BBA 65561

ENZYMATIC SYNTHESIS OF HOMOCYSTEINE OR METHIONINE DIRECTLY FROM *O*-SUCCINYL-HOMOSERINE

MARTIN FLAVIN AND CLARENCE SLAUGHTER
Laboratory of Biochemistry, National Heart Institute, Bethesda, Md. (U.S.A.)

(Received September 26th, 1966)

SUMMARY

The bacterial biosynthesis of methionine has previously been shown to involve formation of cystathionine from cysteine and *O*-succinyl-homoserine, catalyzed by cystathionine γ-synthase, followed by cleavage of cystathionine to yield homocysteine. This report presents evidence that hydrogen sulfide can replace cysteine as a substrate for cystathionine γ-synthase, yielding homocysteine directly. The maximum velocity of homocysteine formation is about half that of cystathionine formation, but the K_m for hydrogen sulfide is 50 times higher than that for cysteine. The leakiness of Salmonella mutants blocked in the conversion of cystathionine to homocysteine suggests that direct synthesis of homocysteine from H_2S may take place in the cell, although only to a limited extent. Cystathionine γ-synthase has also been shown to catalyze formation of methionine directly from *O*-succinyl homoserine and methyl mercaptan. This reaction is presumed to have no role in the utilization of inorganic sulfur compounds for methionine biosynthesis, but may provide an explanation for the ability of *S*-methyl cysteine to support the growth of methionine auxotrophs of some microorganisms.

INTRODUCTION

An essential step in the bacterial biosynthesis of methionine consists in the synthesis of cystathionine[1,2] from L-cysteine and *O*-succinyl-L-homoserine (Reaction 1). The enzyme catalyzing this reaction, cystathionine γ-synthase, has been isolated in pure form from *Salmonella typhimurium*[3], and was found

O-Succinyl-homoserine + cysteine → cystathionine + succinic acid (1)

to be rather specific toward both substrates, although acetyl homoserine could replace succinyl homoserine[4]. In the absence of cysteine the same enzyme catalyzed Reaction 2 with a maximum velocity one-fifth that of Reaction 1.

O-Succinyl-homoserine + H_2O → NH_3 + α-ketobutyrate + succinic acid (2)

We report here on 2 additional reactions which are catalyzed quite effectively* by cystathionine γ-synthase when cysteine is replaced by H_2S (Reaction 3) or by CH_3SH (Reaction 4).

O-Succinyl-homoserine + H_2S → homocysteine + succinic acid (3)

O-Succinyl-homoserine + CH_3SH → methionine + succinic acid (4)

MATERIALS AND METHODS

Reaction 3 was measured by the procedure used by KREDICH AND TOMKINS to assay *O*-acetyl-serine sulfhydrylase[5]. The reaction mixtures contained, in 0.2 ml volume, 50 μmoles of Tris–HCl buffer, pH 7.3, 0.6 μmole of Tris–H_2S, 4 μmoles of amino acid or ester (Table I), and enzyme. At zero time, and after 6 min at 30°, 0.05-ml aliquots were assayed for mercaptan by the color test of KREDICH AND TOMKINS[5]. The molar absorbance of L-homocysteine was 26 000 at 540 mμ.

TABLE I

ENZYMATIC SYNTHESIS OF CYSTEINE AND HOMOCYSTEINE BY EXTRACTS OF METHIONINE AUXOTROPHS OF SALMONELLA

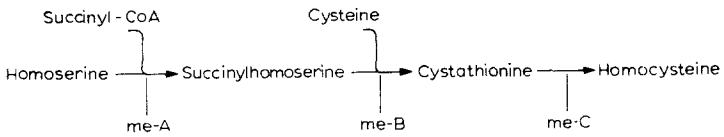
Source of enzyme extract	*Substrate*	*Reaction rates (μmoles/mg protein × min)*		
		H_2S fixation into:		*γ-Elimination (α-ketobutyrate formation)*
		Cysteine	*Homocysteine*	
Me-B-16	*O*-Acetyl-L-serine	0.002		
Me-B-16	*O*-Succinyl-DL-homoserine		0	0
Me-B-16	*O*-Acetyl-DL-homoserine		0	0
Me-A-15	*O*-Acetyl-L-serine	0.006		
Me-A-15	L-Serine	0		
Me-A-15	*O*-Succinyl-DL-homoserine		0.19	0.04
Me-A-15	*O*-Acetyl-DL-homoserine		0.01	
Me-A-15	L-Homoserine		0	

Reaction 4 was measured by the rate of methionine formation. Besides enzyme, the reaction mixtures contained, per 2 ml: 100 μmoles of potassium phosphate, pH 7.3, 0.2 μmole of pyridoxal phosphate, and 40 μmoles each of *O*-succinyl-DL-homoserine and CH_3SH (added from a concentrated solution in ethanol). At zero, 30 and 60 min, 0.5-ml aliquots were assayed for methionine by the nitroprusside procedure[6]. The molar absorbance of methionine was 470 at 510 mμ.

RESULTS AND DISCUSSION

Table I shows the results obtained with crude extracts of 2 methionine auxotrophs of Salmonella, whose metabolic defects are indicated in Scheme I. Extracts of

* The catalysis of Reaction 3 was first observed by Dr. N. KREDICH (personal communication). The enzyme has also been found to catalyze exchange of cysteine into cystathionine, and of succinate into *O*-succinyl-homoserine (M. FLAVIN AND C. SLAUGHTER, unpublished results), but these replacement reactions are relatively slow. The rate of the latter exchange was 1/1000 of that of Reaction 2, and the exchange was inhibited by D-cysteine or β-mercaptopropionate[4].



Scheme 1. Methionine auxotrophs of Salmonella.

mutant me-A catalyzed rapid fixation of H_2S in the presence of *O*-succinyl-homoserine (Table I). The rate of this reaction was 5 times that of Reaction 2, as measured by α-ketobutyrate formation according to "assay A" previously described[4]; it was also much greater than the rate of synthesis of cysteine[5] from H_2S and *O*-acetyl-serine (Table I). In connection with the latter comparison, however, it should be noted that me-A is derepressed for the formation of cystathionine γ-synthase[1,4]. Reaction 3 was not catalyzed by extracts of me-B; another indication that cystathionine γ-synthase was the responsible enzyme was the fact that the relative rates of Reaction 3 with *O*-succinyl-homoserine and *O*-acetyl-homoserine (Table I) were similar to those of Reaction 2 (ref. 4).

Figure 1 shows the extent of H_2S fixation catalyzed by 2 partially purified fractions of cystathionine γ-synthase, in the presence of succinyl homoserine; the fraction of specific activity 14.4 was 76% pure[3]. Equal units of synthase activity, from either fraction, catalyzed the same amount of H_2S fixation (Fig. 1).

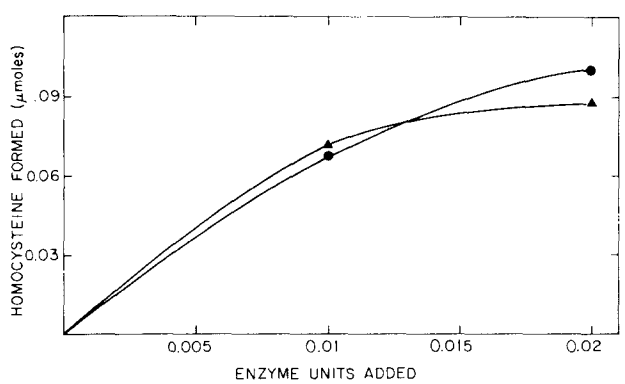


Fig. 1. Homocysteine synthesis catalyzed by fractions of cystathionine γ-synthase purified to specific activities of: ●, 2.5; ▲, 14.4.

The ability of cystathionine γ-synthase to catalyze Reaction 4 was studied in a similar way. Fig. 2 shows the amounts of methionine formed from methyl mercaptan and succinyl homoserine in the presence of 0.8 unit of either of the same enzyme fractions used in Fig. 1.

The identities of the products formed in Reactions 3 and 4 were confirmed by using *O*-succinyl-DL-[2-^{14}C]homoserine (ref. 7). Methionine was isolated by chromatography in 80% phenol[8], and homocysteine, after oxidation to homocysteic acid[1], by paper electrophoresis for 3 h at 3000 V at pH 2 (ref. 9). Inspection of X-ray film exposed to these papers showed conversion of about half the added radioactivity (pre-

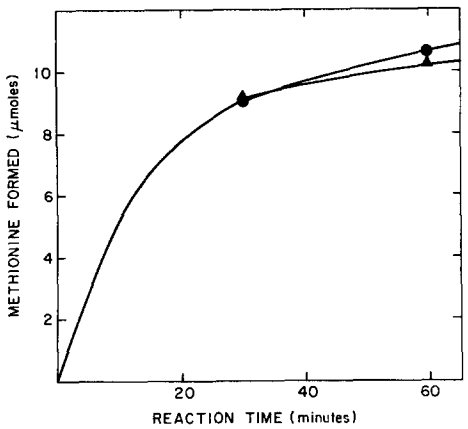


Fig. 2. Methionine synthesis catalyzed by equal units of cystathionine γ-synthase, specific activity: ●, 2.5; ▲, 14.4.

sumably the L isomer of succinyl homoserine) to a component migrating like the expected reaction product.

The above results are taken as proof that Reactions 3 and 4 are catalyzed by cystathionine γ-synthase. Table II outlines kinetic constants for Reactions 1 to 4, as estimated from reciprocal plots. As suggested by the discrepancy between the 2 $v_{max.}$ values for Reaction 3, the error was large in the rate determinations for this reaction, and also for Reaction 4. The source of the error was partly the fact that assays for both reactions failed to give results strictly linear with increasing time or enzyme

TABLE II

KINETIC CONSTANTS FOR CYSTATHIONINE γ-SYNTHASE

The $v_{max.}$ values are expressed as turnover numbers: moles/min per mole of enzyme (160 000 g).

Substrate	*Reaction 2*		*Reaction 1*		*Reaction 3*		*Reaction 4*	
	K_m	$V_{max.}$	K_m	$V_{max.}$	K_m	$V_{max.}$	K_m	$V_{max.}$
O-Succinyl-L-homoserine	$3 \cdot 10^{-4}$	3000	$4 \cdot 10^{-3}$	15 000	$5 \cdot 10^{-3}$	5 000	$9 \cdot 10^{-3}$	2800
L-Cysteine			$7 \cdot 10^{-5}$	15 000				
H_2S					$3 \cdot 10^{-3}$	11 000		
CH_3SH							10^{-1}	2100

concentration, as illustrated in Figs. 1 and 2. The maximum velocities of Reactions 3 and 4 (Table II) are enough to allow these reactions to function effectively in metabolism. However, the K_m values for H_2S, and particularly for CH_3SH, are higher than that for cysteine.

It is now appropriate to consider the possible physiological significance of these various reactions catalyzed by cystathionine γ-synthase. *O*-Acetyl-homoserine has recently been reported to be an intermediate in methionine biosynthesis in Neurospo-

ra[10]. This ester can replace succinyl homoserine in Reactions 1 to 4 and, as shown in Table III, supports the growth of Salmonella mutant me-A-15, which the succinyl ester does not do. Nevertheless, we conclude that acetyl homoserine has no metabolic role in Salmonella, since by several sensitive assays the bacteria have been shown unable to synthesize it (S. NAGAI AND M. FLAVIN, unpublished results).

If Reaction 3 functioned effectively in Salmonella, and homocysteine could be formed directly from succinyl homoserine, the me-C mutants (see Scheme I) should score as wild-type, which is not the case. However, me-C-30 was found to be leaky, as shown in Table III. The slow growth on minimal medium was exponential, and sub-

TABLE III

NUTRITIONAL RESPONSES OF SALMONELLA METHIONINE AUXOTROPHS

Supplement to minimal medium (0.5 μmole/ml)	*Mutant strain:*					
	me-A-15		*me-B-16*		*me-C-30*	
	Growth	*Doubling time (min)*	*Growth*	*Doubling time (min)*	*Growth*	*Doubling time (min)*
DL-Methionine	+	40	+	60	+	40
L-Homocysteine	+	40	+	60	+	
L- + D-*allo*-cystathionine	+	80	+	60	+	
O-Acetyl-DL-homoserine	+	80	–		–	300
O-Succinyl-DL-homoserine	–		–		+	300
None	–		–		±	300

culture showed it not to be due to reversion[1]. It seems likely therefore that homocysteine can be synthesized by Reaction 3 in Salmonella, but very slowly, perhaps because of the high K_m for H_2S. Neurospora extracts have been reported to catalyze a slow synthesis of homocysteine from H_2S and homoserine[11]. This reaction might be analogous to Reaction 3 in Salmonella.

The direct synthesis of methionine from succinyl homoserine and CH_3SH (Reaction 4) would have no metabolic utility in so far as methionine would presumably be a necessary precursor of methyl mercaptan. Reaction 4 is of interest in relation to reports that, again in Neurospora, *S*-methylcysteine[12] could support the growth of some methionine auxotrophs[13–15]. Neurospora[16] and perhaps *Escherichia coli* also[17], can generate methyl mercaptan from *S*-methylcysteine.

The presence in Neurospora of a reaction analogous to Reaction 4 would then explain the growth response to *S*-methylcysteine. It may be significant that a Neurospora me-5 mutant, blocked in the synthesis of *O*-acetyl-homoserine[10], did not respond to *S*-methylcysteine[15].

REFERENCES

1 C. DELAVIER-KLUTCHKO AND M. FLAVIN, *J. Biol. Chem.*, 240 (1965) 2537.
2 M. M. KAPLAN AND M. FLAVIN, *Biochim. Biophys. Acta*, 104 (1965) 390.
3 M. M. KAPLAN AND M. FLAVIN, *J. Biol. Chem.*, 241 (1966) 5781.
4 M. M. KAPLAN AND M. FLAVIN, *J. Biol. Chem.*, 241 (1966) 4463.

5 N. M. KREDICH AND G. M. TOMKINS, *J. Biol. Chem.*, 241 (1966) 4955.
6 F. A. CSONKA AND C. A. DENTON, *J. Biol. Chem.*, 163 (1946) 329.
7 M. FLAVIN AND C. SLAUGHTER, *Biochemistry*, 4 (1965) 1370.
8 M. FLAVIN AND C. SLAUGHTER, *Biochemistry*, 3 (1964) 885.
9 M. FLAVIN AND C. SLAUGHTER, *Biochemistry*, 5 (1966) 1340.
10 S. NAGAI AND M. FLAVIN, *J. Biol. Chem.*, 241 (1966) 3861.
11 J. L. WIEBERS AND H. R. GARNER, *Abstr. 150th Meeting Am. Chem. Soc.*, 77 (1965) 39C.
12 E. C. WOLFF, S. BLACK AND P. F. DOWNEY, *J. Am. Chem. Soc.*, 78 (1956) 5958.
13 J. B. RAGLAND AND J. L. LIVERMORE, *Arch. Biochem. Biophys.*, 65 (1956) 576.
14 J. L. WIEBERS AND H. R. GARNER, *J. Bacteriol.*, 88 (1964) 1798.
15 S. TOKUNO, B. STRAUSS AND Y. TSUDA, *J. Gen. Microbiol.*, 28 (1962) 481.
16 M. FLAVIN AND C. SLAUGHTER, *Biochim. Biophys. Acta*, 132 (1967) 406.
17 C. DELAVIER-KLUTCHKO AND M. FLAVIN, *Biochim. Biophys. Acta*, 99 (1965) 375.