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MECHANISM OF ACTION OF THE NITROSOUREAS: FORMATION OF 1,2-(DIGUANOSIN-7-YL) ETHANE FROM THE REACTION OF BCNU (1,3-BIS-[2-CHLOROETHYL]-1-NITROSOUREA) WITH GUANOSINE

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SUMMARY

A crosslinked dinucleoside, 1,2-(diguanosin-7-yl) ethane, has been isolated from the reaction of guanosine with the antitumor agent, BCNU. The formation of this product suggests that DNA crosslinking, which may be responsible for the cytotoxicity of BCNU, could occur through such dinucleosides.

INTRODUCTION

Previous work from this laboratory has shown that haloethyl nitrosoureas react with nucleosides to form a variety of adducts (1-5). Two of these, 3,N^4-ethanocytidine and 1,N^6-ethanoadenosine, are thought to arise from a two-step reaction: initial alkylation of the base by a haloethyl carbonium ion followed by displacement of the halogen and internal cyclization. As suggested earlier (2), such a reaction between DNA strands could lead to interstrand crosslinks and could explain the cytotoxicity of BCNU.

The DNA crosslinking ability of BCNU has now been demonstrated in Kohn's laboratory by physical means (6) and interest in the possibility that a haloethylnucleoside could react with a second nucleoside has increased accordingly. In this report, we present direct evidence for the formation of a dinucleoside from the reaction of BCNU with guanosine.

MATERIALS AND METHODS

Crystalline BCNU was obtained from Dr. Robert Engle, Drug Research and Development, National Cancer Institute, Division of Cancer Treatment. Guanosine was obtained from Aldrich Chemical Co. and 1,2-dibromoethane, from Fisher Scientific Co.

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Synthesis of 1,2-(diguanosin-7-yl) ethane: 100 mg guanosine was dissolved in 1 ml DMSO and 100 λ 1,2-dibromoethane was added. After reaction at 37°C for 48 hours, cold 0.05 M sodium formate, pH 4.5, was added to precipitate most of the guanosine. The mixture was centrifuged and the supernatant was applied to a SP-Sephadex C-25 column (2 x 60 cm). This was eluted with a 1600 ml linear gradient of sodium formate 0.05 M - 0.5 M, pH 4.5, at a flow rate of 1 ml/min, and 10 min fractions were collected. Fractions corresponding to the last peak were pooled and lyophylized.

The lyophylized material was redissolved in water and the product was purified by reverse phase high pressure liquid chromatography on a μ-Bondapak C_{18} column as follows: 2 ml portions of the solution containing the product were applied to the column and eluted with 2% acetonitrile in 0.05 M KH_2PO_4, pH 4.5. The major peak, which eluted at 11 min, was collected. Most of the salt was removed by an additional chromatography on C_{18} with 3% acetonitrile in 1 mM formic acid as eluent.

Preparation of 1,2-(diguan-7-yl) ethane: 20 A_{260} units of 1,2-(diguanosin-7-yl) ethane were dissolved in 1 ml 0.1 N HCl, heated at 100°C for 15 min, cooled and neutralized by dropwise addition of 1 M NH_4OH. The precipitate that formed was collected by centrifugation, resuspended in cold water and centrifuged again. The pellet was dried overnight under vacuum and used directly for mass spectral analysis.

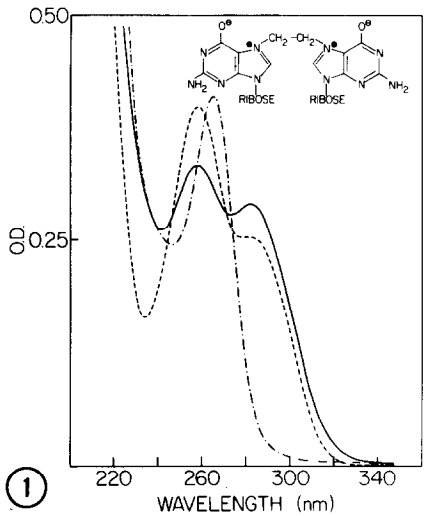
Reaction of guanosine with BCNU: 10 mg guanosine and 10 mg BCNU were dissolved in 0.5 ml DMSO and added to 0.5 ml 0.05 M sodium cacodylate buffer, pH 7.0. After incubation at 37°C for 24 hours, 10 ml cold 0.05 M sodium formate, pH 6.0, was added to precipitate most of the unreacted guanosine. This mixture was centrifuged and the supernatant applied to a SP-Sephadex C-25 column as described in the legend to Figure 3.

Ultraviolet spectra of 1,2-(diguanosin-7-yl) ethane were obtained in 0.1 N HCl, in 0.1 N sodium cacodylate buffer, pH 7.0, and in 0.1 N NaOH on a Beckman Model 35 spectrophotometer. The acid spectrum of 1,2-(diguan-7-yl) ethane was obtained in 0.02 M KH_2PO_4, pH 2.8, and the base spectrum was obtained by adjusting the pH to 13 with 1 M NaOH.

The mass spectrum of 1,2-(diguan-7-yl) ethane was obtained on a Varian MAT 311A field desorption mass spectrometer at 23 ma emitter current. Mr. Marion Kirk, at the Southern Research Institute, Birmingham, Alabama, kindly performed this analysis for us.

RESULTS AND DISCUSSION

The first step in these studies was to isolate and identify marker amounts of 1,2-(diguanosin-7-yl) ethane. The reaction mixture of guanosine and 1,2-dibromoethane described under Methods was separated on SP-Sephadex C-25 into three major peaks. The first peak, which eluted in the void volume, was unreacted guanosine. The second peak was identified as 7-(β-bromoethyl) guanosine (data not presented) and the last peak was identified as 1,2-(diguanosin-7-yl) ethane.



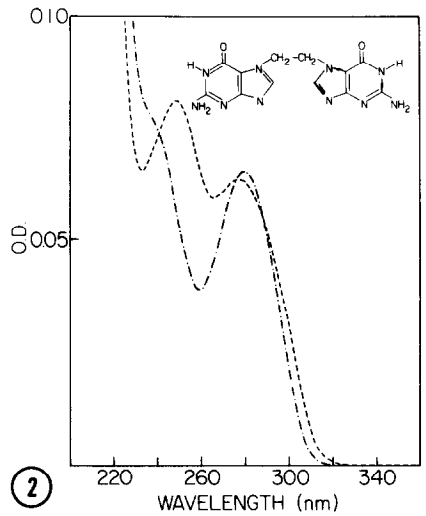
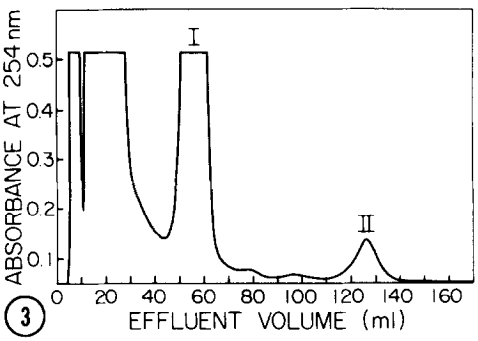


Figure 1. Ultraviolet spectra of 1,2-(diguanosin-7-yl) ethane in acid ----, neutral ——, and base —·—·—.

Figure 2. Ultraviolet spectra of 1,2-(diguan-7-yl) ethane at pH 2.8 ----, and pH 13 —·—·—.

Its structure was deduced from a consideration of its charge and ultraviolet spectra, and by the ultraviolet and mass spectra of its depurinated derivative, 1,2-(diguan-7-yl) ethane. The ultraviolet spectra of 1,2-(diguanosin-7-yl) ethane, shown in Figure 1, are very similar to those of 7-methylguanosine. These spectra and the fact that it was retained on a cation exchange column much longer than compounds bearing one positive charge led to the suggestions that it was 1,2-(diguanosin-7-yl) ethane. This compound was depurinated for mass spectral analysis. The ultraviolet spectra of the depurinated product 1,2-(diguan-7-yl) ethane are shown in Figure 2 and are typical of a 7-substituted guanine. Field desorption mass spectroscopy gave a molecular weight of 328 consistent with its proposed structure.

It was then possible to show that the same dinucleoside was formed when BCNU reacted with guanosine. The BCNU-guanosine reaction mixture described above was chromatographed on SP-Sephadex C-25 as shown in Figure 3. Two peaks were retained on the column: Peak I which corresponds to 7-(β-hydroxyethyl) guanosine, a known product of the reaction (4), and a later Peak II.



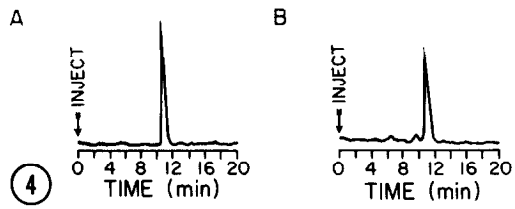


Figure 3. Separation of BCNU-guanosine reaction mixture on SP-Sephadex C-25. The reaction mixture described in the text was applied to an SP-Sephadex C-25 column (0.9 x 20 cm) and eluted at a flow rate of 1 ml/min with a 200 ml linear gradient of sodium formate, pH 6.0, 0.05 M - 0.5 M.

Figure 4. High pressure liquid chromatograms of: (A) a reference peak corresponding to 1,2-(diguanosin-7-yl) ethane synthesized from dibromoethane, and (B) 1,2-(diguanosin-7-yl) ethane isolated as Peak II, Figure 3, from the BCNU-guanosine reaction mixture. A 50 µl sample of each was applied to an Excalibar Sherosorb ODS 5 µm column (4.6 mm x 25 cm) (Laboratory Data Control) and eluted isocratically with 3% acetonitrile in 0.02 M KH_2PO_4, pH 2.85, at a flow rate of 1.0 ml/min.

The ultraviolet spectra of Peak II were identical with the spectra of 1,2-(diguanosin-7-yl) ethane in both acid and base.

The chromatographic behavior of the Peak II product and 1,2-(diguanosin-7-yl) ethane were also compared on reverse phase high pressure liquid chromatography. It can be seen in Figure 4 that the retention times for the two samples are identical.

Since Peak II behaves in the same manner on SP-Sephadex C-25 and reverse phase high pressure liquid chromatography as marker 1,2-(diguanosin-7-yl) ethane, and has the same ultraviolet spectrum, we can conclude that this dinucleoside is indeed a product of the reaction of BCNU with guanosine.

Although it has been reported that BCNU is capable of forming interstrand crosslinks in DNA (6), no crosslinked products have been described previously. In this paper, we have shown that BCNU is capable of reacting with two guanosine molecules to form a dinucleoside. Whether the same product is formed in DNA is not known at this time, but the formation of similar dinucleosides could easily explain the crosslinking which has been observed.

ACKNOWLEDGEMENT

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