Enzymatic Cleavage of S-Adenosylhomocysteine and the Transfer of Labile Methyl Groups *

L.-E. ERICSON**, J. N. WILLIAMS, JR., and C. A. ELVEHJEM

Department of Biochemistry University of Wisconsin Madison 6, Wisconsin, U.S.A.

Evidence has recently been presented that S-(5'-desoxyadenosine-5')-homocysteine, referred to hereafter as S-adenosylhomocysteine, is the primary product resulting from methylations involving S-adenosylmethionine ("active methionine")[1]. S-adenosylhomocysteine has been synthesized by Baddiley and Jamieson [2]. Some experiments with synthetic S-adenosylhomocysteine, kindly supplied by Dr. Baddiley, The Lister Institute of Preventive Medicine, London, have been carried out in our laboratory in order to elucidate the metabolic fate of this compound.

The possibility existed that S-adenosylhomocysteine, and not homocysteine, might be the correct substrate for the methionine-generating enzyme, betaine-homocysteine transmethylase. This enzyme has recently been purified [3] and a comparison could thus be made of the amount of methionine formed from a given amount of S-adenosylhomocysteine (1.1 mg) with that produced by the same preparation of purified enzyme from an equivalent amount of homocysteine (0.35 mg). Methionine was determined by the colorimetric method of McCarthy and Sullivan [4]. It was found, using purified betaine-homocysteine transmethylase, that the yield of methionine from S-adenosylhomocysteine under aerobic or anaerobic conditions was negligible compared to that obtained from homocysteine. On the other hand with whole rat liver homogenate S-adenosylhomocysteine was observed to possess up to 40 % of the activity of homocysteine.

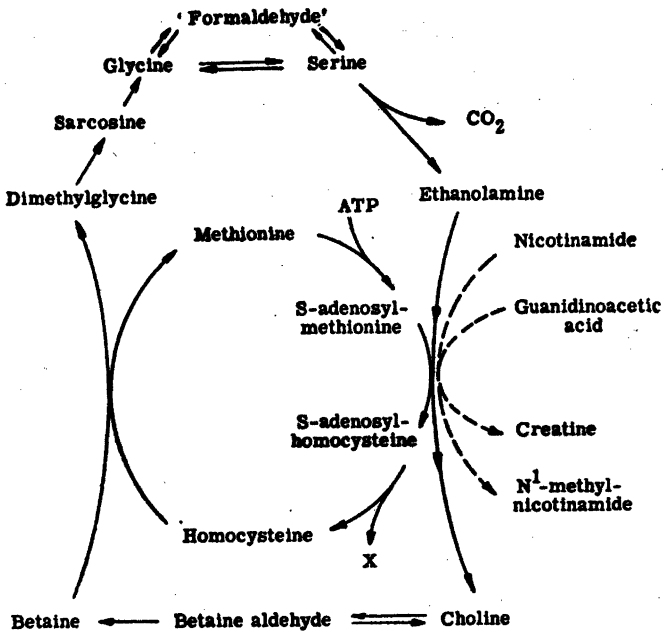
To study the reaction products formed enzymatically during the incubation of S-adenosylhomocysteine with whole rat liver homogenate, 0.55 mg of substrate and 0.1 ml of a 20 % rat liver homogenate (in 0.04 *M* phosphate buffer of pH 7.3) were incubated for 6 hours at 37° C in a total volume of 0.5 ml. Separation of the reaction products by one-dimensional paper chromatography revealed a ninhydrin-positive spot with the same R_F value as homocysteine in two different solvent systems [5,6]. When S-adenosylhomocysteine and rat liver homogenate were incubated together with betaine, it was observed that the spot with the same R_F as homocysteine decreased in intensity, and a spot with an R_F value identical with that of methionine in the two solvent systems appeared. A spot with the same R_F value as α-aminobutyric acid in the two solvent systems referred to above [5,6] as well as in a third system (2-methoxyethanol : propionic acid : water, 60:20:20 v/v) was also detected. The intensity of this spot was slightly reduced when betaine was added to the incubation mixture. Also, a ninhydrin-positive compound with the same R_F value as α-aminobutyric acid appeared on the chromatograms after incubation of either homocysteine or homoserine with rat liver homogenate, and the intensity of this spot was increased by addition of glutamate to the incubation mixture. No homoserine could be found in the incubation mixtures containing S-adenosylhomocysteine and rat liver homogenate. These observations indicate that, in the experiments with S-adenosylhomocysteine, α-aminobutyric acid was formed mainly from the homocysteine resulting from the enzymatic cleavage of S-adenosylhomocysteine. The identification of α-aminobutyric acid as one of the reaction products of the enzymatic degradation of S-adenosylhomocysteine offers an explanation for the finding by Dent [7] that human beings fed a large dose of methionine excrete appreciable amounts of α-aminobutyric acid in the urine.

In separate experiments using the purified apoenzyme, it was found that S-adenosylhomocysteine could not serve as a cofactor [3] for betaine-homocysteine transmethylase.

Considering the results of other investigators (for references, see [8,9]) together with our own [3], an attempt has been made to summarize the present knowledge about transmethylation reactions in animals (Fig. 1). It is likely that the formation of anserine [10] and of adrenalin [11,12] could be

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Nutrition Foundation, Inc., New York.

** Present address: Royal Institute of Technology, Stockholm, Sweden.



included in Fig. 1 in a manner similar to that used for creatine and N′-methylnicotinamide. It is also possible that dimethylthetin and dimethylpropiothetin should be included in this diagram as donors of methyl groups to homocysteine [13] in the same way as shown for betaine.

1. Cantoni, G. L. and Scarano, E. *J. Am. Chem. Soc.* **76** (1954) 4744.
2. Baddiley, J. and Jamieson, G. A. *In press.*
3. Ericson, L.-E., Williams, J. N., Jr. and Elvehjem, C. A. *J. Biol. Chem.* **212** (1955) 537.
4. McCarthy, T. E. and Sullivan, M. X. *J. Biol. Chem.* **141** (1941) 871.
5. Clayton, R. A. and Strong, F. M. *Anal. Chem.* **26** (1954) 1362.
6. Ericson, L.-E. and Lewis, L. *Arkiv Kemi* **6** (1953) 427.
7. Dent, C. E. *Science* **105** (1947) 335.
8. Cantoni, G. L. in McElroy, W. D. and Glass, B. *Phosphorus Metabolism II*, The Johns Hopkins Press, Baltimore 1952, pp. 129—152.
9. Fruton, J. S. and Simmonds, S. *General Biochemistry*, J. Wiley and Sons, Inc., New York 1953, pp. 697—716.
10. Schenck, J. R., Simmonds, S., Cohn, M., Stevens C. M. and du Vigneaud, V. *J. Biol. Chem.* **149** (1943), 355.
11. Fumio, I. *Med. J. Osaka Univ.* **1** (1945) 1.
12. Keller, E. B., Boissonnas, R. A. and du Vigneaud, V. *J. Biol. Chem.* **183** (1950) 627.
13. Dubnoff, J. W. and Borsook, H. *J. Biol. Chem.* **176** (1948) 789.

Received May 2, 1955.