Control Action of Actithiazic Acid on the Biosynthesis of Biotin-vitamers by Microorganisms

Sir:

Actithiazic acid, or acidomycin, (abbreviated as ACM), is an antibiotic which was independently isolated from the culture filtrate of *Streptomyces* sp. by several workers.\(^1\)\(^-\)\(^5\) This antibiotic exhibits the specific high activity against *Mycobacterium tuberculosis* *in vitro*, while is ineffective *in vivo* because of a probable antagonism with biotin present in the tissue.\(^6\) There have been some reports about the antitubercular and antibiotic activities, and the physicochemical properties\(^1\)\(^,\)\(^7\) of ACM. However, nothing has been known about the action of ACM on the pathway of biosynthesis of biotin-vitamers.

During the course of our study on the biosynthesis of biotin-vitamers by microorganisms, we have found that the accumulation of the vitamers was remarkably affected by an addition of ACM to the culture medium. That is, the amount of total biotin increased several to dozens-fold, while that of true biotin markedly decreased. The present communication reports the effect of ACM on the biosynthesis of biotin-vitamers using several microorganisms.

Bacteria, molds, yeasts and actinomycetes were cultivated at 28°C on a reciprocal shaker in a test tube contained 3 ml of the following media; for bacteria, peptone; 20 g, casamino acid; 5 g, FeSO₄·7H₂O; 0.01 g, MnSO₄·4·H₂O; 0.01 g, pimelic acid; 0.5 g in 1000 ml of tap water, pH 7.0: for molds, glucose; 50 g, casamino acid; 5 g, peptone; 5 g, KH₂PO₄; 1 g, MgSO₄·7H₂O; 0.5 g, MnSO₄·4·H₂O; 0.01 g, FeSO₄·7H₂O; 0.01 g, pimelic acid; 0.5 g in 1000 ml of tap water, pH 6.8: for yeasts, glucose; 50 g, peptone; 5 g, K₂HPO₄; 4 g, KH₂PO₄; 2 g, MgSO₄·7H₂O; 0.2 g, yeast extract; 2.5 g, pimelic acid; 0.5 g in 1000 ml of tap water, pH 6.5: for actinomycetes, glycerol; 20 g, peptone; 20 g, casamino acid; 5 g, NaCl; 1 g, MgSO₄·7H₂O; 0.5 g, K₂HPO₄; 0.5 g, FeSO₄·7H₂O; 0.01 g, MnSO₄·4·H₂O; 0.01 g, pimelic acid; 0.5 g in 1000 ml of tap water, pH 7.0. Actithiazic acid, which was kindly provided by Takeda Chemical Industries Ltd., was added to the medium as indicated in table and figures. The biotin-vitamers accumulated in the culture filtrate were quantitatively determined by microbiological assays with *Saccharomyces cerevisiae*\(^8\) (referred to as total biotin) and *Lactobacillus arabinosus*\(^9\) (referred to as true biotin). It was preliminarily confirmed that the assays were not affected.

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2) B. A. Sobin, *ibid.,* 74, 2947 (1952).
5) K. Ogata *et al.,* Presented at the 17th Meeting of the Western Provinces Branch of Japanese Antibiotic Research Association held in Hiroshima, Japan (1951).
TABLE I. EFFECT OF ACM ON BIOTIN-VITAMERS ACCUMULATION BY VARIOUS MICROORGANISMS

<table>
<thead>
<tr>
<th>Strains</th>
<th>ACM</th>
<th>Total biotin (µg/ml)</th>
<th>True biotin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>−a)</td>
<td>+b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−a)</td>
<td>+b)</td>
</tr>
<tr>
<td><em>Escherichia freundii</em></td>
<td>AKU 0011</td>
<td>0.3</td>
<td>22.0</td>
</tr>
<tr>
<td><em>Aerobacter cloacae</em></td>
<td>AKU 0024</td>
<td>0.5</td>
<td>34.0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>IFO 3009</td>
<td>5.3</td>
<td>112.0</td>
</tr>
<tr>
<td>′  sphaericus</td>
<td>IFO 3525</td>
<td>70.0</td>
<td>310.0</td>
</tr>
<tr>
<td><em>Rhizopus oryzae</em></td>
<td>IFO 4783</td>
<td>3.4</td>
<td>9.4</td>
</tr>
<tr>
<td><em>Aspergillus oryzae</em></td>
<td>AKU 3311</td>
<td>0.1</td>
<td>3.2</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>IFO 4626</td>
<td>0.7</td>
<td>3.5</td>
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<td><em>Rhodotorula rubra</em></td>
<td>IFO 0001</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>′  glutinis</td>
<td>IFO 0871</td>
<td>5.0</td>
<td>30.0</td>
</tr>
<tr>
<td><em>Streptomyces albus</em></td>
<td>AKU 2201</td>
<td>trace</td>
<td>0.7</td>
</tr>
<tr>
<td>′  fradiae</td>
<td>IFO 3123</td>
<td>1.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

The incubation was carried out at 28°C for 4 days.

* ACM was not added.
  b) ACM (200 µg/ml) was added to the medium.

![FIG. 1. Effect of ACM Concentration on Accumulation of Total Biotin by B. sphaericus.](image)

The incubation was carried out at 28°C for 4 days.

- O: Pimelic acid, 0 µg/ml
- x−−: 50 µg/ml
- △−−: 100 µg/ml
- ●−−: 500 µg/ml
- △−−: 1000 µg/ml

at all by ACM under the conditions in the present study.

Table I shows that, when 200 µg per ml of ACM was added to the medium, the amounts of total biotin in culture filtrate increased several to dozens-fold compared with that in the absence of ACM, while that of true biotin decreased to trace amount in all the cultures of microorganisms tested. As for *Bacillus sphaericus*, which was previously reported to have a high ability to accumulate biotin-
vitamers, mainly desthiobiotin\(^{10}\), its ability was far more enhanced by an addition of ACM. As shown in Fig. 1, the amount of total biotin accumulated by \textit{B. sphaericus} increased with the increased concentration of ACM in the presence of pimelic acid and reached the maximum amount of 350 \(\mu\text{g}\) per ml with the addition of 200 \(\mu\text{g}\) per ml of ACM and 500 \(\mu\text{g}\) per ml of pimelic acid. The time course of the accumulations of total biotin and true biotin was investigated in the presence or absence of ACM (Fig. 2). By an addition of ACM, the amount of total biotin increased along with the culture time, while that of true biotin was clearly suppressed.

The results described above suggest that ACM is not incorporated into the molecules of the biotin-vitamers, but it may competitively act at a certain site of biotin biosynthesis from desthiobiotin. Then the increase of total biotin and the decrease of true biotin were caused. Further detailed investigation on the mechanism of the action of ACM will be presented in the near future.

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Received October 8, 1970