# *S*-ADENOSYLMETHIONINE:HOMOCYSTEINE METHYLTRANSFERASE AS A REGULATORY ENZYME IN EMBRYOS OF *MUSCA DOMESTICA*

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Received 10 May 1976

## 1. Introduction

Cellular regulation of the tRNA methyltransferases may be mediated, in part, through the action of competing methyltransferase systems [1]. These regulatory methyltransferases compete for the methyl donor *S*-adenosylmethionine and generate *S*-adenosylhomocysteine as a product [2]. *S*-Adenosylhomocysteine has been shown to be a potent inhibitor of tRNA methyltransferases, whereas competing methyltransferases are quite refractory to inhibition by this compound [2]. To date, three competing methyltransferase systems have been described: glycine-*N*-methyltransferase from rabbit, mouse and rat tissues [1]; nicotinamide methyltransferase from rat and porcine liver, and a human tumor cell line [3]; and catechol-*O*-methyltransferase from rat uterus [1].

In this communication we describe the presence of *S*-adenosylmethionine: homocysteine methyltransferase (EC 2.1.1.10) in embryonic extracts of the housefly *Musca domestica.* The enzyme utilizes *S*-adenosylmethionine to transmethylate homocysteine to form methionine and *S*-adenosylhomocysteine [4]. Our experiments suggest that this enzyme may act as a competing methyltransferase, in addition to participating in methionine biosynthesis.

## 2. Materials and methods

*S*-Adenosyl-L[$^{14}CH_3$]methionine (spec. act. 50 mCi/mmole) was purchased from Amersham-Searle. *S*-Adenosylmethionine was purchased from Boehringer-Mannheim Corp. and was also prepared in our laboratory from baker's yeast by the method of Shapiro and Ehninger [5]. *S*-Adenosylhomocysteine was obtained from Sigma Chemical Co. *Escherichia coli* B transfer RNA was a product of General Biochemicals Corp. L-Homocysteine thiolactone·HCl was purchased from Calbiochem.

*Musca domestica* of the Orlando wild-type strain were maintained in cages at 29°C and embryos were collected as previously described [6]. A cell-free extract from one-hour *Musca domestica* embryos was prepared by homogenizing with 4 vol of 0.02 M potassium phosphate (pH 6.8), 1 M dextrose, 0.004 M β-mercaptoethanol and 0.0004 M EDTA in a Tenbroeck homogenizer. The 20 000 *g* supernatant contained the two methyltransferases. The tRNA methyltransferases were assayed by the method of Sharma et al. [7]. For assaying *S*-adenosylmethionine: homocysteine methyltransferase, the basic procedures were those of Shapiro and Yphantis [8], except that a sodium column was used instead of a lithium column.

## 3. Results

The data in fig.1 illustrate the inhibition of the tRNA methyltransferases and the *S*-adenosylmethionine:homocysteine methyltransferase, from one-hour embryos of *M. domestica,* by *S*-adenosylhomocysteine. At a substrate concentration of 4 μM *S*-adenosylmethionine, the tRNA methyltransferases were 76% inhibited in the presence of 10 μM *S*-adenosylhomocysteine, while greater than 95% inhibitor was observed at a 50 μM concentration of the inhibitor. Under identical conditions, the *S*-adenosylmethionine:

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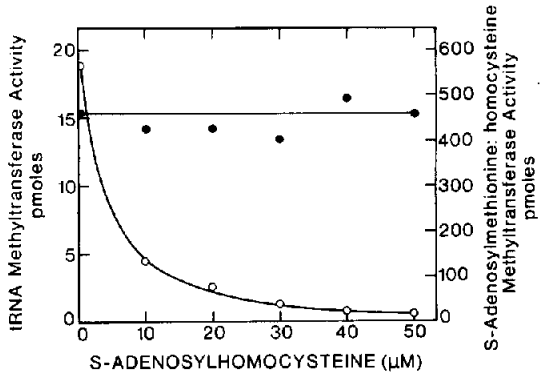


Fig.1. The effect of increasing concentrations of *S*-adenosylhomocysteine on the rate of methylation of tRNA and homocysteine. *S*-Adenosylmethionine:homocysteine methyltransferase activity (•) is expressed as pmoles/mg protein/h while tRNA methyltransferase activity (○) is expressed as pmoles/mg protein/30 min. In each case the reaction mixture contained 4 μM *S*-adenosylmethionine and 1.0 mg of *M. domestica* embryo protein.

homocysteine methyltransferase was not inhibited in the range of 10–50 μM *S*-adenosylhomocysteine.

Since a marked difference was observed in the behavior of tRNA methyltransferases and *S*-adenosylmethionine:homocysteine methyltransferase towards product inhibition by *S*-adenosylhomocysteine, it was of interest to investigate their reaction kinetics for the substrate *S*-adenosylmethionine. Double reciprocal plots resulted in apparent $K_M$ values of $3.6 \times 10^{-6}$ M for the tRNA methyltransferases (fig.2) and $2.8 \times 10^{-3}$ M for the *S*-adenosylmethionine:homocysteine methyltransferase (fig.3).

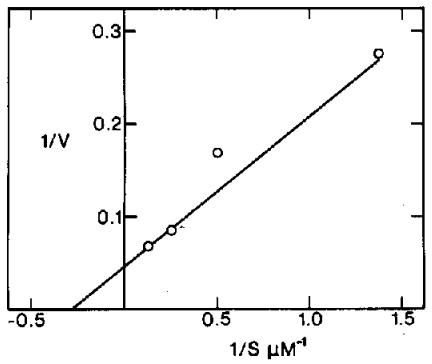


Fig.2. Double reciprocal plot of the tRNA methyltransferase reaction. The concentration of *S*-adenosylmethionine was varied from 0.8 μM to 8.0 μM. The velocity represents pmoles of methyl groups transferred per 100 μg *E. coli* B tRNA. The assay mixture contained 1.0 mg of *M. domestica* embryo protein.

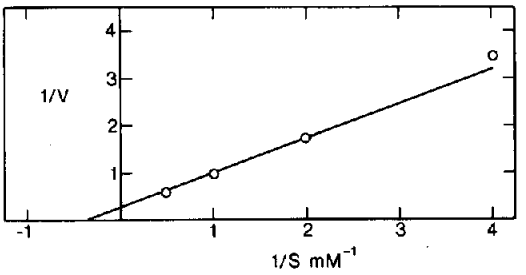


Fig.3. Double reciprocal plot of the *S*-adenosylmethionine: homocysteine methyltransferase reaction. The concentration of *S*-adenosylmethionine was varied from 0.25 mM to 2.0 mM. The velocity represents nmoles of methionine formed in one hour. The assay mixture contained 1.0 mg of *M. domestica* embryo protein.

## 4. Discussion

The present data suggest that *S*-adenosylmethionine homocysteine methyltransferase participates in the cellular regulation of the levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine and thereby influences the physiological expression of the tRNA methyltransferases. This enzyme shares with other competing methyltransferases the important property of being insensitive to inhibition by *S*-adenosylhomocysteine at concentrations which totally inhibit the tRNA methyltransferases. At concentrations of 10–50 μM, *S*-adenosylhomocysteine produces 50% inhibition of tRNA methyltransferases from extracts of rat liver and kidney [9]. Our results with extracts of *M. domestica* embryos agree well with these values. No information exists concerning the intracellular concentration of *S*-adenosylmethionine and *S*-adenosylhomocysteine in insects; however, values of 0.045–0.060 μmol/g wet weight have been reported in rat liver for both compounds [10]. At such physiological concentrations of *S*-adenosylhomocysteine, the tRNA methyltransferases would be subject to inhibition by *S*-adenosylhomocysteine, while the *S*-adenosylmethionine:homocysteine methyltransferase would remain uninhibited.

Competing methyltransferases may also regulate tRNA methyltransferases by competing for the

substrate *S*-adenosylmethionine. We have found an apparent $K_M$ of 3.6 $\mu$M for the tRNA methyltransferases in our system. The *S*-adenosylmethionine: homocysteine methyltransferase from *M. domestica* embryos exhibits an apparent $K_M$ of 2.8 mM. The purified homocysteine methyltransferases from the yeast *Sacchromyces cerevisiae* and jack bean meal exhibited $K_M$ values of 625 $\mu$M and 55 $\mu$M respectively [11,12]. The *S*-adenosylmethionine:homocysteine methyltransferase is similar to other competing methyltransferases in possessing a much higher $K_M$ than the tRNA methyltransferases.

Several properties of the *S*-adenosylmethionine; homocysteine methyltransferase distinguish it from other competing methyltransferases. The glycine-*N*-methyltransferase is found in appreciable quantities only in adult organs being virtually absent from fetal organs [2]. *S*-Adenosylmethionine:homocysteine methyltransferase, however, is present in significant quantities in *M. domestica* embryonic tissue. The products of the glycine-*N*-methyltransferase and the nicotinamide methyltransferase are distal to methionine and *S*-adenosylmethionine biosynthetic pathways. *S*-Adenosylmethionine:homocysteine methyltransferase, on the other hand, has been postulated to participate in the regulation of the internal methionine and *S*-adenosylmethionine levels [13]. This enzyme, therefore, may have a dual function as both a competing methyltransferase and as a regulatory enzyme in the *S*-adenosylmethionine biosynthetic pathway.

## Acknowledgments

This research was supported by Public Health Service Grant AM 14133 from the National Institute of Arthritis and Metabolic Diseases and by National Science Foundation Grant GB 36861. The authors wish to express their appreciation to Dr Fritz Schlenk for his critical reading of this manuscript.

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