Biosynthesis of Pimeloyl-CoA, a Biotin Precursor in *Escherichia coli*, Follows a Modified Fatty Acid Synthesis Pathway: ^{13}C-Labeling Studies

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The vitamin biotin, which functions as a cofactor in certain carboxylation reactions, is synthesized by a multistep pathway in microorganisms and plants.[1-4] The pathway as worked out in *Escherichia coli* is shown in Figure 1.[1] The enzymes catalyzing some of the steps in this pathway are encoded by genes in the *bio* operon.[1,5] As indicated in Figure 1, little is known about the initial steps that lead to pimeloyl-CoA with the exception that at least two *E. coli* genes, *bioC* and *bioH*, encode enzymes that appear to be involved.[1] The ^{13}C-labeling experiments reported in this communication indicate that pimeloyl-CoA is made by a pathway similar to that of fatty acid and polyketide synthesis.

E. coli KS302/pBOP cells which carry the *bio* operon on a multicopy plasmid (obtained from Gerald Cohen, University of Tel Aviv) were grown in minimal media with the following carbon sources: (1) [1-^{13}C]acetate, (2) [2-^{13}C]acetate, and (3) [1,2-$^{13}C_2$]acetate mixed 1:4 with unlabeled acetate.[6] Biotin released into the media (about 1 mg L^{-1}) during growth on each carbon source was purified by absorbance on charcoal, ion-exchange chromatography, and reverse-phase HPLC.[7] ^{13}C-NMR spectra (75.5 MHz, DMSO-d_6) of the purified biotin samples were measured. The spectra when [1-^{13}C]acetate and [2-^{13}C]acetate were used as the carbon sources are shown in Figure 2, along with peak assignments based on prior natural abundance 2D NMR studies.[8] Biotin from cells grown on [1-^{13}C]acetate displays three prominent ^{13}C peaks at δ = 24.5, 28.0 and 61.0, which correspond to the C-8, C-6, and C-3 carbons, respectively. Biotin from cells grown on [2-^{13}C]acetate displays three prominent peaks at δ = 28.1, 33.5, and 55.4, corresponding to the C-7, C-9, and C-2 carbons, respectively. We conclude from these labeling studies that C-3, C-6, and C-8 of biotin come from C-1 of acetate and that C-2, C-7, and C-9 of biotin come from C-2 of acetate. It is known that the C-4 and C-5 carbons of biotin come from L-alanine and that the ureido carbon of biotin comes from CO_2.[9] Thus, only the origin of the C-10 of biotin remains in question.

In the ^{13}C-NMR spectrum of biotin from cells grown on [1,2-$^{13}C_2$]acetate, the resonances from C-2, C-3, C-6, C-7, C-8, and C-9 all appeared as doublets with coupling constants (J_{2-3} = 36 Hz; J_{6-7} = 33 Hz; J_{8-9} = 34.5 Hz) indicating that three intact acetate units are directly incorporated into biotin similar to fatty acid and polyketide synthesis.[10,11] This labeling pattern supports the biosynthetic pathway shown in Figure 3 (in this figure and ensuing text, TE refers to a group attached to the intermediate in a thioester linkage, e.g., coenzyme A, acyl carrier protein, condensing enzyme. The labeling pattern is not consistent with the synthesis of pimeloyl-CoA from octanoate by α- and ω-oxidation, nor is it consistent with the formation of pimelate from tryptophan, lysine, diaminopimelate or by the α-keto acid elongation of 2-oxoglutarate. While the pathway shown in Figure 3 has been proposed previously,[12] this work provides the first evidence for it.

An early step in Figure 3 is the condensation of two molecules of malonyl-TE followed by a single decarboxylation to form 3-oxoglutaryl-TE, i.e., malonate is the starter unit. The CO_2 incorporated into the malonate acting as a starter unit would be permanently "fixed" and become C-10 of biotin. There is a precedent for malonate (or malonamide) being a starter unit in the biosynthesis of oxytetracycline via a polyketide.[13] An attempt to demonstrate the incorporation of carbon from ^{13}C-malonate into C-10 failed, apparently because exogenous malonate is not activated by *E. coli* (malonate cannot be used by *E. coli* as a carbon source). Additionally, no label was incorporated in C-10 (or C-2′, the ureido carbon that is known to come from CO_2) when cells were grown in [^{13}C]CO_2. Apparently the labeled CO_2 was swept out of the media as the culture was aerated. Although it seems likely that the source of C-10 is CO_2, it remains to be proven.

It should be noted that the labeling pattern found requires the two carboxyls of pimelate to be metabolically distinct. This rules out free pimelic acid as an intermediate, a result consistent with the literature reports that free pimelic acid is not a biotin precursor in *E. coli*.[1,14]

The results shown in this paper combined with the recent report that the sequence of the *bioX* gene in *Bacillus sphaericus* contains a consensus phosphopantetheine attachment site[2] raise the possibility that a novel acyl carrier protein is involved in the synthesis of the pimeloyl-CoA, perhaps as a carrier for 3-oxoglutarate. If this were the case, a gene analogous to *bioX* must be present in *E. coli*. In this regard, it is of interest that the ability to make pimelic acid for biotin biosynthesis seems to be limited to organisms with soluble fatty acid biosynthesis complexes. Finally, if biotin is made by the modified fatty acid synthesis pathway outlined here, we are left with the evolutionary conundrum that the vitamin biotin is directly required for the biosynthesis of itself.[10a]

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(6) *E. coli* strain KS302/pBOP was grown in minimal media with addition of thiamine-HCl (0.003%), vitamin-free casamino acid (2%), ampicillin (0.01%), and ^{13}C-labeled acetate (0.05%) at 37 °C for ~20 h. At the end of the run, the cells were separated from the media by centrifugation.

(7) Biotin containing extra cellular fluid was stirred with charcoal (10 g L^{-1}) for 5 h at pH 3.0 and filtered. The charcoal was eluted with a 18:1 mixture of ethanol/ammonia. The eluate was absorbed onto a QAE Sephadex column and eluted with NH_4HCO_3. The biotin-containing fractions were identified by bioassay. These were absorbed onto a C-18 column and eluted with acetonitrile and then absorbed onto a Mono-Q column and eluted with NH_4HCO_3.

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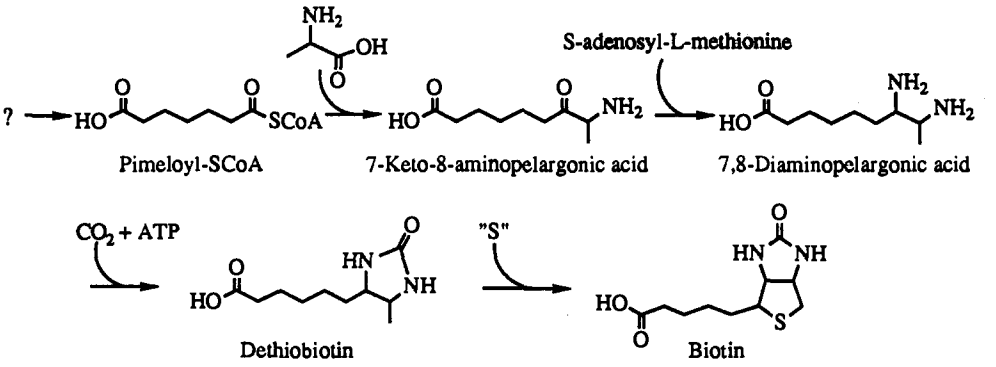

Figure 1. Pathway for the biosynthesis of biotin in *E. coli*. The question mark indicates that the pathway for the synthesis of the intermediate pimeloyl CoA is not known. The "S" indicates the sulfur donor for the last reaction, which is unknown.

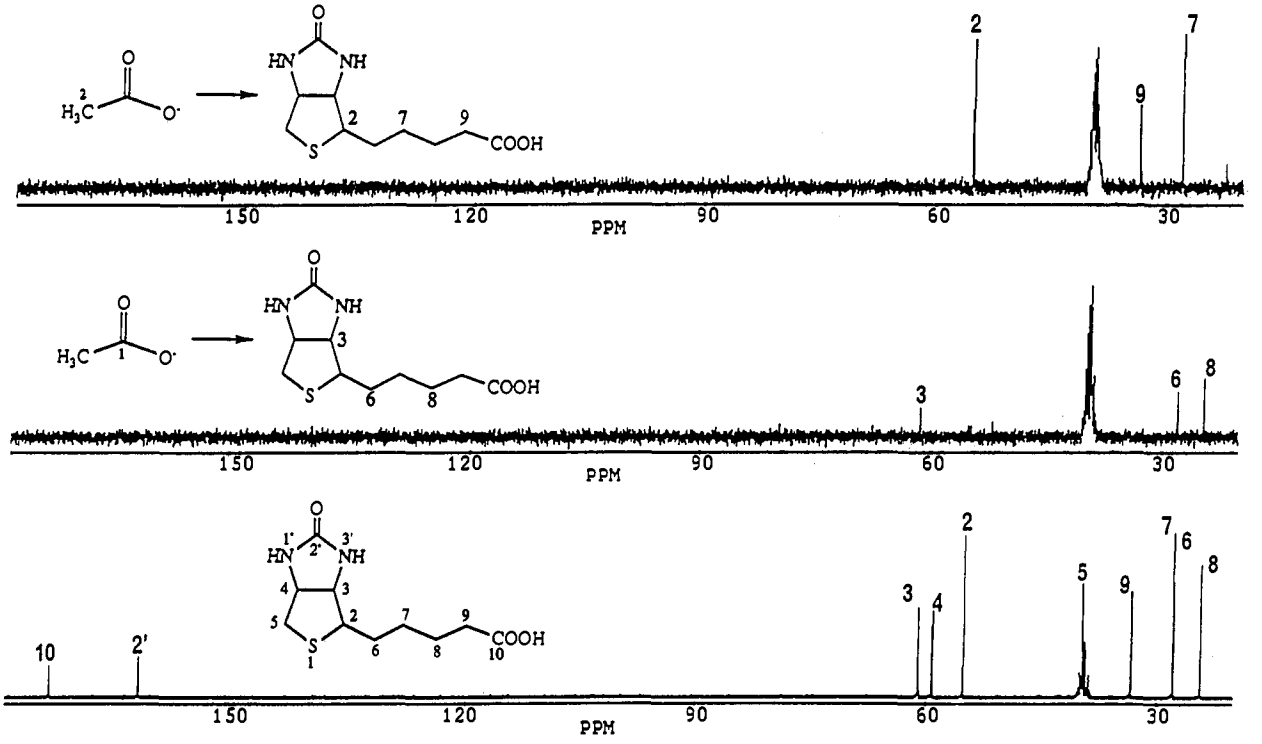

Figure 2. ^{13}C-NMR spectra of biotin (natural abundance, bottom spectrum); biotin purified from cells grown with $CH_3{}^{13}COO^-$ (middle spectrum); and biotin purified from cells grown with $^{13}CH_3COO^-$ (top spectrum) in DMSO-d_6 at 75.5 MHz.

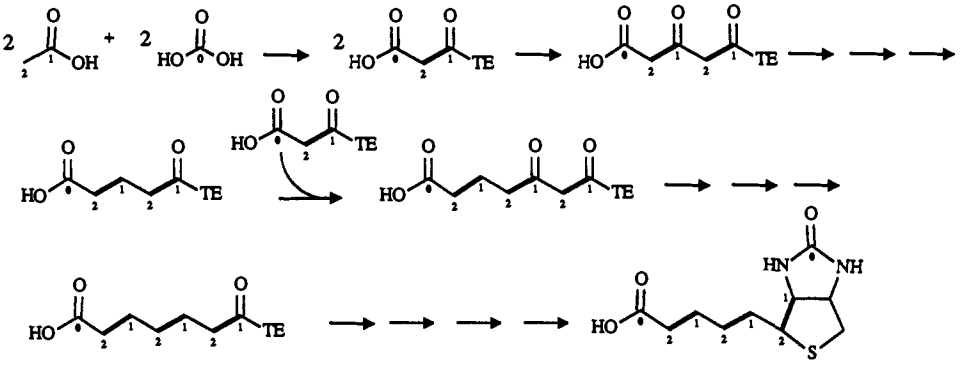

Figure 3. Proposed modified fatty acid synthesis pathway for formation of pimeloyl-CoA from acetate in *E. coli*. The designations 0, 1, and 2 adjacent to the carbon atoms indicate the origin of those atoms with 0 being bicarbonate, 1 being C-1 of acetate, and 2 being C-2 of acetate. The darker bonds indicate intact acetate units. No label indicates that the origin of the carbon was from the alanine supplied in the casamino acids. Three arrows in a row indicates reduction, dehydration, and reduction typical of fatty acid biosynthesis. Four arrows in a row indicates the four reactions in the conversion of pimeloyl-CoA to biotin.

Supplementary Material Available: Schemes showing biotin labeling patterns expected from several pathways (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.