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Biotin biosynthesis

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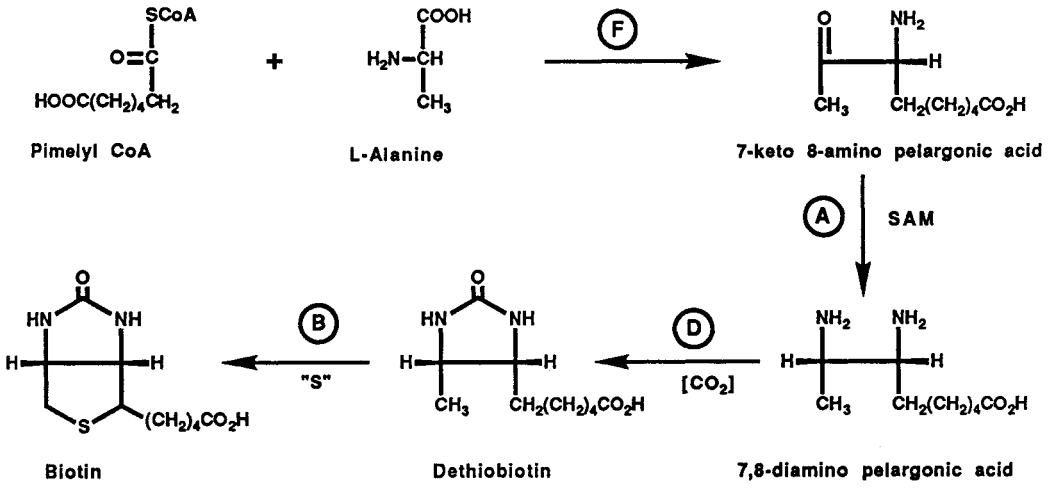
Abstract. Biotin is biosynthesized in four steps from pimeloyl CoA. KAPA synthase which catalyzes the condensation fo pimeloyl CoA with alanine has been purified, kinetically characterized and its mechanism is discussed. The insertion of sulfur into dethiobiotin could be studied only *in vivo*. Experiments with different precursors indicate that the first step is very likely the introduction of sulfur on the methyl group, followed by cyclization.

Biotin, as the coenzyme of carboxylases which carry out essential metabolic reactions, is an important molecule and its biosynthesis has been studied since 1937, soon after its discovery in several microorganisms (1). The main features of the biosynthetic pathway have been established (Scheme I), but many problems remain unsolved.

Scheme I

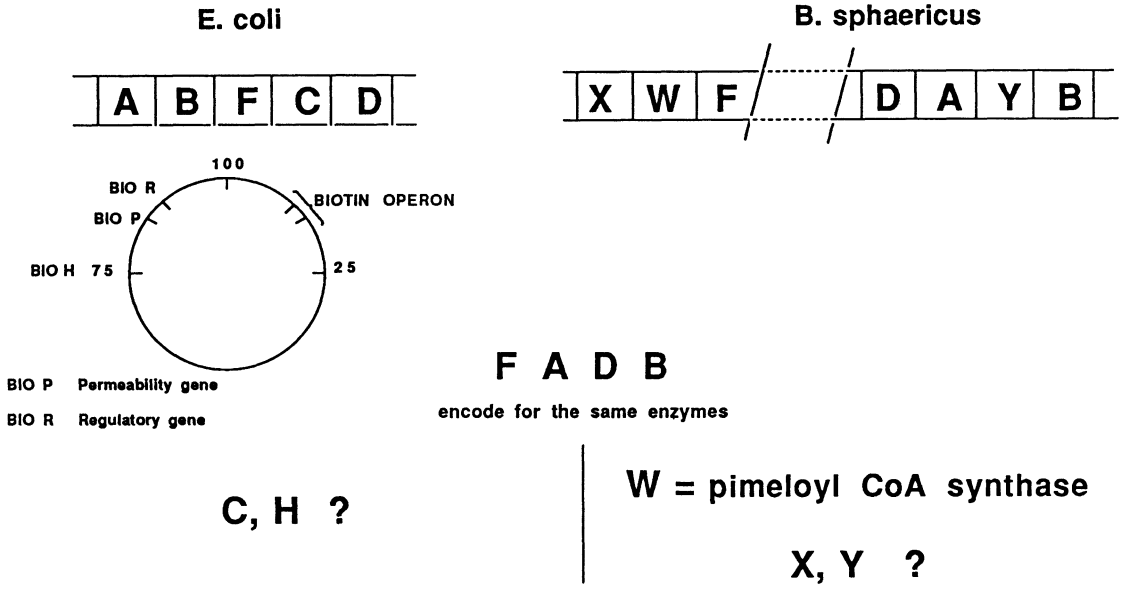


The most fascinating one is certainly the last one, namely the insertion of sulfur in dethiobiotin, but there are still many questions concerning the first steps.

The efforts of many companies to produce biotin with recombinant bacteria have renewed the interest for the genetic and mechanistic aspects of the pathway. Furthermore, a better understanding of the mechanism of action of these enzymes should help to design inhibitors. These inhibitors could have interesting selective properties, since the intermediates involved in the formation of biotin are quite unique in the metabolism.

The genes organization has been well characterized in *Escherichia coli* (2) and *Bacillus sphaericus* (3) (Scheme II). They code for enzymes, as reported on Scheme I.

Scheme II



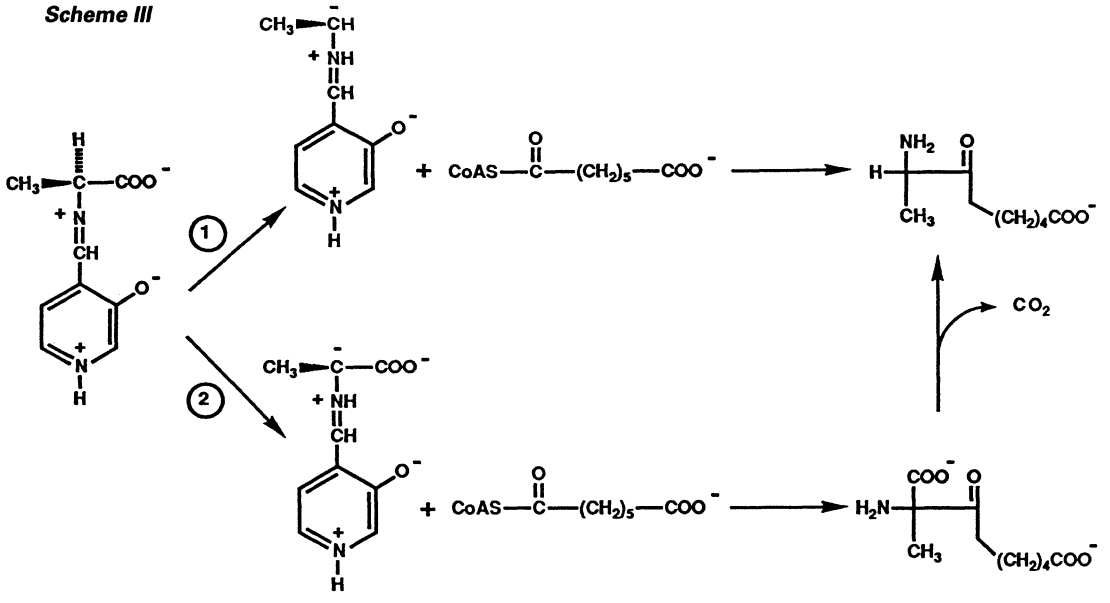
However, some gene products have not yet been identified. In *B. sphaericus*, we have recently reported that Bio W corresponded to pimeloyl CoA synthase (4), catalyzing the reaction :

$$^{-}OOC\text{-}(CH_2)_5\text{-}COO^{-} + ATP + CoASH \xrightarrow{Mg^{2+}} {}^{-}OOC\text{-}(CH_2)_5\text{-}COSCoA + AMP + PP_i$$

But the function of Bio X is still completely unknown.

In *E. coli*, the origin of pimeloyl CoA is still a mystery. Its formation from pimelate is highly questioned (3b). Bio C and Bio H code for enzymes which are involved in the early steps leading to pimeloyl CoA but which are chemically unexplored (2).

Scheme III



The Bio F product is KAPA synthase, already partially purified by Izumi et al. (5) from a wild strain of *B. sphaericus*. We have completely purified this *B. sphaericus* enzyme from an *E. coli* overproducing strain and determined its kinetic parameters (6). KAPA synthesis is quite analogous to the synthesis of aminolevulinic acid from glycine and succinyl CoA, catalyzed by ALA synthase. Our preliminary results showing that the enzyme catalyzes the exchange of the α proton of (L)-Alanine indicate a probable identity of mechanism : the carbanion on the Schiff base with PLP is produced by αH abstraction and not by decarboxylation. Decarboxylation follows the condensation (8) (Scheme III).

The following enzyme, DAPA aminotransferase (Bio A) is an interesting one in the sense that the amino donor in the transamination is not glutamate or another amino-acid, but S. Adenosyl Methionine (9).

Dethiobiotin synthase (Bio D) catalyzes the ureido ring formation as follows (10) :

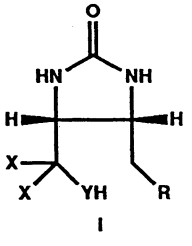
$$\text{DAPA} + CO_2 + \text{ATP} \xrightarrow{Mg^{2+}} \text{DTB} + \text{ADP} + PP_i$$

All these enzymes, although worth of further studies, catalyze however known types of reactions.

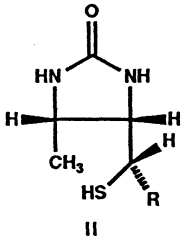
That is not the case for the last one, biotin synthase (Bio B). We have been interested for many years in this reaction which presents analogy with the formation of lipoic acid from octanoic acid (10) and, may be, with the cyclization of ACV tripeptide into penicillin (11).

We could show, several years ago that only the two hydrogens which are replaced by sulfur are involved in the transformation (12). This rules out any unsaturated intermediate. The putative hydroxylated products can also very likely be ruled out (13) and the only reasonable hypothesis is a direct sulfur insertion.

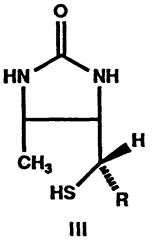
We have synthesized the three possible thiols, I, II, III and we have observed that I, labelled with ^2H(Ib), ^{35}S(Ic) or ^{34}S(Id), is transformed into biotin without loss of the label by resting cells of *B. phaericus*.



I



II



III

a X = H ; Y = ^{32}S
b X = ^2H ; Y = ^{32}S
c X = H ; Y = ^{35}S
d X = H ; Y = ^{34}S

R = $(CH_2)_4$-COOH

In growing cells, a complex pathway is observed, the significance of which is not yet understood. For instance, Ib and Ic are transformed into biotin by *E. coli C124*, a Bio A^- mutant, with the label being almost completely washed out[14].

It is reasonable to assume that the insertion of sulfur at a saturated carbon of dethiobiotin is catalyzed by a metalloenzyme, through a radical mechanism. An homolytic pathway is stongly suggested by the stereochemical results obtained by Arigoni's group, which show that dethiobiotin with a chiral methyl group is converted into biotin with racemisation (15). It is also reasonable to assume that the enzyme which inserts the sulfur of lipoic acid is of the same type. Indeed, very recently, Hayden et al. (16) sequenced the *lip* gene of *E. coli* and found a region presenting a large similarity with biotin synthase.

A tempting working hypothesis is to consider that these enzymes are non-heme iron-proteins like Isopenicillin N Synthase (IPNS). However, no homology was detected between Bio B or *lip* and IPNS.

Indeed the situations are not identical in the three cases. If the type of reaction to catalyze is the same, sulfur is present in the precursor of penicillin, whereas an external sulfur donor is necessary for biotin or lipoic acid.

Further work requires an active *in vitro* system which could not be obtained till now. Formation of biotin, either from DTB or from I, which is observed in whole cells or in protoplasts is completely abolished in cell-free systems even by smooth breaking of the protoplasts by osmotic shock. Work is going on to find out proper conditions.

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