METHYL GLYOXAL BIS(GUANYLHYDRAZONE) AS A POTENT INHIBITOR OF MAMMALIAN AND YEAST S-ADENOSYLMETHIONINE DECARBOXYLASES

H.G. Williams-Ashman and Amelia Schenone

The Ben May Laboratory for Cancer Research and Department of Biochemistry
University of Chicago, Chicago, Illinois 60637

Received November 1, 1971

Abstract: The putrescine-activated S-adenosylmethionine decarboxylases of rat ventral prostate and baker's yeast are strongly inhibited by methyl glyoxal bis(guanylhydrazone), a substance which has been reported to exhibit anti-leukemic actions. Low concentrations of the drug preferentially inhibit the enhancement by putrescine of the decarboxylation of S-adenosylmethionine by the prostate enzyme. The magnesium ion-activated and putrescine insensitive S-adenosylmethionine decarboxylase of E. coli is inhibited only at very high concentrations of methylglyoxal bis(guanylhydrazone). Some observations on the specificity of activation of the yeast and mammalian S-adenosylmethionine decarboxylases by short chain aliphatic diamines are also discussed.

Recent investigations have indicated that in a variety of animal tissues (1-7) and baker's yeast (8,9), the major if not sole pathway for the biosynthesis of spermidine and spermine involves the following enzymes that are readily separable from one another: (i) an S-adenosylmethionine (S-Ado-met) decarboxylase that is markedly activated by putrescine (ii) an enzyme (spermidine synthase) that catalyzes the formation of spermidine and 5'-methyl thioadenosine (MTA) from decarboxylated S-Ado-met and putrescine; and (iii) an analagous spermine synthase, which promotes the synthesis of spermine from spermidine and decarboxylated S-Ado-met. An availability of powerful and specific inhibitors of one or more of these reactions of polyamine biosynthesis would be of interest not only from an enzymological point of view, but also because such inhibitors (provided that they could penetrate living cells) might depress the formation of spermidine and/or spermine in vivo, and thus might help to throw light on the validity of many current hypotheses concerning the functions of polyamines in animal tissues (3,6,10).

It will be shown here that methyl glyoxal bis(guanylhydrazone) in concentrations of a few micromolar inhibits the putrescine-activated S-Ado-met decarboxylases of rat ventral prostate and yeast, whereas very much higher levels of the drug are required to inhibit the magnesium ion-dependent and putrescine-insensitive S-Ado-met decarboxylase of Escherichia coli. Methyl glyoxal bis(guanylhydrazone) is known to be a toxic substance that inhibits the growth of certain animal and human leukemias (11,12); the effects of the drug against mouse leukemia L1210 can be prevented by concurrent administration of spermidine (11). During the course of the studies to be presented, it was observed that high concentrations of some other short chain aliphatic diamines besides putrescine cause marked activation of rat prostate but not yeast S-Ado-met decarboxylase. Some aspects of the latter phenomena and the influence of methyl glyoxal bis(guanylhydrazone) thereon are also considered.

EXPERIMENTAL PROCEDURES

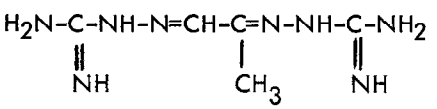
S-Ado-met decarboxylases were purified from rat ventral prostate (4,5) and baker's yeast (8) according to the methods described in the cited references. Unless otherwise stated, the activity of the latter enzymes was determined by following release of $^{14}CO_2$ from 0.2 mM S-Ado-met-^{14}COOH in the presence of 100 mM sodium phosphate pH 7.1, 5 mM dithiothreitol, and, if added, 2.5 mM putrescine at 37° for the times indicated, according to previously described procedures (4,5). The source of S-Ado-met decarboxylase from E. coli was a dialyzed ammonium sulfate (0-80% saturation) fraction obtained from an ultracentrifuged supernatant of a sonicated extract of this organism prepared in 10 mM phosphate buffer pH 7.2-0.1 mM EDTA.

Authentic samples of methyl glyoxal bis(guanylhydrazone) and related compounds were generously donated by Dr. Enrico Mihich (11). Commercial specimens of the dihydrochlorides of propane-1,3-diamine and pentane-1,5-diamine (cadaverine) were recrystallized with the aid of acid-washed Norit using a solvent consisting of 15 parts of ethanol and 1 part of water. These amines were completely separated from one

another, as well as from butane-1,4-diamine (putrescine) by the procedure of Weaver and Herbst (13), involving electrophoresis on paper for 5 hr at 300 v. in 0.03 M citrate buffer of pH 6.5; the various amines were located on the paper strips was determined with the cadmium-ninhydrin reagent (14). Experiments involving application of large quantities of recrystallized propane-1,3-diamine and cadaverine to the paper strips failed to indicate the presence of any putrescine in the samples of the two other aliphatic diamines under conditions where a contaminating level of 0.2% of putrescine could have been readily detected.

RESULTS AND DISCUSSION

Extensive tests on many substances that structurally resemble putrescine and spermidine revealed that remarkably low concentrations of methyl glyoxal bis(guanyl-hydrazone) (MGBG) caused strong inhibition of the putrescine-activated S-Ado-met decarboxylases of rat ventral prostate and liver, and of baker's yeast. The first



methyl glyoxal bis(guanylhydrazone)

experiment depicted in Table 1 shows that the degree of inhibition of the prostate enzyme by 0.002 mM and 0.005 mM MGBG was more or less constant over the pH range of 6.5 to 8.5. In this instance, the activator putrescine was present in a saturating concentration of 2.5 mM; noteworthy in this connection is that the extent of enhancement of prostatic (4) S-Ado-met decarboxylation by putrescine increases greatly as the pH is lowered over the aforementioned range. With 2.5 mM putrescine as activator at pH 7.1, the inhibition of both rat ventral prostate and baker's yeast S-Ado-met decarboxylases was observed to rise steeply over a range of MGBG concentration from 0.0005 mM to 0.005 mM, 50% depression of carbon dioxide release being attained at roughly 0.0015 mM MGBG. In the

absence of putrescine, considerably higher concentrations of MGBG are required to depress decarboxylation of S-Ado-met by the prostate enzyme (experiment 2 of Table 1). Yeast S-Ado-met decarboxylase is virtually inactive without addition of putrescine (8),

TABLE 1

INHIBITION OF RAT VENTRAL PROSTATE S-ADENOSYL METHIONINE DECARBOXYLASE BY METHYL GLYOXAL BIS(GUANYLHYDRAZONE)

pH	Concentration of methyl glyoxal bis(guanylhydrazone)	Concentration of putrescine	CO_2 released from S-Ado-met-^{14}COOH (nmoles/30 min)
		Experiment 1	
6.5	0	2.5 mM	0.80
6.5	0.002 mM	2.5 mM	0.28
6.5	0.005 mM	2.5 mM	0.11
7.3	0	2.5 mM	1.18
7.3	0.002 mM	2.5 mM	0.29
7.3	0.005 mM	2.5 mM	0.10
8.5	0	2.5 mM	0.48
8.5	0.002 mM	2.5 mM	0.17
8.5	0.005 mM	2.5 mM	0.05
		Experiment 2	
7.1	0	0	0.13
7.1	0.04 mM	0	0.05
7.1	0.20 mM	0	0.04
7.1	0	2.5	1.02
7.1	0.04 mM	2.5	0.03
7.1	0.20 mM	2.5	0.03

Each vessel contained 100 mM sodium phosphate of specified pH, 0.2 mM S-Ado-met labeled in the carboxyl group, 5 mM dithiothreitol and purified rat ventral prostate enzyme (4). Incubated at 37^0 for 30 min. The pH values shown were of the whole reaction mixture and were checked with a glass electrode.

so that it is difficult to study the action of inhibitors on the yeast enzyme unless putrescine is in the reaction mixture.

The activity of S-Ado-met decarboxylases from rat ventral prostate, yeast, and E. coli is depressed by substances such as phenylhydrazine, hydrazine and sodium borohydride in concentrations of several millimolar (1,4,8,15). It would appear unlikely, however, that very small amounts of MGBG inhibit the yeast and prostate enzymes simply by virtue of acting as a carbonyl reagent. In this connection it is noteworthy that at pH 7.1, aminoguanidine (the elements of which are present at both ends of the MGBG molecule) at 1 mM does not inhibit the yeast enzyme when putrescine is present, or the prostate decarboxylase in the absence or presence of putrescine, or the E. coli enzyme tested with 20 mM $MgCl_2$ and without putrescine, although higher concentrations of aminoguanidine inhibit S-Ado-met decarboxylase from all three sources. Much higher concentrations of MGBG are required to depress the E. coli as compared with the yeast or prostate S-Ado-met decarboxylase; in the presence of 20 mM $MgCl_2$ at pH 7.1, the E. coli enzyme tested with saturating levels of the S-Ado-met substrate is inhibited 33% and 81% by MGBG in concentrations of 0.1 mM and 1 mM respectively. In all of the aforementioned cases, the inhibitions of enzymic S-Ado-met decarboxylation were practically constant over an initial incubation period of 30 min at 37°. Nearly the same extent of inhibition by 0.002 mM MGBG was observed at both 20° and 40° when the prostate enzyme was determined in the presence of 2.5 mM putrescine. Reduction of the concentration of the putrescine activator from 2.5 mM to a sub-saturating level of 0.1 mM had little influence on inhibition of the prostate enzyme by 0.005 mM MGBG when the initial concentration of S-Ado-met was either 0.1 mM or 0.2 mM. Taken together, these observations are consistent with the view that low concentrations of MGBG interact with the putrescine binding site of yeast and prostate S-Ado-met decarboxylase which is apparently distinct from the substrate (S-Ado-met) binding site. The inhibitory activity of MGBG is greatly reduced if the primary

amino groups of the aminoguanidine moieties at each end of the molecule are substituted with a methyl group.

In earlier experiments (1) it was observed that of a variety of amines and amino acids tested, only putrescine and to a lesser extent spermidine could markedly enhance the S-Ado-met decarboxylase of rat ventral prostate. Using somewhat different incubation media and enzyme preparations, we have now found that high concentrations of cadaverine (1,5-diaminopentane) and of 1,3-diaminopropane will stimulate the decarboxylation of S-Ado-met by relatively crude or highly purified preparations of the prostate enzyme, although the apparent affinity of the prostate decarboxylase for the latter two aliphatic diamines as well as the relative maximal velocities in the presence of an excess of these activators are lower than those exhibited by putrescine (1,4-diaminobutane). A typical experiment illustrating these points is shown in Table 2. The specimens of cadaverine and 1,3-diaminopropane used in these experiments were highly purified, and the results could not be ascribed to their contamination with putrescine. Table 2 additionally shows that MGBG also inhibits the prostate S-Ado-met decarboxylase when either cadaverine or 1,3-diaminopropane are used as activators in place of putrescine. Neither cadaverine nor 1,3-diaminopropane in the concentration of 0.5 mM to 10 mM can replace putrescine as a stimulator of baker's yeast S-Ado-met decarboxylase. In the course of these experiments, we corroborated previous findings (1) that the prostate enzyme is not stimulated by spermine, L-lysine or L-arginine.

The potent inhibition of the putrescine-activated S-Ado-met decarboxylases of rat ventral prostate and yeast by minute concentrations of MGBG raises the question as to whether these substances may depress the formation of decarboxylated S-Ado-met, and hence the biosynthesis of spermidine and spermine, in living eukaryotic cells. We are currently undertaking experiments to examine this possibility. The demonstration that the anti-proliferative action of MGBG against some animal leukemic cells may be reversed by administration of spermidine (11) hints that this substance might conceivably act by inhibiting the formation of polyamines in malignant cells rather than competing with the actions

TABLE 2

Activation of rat ventral prostate S-adenosylmethionine decarboxylase by short chain aliphatic diamines and inhibition of the enzyme by methylglyoxal bis(guanylhydrazone)

Concentration of diamine activator	CO_2 release from S-Ado-met (nmoles/30 min)	Percentage inhibition by 0.01 mM methylglyoxal bis(guanylhydrazone)
0	0.20	43
Putrescine 1 mM	2.46	93
Putrescine 5 mM	2.36	n.d.
Cadaverine 0.5 mM	0.88	n.d.
Cadaverine 1 mM	1.15	n.d.
Cadaverine 5 mM	1.53	95
1,3-diaminopropane 0.5 mM	0.36	n.d.
1,3-diaminopropane 1 mM	0.52	n.d.
1,3-diaminopropane 5 mM	0.92	85

Experimental conditions as in Table 1 except that a cruder preparation of the rat ventral prostate enzyme, taken from stage 2 of a previously described procedure (4), was employed. The inhibitions by methylglyoxl bis(guanylhydrazone) shown in the right hand column were determined in a separate experiment but using enzyme from step 6 of the method J. Jänne and Williams-Ashman (4), which responded in a similar fashion to additions of cadaverine and 1,3-diaminopropane. "n.d." signifies: not determined.

---

of spermidine. Cohen (10) has recently pointed out that the actions of a number of other quite different drugs that can be regarded as substituted diamines are also known to be reversed by spermidine.

ACKNOWLEDGEMENTS

We are greatly indebted to Dr. E. Mihich for suggesting that we examine

the effects of methyl glyoxal bis(guanylhydrazone) on polyamine biosynthetic enzymes, and for providing us with samples of this and related compounds. The investigations were supported in part by a research grant (HD-01453) from the U.S. Public Health Service.

REFERENCES

(1) Pegg, A.E., and Williams-Ashman, H.G., J. Biol. Chem., 244, 682 (1969).
(2) Pegg, A.E., and Williams-Ashman, H.G., Arch. Biochem. Biophys., 137, 156 (1970).
(3) Williams-Ashman, H.G., Pegg, A.E., and Lockwood, D.H., Advances in Enzyme Regulation, 7, 291 (1969).
(4) Jänne, J., and Williams-Ashman, H.G., Biochem. Biophys. Res. Communs., 42, 222 (1971).
(5) Jänne, J., Schenone, A., and Williams-Ashman, H.G., Biochem. Biophys. Res. Communs., 42, 758 (1971).
(6) Raina, A., Jänne, J., Hannonen, P., and Höltta, E., Ann. N.Y. Acad. Sci., 171, 697 (1970).
(7) Raina, A., and Hannonen, P., Fed. Europ. Biochem. Soc. Letters, 16, 1 (1971).
(8) Jänne, J., Williams-Ashman, H.G., and Schenone, A., Biochem. Biophys. Res. Communs., 43, 1362 (1971).
(9) Coppoc, G.L., Kallio, P., and Williams-Ashman, H.G., Int. J. Biochem., in press.
(10) Cohen, S.S., Introduction to the Polyamines, Prentice-Hall, New Jersey (1971)
(11) Mihich, E., Cancer Research, 23, 1375 (1963).
(12) Freedlander, B.L., and French, F.A., Cancer Research, 18, 360 (1958).
(13) Weaver, R.H., and Herbst, E.J., J. Biol. Chem., 231, 637, 647 (1958).
(14) Pegg, A.E., Lockwood, D.H., and Williams-Ashman, H.G., Biochem. J., 117, 17 (1970).
(15) Wicker, R.B., Tabor, C.W., and Tabor, H., J. Biol. Chem., 245, 2132 (1970).