THE ENZYMIC FORMATION OF O-ACETYLHOMOSERINE IN BACILLUS SUBTILIS AND ITS REGULATION BY METHIONINE AND S-ADENOSYLMETHIONE*

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Summary: Cell-free extracts of Bacillus subtilis catalyze the formation of O-acetylhomoserine from acetyl-CoA and L-homoserine, while a corresponding reaction with succinyl-CoA is not observed. The formation of O-acetylhomoserine is subject to multivalent feedback inhibition by L-methionine and S-adenosylmethionine, implicating it as a step in methionine biosynthesis. Thus, in Bacilli the biosynthesis of both lysine and methionine appears to involve acetylated intermediates, in contrast to Escherichia coli where the corresponding succinylated derivatives are used.

The biosynthesis of lysine and methionine from aspartate involves two types of acylated intermediates, N-acyl-α-amino-ε-ketopimelate and O-acylhomoserine. In the former, the acyl moiety serves as a protecting group to prevent the cyclization of the intermediate, while in the latter it acts as an anionic leaving group to facilitate the pyridoxal-P dependent conversion to cystathionine. In Escherichia coli, the acyl

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group of both intermediates derives from succinyl-CoA (1, 2), whereas Bacillus megaterium employs N-acetyl-α-amino-ε-ketopimelate in lysine biosynthesis (3). It was therefore of interest to determine whether Bacilli utilize acetyl-CoA also for the acylation of homoserine, a reaction that has previously been observed only in fungi (4).

EXPERIMENTAL PROCEDURE

Materials - Acetyl-CoA and succinyl-CoA were prepared by the reaction of CoA with the corresponding acyl anhydrides (5). CoA and S-adenosylmethionine were obtained from P-L Biochemicals and L-(U-^{14}C)-homoserine from Amersham.

Bacterial Extracts - Bacillus subtilis ATCC 6051 was grown in a synthetic medium as described previously (6). Cells were harvested in mid-exponential phase, suspended in 2 volumes of a buffer containing 80 mM potassium phosphate, pH 7.5, 1 mM β-mercaptoethanol, and 20% ethylene glycol, and disrupted by passage through a French pressure cell at 7,500 p.s.i. The supernatant fraction (20 mg of protein per ml) obtained after centrifugation at 100,000 x g for 1 hour was used for all experiments.

Enzyme Assay - The acylation of homoserine was assayed by a slight modification of the method of Nagai and Flavin (4). The incubation mixtures contained in a final volume of 0.5 ml: 50 μmoles of triethanolamine hydrochloride, pH 7.6, acetyl-CoA and L-(U-^{14}C)-homoserine as indicated, and 0.05 ml of extract. After incubation at 25° for 10 minutes, the reaction was terminated by the addition of 0.08 ml of 1 M KOH. The mixtures

were placed in a boiling water bath for 1 minute, cooled, and passed over small columns (0.5 ml) of Dowex-50 (H^+). The columns were washed with 2 ml of water and a portion (1 ml) of the combined eluates was counted in a liquid scintillation spectrometer using the scintillation fluid of Bray (7). The values obtained were corrected by the use of blank incubations from which acetyl-CoA had been omitted.

RESULTS

When extracts of B. subtilis were incubated with succinyl-CoA, little acylation of L-homoserine could be detected. On

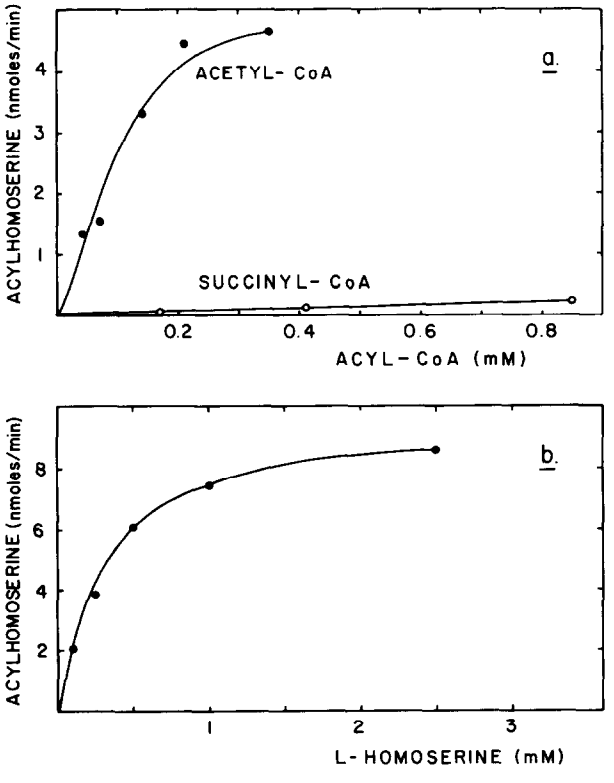

Figure 1. Effect of substrate concentrations on the rate of O-acylhomoserine formation. (a) The concentration of acyl-CoA was varied at 0.5 mM L-homoserine. (b) The concentration of L-homoserine was varied at 0.35 mM acetyl-CoA. The conditions of the assay were as described under 'Experimental Procedure'.

the other hand, in the presence of acetyl-CoA a rapid formation of O-acetylhomoserine was observed. Similar results were obtained with B. polymyxa. The dependence of the acetylation reaction on substrate concentration is shown in Fig. 1. At 0.5 mM L-homoserine, the apparent K_m for acetyl-CoA was 0.16 mM, while at 0.35 mM acetyl-CoA the corresponding parameter for L-homoserine was 0.4 mM. At lower concentrations of the co-substrate, the values of the apparent K_m decreased for both compounds (not shown).

The enzymic acetylation of L-homoserine was inhibited both by L-methionine and S-adenosylmethionine at relatively

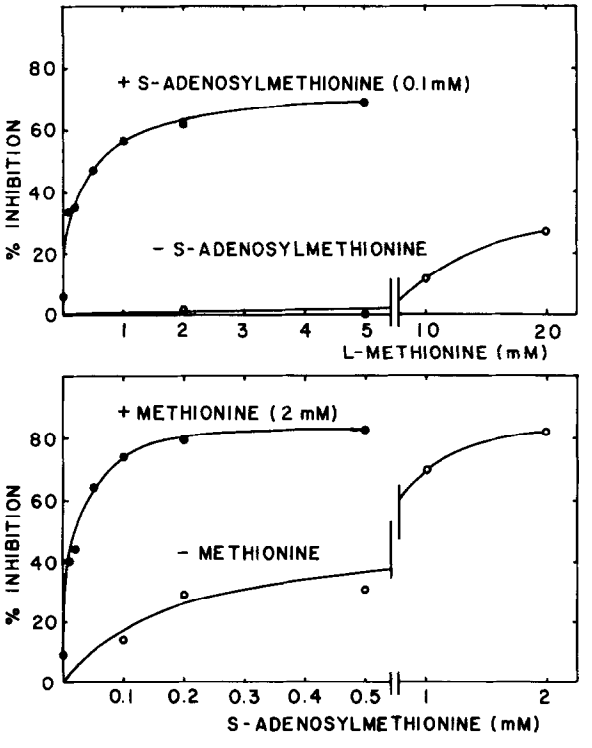

Figure 2. Inhibition of O-acetylhomoserine formation by methionine and S-adenosylmethionine. The concentrations of the inhibitors were varied as indicated. The assays were carried out at 0.14 mM acetyl-CoA and 0.5 mM L-homoserine as described under 'Experimental Procedure'.

high concentrations (Fig. 2). However, a striking synergistic effect of the two inhibitors was observed. As shown in Fig. 2, combinations of methionine and S-adenosylmethionine at low concentrations strongly inhibited the enzyme.

DISCUSSION

An interesting dichotomy exists in the aspartate pathway of amino acid biosynthesis. In bacteria, algae and higher plants this pathway participates in the biosynthesis of lysine, while in fungi and euglenids lysine derives from glutamic acid (8). The discovery by Nagai and Flavin (4) that acetylhomoserine is an intermediate in methionine biosynthesis in Neurospora, while in E. coli (1) and Salmonella typhimurium (9) the corresponding succinylated derivative is used, suggested that the same phylogenetic division also applied to the methionine branch of the pathway. However, the observation that O-acetylhomoserine was accumulated by certain methionine auxotrophs of Arthrobacter paraffineus and of a Bacillus species raised the possibility that the acetylated intermediate was not restricted to fungi (10). The results presented in this communication support this conclusion. Extracts of B. subtilis and B. polymyxa catalyze the acetylation but not the succinylation of L-homoserine. Like the homoserine transsuccinylase of E. coli (11), this reaction is inhibited by combinations of methionine and S-adenosylmethionine, undoubtedly a feedback mechanism which implicates the homoserine transacetylase in the biosynthesis of these compounds.

This observation and the fact that B. megaterium employs

an acetylated derivative also in the biosynthesis of lysine (3) while E. coli uses the corresponding succinylated compound suggest that acyl donor specificity in the aspartate pathway actually represents an evolutionary divergence between Enterobacteriaceae and Bacillaceae. Since the chemical roles of the acyl groups in lysine and in methionine biosynthesis are quite different and since the two acylation reactions must be carried out by different enzymes, the reason for the parallel change in specificity is not obvious. Perhaps subtle differences in the regulation of intermediary metabolism in the two groups of bacteria make in one case acetyl-CoA, in the other succinyl-CoA the more efficient acyl donor. Indeed, in some strains of E. coli (12) and Aerobacter aerogenes (13) the use of succinyl-CoA seems to provide a selective disadvantage as evidenced by their simultaneous growth requirement for lysine and methionine.

At present, only a few examples of multivalent feedback inhibition have been described (14). It is therefore of interest that the homoserine transacetylase of B. subtilis and the homoserine transsuccinylase of E. coli share this type of control, suggesting a similarity between the two enzymes in spite of their different substrate specificities. Certain striking similarities have also been observed between one of the aspartokinases of the two organisms (6). More detailed structural studies of enzymes of this type may provide interesting information concerning the evolutionary relationship between these two bacterial species.

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