**, 186 (1942).

8) E. E. Snell, R. E. Eakin and R. J. Williams, *J. Am. Chem. Soc.*, **62**, 175 (1940).

9) L. D. Wright and H. R. Skeggs, *Proc. Soc. Exptl. Biol. Med.*, **56**, 95 (1944).

As shown in Table I, glutaric acid was found to be essential for the biosynthesis of biotin-vitimers among dicarboxylic acids tested by all the three strains of *Agrobacterium*, while pimelic acid was not effective at all. Further investigation was carried out about the effect of amino acids and other compounds on the biosynthesis of biotin-vitimers. As shown in Table II, it was found that L-lysine was also

TABLE II. EFFECT OF AMINO ACIDS ON THE BIOSYNTHESIS OF BIOTIN-VITIMERS BY *Agrobacterium* sp.

Addition	Biotin-vitimers synthesized ($\mu\text{g/ml}$) by		
	<i>A. tumefaciens</i> IAM 1525	<i>A. radiobacter</i> IAM 1526	<i>A. radiobacter</i> IAM 1527
L-Alanine	trace	0.33	trace
L-Leucine	"	trace	"
L-Glutamic acid	"	0.34	"
L-Aspartic acid	"	trace	"
L-Lysine	2.70	0.56	0.48
L-Arginine	trace	trace	trace
L-Ornithine	"	"	"
L-Threonine	"	"	"
L-Cysteine	"	"	"
DL-Methionine	"	"	"

Reaction mixture was the same as described in Table I except that 2 mg of amino acid was added instead of dicarboxylic acid.

effective for the biosynthesis of the vitimers. L-Alanine and L-glutamic acid were somewhat effective for *A. radiobacter* IAM 1526, but these amino acids were scarcely effective for other two strains. Figure 1 showed that the amount of biotin-vitimers synthesized in the reaction mixture increased with the increased concentration of glutaric acid or L-lysine till the concentration of 3 to 4 mg per ml. The relation of the these compounds in the biosynthesis of biotin-vitimers may be possible to explain with a bacterial metabolic pathway in which

FIG. 1. Effect of Concentration on Glutaric Acid or L-Lysine-HCl on the Biosynthesis of Biotin-Vitimers by *A. tumefaciens* IAM 1525.

Reaction mixture, containing about 35 mg (as dry matter) of resting cells, indicated amounts of glutaric acid or L-lysine-HCl and 200 μmoles of Tris-maleate buffer, pH 7.0, in a total volume of 1 ml, was aerobically incubated for 5 hr.

○—○, glutaric acid was added; △—△, L-lysine-HCl was added.

L-lysine was degraded to glutaric acid. The main component of biotin-vitimers synthesized from glutaric acid or L-lysine by these *Agrobacterium* was characterized as desthiobiotin by the bioautography with *S. cerevisiae*. This was also confirmed from the observation that the vitimers could not be detected by microbiological assay with *L. arabinosus*.

The results described above show the presence of unique biosynthesis of biotin-vitimer from glutaric acid which has never so far been known, in *Agrobacterium* sp. The details of this work will be published in the near future.

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