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Review
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Biotin Biosynthesis in Microorganisms

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Studies on the biosynthesis of biotin and its regulation in microorganisms are reviewed. The pathway of biotin biosynthesis is as follows: pimelic acid→pimelyl-CoA→7-keto-8-aminopelargonic acid→7,8-diaminopelargonic acid→dethiobiotin→biotin. All the enzymes which were involved in the reactions from pimelic acid to dethiobiotin were elucidated and found to be of new types. Recent studies on the last step of the pathway are progressing with intact cells to give a clue to elucidation of the conversion mechanism from dethiobiotin to biotin. The biosynthesis of biotin was found to be regulated by biotin through feedback repression.

KEY WORDS: Biotin biosynthesis/ Pimelyl-CoA synthetase/ 7-Keto-8-aminopelargonic acid synthetase/ 7,8-Diaminopelargonic acid aminotransferase/ Dethiobiotin synthetase/ Feedback repression/

INTRODUCTION

Biotin, or vitamin H, can be synthesized by a great number of microorganisms, while some organisms which cannot synthesize biotin require it for growth. It is also well known that biotin widely distributes in plant and animal tissues. Main biochemical function of biotin is as the cofactor for a variety of enzymic reactions catalyzed by carboxylases, decarboxylases and transcarboxylases.[1-3] Consequently, the vitamin plays an important role in fatty acid metabolism *in vivo*. On the other hand, biotin plays a vital role in microbial production of amino acids, particularly in that of glutamic acid, as regulation factor. The action mechanism of biotin in glutamic acid production, which is closely related to the membrane permeability of the producing organisms toward glutamic acid, has been elucidated by extensive studies in a variety of ways.[4]

Studies on the biosynthesis of biotin in microorganisms started directly after the determination of its chemical sturcture[5] with studies on the nutritional requirements of microorganisms, contemporaneious with studies on its overall chemical synthesis.[6-9] Subsequently, through radiobiochemical and genetic studies, and more recently through enzymic studies, the biosynthetic pathway is at present thought to be from pimelic acid *via* dethiobiotin (DTB) as shown in Fig. 1. The present review covers the biosynthesis of biotin and its regulation in microorganisms. Concerning the microbial production and biodegradation of biotin, our recent review[10] is available.

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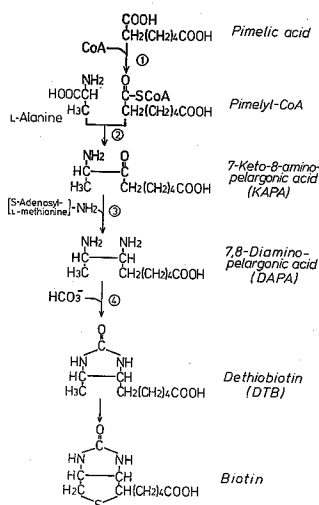
COOH
CH₂(CH₂)₄COOH   Pimelic acid
CoA— ①

NH₂   O
HOOCCH   C-SCoA
L-Alanine H₃C   CH₂(CH₂)₄COOH   Pimelyl-CoA
②

NH₂   O
HC——C
H₃C   CH₂(CH₂)₄COOH   7-Keto-8-amino-pelargonic acid (KAPA)
[S-Adenosyl-L-methionine]-NH₂ ③

NH₂   NH₂
HC——CH
H₃C   CH₂(CH₂)₄COOH   7,8-Diamino-pelargonic acid (DAPA)
HCO₃⁻ ④

O
C
HN   NH
HC——CH
H₃C   CH₂(CH₂)₄COOH   Dethiobiotin (DTB)

O
C
HN   NH
HC——CH
H₂C   CH(CH₂)₄COOH   Biotin
S

Fig. 1. Biosynthetic pathway of biotin in microorganisms.
①, pimelyl-CoA synthetase; ②, KAPA synthetase;
③, DAPA aminotransferase; ④, DTB synthetase.

PATHWAY OF BIOTIN BIOSYNTHESIS

1) Role of Pimelic Acid and Pelargonic Acid Dervatives as Precursors

Mueller[11,12] discovered that the growth of *Corynebacterium diphtheriae* is promoted by pimelic acid. Subsequently, du Vigneaud et al.[13] found that the growth of this organism was also promoted by the addition of biotin instead of pimelic acid, and predicted for the first time that pimelic acid is a precursor in the biosynthesis of biotin. Later, Wright et al.,[14-16] on finding that the biotin-vitamer accumulated in culture broth of *Aspergillus niger* increased on addition of pimelic acid, isolated this substance and identified it as biotin *l*-sulfoxide. Genghof[17] reported that a biotin-vitamer formed from pimelic acid by *Corynebacterium xerose* is DTB.

Using about 1,000 strains of molds, bacteria and actinomycetes, Ogata et al. investigated the accumulation of biotin-vitamers from pimelic acid and found the promoting effect of pimelic acid in a large part of strains (Table I).[18] "True biotin" in the table is the amount of biotin-vitamer given by the bioassay using *Lactobacillus plantarum*, and includes biotin and biotin sulfoxide. "Total biotin" is the amount of biotin-vitamer given by the bioassay using *Saccharomyces cerevisiae*, and includes DTB, 7-keto-8-aminopelargonic acid (KAPA) and 7,8-diaminopelargonic acid (DAPA) as well as biotin. As shown in Table I, on addition of pimelic acid to the medium of *Bacillus sphaericus*, 20 to 200 µg/ml of total biotin were accumulated. This is several hundred times more than has hitherto been reported in microorganisms.[19] The main component of the biotin-vitamers formed from pimelic acid was DTB, which was purified in a crystalline form from the culture broth.[20] Large quantities of DTB were accumulated from pimelic acid using even resting cells of this bacterium.[21] Finally, radioactive DTB was synthesized from ¹⁴C-labeled pimelic acid with the resting cells.[22] Eisenberg[23] and Elford and Wright[24] also

Table I. Accumulation of Biotin-vitamers by Various Microorganisms

Microorganism	Biotin-vitamers accumulated ($\mu g/ml$)			
	True biotin		Total biotin	
	a)*	b)*	a)*	b)*
Molds				
Mucor circinelloides	0.10	0.14	0.40	5.00
Rhizopus oryzae	0.16	0.26	0.19	1.50
Penicillium chrysogenum	0.24	0.40	0.35	0.80
Aspergillus nidulans	0.04	0.04	0.25	1.00
Unidentified strain H-38-S-2	0.24	0.24	0.68	1.80
" H-56	0.09	0.31	0.15	1.30
Bacteria				
Escherichia coli	trace	trace	trace	0.07
Aerobacter cloacae	trace	trace	trace	0.05
Alcaligenes faecalis	trace	trace	trace	0.40
Bacillus sphaericus	trace	trace	0.07	20-200
Sarcina lutea	trace	trace	trace	0.40
Pseudomonas fluorescens	trace	trace	trace	2.00
Bacillus sp. K-681-UV-134	trace	trace	0.04	20-50
Actinomycetes				
Streptomyces sp. A-1014-2	0.06	0.18	0.13	1.50
" A-1031-7	0.03	0.07	0.03	0.50

* a) Pimelic acid was not added.
   b) Pimelic acid (500 $\mu g/ml$) was added to the medium.

confirmed the incorporation of ^{14}C-labeled pimelic acid into biotin-vitamers during growth of molds.

Thus, it was determined that pimelic acid is a precursor of biotin. Janota-Bassalik and Wright,[25,26] Ohsugi et al.[27] and Ogata et al.[28] showed that azelaic acid, which has two carbon atoms more than pimelic acid, can serve as a precursor of biotin, probably because it can be converted to pimelic acid by β-oxidation.

Dittmer and du Vigneaud[29] found that DAPA shows one tenth of the activity of biotin toward *S. cervisiae*. From genetic analyses of *Aspergillus nidulans,* Pontecorvo[30] and Ryan[31] proposed the pathway pimelic acid→DAPA→DTB→biotin. In glutamic acid production using biotin auxotrophic bacteria, *Brevibacterium lacto-fermentum* and *Br. flavum,* Okumura et al.[32-36] found that DTB, DAPA, KAPA, 7-amino-8-ketopelargonic acid, 7,8-diketopelargonic acid, and also oleic acid, satisfied the biotin requirement. They strongly suggested that these compounds might be precursors in the biotin biosynthesis from the relation in the chemical structure. They also investigated the amount of each biotin-vitamer added which gave a maximum yield of glutamic acid, and the amount of intracellular biotin formed on incubation in the presence of each of these biotin-vitamers. Besides biotin and DTB, Iwahara et al. found and isolated another biotin-vitamer accumulated in the pimelic acid-supplemented culture broth of *Bacillus cereus,* and identified it as KAPA. Resting cells of *B. sphaericus* synthesized ^{14}C-labeled DTB from the KAPA which was

formed from ^{14}C-labeled pimelic acid by *B. cereus*.[38] From these facts, KAPA was deduced to be an intermediate in the biosynthesis of biotin. Eisenberg also isolated KAPA in crystalline form from the culture broth of *Phycomyces blakesleeanus*.[39,40]

The authors found that various bacteria and yeasts could converts DAPA to other biotin vitamers, which responded to *B. subtilis*.[41] They isolated them in crystalline form from culture filtrate of *Pseudomonas graveolens*, and identified them as DTB and its β-oxidation product, bisnordethiobiotin. This fact strongly supported the possibility of DAPA as a biotin intermediate.

Rolfe and Eisenberg[42] and Pai[43] obtained biotin auxotrophic mutants of *Escherichia coli*, with which they could confirm the proposed pathway of biotin biosynthesis to be pimelic acid→KAPA→DAPA→DTB→biotin by means of cross-feeding tests, the identification of biotin-vitamers accumulated and growth experiments with each vitamer.

(2) Enzymic Reactions

i) Pimelyl-CoA synthetase

The authors[44] showed for the first time the enzyme activity to form pimelyl-CoA from pimelic acid and CoA in microbial cell-free extracts by the coupling reaction of the KAPA synthetase system as mentioned below (Section II.(2).ii)) which forms KAPA from pimelyl-CoA and L-alanine through participation of pyridoxal 5′-phosphate (PLP). On investigation of the distribution of pimelyl-CoA synthetase activity in cell-free extracts of bacteria, high activities were detected in *Bacillus megaterium*, *Pseudomonas fluorescens*, *Micrococcus roseus* etc. With these cell-free extracts, this enzyme reaction was found to require ATP, and Mg^{2+} other than pimelic acid and CoA. The enzyme was purified from cell-free extract of *B. metaterium*. The purified pimelyl-CoA synthetase was characterized. *Km* values for the substrate and cofactors with respect to pimelic acid, CoA, ATP and Mg^{2+} were $2.4^{-4} \times 10^{-4}$M, 5.5×10^{-4}M, 1.5×10^{-3}M and 1.5×10^{-3}M, respectively. Opimum temperature was 32°C and optimum pH was 7.0. The pimelyl-CoA formed by this reaction was converted to its hydroxamate, and it was identical with the hydroxamate of synthetic pimelyl-CoA on paper chromatography. Metal chelating agents like ethylenediamine tetraacetic acid (EDTA), *o*-phenanthroline and α,α′-dipyridyl, and ferric ion markedly inhibited this enzyme reaction. Mn^{2+} and ADP could be substituted for Mg^{2+} and ATP, respectively.

ii) KAPA synthetase

Eisenberg and Star,[46] using crude cell-free extract from a biotin auxotrophic mutant of *E. coli*, reported that KAPA was synthesized from pimelyl-CoA and L-alanine through participation of PLP. This was the first report to elucidate the biosynthetic pathway of biotin in cell-free system.

The authors[47,48] purified the KAPA synthesizing enzyme from the cell-free extract of *B. sphaericus*, a DTB producing bacterium shown in Table I. Using the purified enzyme, it was found that L-alanine was only amino acid condensed with pimelyl-CoA. This enzyme reaction required PLP as coenzyme, and was strongly

inhibited by various carbonyl reagents which are inhibitors peculiar to vitamin B6 enzymes. The reaction suffered strong competitive inhibition by a few amino acids including L-cysteine, the most inhibitory, D-alanine, L-serine, glycine, and D- and L-histidine. The authors also detected this enzyme activity in cell-free extracts of many bacteria among about 100 strains tested.[49]

Thus, KAPA synthetase, which catalyzes the condensation reaction possibly accompanying decarboxylation to form a ketoamino acid from acyl-CoA and amino acid with PLP as coenzyme, should be a new type of enzyme like δ-aminolevulinic acid synthetase, and was named pimelyl-CoA:L-alanine 2-C-pimelyltransferase or L-alanine pimelyltransferase (EC 2.3.1.47).

iii) DAPA aminotransferase

Pai,[50] using cell-free extract from *E. coli*, found that DAPA was formed from KAPA, and pointed out the existence of a DAPA aminotransferase. He reported that methionine and PLP were amino donor and coenzyme, respectively, in this enzyme reaction. Later, Eisenberg and Stoner[51] also demonstrated this enzyme activity in *E. coli*. The subsequent investigation of amino donors showed that L-methionine was effective in the reactino with resting cells, but required the addition of ATP and Mg^{2+} with the cell-free extract. They found that *S*-adenosyl-L-methionine (SAM) could substitute as the amino donor instead of L-methionine, ATP and Mg^{2+}.

The authors[52] investigated the activity of this enzyme in the cell-free extracts of about 100 strains of bacteria preserved in our laboratory. The method of measurement of the activity was to convert the DAPA formed to DTB by the coupling reaction of DTB synthetase, and to determine DTB by bioassay with *B. subtilis*. The DTB synthetase used was the partially purified enzyme preparation from *Pseudomonas graveolens* described below (Section II.(2). iv)). The authors found *Brevibacterium divaricatum*, *Salmonella typhimurium*, *Bacillus roseus*, *Micrococcus roseus*, and *E. coli* to have the high activity. From the cell-free extract of *Br. divaricatum*, which showed the highest activity, the enzyme was purified about 5,000-fold. The purified enzyme preparation gave a single band on disc gel electrophoresis. This enzyme was found to be a typical PLP enzyme having absorption maxima in the region of 320 nm and 410 nm as well as at 280 nm. The enzyme was confirmed to be a new aminotransferase showing specificity only for SAM as amino donor. As amino acceptor, 7-amino-8-ketopelargonic acid, an isomer of KAPA, had only one-hundredth of the activity of KAPA. Pyridoxamine 5'-phosphate (PMP), as well as PLP, could act as coenzyme. Km values were 0.69×10^{-4} M for KAPA, 0.55×10^{-3} M for SAM, and 0.83×10^{-6} M for PLP. It would be interesting that KAPA shows strong substrate inhibition in concentration higher than 0.1 mM, suggesting that this might serve as a control mechanism.

From the isolation of the reaction product, the reaction mechanism was proposed as shown in Fig. 2, that is, the expected keto product of SAM, *S*-adenosyl-2-oxo-4-methylthiobutyric acid, seems to be nonenzymically decomposed to yield 5'-methylthioadenosine and 2-oxo-3-butenoic acid.[53]

7-keto-8-amino-   7,8-diamino-
pelargonic acid   pelargonic acid

PMP-enzyme   PLP-enzyme

S-adenosyl-2-oxo-4-   S-adenosyl-
methylthiobutyric acid L-methionine

nonenzymic

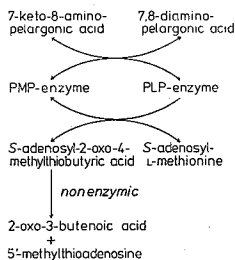2-oxo-3-butenoic acid
+
5'-methylthioadenosine

Fig. 2. Proposed mechanism for DAPA aminotransferase reaction.

(iv) DTB synthetase

Eisenberg and Krell[54] found that DTB was formed from DAPA by resting cells of *E. coli*.   This reaction was accelerated 2- to 3-fold by addition of L-serine, $NaHCO_3$, and glucose.   Subsequently, Eisenberg and Krell,[55] Pai[56] and Cheeseman and Pai,[57] using *E. coli*, and the authors,[58] using *Pseudomonas graveolens*, observed the formation of DTB from DAPA by cell-free extracts, and clarified that HCO_3^-, ATP and Mg^{2+} as well as DAPA are necessary for this enzyme reaction.

Krell and Eisenberg[59] purified the enzyme (DTB synthetase or ureido ring synthetase) about 200-fold from the cell-free extract of *E. coli* and obtained an enzyme preparation of over 90 % purity.   The enzyme has a molecular weight of 42,000 and is composed of two subunits.   The authors[58,60] also purified the enzyme about 2,000-fold from the cell-free extract of *P. graveolens*, and obtained an enzyme preparation showing a nearly symmetric peak upon ultracentrifugation.   The sedimentation coefficient $(s_{20,w})$ was 3.496×10^{-3} cm/sec.   The optimum pH was 7.0–8.0 and the optimum temperature was almost 50°C.   The activity of biotin diaminocarboxylic acid, a compound lacking the ureido part of biotin, as substrate for this enzyme was about one tenth that of DAPA, in which case biotin was formed as a product.[58,60] CO_2 had higher activity than HCO_3^-.[59]   Of metal ions tested, Mn^{2+} showed activity of 95–136 % and Fe^{2+} 71–91 % against Mg^{2+}.[60]   CTP, UTP, GTP and ITP showed 10–20 % of the activity of ATP.[61]   The enzyme reaction was strongly inhibited by chelating agents, such as EDTA, α,α'-dipyridyl and *o*-phenanthroline.
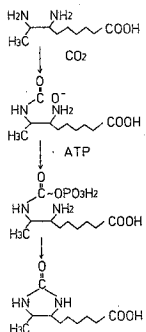
Fig. 3. Proposed mechanism for DTB synthetase reaction.

Moreover, it was established that ADP shows competitive inhibition toward ATP[59,61] and that the substrates DAPA and BDC are competitive with each other.[61]

Investigation of stoichiometry of the enzyme reaction proved that equimolar amounts of DTB and ADP were formed.[59,61] Based on this observation, a reaction mechanism for DTB synthetase was proposed as shown in Fig. 3. Thus, DTB synthetase is a new kind of carboxylase (EC 6.3.3.3) because it catalyzes carboxylation accompanying the formation of ureido ring.

v) Biotin synthesizing reaction

Since early studies of biotin biosynthesis, it has been recognized that DTB is converted to biotin during growth of various microorganisms.[62-67] Using resting cells, biotin biosynthesis from DTB was demonstrated with some bacteria[64,68-70] and yeasts.[71-74] There have been no studies on enzymic synthesis of biotin from DTB. However, there have been some reports on potential sulfur donor for biotin synthesis using resting cells of yeasts. In addition, interesting studies has been recently published on the conversion mechanism of DTB to biotin and on a possible intermediate.

In the investigation of sulfur sources for biotin biosynthesis from DTB, using resting cells of *Saccharomyces cervisiae*, Niimura *et al.*[72] found methionine sulfoxide and methionine to be most effective, and secondly that Na_2SO_3, Na_2S, Na_2SO_4, homocysteine, SAM and methylmercaptan were also effective. They used ^{35}S-methionine and detected the reaction products by radioautography. Radioactive biotin, biocytin and biocytin sulfoxide were formed. The authors[74] also tested the effect of various sulfur compounds using resting cells of *Rhodotorula glutinis* which forms appreciable amounts of biotin from DTB. In the presence of DTB, this yeast formed hardly biotin on addition of inorganic sulfates and sulfites or L-cysteine etc., but formed considerable amounts of biotin on addition of DTB and methionine and in particular the L-form of methionine. Next, after reaction using ^{35}S-L-methionine, the authors isolated radioactive biotin by cation and anion exchange column chromatographies, avidin treatment and dialysis, and identified it by radiochromatography and radioautography. As a result, it was confirmed that sulfur contained in 1 molecule of L-methionine was incorporated into 1 molecule of biotin.

Li *et al.*[75] carried out experiments with *Aspergillus niger* on the incoroporation of carbonyl-^{14}C-DTB and carboxyl-^{14}C-DTB, which were also labeled randomly with ^3H, into biotin to measure a change of the number of hydrogen atoms during the conversion of DTB to biotin. The ratio of $^3H/^{14}C$ for DTB and for the biotin formed showed that the ^3H radioactivity of biotin was 15 to 20 % lower than that of DTB. Thus, they considered DTB might be converted to biotin with the loss of 3 of 4 hydrogen atoms.

More recently, Parry and Kunitani[76] have developed a new stereospecific synthesis of DTB and reexamined the mechanism of the conversion of DTB to biotin by using specifically labeled ^3H-DTB. The samples of tritiated DTB synthesized were each mixed with *dl*-[10-^{14}C]-DTB and the doubly labeled precurosors were then administered to cultures of *A. niger*. After incubatfon, the biotin synthesized from

each doubly labeled precursor was isolated as *d*-biotin sulfone, and converted to biotion sulfone methyl ester. The methyl esters were purified by chromatography and then recrystallized to give constant activity and constant ratio of $^3H/^{14}C$. From the results shown in Table II, it appears that the introduction of sulfur at C-1 and

Table II. Incorporation of Specifically Tritiated Dethiobiotin into Biotin[a]
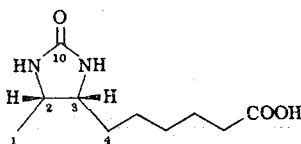
Expt. no.	Precursor	$^3H/C^{14}$ for precursor	$^3H/C^{14}$ for biotin sulfone methyl ester	Percent ^3H retention
1	*dl*-2, 3-H; 10-^{14}C-DTB[b]	6.05	5.74	95
2	*dl*-[3^3-H; 10-^{14}C]-DTB[c]	2.89	3.04	105
3	*dl*-[1^3-H; 10-^{14}C]-DTB	6.88	4.81	70
4	*dl*-[4(*RS*)-^{3}H;10-^{14}C]-DTB	5.88	3.10	53

a) From Parr3 and Kunitani[76]
b) Precursor had 58W ^{3}H at C-2,
c) 42% at C-3. Precursor had 17% ^{3}H at C-2, 83% at C-3.

C-4 of DTB takes place without the loss of hydrogen from C-2 or C-3, suggesting that unsaturation is not introduced at C-2 or C-3 like type (ii) in Fig. 4 during the biosynthesis of biotin from DTB. However, they consider that the possibility of enzymic removal of hydrogen from C-2 or C-3 followed by replacement of the hydrogen without exchange cannot be excluded. Moreover the result that the incoporation of *dl*-[1-^{3}H]-DTB into biotin proceeds with 30 % tritium loss is consistent with the removal of one hydrogen atom from the methyl group of DTB. The result that *dl*-[4-(*RS*)-^{3}H]-DTB is incorporated into biotin with 47 % tritium loss suggests that the stereospecific removal of one hydrogen atom from C-4 of DTB may occur during the formation of biotin. Guillerm *et al.*[77] also proved that there is no loss of tritium in C-2 position during the conversion in *E. coli*. Thus, these results clearly demonstrate that two hydrogen atoms are removed from *d*-DTB during its conversion to *d*-biotin.

The next step in elucidating the mechanism of the biosynthetic conversion of DTB to biotin should be to investigate how the methyl group at C-1 and the methylene group at C-4 of DTB are converted and determine the order of functionalization of C-1 and C-4 in DTB during its conversion to biotin. Frappier *et al.*[78] have recently shown that the three DTB derivatives hydroxylated at position 1, 4 or 1 and 4, are not intermediates from the growth test and transport experiments with a biotin auxotrophic mutant of *E. coli*.

All these observations strongly support a saturated intermediate of type (ii), but not an unsaturated one (i) as shown in Fig. 4. Recently, Salib *et al.*[79] have isolated a possible intermediate between DTB and biotin from the incubation medium
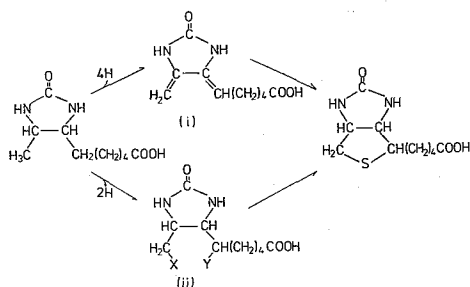
Fig. 4. Hypothetic intermediates between DTB and biotin.

of resting cells of a biotin auxotrophic mutant of *E. coli*. The compound contains sulfur, and it promoted the growth of *E. coli* strains which are blocked between DTB and biotin. The conversion of the labeled intermediate into biotin by growing cells of the *E. coli* was also established. However, complete purification and structure determination of the compound have not yet been established.

REGULATION OF BIOTIN BIOSYNTHESIS

Pai and Lichstein[80,81] cultured *E. coli* in medium supplemented with various concentrations of biotin, and measured the biotin-vitamer activity in the medium toward *S. cervisiae*. In contrast to the constant intracellular content, the amount of biotin-vitamers formed extracellularly decreased remarkably with biotin added. This inhibition of biotin-vitamer biosynthesis was specific for biotin, hardly occurring with DTB, oxybiotin and biocytin. Using resting cells of this organism, they demonstrated that this inhibition by biotin was not feedback inhibition but repression.[82] Moreover, biotin showed repression even toward the biosynthesis of biotin from DTB,[83] and this repressive action of biotin was observed in several strains of bacteria.[84] Iwahara[85] also found the accumulation of biotin-vitamers to be almost completely inhibited by the addtion of biotin to the culture medium of various bacteria, whereas this inhibitory action was not observed at all with fungal species. Resting cells of *B. sphaericus*, a DTB producing bacterium, which were harvested from medium not supplemented with biotin were able to biosynthesize DTB from pimelic acid. While the biosynthetic ability in cells obtained from medium supplemented with biotin was extremely feeble. These facts also bore out the suggestion of Pai *et al.* that inhibitory action of biotin depends on repression.

Recently, the regulation mechanism of the biosynthetic pathway of biotin is also being elucidated at the enzymic level. Eisenberg and Krell[55] have reported that KAPA synthetase and DTB synthetase of *E. coli* are almost completely repressed by the addition of 1 ng/ml of biotin to the medium. Pai observed similar repression of DTB synthetase[57] and DAPA aminotransferase[50] of *E. coli* by biotin. The authors[49] found KAPA synthetase of *B. sphaericus* and *B. subtilis* was repressed by biotin. As shown in Fig. 5(A), the authors demonstrated that KAPA synthetase of *B. sphaericus* and DAPA aminotransferase of *Br. divaricatum* were repressed by the ad-
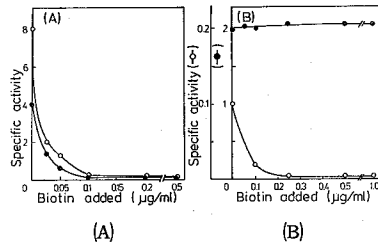
(A)   (B)

Fig. 5. Effect of biotin on the synthesis of biotin biosynthetic enzymes.
(A), KAPA synthetase of *Bacillus sphaericus* (●) and DAPA amino-
transferase of *Brevibacterium divaricatum* (○); (B), pimelyl-CoA
synthetase (●) and DTB synthetase (○) of *Bacillus megaterium*.

dition of 0.1 μg/ml of biotin to the medium.[49,52] Furthermore, the authors found
that, in contrast to the complete repression of DTB synthetase of *B. megaterium* by
0.25 μg/ml of biotin, as can be seen in Fig. 5(B), pimelyl-CoA synthetase was not
repressed even by the addition of 1 μg/ml of biotin.[44]

In this way, a strong repressive action of biotin has been demonstrated on all
the enzymes between pimelyl-CoA and DTB and on part of the boisynthetic system
between DTB and biotin (Fig. 6). This repression is thought to be the main reason
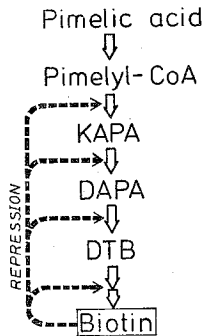

Fig. 6. Regulation of biotin biosynthesis *via* feedback
repression by biotin.

for the minute amounts of biotin produced by a large number of microorganisms.

During the studies on regulation of biotin biosynthesis, the authors[86,87] found
the controlling action on biotin biosynthesis of actithiazic acid (ACM), an antibiotic
for tuberculosis which is an antagonist of biotin and resembles biotin in chemical
structure as shown in Fig. 7. On incubation with pimelic acid, a large number of
yeasts, molds, bacteria and actinomycetes formed remarkably increased amounts of
DTB when ACM was added to the medium. Conversely, the amount of biotin
formed dropped considerably when ACM was added. In the case of *B. sphaericus*,
the amount of DTB formed increased about 5-fold by addition of 200 μg/ml of ACM,
and reached a maximum of 350 μg/ml (Fig. 7). As a result of investigation of the
action of ACM on the biosynthesis of biotin, it is thought that ACM suppresses for-
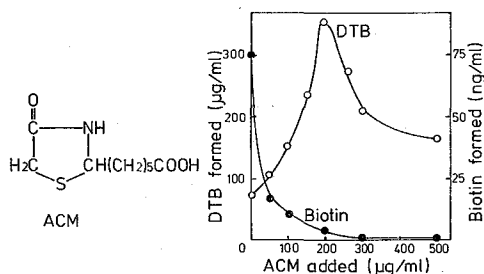mation of biotin by inhibition of a part of the biosynthetic system of biotin form

Fig. 7. Structure of actithiazic acid (ACM) and its effect on the accumulation of biotin-vitamers by *Bacillus sphaericus*.

DTB, resulting in releasing the repression by biotin of the biosynthetic system of DTB from pimelic acid. Eisenberg[88] also investigated the action of ACM using resting cells of *E. coli* and confirmed that ACM showed competitive inhibition on the biosynthetic system of biotin from DTB. Further, he found the enzyme activities of both DAPA aminotransferease and DTB synthetase to be considerably higher from cells cultured in ACM-supplemented medium.

It was thought that, if mutants which did not suffer this repression by biotin could be obtained, large amount of biotin-vitamers could be accumulated. Pai[89] derived a derepressed mutant of *E. coli* of which the amount of biotin formed was 1,000-fold (16 ng/ml) that of the wild strain. The activity of each enzyme in the biotin biosynthetic system was also 3–20 times that of the parent strain, and no repression by biotin was observed.

CONCLUDING REMARKS

The complete picture of the enzyme system of biotin biosynthesis particularly in the pathway from pimelic acid to DTB has been built up despite of the fact that a large number of microorganisms synthesize biotin in merely minute amounts. All the enzymes involved in the pathway have been made clear to be of new types. We believe the information which has been obtained through such investigations will also contribute to a field of enzyme chemistry.

In addition, studies on the final step, that is, the synthesis of biotin from DTB, are now progressing to elucidate a sulfur containing intermediate and conversion mechanism. This elucidation may provide a valuable clue to the accumulation of large quantities of biotin, which is recently attracting the interest relating to a food and fodder supplement of biotin.

So far, there has been no microorganism reported of which all the enzyme activities involved in DTB synthesis from pimelic acid are detected. Recently, the authors[90] has been able to detect for the first time all the enzyme activities in *B. sphaericus*. This bacterium has also been found to have a considerably high ability to synthesize biotin from DTB in resting cell system.

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