8. P. Modéus, J. Hogberg and S. Orrenius, in *Methods in Enzymology*, Vol. 52 (Eds. S. Fleischer and L. Packer), p. 60. Academic Press, New York (1978).
9. H. Morrison, V. Hammarskiöld and B. Jernström, *Chem.-Biol. Interact.* **45**, 235 (1983).
10. A. Karakaya and D. E. Carter, *J. Chromatogr.* **195**, 431 (1980).
11. M. H. Grant, M. A. L. Melvin, P. Shaw, W. T. Melvin and M. D. Burke, *FEBS Lett.* **190**, 99 (1985).
12. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
13. P. J. Hissin and R. Hilf, *Analyt. Biochem.* **74**, 214 (1976).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. W. J. Dixon (Ed.), *Biomedical Computer Programs*, University of California Press (1983).
16. T. Croci and G. M. Williams, *Biochem. Pharmac.* **34**, 3029 (1985).
17. A. R. Steward, G. A. Dannan, P. S. Guzelian and F. P. Guengerich, *Molec. Pharmac.* **27**, 125 (1985).
18. F. J. Wiebel, J. Singh, E. Schindler and K-H. Summer, *Toxicology*, **17**, 123 (1980).
19. J. L. Driscoll, N. T. Hayner, R. Williams-Holland, G. Spies-Karotkin, P. M. Galletti and H. O. Jouregui, *In Vitro*, **18**, 835 (1982).
20. M. H. Grant, W. T. Melvin and M. D. Burke, *Proceedings of International Conference on Practical* In Vitro *Toxicology*, p. 5.13 (1985).
21. N. W. Toribara, R. B. Kirkpatrick, C. N. Falany, D. R. La Brecque and T. R. Tephly, *Hepatology*, **4**, 1054 (1984).

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Methylglyoxal bis(butylamidinohydrazone), a new inhibitor of polyamine biosynthesis that simultaneously inhibits ornithine decarboxylase, adenosylmethionine decarboxylase and spermidine synthase

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Aliphatic polyamines, putrescine, spermidine and spermine, seem to be essential for cell growth, and they are considered to serve as control intermediates in cellular responses to hormones, growth factors and other environmental signals [1]. For this reason there has been a great deal of interest in determining the regulatory mechanisms of polyamine biosynthesis. Additionally, considerable efforts have also been made to develop and examine inhibitors for polyamine biosynthesis. These inhibitors appear to be clinically important in the treatment of cancer [2], and they are useful in determining the functions of the polyamines in normal cell growth and differentiation [3].

The most widely used inhibitors of polyamine biosynthesis are difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC; EC 4.1.1.17) [4] and methylglyoxal bis(guanylhydrazone) (MGBG), a potent inhibitor of putrescine-activated adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50) [5]. MGBG is used very often in polyamine researches [6, 7], but the severe side effects [8, 9] are compromising its usefulness.

In view of the obvious advantages afforded by more specific inhibitors of polyamine synthesis, we discovered dicyclohexylamine [10] and recently synthesized *N*-chlorosulfonyl-dicyclohexylamine [11] as the inhibitors for spermidine synthase. These compounds have been proven effective in altering polyamine metabolism in several mammalian and bacterial systems [11–15].

In the present paper we report that methylglyoxal bis(butylamidinohydrazone) (MGBB) exerts its inhibitory effects on three different enzymes in the polyamine biosynthetic pathway.

Materials and methods

Chemicals. MGBB was synthesized as described elsewhere [16], principally according to the method previously published [17]. DL-[1-^{14}C]ornithine (sp. act. 57.6 mC$_i$/mmole), *S*-adenosyl-L-[carboxy-^{14}C]methionine (sp. act. 58 mC$_i$/mmole) and *S*-adenosyl-L-[methyl-^{14}C]methionine (sp. act. 53.6 mC$_i$/mmole) were purchased from New England Nuclear Corp. (Boston, MA). Decarboxylated *S*-adenosylmethionine, both unlabeled and labeled in the methyl group, was prepared by the action of AdoMetDC from *Escherichia coli* (strain B) and purified by chromatography on Dowex-50-H$^+$ and paper electrophoresis [18]. All other chemicals were products of Nakarai Chemicals Ltd.

Enzyme preparations. ODC from Ehrlich ascites tumor cells [19], AdoMetDC from rat liver [20] and spermidine and spermine synthases from rat ventral prostate [10] were prepared as described in previous publications. Protein was determined by the method of Bradford [21] using bovine serum albumin as a standard.

Enzyme assays. The activities of ODC [22], AdoMetDC [23], spermidine and spermine synthases [24] were measured as described earlier.

Results and discussion

MGBB showed the inhibition of ODC, AdoMetDC and spermidine synthase activities. To our surprise, this compound, a derivative of AdoMetDC inhibitor MGBG, inhibited ODC more sensitively than AdoMetDC. The effect of the concentration of ornithine on the inhibition of ODC by MGBB is shown in Fig. 1. This inhibition was competitive with ornithine, and the calculated K_i for MGBB was 3.5 μM. The K_m value for ornithine was estimated to be 0.33 mM.

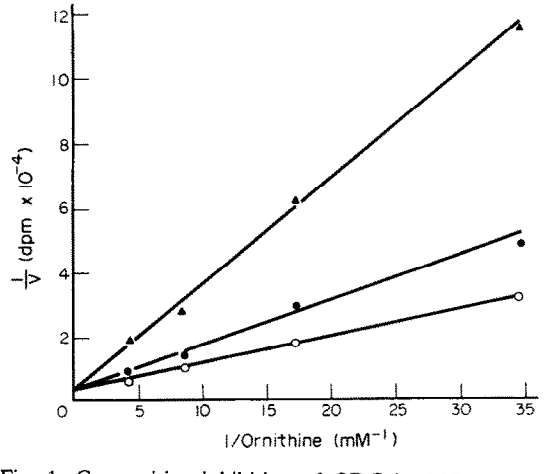

Fig. 1. Competitive inhibition of ODC by MGBB with ornithine as the variable substrate. ODC activity was assayed in the absence (○) or presence of 2 (●) or 10 μM (▲) MGBB, with 0.029–0.232 mM ornithine and 72 μg enzyme protein.

Figure 2 shows the effect of the concentration of AdoMet on the inhibition of AdoMetDC by MGBB. The inhibition was competitive with AdoMet. The K_i value for MGBB and K_m for AdoMet were calculated to be 18 μM and 0.20 mM, respectively.

Spermidine synthase, but not spermine synthase, was inhibited noncompetitively by MGBB (Fig. 3). The K_i and K_m values for MGBB and putrescine were 80 and 23 μM, respectively. Removal of MGBB from the active form of spermidine synthase by dialysis restored the activity to that found in preparations treated similarly except for exposure to the inhibitor. Thus, the inhibition of spermidine synthase by MGBB was reversible.

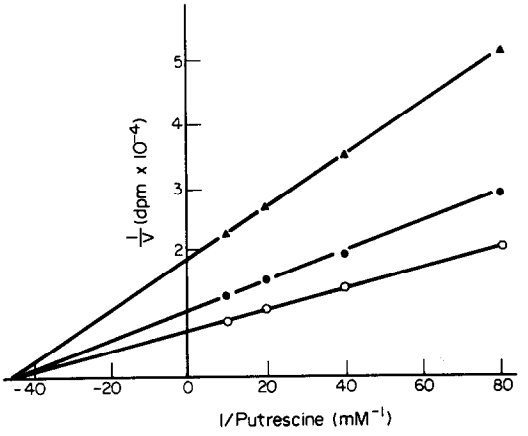

Fig. 2. Competitive inhibition of AdoMetDC by MGBB with AdoMet as the variable substrate. ADoMetDC activity was assayed in the absence (○) or presence of 20 (●) or 100 μM (▲) MGBB, with 0.025–0.2 mM AdoMet and 56 μg enzyme protein.

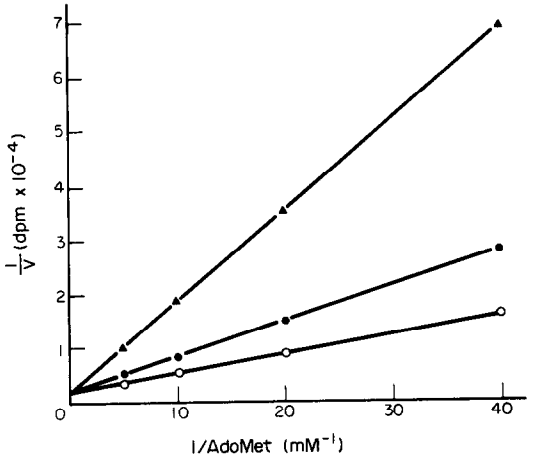

Fig. 3. Noncompetitive inhibition of spermidine synthase by MGBB with putrescine as the variable substrate. Spermidine synthase activity was assayed in the absence (○) or presence of 50 (●) or 200 μM (▲) MGBB, with 40 μM decarboxylated AdoMet, 0.0125–0.1 mM putrescine and 82 μg enzyme protein.

MGBB may construct the homologous conformation to L-α-ornithine as well as to AdoMet, resulting in competitive inhibitions of ODC and AdoMetDC. Noncompetitive inhibition of spermidine synthase using putrescine as the variable substrate suggested that MGBB bound to the decarboxylated AdoMet-binding site.

In summary, MGBB was shown to be a potent competitive inhibitor of ODC (K_i = 3.5 μM) and AdoMetDC (K_i = 18 μM), and, to a lesser extent, it also inhibited spermidine synthase (K_i = 80 μM). Spermine synthase was not inhibited by this drug.

Department of Biochemistry
Mie University School of Medicine
Tsu, Mie 514, Japan

HIROSHIGE HIBASAMI
TETSUYA TSUKADA
SATORU MAEKAWA
KUNIO NAKASHIMA

REFERENCES

1. C. W. Tabor and H. Tabor, *A. Rev. Biochem.* **53**, 749 (1984).
2. A. Sjoerdsma and P. J. Shechter, *Clin. Pharmac. Ther.* **35**, 287 (1984).
3. A. E. Pegg and P. P. McCann, *Am. J. Physiol.* **243**, C212 (1982).
4. B. W. Metcal, P. Bey, C. Danzin, M. J. Jung, P. Casara and J. P. Vevert, *J. Am. chem. Soc.* **100**, 2551 (1978).
5. H. G. Williams-Ashman and A. Schenone, *Biochem. biophys. Res. Commun.* **46**, 288 (1972).
6. P. S. Mamont, M.-C. Duchesne, J. Grove and P. Bey, *Biochem. biophys. Res. Commun.* **81**, 58 (1978).
7. J. Jänne, H. Pösö and A. Raina, *Biochim. biophys. Acta* **473**, 241 (1978).
8. J. Jänne, E. Hölttä, A. Kallio and K. Käpyaho, *Spec. Top. Endocrin. Met.* **5**, 227 (1983).
9. C. W. Porter, F. Mikles-Robertson, D. Kramer and C. Dave, *Cancer Res.* **39**, 2414 (1979).
10. H. Hibasami, M. Tanaka, J. Nagai and T. Ikeda, *FEBS Lett.* **116**, 99 (1980).
11. H. Hibasami, T. Tsukada and K. Nakashima, in *Polyamines: Basic and Clinical Aspects* (Eds. K. Imahori, F. Suzuki, O. Suzuki and U. Bachrach), p. 243. VNU Science Press, Utrecht (1985).
12. H. Hibasami and H. Ito, *Jpn. J. Cancer Res. (Gann)* **72**, 512 (1981).
13. A. J. Bitonti, P. P. McCann and A. Sjoerdsma, *Biochem. J.* **208**, 435 (1982).
14. R. Porta, M. Camardella, V. Gentile and A. DeSantis, *J. Neurochem.* **42**, 321 (1984).
15. A. E. Pegg, A. J. Bitonti, P. P. McCann and J. K. Coward, *FEBS Lett.* **155**, 192 (1983).
16. H. Hibasami, T. Tsukada, S. Maekawa and K. Nakashima, *Cancer Lett.* **30**, 17 (1986).
17. H. Nishimura, H. Toku, C. Yamazaki, K. Hasegawa, H. Sakaguchi and D. Nagaki, *Kitasato Archs Exp. Med.* **48**, 31 (1975).
18. H. Pösö, P. Hannonen and J. Jänne, *Acta chem. scand.* **B30**, 807 (1976).
19. A. Kallio, H. Pösö, S. K. Guha and J. Jänne, *Biochem. J.* **166**, 89 (1977).
20. A. E. Pegg, *Biochem. J.* **141**, 581 (1974).
21. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
22. J. E. Seely, H. Pösö and A. E. Pegg, *Biochemistry* **21**, 3394 (1982).
23. A. E. Pegg, H. G. Williams-Ashman, *J. biol. Chem.* **244**, 682 (1969).
24. H. Hibasami and A. E. Pegg, *Biochem. J.* **169**, 709 (1978).