Judging from the reducting power of ferric ions described here, the antioxidative potency increased in the order PG, NDGA, BHA, EP and BHT. It has been reported that the combinations of BHA with BHT or of BHA with PG showed synergism in the protection of lard from peroxidation.[5] In this study, the combination of BHA and BHT showed synergism, while that of BHA and PG showed inhibitory effects. Thus, the potencies of these antioxidants towards the reduction of ferric ions were not in accord with their antioxidative effects on lard.

As early as 1938, Emmerie and Engel[6] proposed an elegant colorimetric determination of α-tocopherol on the basis of its reducing power against ferric chloride, and this method has been applied to the analysis of several antioxidants with slight modifications.[7,8,14,15] It was noted that application of the Emmerie–Engel method to the estimation of antioxidants was not suitable when more than two antioxidants are present, since several combinations greatly modified the reducing potency of the antioxidants against ferric ions.

14) M.H. Stern and J.G. Baxter, *Anal. Chem.*, **19**, 902 (1947).
15) C.C. Tsen, *Anal. Chem.*, **33**, 849 (1961).

[Chem. Pharm. Bull. 28(7)2232—2234(1980)]

Inhibition of S-Adenosylmethionine Decarboxylase from Rat Liver by Synthetic Decarboxylated S-Adenosylmethionine and Its Analogs

Banri Yamanoha and Keijiro Samejima

Tokyo Biochemical Research Institute[1]

(Received February 1, 1980)

Synthetic decarboxylated S-adenosylmethionine and six analogs were tested in a decarboxylating system based on rat liver S-adenosylmethionine decarboxylase. All the compounds inhibited the putrescine-activated decarboxylase activity and were competitive with respect to S-adenosylmethionine. Among the compounds tested, S-5′-deoxyadenosyl-(5′)-2-methylthioethylamine was the most potent inhibitor. The K_i value of the inhibitor was seven times lower than that of decarboxylated S-adenosylmethionine.

Keywords——S-adenosylmethionine decarboxylase; polyamines; new inhibitor; product inhibition; decarboxylated S-adenosylmethionine analogs

S-Adenosylmethionine decarboxylase (EC 4.1.1.50) is one of the two key decarboxylases in polyamine biosynthesis. The enzyme from rat liver has been purified and characterized as having a pyruvoyl prosthetic group.[2,3] The product, decarboxylated S-adenosylmethionine, is known to inhibit the enzyme reaction activated by putrescine *in vitro*,[4] since it binds to the enzyme more tightly than the substrate. Recently, we succeeded in synthesizing various analogs of decarboxylated S-adenosylmethionine,[5] and we have studied the effects of these analogs in a spermidine synthesizing system from rat prostate.[6] The present paper deals with the effects of these analogs in the decarboxylating system.

1) Location: *Takada 3-41-8, Toshima-ku, Tokyo 171, Japan.*
2) A.E. Pegg, *FEBS Lett.*, **84**, 33 (1977).
3) A.A. Demetriou, M.S. Cohn, and H. Tabor, *J. Biol. Chem.*, **253**, 1684 (1978).
4) *a*) J. Jänne, A. Schenone, and H.G. Williams-Ashman, *Biochem. Biophys. Res. Commun.*, **42**, 758 (1971); *b*) J. Jänne, H.G. Williams-Ashman, and A. Schenone, *Biochem. Biophys. Res. Commun.*, **43**, 1362 (1971); *c*) H. Pösö, R. Sinervirta, and J. Jänne, *Biochem. J.*, **151**, 67 (1975).
5) K. Samejima, Y. Nakazawa, and I. Matsunaga, *Chem. Pharm. Bull.*, **26**, 1480 (1978).
6) K. Samejima and Y. Nakazawa, *Arch. Biochem. Biophys.*, **201**, 241 (1980).

Experimental

Materials——Decarboxylated S-adenosylmethionine (S-5′-deoxyadenosyl-(5′)-3-methylthiopropylamine), S-5′-deoxyadenosyl-(5′)-2-methylthioethylamine, S-5′-deoxyadenosyl-(5′)-2-ethylthioethylamine, S-5′-deoxyadenosyl-(5′)-3-ethylthiopropylamine, S-5′-deoxyadenosyl-(5′)-3-propylthiopropylamine, S-5′-deoxyadenosyl-(5′)-3-butylthiopropylamine, and S-5′-deoxyadenosyl-(5′)-4-methylthiobutylamine were prepared by the reported methods.[5] S-Adenosyl-L-methionine hydrogensulfate (from yeast) was purchased from Boehringer Mannheim, Germany, and S-adenosyl-L-(carboxyl-^{14}C)-methionine (60 mCi/mmol) from New England Nuclear, U.S.A. Putrescine dihydrochloride was from Tokyo Kasei Co., Japan. Methylglyoxal bis(guanylhydrazone) (MGBG) was obtained from Aldrich Chem. Inc., U.S.A.

Enzyme Preparation and Assay Procedures——The procedures for purifying S-adenosylmethionine decarboxylase from rat liver were the same as those of Pegg.[7] The DEAE-cellulose eluate (step 5) after affinity chromatography twice using columns of MGBG linked to Sepharose was stocked at −20° until used. The enzyme was purified about 1600-fold as compared with 105000 ***g*** supernatant of step 1.

S-Adenosylmethionine decarboxylase was assayed by the method of Pegg and Williams-Ashman[8] except for the use of 0.5 μM S-adenosyl-L-(carboxyl-^{14}C)-methionine (40000 cpm per milliliter of incubation mixture). Various concentrations of the substrate (0.5 to 41.7 μM) were prepared by adding unlabelled S-adenosyl-L-methionine to the incubation mixtures. Preliminary data showed that a remarkable product inhibition occurred under incubation conditions producing decarboxylated S-adenosylmethionine at a final concentration of about 5 μM. Hence, a reduced amount of the enzyme (0.6 μg) was used, producing 0.86 μM decarboxylated S-adenosylmethionine after incubation for 30 min at 37° in the presence of 41.7 μM S-adenosyl-L-methionine. The inhibition by the final concentration of 0.86 μM product could be neglected.

Results and Discussion

The effects of the seven compounds listed in Table I on the decarboxylation were examined. All the compounds inhibited the enzyme reaction, and were competitive with respect to S-adenosyl-L-methionine. The apparent K_m value for the substrate was 27.4 μM, which is similar to that reported.[8] The K_i value for decarboxylated S-adenosylmethionine was 6.3 μM, which is higher than the reported value of about 1 μM.[4c] This difference might be due to the fact that the synthetic sulfonium compounds used here are all diastereoisomers with respect to the triply substituted sulfur atom.

Table I. K_i Values for Decarboxylated S-Adenosylmethionine and Its Analogs[a]

Compounds	K_i (μM)[b]
S-5′-Deoxyadenosyl-(5′)-	
2-methylthioethylamine	0.9±0.2[c]
2-ethylthioethylamine	3.0±0.5[c]
3-methylthiopropylamine	6.3±0.6[c]
3-ethylthiopropylamine	21.6±0.6[d]
3-propylthiopropylamine	21.1
3-butylthiopropylamine	23.6±4.4[d]
4-methylthiobutylamine	22.1±3.0[c]

a) Assay conditions are described in the text. K_i values were calculated from the double-reciprocal lines obtained by the least-squares method.
b) Mean±standard error of K_i values obtained at three different concentrations (10, 20 and 40 μM).
c) Two different concentrations (40 and 100 μM).
d) 40 μM S-5′-deoxyadenosyl-(5′)-3-propylthiopropylamine.

S-5′-Deoxyadenosyl-(5′)-2-methylthioethylamine and S-5′-deoxyadenosyl-(5′)-2-ethylthioethylamine were more potent inhibitors than decarboxylated S-adenosylmethionine, and the other compounds were found to be weaker inhibitors. These results indicate that decarboxylated S-adenosylmethionine analogs carrying an aminoethyl group in place of the

7) A.E. Pegg, *Biochem. J.*, **141**, 581 (1974).
8) A.E. Pegg and H.G. Williams-Ashman, *J. Biol. Chem.*, **244**, 682 (1969).

aminopropyl (or the aminobutyl) group can act as potent inhibitors, while the analogs carrying an ethyl, propyl, or butyl group in place of the methyl group are less effective.

Several inhibitors of mammalian S-adenosylmethionine decarboxylase are now available, such as MGBG,[4b,9] 1,1′-(methylethylethanediylidenedinitrilo)-bis-(3-aminoguanidine),[10] and S-adenosyl-L-ethionine.[11] The K_i value for MGBG was 0.1 μM, which is about 10 times lower than that for S-5′-deoxyadenosyl-(5′)-2-methylthioethylamine. MGBG, however, was a non-specific inhibitor, since it noncompetitively affected thymic diamine oxidase activity with a K_i value of less than 1 μM.[12] The other two inhibitors were also non-specific. A more specific inhibition of polyamine biosynthesis by S-5′-deoxyadenosyl-(5′)-2-methylthioethylamine might be expected, because decarboxylated S-adenosylmethionine is not known to participate in any other biochemical pathways apart from the biosynthesis of spermidine and spermine.[13]

S-5′-Deoxyadenosyl-(5′)-2-methylthioethylamine also inhibited spermidine synthase activity from rat prostate in a competitive fashion with respect to decarboxylated S-adenosylmethionine, and had no substrate activity.[6] Hence, if the inhibitor penetrates into cells and has a sufficient half-life in the cells, synthesis of spermidine should be influenced by the inhibition of the two enzymes. These possibilities must be examined.

Acknowledgement This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan. The authors are very grateful to Dr. Masashi Okada, Director of this Institute, for his support during this work.

9) H.G. Williams-Ashman and A. Schenone, *Biochem. Biophys. Res. Commun.*, **46**, 288 (1972).
10) A.E. Pegg and C. Conover, *Biochem. Biophys. Res. Commun.*, **69**, 766 (1976); A.E. Pegg, *J. Biol. Chem.*, **253**, 539 (1978); L. Wiegand and A.E. Pegg, *Biochem. Biophys. Acta*, **517**, 169 (1978).
11) A.E. Pegg, *Biochem. Biophys. Acta*, **177**, 361 (1969).
12) E. Hölttä, P. Hannonen, J. Pispa, and J. Jänne, *Biochem. J.*, **136**, 669 (1973).
13) H.G. Williams-Ashman, A. Corti, and B. Tadolini, *Ital. J. Biochem.*, **25**, 5 (1976).