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STRUCTURE OF A NEOTHRAMYCIN-2'-DEOXYGUANOSINE ADDUCT

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SUMMARY

The interaction of neothramycin, a pyrrolo(1,4)benzodiazepine antibiotic with antitumor activity, with deoxyribonucleosides was studied as a model of the drug-DNA binding. The antibiotic was demonstrated to react with 2'-deoxyguanosine in aqueous dimethyl sulfoxide. Two reaction products were found on thin-layer plates. The major one was purified with a low yield, and the chemical structure was determined by PMR and mass spectrometry to be a one-to-one adduct of neothramycin and 2'-deoxyguanosine. A covalent bond was formed between C-3 of the antibiotic and N-2 of 2'-deoxyguanosine by dehydration.

Neothramycin1), a potent antitumor agent, is a new member of the pyrrolo(1,4)benzodiazepine group of antibiotic, which anthramycin, sibiromycin, and tomaymycin belong to. We have investigated the mechanism of action of neothramycin, and found that the drug binds to double strand DNA and preferentially inhibits RNA synthesis over DNA synthesis in vivo and in vitro2,3). The binding to DNA is unusually slow, and requires the double strand structure and the presence of guanine base. Neothramycin is a mixture of diastereomers A and B at C-3. The 10,11-azomethine group is hydrated to give a carbinolamine and the carbinolamine carbons at C-3 and C-11 easily epimerize in an aqueous solution (Fig. 1).

Hurley et al.4) have proposed a CPK (Corey, Pauling and Koltun) molecular model for the anthramycin-DNA complex, in which a labile covalent aminal linkage is formed between C-11 of the antibiotic and N-2 of DNA guanine, and is stabilized by secondary hydrogen bonding interactions.

The isolation of drug-DNA complex and determination of its chemical structure are essential for the elucidation of molecular mechanism of drug-DNA reaction. The chemical structure of complexes

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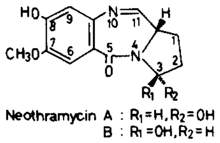

Fig. 1. Structure of neothramycin.

of some mutagenic and/or oncogenic agents with nucleotides or bases has been determined by hydrolysis of drug-reacted DNA with acid or nucleases[5,6]. However, the procedure cannot be applied for neothramycin by the following reasons: (1) The drug-DNA complex is decomposed during hydrolysis, because it is labile to acid and alkali. (2) The reaction of neothramycin with double strand DNA is reversible, and the association constant is extremely low; and the antibiotic hardly reacts with single strand DNA or synthetic nucleotides. The neothramycin-DNA adduct tends to dissociate during hydrolysis of DNA[3,7].

We have prepared a neothramycin-2'-deoxyguanosine adduct in aqueous dimethyl sulfoxide. The adduct has been purified and the structure has been determined by PMR and mass spectrometry. The results are presented in this publication.

MATERIALS AND METHODS

Chemicals.

Neothramycin was a gift of Meiji Seika Kaisha, Ltd., Tokyo, Japan. Thymidine, 2'-deoxycytidine, 2'-deoxyadenosine, 2'-deoxyinosine, and 2'-deoxyguanosine were purchased from Sigma, St. Louis, Missouri, Sephadex LH-20 from Pharmacia Fine Chemicals, Uppsala, Sweden, and silica gel thin-layer plates from Merck, Darmstadt, Germany. All other reagents and solvents were of the highest grade available.

Reaction of neothramycin with 2'-deoxynucleosides.

Neothramycin and 2'-deoxynucleoside (each 5 mg) were dissolved in 0.03 ml of 30 % aqueous dimethyl sulfoxide, and kept at room temperature for 48 hours. The reaction mixture (1 μl) was spotted on a silica gel thin-layer plate, and developed with chloroform-methanol-water (43 : 36 : 10, solvent I) or chloroform-methanol (4 : 1, solvent II). After development, the antibiotic and 2'-deoxynucleoside were stained with fast blue B and diphenylamine-aniline reagents, respectively.

Isolation of a neothramycin-2'-deoxyguanosine adduct.

Neothramycin and 2'-deoxyguanosine, each of 1 g, were dissolved in 5 ml of 20 % aqueous dimethylsulfoxide, and kept for 48 hours at room temperature. The reaction mixture was diluted with 45 ml of cold methanol, applied on Sephadex LH-20 column (2 x 150 cm), and eluted with 2.5 l of methanol at a flow rate of 2.0 ml/min. in a cold room. The fractions containing a neothramycin-2'-deoxyguano-

Table 1. Thin-layer chromatography of neothramycin-2'-deoxyguanosine adducts.

	Rf values	
	Solvent I	Solvent II
Neothramycin A	0.80	0.56
Neothramycin B	0.80	0.45
Minor adduct	0.61	0.05
Major adduct	0.58	0.05
2'-Deoxyguanosine	0.51	0.05

sine adduct, which were detected by thin-layer chromatography as described above, were combined and concentrated to dryness under reduced pressure. The residue was then purified by preparative thin-layer chromatography on silica gel plates (0.25 mm, 20 cm x 20 cm), using a solvent system of chloroform-methanol-30 mM phosphate buffer, pH 7.0, (43 : 36 : 10) in a cold chamber. The area (Rf 0.57 - 0.59) containing the adduct was detected by fluorescence, and eluted with methanol.

Spectral data.

UV absorption spectra were measured in methanol on a Hitachi 124 spectrometer. Field desorption mass spectra were determined by a Hitachi RMU-7M spectrometer at 260 mA emitter current. Fourier-transform PMR spectra were recorded on a Varian XL-100- DISK spectrometer, using deuterodimethyl sulfoxide as a solvent and tetramethylsilane as an internal standard.

RESULTS

Reaction of neothramycin with five sorts of 2'-deoxynucleosides.

The reaction of neothramycin with 2'-deoxyguanosine produced two kinds of adducts, which were stained with both fast blue B and diphenylamine aniline reagents on thin-layer plates. The Rf values are shown in Table 1. The antibiotic interacted with 2'-deoxyguanosine in aqueous dimethyl sulfoxide, but not in anhydrous dimethyl sulfoxide. The optimal H_2O concentration was approximately 30 %. The reaction was promoted by hydrogen ion in a range of pH 4 - 7.

In the reaction with 2'-deoxyadenosine, less amounts of two reaction products were detected on thin-layer plates (Rf 0.26 and 0.30 by solvent II, 2'-deoxyadenosine Rf 0.19). However, 2'-deoxycytidine, 2'-deoxyinosine and thymidine did not react with neothramycin in 30 % aqueous dimethyl sulfoxide.

Isolation of a neothramycin-2'-deoxyguanosine adduct.

Approximately 6 mg of the major adduct (Table 1) was obtained as a colorless powder from 1 g of neothramycin and 1 g of 2'-deoxyguanosine, and the yield was less than 1 %. The poor yield seemed to be due to the low association constant of the reaction.

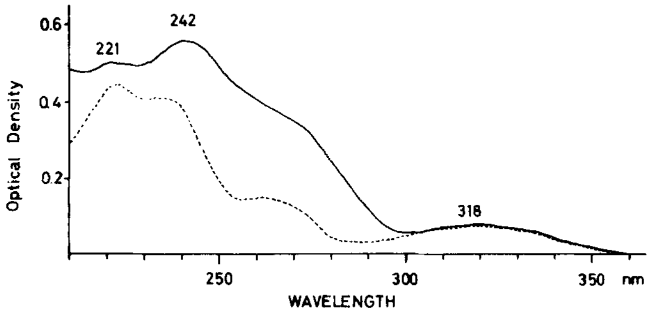

Fig. 2. UV absorption spectra of neothramycin (-----) and the antibiotic-2'-deoxyguanosine adduct (———).

Structure of the neothramycin-2'-deoxyguanosine adduct.

The UV absorption spectra revealed that the adduct consisted of neothramycin and 2'-deoxyguanosine in a ratio of 1 : 1, because the spectrum of the adduct could be dissected into those of the antibiotic and 2'-deoxyguanosine8) (Fig. 2).

The field-desorption mass spectrum of the adduct showed M^+(m/z 511), which coincided with a calculated value for the one-to-one adduct of neothramycin and 2'-deoxyguanosine. Another molecular ion at m/z 534 corresponded to $(M + Na)^+$.

The PMR spectrum of the adduct was compared with that of 3-O-butylneothramycin A in the region of 4 - 8 ppm (Fig. 3). A doublet signal (δ 7.57, J=7.0 Hz), which could be assigned to the 2-NH of the 2'-deoxyguanosine moiety, was observed in a low field. A doublet signal (3-H) at δ 5.56 in the PMR of 3-O-butylneothramycin A changed to a multiplet signal at δ 5.94 in that of the adduct, while a doublet signal of the 11-H (δ 7.82, J=4.2 Hz) did not shift.

The results suggested that neothramycin covalently binds at C-3, but not at C-11, to the 2-NH_2 group of 2'-deoxyguanosine by dehydration. The stereochemistry at C-3 in the neothramycin moiety of the adduct remained to be determined.

DISCUSSION

The current experiments show that neothramycin reacts with 2'-deoxyguanosine, and the reaction is catalyzed by acid, involving the nucleophilic attack of the 2-amino group of 2'-deoxyguanosine at C-3 of the antibiotic in aqueous dimethyl sulfoxide. Although

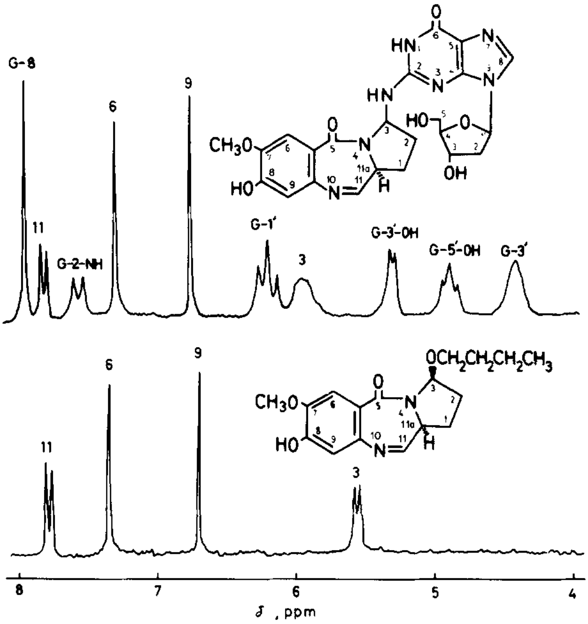

Fig. 3. PMR spectra of 3-O-butylneothramycin A and the adduct of neothramycin and 2'-deoxyguanosine.

the structure was not determined, the other reaction product was obtained in an extremely low yield, and was identified to be an adduct of neothramycin and 2'-deoxyguanosine.

The neothramycin-2'-deoxyguanosine reaction is similar to the antibiotic-DNA interaction in the following characteristics: (1) the same base specificity, (2) reversible reactions, (3) slow binding rates, (4) low yields, and (5) acid catalysis[3,7]. It suggests that neothramycin covalently binds to the 2-amino group of the guanine moiety of DNA to produce an aminal linkage in a similar manner to that of the drug-2'-deoxyguanosine adduct formation.

The fluorescence intensity of neothramycin is markedly increased by the presence of native DNA[3], but not significantly altered by 2'-deoxyguanosine (Data are not shown). The results are in accord with the current assumption that the antibiotic binds through C-3 to 2'-deoxyguanosine but through C-11 to double strand DNA[3,4].

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