Biochemical and Chemical Characterization of Trifluoromethylglyoxal Bis(guanylhydrazone), a Close Analog of the Antileukemic Drug Mitoguazone

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Adenosylmethionine Decarboxylase Inhibition, *E-Z* Isomerism, Internal Hydrogen Bond, L1210 Leukemia Cells, Structure-Activity Relationships

In order to study the structure-activity relationships of bis(guanylhydrazone) type polyamine antimetabolites, trifluoromethylglyoxal bis(guanylhydrazone) (CF_3-GBG), a close analog of the antileukemic drug methylglyoxal bis(guanylhydrazone) (mitoguazone, MGBG) was synthesized according to a novel modification of previous methods, yielding single crystals. Single-crystal X-ray crystallography revealed the presence of an isomer different from the one detected in the case of MGBG and all other bis(guanylhydrazones) so far studied. In contrast to MGBG, CF_3-GBG was shown to be a very weak inhibitor of yeast adenosylmethionine decarboxylase, being thus devoid of value as a polyamine antimetabolite. In addition, the compound did not have antiproliferative activity against mouse L1210 leukemia cells *in vitro*. As long as analogous isomers of the two compounds are not available, no conclusions can be drawn about the reasons lying behind the drastical differences between their biological properties.

Introduction

The bis(guanylhydrazones) GBG [1] and MGBG [1] (see Fig. 1) are potent antileukemic and antiproliferative agents [2–5]. In addition, they are powerful competitive inhibitors of AdoMetDC, one of the two rate-limiting enzymes of polyamine biosynthesis [5–7], this property obviously being due to the structural resemblance between them and adenosylmethionine, the natural substrate of AdoMetDC [5, 7]. Oddly enough, they are simultaneously structural analogs also of spermidine, a natural polyamine [5, 7] (Fig. 1). The latter property is very important, since it seems to be the reason for the fact that the uptake of GBG and MGBG into tumor cells can be drastically enhanced by a prior treatment with polyamine depleting agents such as DFMO, an inhibitor of the other rate-limiting enzyme of polyamine biosynthesis, namely ornithine decarboxylase [5, 8, 9]. Obviously because of their enhanced uptake, GBG and MGBG act synergistically with DFMO *in vitro, in vivo,* and also when used clinically [5, 7,

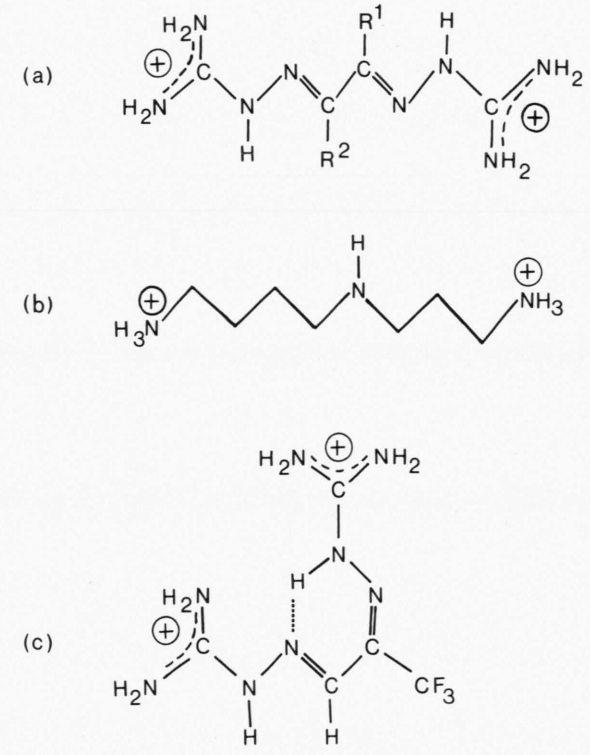

Fig. 1 (a) A generalized structural formula of the diprotonated forms (dications) of GBG ($R^1 = R^2 = H$), MGBG ($R^1 = CH_3$, $R^2 = H$) and various alkyl- and dialkylglyoxal analogs thereof. (b) The structural formula of the dication form of spermidine. (c) The structural formula of the dication form of CF_3-GBG. This formula is based on the present X-ray results.

Abbreviations: AdoMetDC, adenosylmethionine decarboxylase; CF_3-GBG trifluoromethylglyoxal bis(guanylhydrazone); DFMO, α-difluoromethyl ornithine; GBG, glyoxal bis(guanylhydrazone); MGBG, methylglyoxal bis(guanylhydrazone), also known as mitoguazone.

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9–12]. These findings have renewed the interest also in various analogs of GBG and MGBG, even those ones that do not have antiproliferative activity *per se*, since also they might act synergistically with DFMO, if they inhibit AdoMetDC.

Studies on different kinds of analogs are warranted also because the ultimate mechanism of the antiproliferative action of GBG and MGBG is not known and may be unrelated to the effects of the compounds on polyamine metabolism [13–16]. If a large variety of analogs are studied, valuable information on structure-activity relationships may be obtained, which in turn may be useful in the study of the mechanism of action of GBG and MGBG. In recent years, studies on the analogs of the compounds have, however, been largely concentrated on various C- and N-alkylated derivatives only [7, 17–22]. In this paper, we outline some biochemical and chemical characteristics of a different kind of MGBG analog, namely the trifluoromethylglyoxal derivative CF_3-GBG. This compound was chosen for study because the steric properties of the CF_3 group obviously are close to those of the methyl group of MGBG, while, on the other hand, the electronegativity of the fluorine atoms would be expected to profoundly affect the electronic structure of the molecule.

Materials and Methods

Synthesis of CF_3-GBG sulfate

1,1,1-Trifluoromethyl acetone (Aldrich-Chemie, Steinheim, F.R.G.) was converted to 1,1-dibromo-3,3,3-trifluoropropanone essentially as described by McBee and Burton [23] and was purified by fractional distillation.

4.03 g (0.030 mol) of analytical grade sodium acetate trihydrate was dissolved in 10 ml of water, and 4.01 g (0.015 mol) of 1,1-dibromo-3,3,3-trifluoropropanone was added to the solution. The mixture was heated with stirring for 70 min in a boiling water bath. Then, it was added dropwise to a solution prepared from 4.05 g (0.030 mol) of aminoguanidine bicarbonate (Aldrich-Chemie), 7 ml of 2.5 M aqueous H_2SO_4 and 13 ml of water. The mixture was heated in a boiling water bath for 1.5 h. After standing overnight at room temperature, the mixture was again heated for 3 h. Then, it was concentrated to a volume of about 5–10 ml by flash distillation on an oil bath. The mixture was allowed to cool and was then put onto an ice bath. The yellow precipitate formed was filtered off and washed with a few hundred millilitres of absolute ethanol and finally a small amount of water. Thus, a white crystalline material was obtained, but it appeared to consist of aminoguanidine sulfate. The washings, divided into two fractions of roughly equal volume, were kept in closed flasks in a dark cupboard for several months. During that time, a small amount of yellowish needle-like single crystals were formed in the fraction that contained water in addition to ethanol. The crystals were filtered off and air-dried, yielding ca. 71 mg (ca. 1.4% of theoretical) of the desired product CF_3-GBG · H_2SO_4 · H_2O. The product was identified by X-ray crystallography. It remains to be studied, whether more product could be obtained from the filtrate. When kept in a closed vessel for a total of ca. one year, also the first fraction of the washings yielded a solid precipitate whose identity has, however, not yet been determined.

Biochemical measurements

AdoMetDC inhibition was studied essentially according to previously published procedures [17, 24]. The measurements were carried out in a potassium phosphate buffer (pH 7.4, final concentration 0.1 M). The AdoMetDC used was partially purified from baker's yeast by the method of Seppänen *et al.* [25]. The S-adenosyl-L-[1-^{14}C]methionine (specific activity 42.7 Ci/mol) used was prepared enzymically from L-[1-^{14}C]methionine (Amersham International, Amersham, Bucks., U.K.) as described by Pegg and Williams-Ashman [26]. Unlabelled S-adenosyl-L-methionine was prepared from L-methionine according to the same method.

Cell culture

Mouse L1210 leukemia cells were grown in Gibco's medium RPMI 1640 supplemented with 5% (v/v) of pooled human AB serum (Finnish Red Cross Transfusion Service, Helsinki, Finland), 2 mM glutamine, and antibiotics (streptomycin and the sodium salt of penicillin G, 50 mg of each per litre). Cells were counted using an electronic particle counter (Coulter Counter, model Industrial D).

Results and Discussion

One previous report has appeared on CF_3-GBG, namely that of Podrebarac and Cheng [27], but it

dates back to the time when neither the ability of bis(guanylhydrazones) to inhibit AdoMetDC nor AdoMetDC itself had been discovered. Therefore, further studies were warranted. Podrebarac and Cheng reported that the treatment of 1,1-dibromo-3,3,3-trifluoropropanone with aminoguanidine hydrobromide for an extended period (several weeks) gave a very low yield of CF_3-GBG dihydrobromide. The product was difficult to purify. The sulfate salt was prepared analogously but failed to give a satisfactory analysis. It was, however, successfully prepared by oxidizing 1,1,1-trifluoroacetone with selenium dioxide and treating the product, trifluoromethylglyoxal, *in situ* with aminoguanidine sulfate. The yield was, however, low and the procedure was obviously hazardous and quite complicated [27]. Therefore, we wished to test new modifications of the above methods.

Since it had been reported [28] that refluxing 1,1-dibromo-3,3,3-trifluoropropanone with sodium acetate in water yielded an intermediate that probably was trifluoromethylglyoxal and that could easily be used for further syntheses *in situ,* we decided to try to combine this approach and the methods of Podrebarac and Cheng [27]. As is evident from the Materials and Methods section, an even lower yield of CF_3-GBG sulfate was obtained but fortunately in the form of single crystals that were large and good enough to permit a single-crystal X-ray analysis. This was important not only because now an unambiguous identification of the product could be performed but also because it became possible to study, whether there are any major differences between the structures of CF_3-GBG and MGBG.

Fig. 2 shows a ball-and-stick drawing of the diprotonated form (dication) of CF_3-GBG. It is evident from the figure that the product consisted of a geometrical isomer that is not the same as has been reported for the salts of GBG [21], MGBG [29] and all other bis(guanylhydrazones) so far studied [20, 22, 30] (see also Fig. 1). One further difference from all other bis(guanylhydrazones) whose structures have so far been reported is constituted by the presence of a strong internal hydrogen bond whose formation is not possible in the case of the isomer shown in Fig. 1a. Otherwise, the structure of CF_3-GBG did not differ drastically from the structures of other bis(guanylhydrazones) so far studied. With the exception of the fluorine atoms, the dication of CF_3-GBG was found to be practically planar, just like the di-

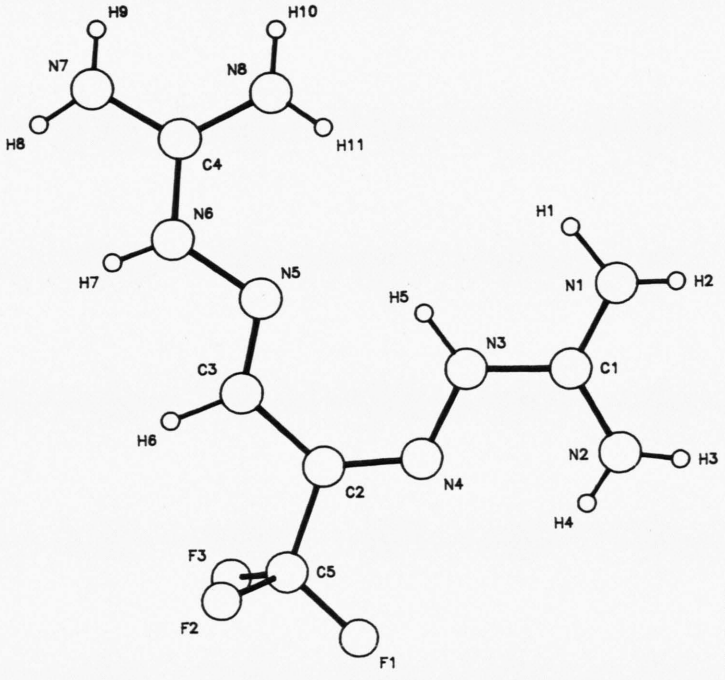

Fig. 2. A ball-and-stick drawing of the dication form of CF_3-GBG, as present in CF_3-GBG·H_2SO_4·H_2O. There is an internal hydrogen bond between N5 and H5.

Table I. Inhibition of yeast AdoMetDC by CF_3-GBG. The concentration of the substrate, adenosylmethionine, was 0.2 mM. Each value represents the mean of two determinations.

Concentration of CF_3-GBG [μM]	Activity of AdoMetDC (% of uninhibited control)
0	100
0.5	104
5	93
50	93
500	66

cations of GBG [21], MGBG [29], and their dimethylglyoxal analog [30]. A detailed report of the results of the X-ray study will be given elsewhere.

From the biochemical point of view, the most interesting question was, whether CF_3-GBG is a potent inhibitor of AdoMetDC. As is shown in Table I, even high concentrations (500 μM) of CF_3-GBG caused only a limited inhibition of yeast AdoMetDC. Therefore, no attempt was made to determine the K_i value or the mode of inhibition. The results indicate that with all probability CF_3-GBG, or at least the isomer of it shown in Fig. 1c, is devoid of value as an AdoMetDC inhibitor, the difference from GBG, MGBG and their alkyl- and dialkylglyoxal analogs being striking. Whether the lack of inhibitory power is mainly due to the effects of the fluorines *per se* or to the fact that the compound consisted of an isomer different from the one detected in the case of GBG, MGBG and other congeners with potent inhibitory properties, remains to be studied. One more point worth further studies is, whether other isomers of CF_3-GBG could be synthesized using different synthetic strategies.

When mouse L1210 leukemia cells were grown *in vitro* in the presence of 50 μM CF_3-GBG, no distinct inhibition of growth could be detected, as compared to an untreated control (data not shown). This result is in line with the observations of Podrebarac and Cheng, who reported [27] that CF_3-GBG does not inhibit this tumor line *in vivo*. Our very preliminary studies suggest, however, that CF_3-GBG (50 μM) and DFMO (2 mM) may have a slight synergistic antiproliferative effect against L1210 cells *in vitro*. Further studies are needed on this topic. Since there is evidence suggesting that the antiproliferative effects of bis(guanylhydrazones) may vary considerably depending on the cell line studied (H. Elo, unpublished results), further testing of CF_3-GBG against a variety of cell lines might also be worth while.

In conclusion, the present results indicate that the biological properties of CF_3-GBG are drastically different from those of the close congener MGBG. As long as analogous isomers of the two compounds are not available, no conclusions can, however, be drawn about the reasons lying behind these differences.

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[1] The systematic names of the compounds are: GBG = 2,2′-(1,2-ethanediylidene)bis(hydrazinecarboximidamide), MGBG = 2,2′-(1-methyl-1,2-ethanediylidene)bis(hydrazinecarboximidamide), and CF_3-GBG = 2,2′-(1-(trifluoromethyl)-1,2-ethanediylidene)bis(hydrazinecarboximidamide).
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