BIOSYNTHESIS OF METHIONINE FROM S-ADENOSYLMETHIONINE IN ESCHERICHIA COLI[1]

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It has been found that the homocysteine methyltransferases of various microorganisms, mammalian livers, and plant tissues (Shapiro, 1956, 1958, 1962; Shapiro and Yphantis, 1959; Turner and Shapiro, 1961; Abrahamson, 1962) utilize S-adenosylmethionine[2] as a methyl donor in the synthesis of methionine according to the reaction:

L-Homocysteine + S-Adenosyl-L-methionine ——> L-Methionine + S-Adenosyl-L-homocysteine + H^+ (1)

S-AM also acts as a precursor of methionine in Escherichia coli, but the mechanism of its action in this instance has now been demonstrated to differ markedly.

METHODS

The microorganisms used were E. coli (Texas) and a mutant thereof, E. coli (Texas) M, which had originally been described as a methionine auxotroph (McRorie et al. 1954). Growth response studies were undertaken with cultures grown for 18 hr at 37°C in 10 ml of appropriately supplemented (see Table 1) minimal medium (Shapiro, 1962) on a reciprocal shaker. In order to obtain large masses of cell material, E. coli (Texas) was grown on unsupplemented minimal medium whereas the auxotroph was grown on minimal medium supplemented with 50 mμM/ml of L-methionine. Cell-free extracts were prepared by exposing a 30% suspension of washed cells in 0.1 M phosphate buffer, pH 6.8, for 15 min to a 10 KC Raytheon sonic oscillator. Debris was removed by centrifugation for

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[2]Abbreviations are: S-AM, S-adenosylmethionine; S-AH, S-adenosylhomocysteine.

30 min at 8000 x g and the extract stored in a freezer. Harvested cells were fractionated by the method of Roberts et al. (1955) and specific activity of the protein methionine determined (Shapiro et al., 1962).

Methionine biosynthetic activity of the cell-free extracts was determined by the assay technique of Shapiro and Yphantis (1959). The various reaction products present in cell-free reaction mixtures were isolated and identified by paper chromatography (Shapiro et al., 1962).

S-AM and the three radioactive forms of S-AM were isolated from Saccharomyces cerevisiae by the procedure of Schlenk et al. (1959); S-AH was prepared enzymatically from rat-liver homogenates by the method of Duerre (1962). For use in bacteriological media, heat labile compounds were sterilized by filtration through millipore filters.

RESULTS AND DISCUSSION

The results of the growth response studies with E. coli (Texas) M are summarized in Table 1. All the compounds supporting growth are either

TABLE 1

Growth responses of E. coli (Texas) M

Supplement (200 mμM/ml)[a]	Optical Density[b]
None	0
L-Methionine	0.51
D-Methionine	0.56
S-Adenosyl-L-methionine	0.37
S-Methyl-L-methionine	0.49
S-Methyl-D-methionine	0.44
Dimethyl-β-Propiothetin	0.02
S-Adenosyl-L-homocysteine	0.40
S-Methyl-L-cysteine	0.17
L-Ethionine	0.09

[a] Dimethyl-β-acetothetin, betaine, L-serine, S-ethyl-L-cysteine, 5'-methylthioadenosine, L-homocysteine and DL-cystathionine do not support growth. Addition of either 200 mμM/ml L-homocysteine or 400 mμM/ml DL-cystathionine to any of the supplements has no significant effect.

[b] Klett-Summerson colorimeter with No. 59 filter.

sulfonium derivatives or are capable of conversion to sulfonium derivatives. The possibility existed that these compounds were directly incorporated into proteins, as Levine and Tarver (1951) had found for ethionine in rats, or that those compounds containing methionine moieties were simply degraded to yield methionine. These possibilities were not supported by the data summarized in Table 2. L-Methionine and S-AM support the synthesis of a quantity of cell material which contains many times the methionine directly available from the S-AM or added methionine. Exogenous methionine must act as a precursor of a compound which is itself involved in the biosynthesis of methionine. This compound presumably acts in a catalytic manner, being regenerated. The specific activities of the protein methionine demonstrate that only a very small percentage of protein methionine is derived from the substrate and that the rest must be synthesized de novo. Thus, in a strict sense, E. coli (Texas) M is not a methionine auxotroph.

TABLE 2

Biosynthesis of methionine by E. coli (Texas) M

Substrate	Total uptake (μM)	Protein methionine (μM)	Specific activity (cpm/μM)		Label incorporated into protein methionine (%)
			Substrate ($\times 10^{-5}$)	Protein methionine ($\times 10^{-2}$)	
L-Met-$C^{14}H_3$	2.50	34.5	33	62	0.19
L-Met-S^{35}	3.75	22.6	4.6	175	3.80
S-AM-$C^{14}H_3$	3.40	78.1	1.6	3.14	0.02
S-AM-S^{35}	3.36	34.3	5.3	< 0.08	< 0.02

The most direct explanation of the activity of S-AM would be that it is acting as a methyl donor, as described in reaction (1) but this proved not to be the case. As can be seen in Table 3, not only the labeled methyl group, but also the sulfur and C_2 of S-AM are incorporated into the methionine produced by the cell-free extract. This is presumably not a simple cleavage of

TABLE 3

Biosynthesis of methionine by cell-free extracts of E. coli, mμM/ml/hr

Substrate (0.2 μM/ml)	E. coli (Texas)	E. coli (Texas) M
S-AM-$C^{14}H_3$	25.2 ± 1.9[a,b]	24.0 ± 2.4
S-AM-2-C^{14}	24.3 ± 1.1	19.8 ± 4.1
S-AM-S^{35}	20.7 ± 4.7	21.1 ± 4.3

[a]Methionine determined by measurement of radioactivity (Shapiro and Yphantis, 1959).
[b]Values represent means ± standard deviations.

S-AM into methionine and adenosine, since the reaction does not occur unless homocysteine is present. The synthesis of methionine in the assay system containing homocysteine and its absence in the homocysteine-less system was demonstrated by paper chromatography. These observations lead us to postulate the following reaction which, for clarity, is formulated here with one of the labeled forms of S-AM:

$$CH_3\overset{+}{S}<^{\text{Adenosine}}_{CH_2CH_2C^{14}H(NH_2)COOH} + \text{homocysteine} \longrightarrow CH_3SCH_2CH_2C^{14}H(NH_2)COOH + \text{S-adenosylhomocysteine} + H^+ \quad (2)$$

The products of this reaction are identical with those in reaction (1) although the mechanism involves transadenosylation, instead of transmethylation.

Cleavage of the ribose-sulfur bond of S-AM in an enzymatic reaction has not been described before. The genetic block in E. coli (Texas) M may be in the reaction:

$$\text{Adenosine} + \text{homocysteine} \longrightarrow \text{S-AH} + \text{HOH} \quad (3)$$

This reaction was first described in rat liver (de la Haba and Cantoni, 1959), and later in S. cerevisiae (Duerre and Schlenk, 1962). Since E. coli (Texas) M shows a growth response to S-adenosylhomocysteine it seems probable either that this compound is being regenerated to S-AM through remethylation by N^5-

methyltetrahydrofolic acid (Larrabee et al., 1961; Sakami and Ukstins, 1961), or that regeneration is being carried out on the enzymatic degradation product of S-AH, S-ribosylhomocysteine (Duerre, in press). Evidence for the formation of S-AM from S-AH has been obtained with S. cerevisiae (Duerre and Schlenk, 1962; Pigg et al., 1962).

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