Communications to the Editor

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TRANS-4-METHYLCYCLOHEXYLAMINE, A POTENT NEW INHIBITOR OF SPERMIDINE SYNTHASE[1)]

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Trans-4-methylcyclohexylamine was found to inhibit pig spermidine synthase potently in assay conditions containing 50 μM of decarboxylated S-adenosylmethionine. It showed 50% inhibition at 1.7 μM compared to 8.1 μM for cyclohexylamine, and 2.5 μM for S-adenosyl-1,8-diamino-3-thiooctane, known as the most potent inhibitor. The inhibition was competitive with putrescine, similar to cyclohexylamine, and the Ki was 40 nM.

KEYWORDS—spermidine synthase; inhibitor; cyclohexylamine; S-adenosyl-1,8-diamino-3-thiooctane; 4-methylcyclohexylamine

Spermidine synthase, an aminopropyltransferase, catalyzes the tranfer of the aminopropyl moiety from decarboxylated S-adenosylmethionine (decarboxy AdoMet) to putrescine to form spermidine. Recent advances in polyamine research have suggested reevaluation of the enzyme as a target one to deplete cellular polyamine by inhibiting it.[2)]. As a part of our studies on spermidine synthase, we have so far tested more than a hundred compounds of a series structurally related to putrescine for their substrate and/or inhibitor activity. This was to obtain some spatial information on the putrescine binding site of the enzyme. In the course of these studies, we have found a new potent inhibitor, trans-4-methylcyclohexylamine. Here we report its inhibitory properties compared with those of the available inhibitors S-adenosyl-1,8-diamino-3-thiooctane (AdoDATO),[3)] a multisubstrate adduct inhibitor known as the most potent and specific inhibitor to the enzyme, and cyclohexylamine,[4)] a putrescine-competitive inhibitor recently found to specifically inhibit cellular spermidine synthase much like AdoDATO.[5)]

Trans-4-methylcyclohexylamine was prepared in a hydrochloride form by repeated crystallization from a mixture of cis/trans free base (Aldrich). The authentic cis or trans isomer was synthesized using the Mitsunobu reaction[6)] with trans- or cis-4-methylcyclohexanol, respectively. Each isomer was confirmed by NMR. The AdoDATO was kindly donated by Professor James K. Coward, University of Michigan, Ann Arbor. Cyclohexylamine hydrochloride was purchased from Wako Pure Chemical, Osaka, Japan. Tritiated decarboxy AdoMet was prepared from [methyl-^{3}H]-S-adenosyl methionine(73.8 Ci/mmol, New England Nuclear) by enzymatic decarboxylation with *E. coli* S-adenosylmethionine decarboxylase.[7)] Unlabeled decarboxy AdoMet was prepared as described previously[8)] and used to dilute the labeled one. Since the synthetic decarboxy AdoMet is a mixture of stereoisomers, the concentration is expressed as that of the S

isomer that is the substrate of the enzyme. Spermidine synthase was purified to homogeneity from pig liver according to the reported method.[9] After the enzyme reaction we measured the stoichiometric release of labeled 5'-methylthioadenosine from labeled decarboxy AdoMet in the presence of putrescine.[10]

As commercially available 4-methylcyclohexylamine, which is a mixture of cis and trans form, was the most potent inhibitor of the reaction of pig spermidine synthase among tested compounds, we first examined which isomer was most inhibitive. The results using the authentic samples are summarized in Table I. Under the standard assay conditions where the reaction mixture contained 1 mM putrescine and 10 μM decarboxy AdoMet, the trans form showing an IC_{50} at 1.7 μM was over 200 times more potent than the cis form. Obviously the trans form plays a leading role in the inhibition. To compare the inhibitory activities of the trans form and the available inhibitors, we next examined AdoDATO and cyclohexylamine under the same standard assay conditions. As was expected, AdoDATO had the lowest IC_{50} value of the three inhibitors (Table I). However, under a modified assay condition where the reaction mixture contained a higher concentration of decarboxy AdoMet (50 μM), AdoDATO alone decreased its inhibitory activity, and the inhibition was next to trans-4-methylcyclohexylamine which was the most potent in this case. Like cyclohehxylamine, trans-4-methylcyclohexylamine was competed with putrescine. The Ki value for trans-4-methylcyclohexylamine and Km for putrescine were 40 nM and 100 μM respectively.

Table I. Inhibition of Spermidine Synthase

Compound	IC_{50}[a] (μM) in the presence of 1 mM putrescine and decarboxy AdoMet,	
	10 μM	50 μM
Trans-4-methyl-cyclohexylamine	1.7	1.7
Cis-4-methyl-cyclohehxyamine	430	
Cyclohexylamine	8.7	8.1
AdoDATO	0.45	2.5

a) Concentration exhibiting 50% inhibition.

Many attempts to deplete cellular polyamine have been made by using α-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase.[2] It became clear through a number of these experiments that a marked

decrease of cellular putrescine and spermidine caused by DFMO leads to a large compensatory increase in the amount of ornithine decarboxylase and S-adenosylmethionine decarboxylase in the polyamine biosynthesis, followed by a large increase in decarboxy AdoMet.[11] For investigating the effects of inhibition of spermidine synthase on the cellular polyamine level and cell growth in these DFMO-treated cells, the present inhibitor, trans-4-methylcyclohexylamine which is much more potent than cyclohexylamine, should be more useful than AdoDATO, since the inhibition is competitive with putrescine and is not affected by the higher concentrations of decarboxy AdoMet.

REFERENCES AND NOTES

1) This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.
2) A.E. Pegg, Cancer Res., 48, 759(1988).
3) K-C. Tang, A.E. Pegg, and J.K. Coward, Biochem.Biophys.Res.Commun.,96,1371(1980).
4) H. Hibasami, M. Tanaka, J. Nagai, and K. Ikeda, FEBS Lett., 116, 99(1980).
5) J.L.A. Mitchell, D.W. Mahan, P.P. McCann, and P.Qasba, Biochim.Biophys.Acta, 840, 309(1985).
6) O. Mitsunobu, M. Wada, and T. Sano, J.Am.Chem.Soc., 94, 679(1972).
7) V. Zappia, P. Galletti, A. Oliva, and M. Porcelli, Methods Enzymol., 94, 73(1983).
8) K. Samejima, Y. Nakazawa, and I. Matsunaga, Chem.Pharm.Bull., 26, 1480(1978).
9) B. Yamanoha, K. Samejima, T. Nakajima, and T. Yasuhara, J.Biochem., 96,1273(1984).
10) A. Raina, T. Erolanta, and R-L. Pajula, Methods Enzymol., 94, 257(1983).
11) A.E. Pegg, H. Poso, K. Shuttleworth, and R.A. Bennet, Biochem.J., 202, 519(1982); P.S. Mamont, C. Danzine, J. Wagner, M. Siat, A.-M. Joder-Ohlenbusch, and N. Claverie, Eur. J. Biochem., 123,499(1982).

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