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STRUCTURAL ANALYSIS BY NMR OF ANTITUMOR DRUG-DNA COMPLEXES: 9-AMINOANTHRACYCLINE (SM-5887)

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Abstract: SM-5887 is an anthracycline antibiotic with a potent antitumor activity and low toxicity. The structure of the 2:1 SM-5887-d(CGTACG) complex has been studied in solution by 2D NMR spectroscopy. These data revealed that SM-5887 intercalated between the cytosine and guanine residues and the sugar moiety lay in the minor groove. The intercalation geometry of SM-5887 was a hybrid between those of adriamycin and nogalamycin.

Introduction

The intercalation of planar aromatic molecules with the DNA double helix[1] is considered to be important in mutagenesis, carcinogenesis[2], and the medicinal action of antibacterial[3], antiparasitic[4,5], and antineoplastic[6] drugs. A large number of natural and synthetic analogs of the daunomycin family, consisting of an aromatic aglycon chromophore and an amino sugar, have been tested[7] for biological activity. Chemical substituents on the fused ring system and on the amino sugar attenuate the therapeutic properties of the drugs. A slight change in chemical structure causes significant changes in clinical properties. For example, daunomycin is effective in the treatment of leukemias, while the closely related adriamycin is effective in the treatment of solid tumors[8].

(+)-9-amino-4-demethoxy-9-deoxy-7-O-(2-deoxy-β-D-erythro-pentopyranosyl)daunomycinone (SM-5887[9], Fig. 1) is an anthracycline antibiotic with a potent antitumor activity. The toxic effects of SM-5887 were much less than those of adriamycin in terms of delayed-type toxicity, local tissue toxicity, and cardiotoxicity[10]. Even though SM-5887 is expected to intercalate DNA, the molecular details of its binding mode are unknown. A detailed understanding of the drug-DNA intercalations at the molecular level is the first step to realize potent activity and low toxicity. In this paper, we analyzed the binding of SM-5887 to the DNA hexamer d(CGTACG) by 2D NMR spectroscopy using nuclear Overhauser effect data and related information.

Materials and Methods

The solutions of SM-5887-CGTACG complexes for NMR studies were prepared by dissolving the DNA hexamer plus the appropriate amounts of SM-5887 in 0.6 ml of phosphate buffer solution (50 mM sodium phosphate, pH 7.0, 150 mM NaCl in 99.8% D_2O) to prepare a final concentration of 4 mM duplex. The solution was lyophilized twice with 99.8% D_2O and then dried in an NMR tube with a stream of nitrogen gas, and finally 0.6 ml of 99.996% D_2O was added to prepare the sample.

Both 1D and 2D NMR spectra were recorded using a JEOL A 500 500-MHz spectrometer. The chemical shifts (in ppm) were referenced to the 2-trimethylsilyl-2,2,3,3-tetradeuteriopropionic acid, sodium salt as the internal standard. Phase sensitive DQF-COSY and phase sensitive NOESY spectra were recorded as 512 t_1 blocks of 2048 complex points each in the t_2 dimension and averaged 32 scans per block during the recycle delay of 1.0

sec. for the DQF-COSY and 4.0 sec. for the NOESY. The mixing time for the NOESY experiments was 100 ms. The 2D data sets were processed with the program EDL (JEOL Co., Tokyo, Japan) using VAX 3200 workstation. The 512 complex points in the t_1 dimension were zero-filled to 1024 points prior to the FT.

Results and Discussion

1D spectra

To determine the feasibility of NMR studies on SM-5887 bound to DNA, we titrated SM-5887 with d(CGTACG) at different ratios. One dimensional ^{1}H-NMR spectra of SM-5887, the DNA duplex alone, and the complexes in the presence of 0.5, 1.0, and 2.0 equivalents of SM-5887 are shown in Fig. 2. The free SM-5887 and free DNA have sharp resonances. When the ratio of SM-5887 to d (CGTACG) are 0.5:1 and 1:1, the spectra show complex patterns. However, when the ratio reaches 2:1, the spectra become considerably simplified, showing that a stable and symmetric 2:1 complex is formed. Thus, the titration experiments indicated that full assignments of a 2:1 SM-5887-DNA complex would be possible.

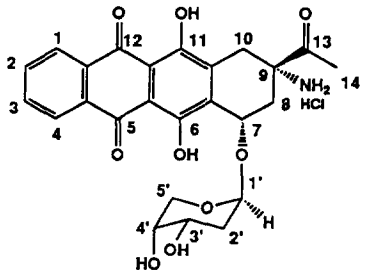
The resonances in the NMR spectrum of the SM-5887-d(CGTACG) complex are broad, with their line widths in the range of 15-20 Hz at 300 K (Fig. 2). In comparison, the line width of the resonances of the free DNA duplex is about 5 Hz at 300 K. About 10 Hz line widths of resonances of the 0.5:1 and 1:1 complexes are noteworthy. The drug is thus in slow exchange among DNA hexamers on the NMR chemical shift time scale. The 1D NMR spectra of the solution containing the 0.5:1 and 1:1 mixtures of the SM-5887-CGTACG complex show more resonances than those of the 2:1 complex (Fig. 2). This is likely the result of the varying population of the three molecular species (free DNA, 1:1 complex and 2:1 complex), based on the observation that the spectrum is nearly a direct superposition of the spectra of the three species.

Resonance Assignment

The assignment of the resonance of the complex was established by starting with the aromatic-H1' fingerprint region using the sequential assignment strategy. Fig. 3 shows the expanded region consisting of the aromatic-to-H1' crosspeaks. The sequential connectivity of the aromatic to H1' pathway can be treated without interruption. However, it is clear that the intensities between the C1H1'-G2H8 and C5H1'-G6H8 crosspeaks are significantly weaker than those of the other internucleotide crosspeaks. These results suggest that the dinucleotide C1pG2 and C5pG6 steps are distorted and no longer have the normal B-DNA backbone conformation. The aromatic-to-H2'/H2" crosspeaks possess the same intensity pattern. It should be noted that the G2H8-C1H2'/H2" and G6H8-C5H2'/H2" crosspeaks are significantly weaker than the remaining corresponding crosspeaks. The aromatic-aromatic crosspeaks, shown in Fig. 4, also support the contention that the C1pG2 and C5pG6 steps are distorted. For example, the C1H6-G2H8 and C5H6-G6H8 crosspeaks are absent, indicating that the normal stacking interactions in free B-DNA are gone. Moreover, COSY spectra provide evidence for a notable change in the CG steps of the recognition sequence[11]. All the ribose sugars in the free DNA duplex fit the pattern expected for the B form DNA. In the bound duplex, however, the COSY crosspeak between C6H1' and C6H2" is present while that between C6H1' and C6H2' is not.

SM-5887

The bound conformation of SM-5887 was evaluated based on the DQF-COSY and NOESY spectra and compared to its free conformation. The COSY crosspeak intensities were analyzed for information on sugar ring



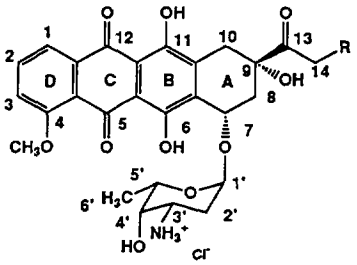


Figure 1 : Molecular formulas of SM-5887, adriamycin, and daunomycin with the numbering system used in this paper.

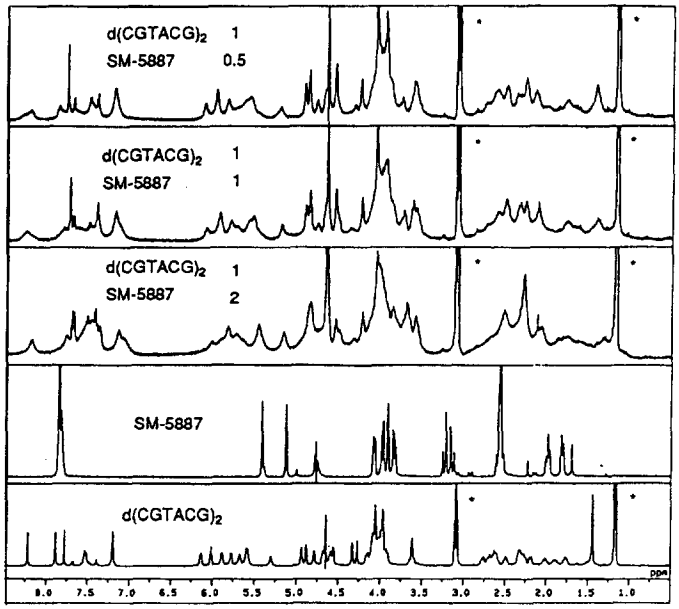


Figure 2: One-dimensional ^{1}H-NMR of the free DNA, 0.5:1, 1:1, and 2:1 SM-5887-d(CGTACG)$_2$ complexes and free SM-5887. The spectra were recorded at 300 °K. Resonances marked with asterisks are from the impurities of the DNA samples.

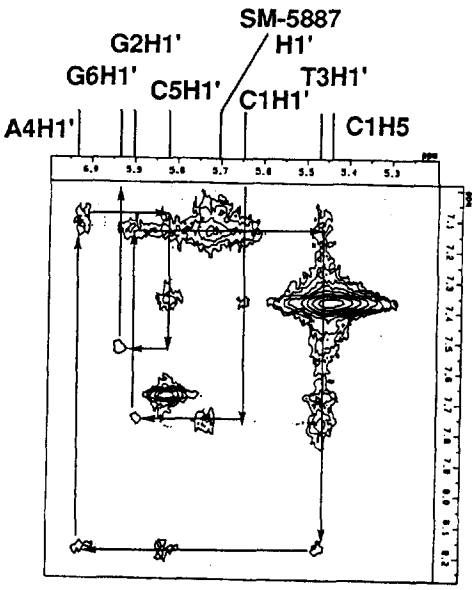


Figure 3: Expanded aromatic to H1' region of 2D NOESY spectra which provided information in the assignments of resonances. The sequential assignment pathway is indicated.

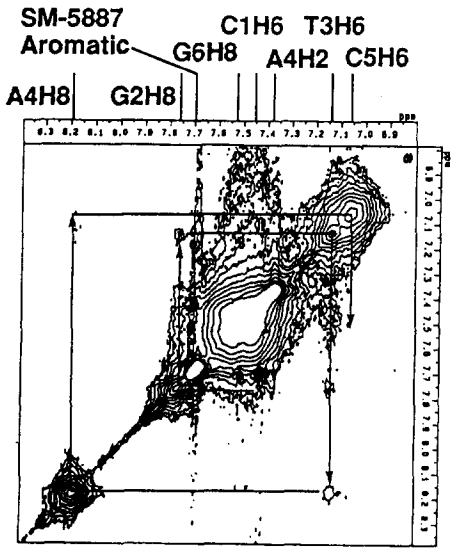


Figure 4: Expanded aromatic to aromatic region of 2D NOESY spectra. The internucreotide conectivity pathway is indicated.

conformation while the NOESY crosspeak intensities were analyzed for information on the orientation of the residue with respect to each other and the floor of the minor groove. Large (~9 Hz) vicinal coupling constants and correspondingly large crosspeaks show that the sugar ring of SM-5887 is in the $^4C_1$ (chair) conformation in solution. When the drug is bound to DNA, relative intensities of the COSY crosspeaks for the sugar ring are similar to what they are in solution, indicating that the conformation does not significantly change. The 2D NOESY data showed abundant NOE crosspeaks despite the small size of the drug. The same intraresidue NOEs are observed in the complex and in the unbound drug. We note that the NOE cross peaks among the protons that are the glycosidic part, define the relative orientation between the two participating moieties. For example, the H8 and H7 protons have a strong NOE crosspeak to H1' but no NOE crosspeak to H2' and H2" (Fig. 5), suggesting that the atom of H7, C7, O7, C1', and H1' are nearly coplanar.

From these data we conclude that the conformation of free SM-5887 is similar to that of the bound form, at least qualitatively. This observation of a rigid saccharide conformation seems to be quite common, as revealed in the strcture of several oligosaccharide antibiotics, including chromomycin A3[12] and calicheamicin Y[13].

Complex

To clarify how the SM-5887 binds at these intercalation cavities, we needed to have a complete assignment of all protons. All resonances have been assigned, and their chemical shifts are tabulated in Table 1. The largest changes in chemical shift between the free and the bound forms are associated with the C1H5 ($\Delta\delta$=+0.51ppm) and C5H5 ($\Delta\delta$=-0.60ppm) protons. These data supported the idea that the anthracycline aglycon is sandwiched between the two cytosines so that the ring current effects matually affected their chemical shifts. Many intermolecular NOEs were idetified from the NOESY data (Table 2). Careful inspection of the 2D NOESY spectra revealed that the H1 and H3 protons of SM-5887, respectively, are spacially very close to C1H5 and C5H5, whereas H2 is spacially close to both C1H5 and C5H5. But two proton pairs are farther apart than those of KH1 to C1H5 and KH3 to C5H5. This suggests that ring D of anthracycline aglycon is sandwiched between C1 and C5. The position of the anthracycline aglycon ring is further defined by the NOE crosspeak between the KH11 proton and many DNA protons, in particular, those from the C1 and G2 residues. This clearly indicates that the aglycon is oriented such that the ring edge containing KH11 is facing toward the backbone of C1pG2.

We developed an initial model for the SM-5887-DNA (2:1) complex by docking SM-5887 into the B form DNA in an orientation that satisfies the qualitative NOE constraints (Fig. 6). The model shows the bound conformation of SM-5887 and the location of SM-5887 with respect to the recognition sequences. The model has not yet been completely refined, however many NOEs in the observed experimental NOESY spectrum are satisfied. In comparison with adriamycin and daunomycin[14], the model of the 2:1 SM-5887-CGTACG complex has its aglycon sliding toward the minor groove. We considered that the repulsion of the amino group at C9 of SM-5887 between those of C2 of guanine under neutral conditions would make the aglycon of SM-5887 move toward the minor groove.

This reported work established the feasibility and relevance of NMR studies of SM-5887 bound DNA and has led to the development of an initial model for the complex. The results suggested that the CpG step is the preferred binding site for SM-5887. However, this should await the results from a more careful analysis of drug-DNA complexes involving several DNA sequences. The intercalation geometry of SM-5887 was a hybrid between those of adriamycin and nogalamycin[15] similar to aclacinomycin[16]. Nogalamycin has its ring D stacked between the CpG steps. The next step is to refine the structure of the complex with the aid of molecular dynamics.

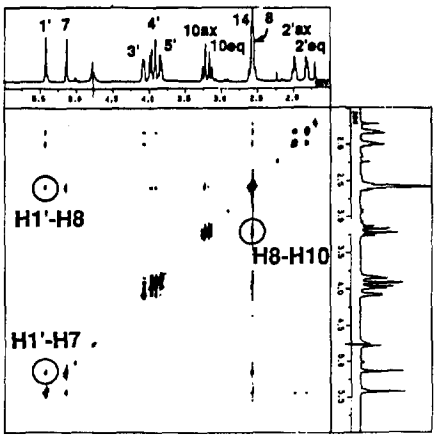


Figure 5: Expanded region of the experimental 2D NOESY spectrum of SM-5887 in D_2O. Some key distances between different parts of the molecules are marked.

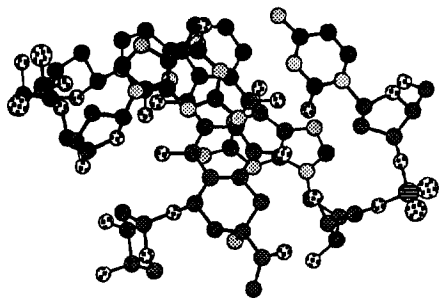


Figure 6: Model for the SM-5887-d(CGTACG) complex, looking perpendicular to the anthracycline aglycon of SM-5887. The model was generated by using the Sybyl molecular graphics package.

Table 1: ^{1}H-NMR Chemical Shifts (ppm) of d(CGTACG), and SM-5887 at 300 °K

Nonexchangeable Proton Chemical Shifts of Free and Bound d(CGTACG),

		H8/6	H5,H2,Me	H1'	H2'	H2"	H3'	H4'	H5'	H5"
C1	f-DNA	7.68	5.95	5.79	2.01	2.44	4.75	4.00		
	b-DNA	7.45	5.44	5.65	2.03	2.33	4.47	4.35	3.65	3.81
	Δ	0.23	0.51	0.14	-0.02	0.11	0.18	-0.35		
G2	f-DNA	8.00		5.98	2.70	2.81	4.88	4.38		
	b-DNA	7.77		5.90	2.80	2.82	4.98	4.44	3.95	4.07
	Δ	0.23		0.08	-0.10	-0.01	-0.10	-0.06		
T3	f-DNA	7.33	1.58	5.75	2.10	2.45	4.92	4.22		
	b-DNA	7.14	1.25	5.47	1.90	2.17	4.74	4.09	4.11	4.08
	Δ	0.19	0.33	0.28	0.20	0.28	0.18	0.13		
A4	f-DNA	8.35	7.67	6.28	2.75	2.87	5.05	4.45		
	b-DNA	8.19	7.38	6.04	2.50	2.73	4.93	4.32	3.87	4.08
	Δ	0.16	0.29	0.24	0.25	0.14	0.12	0.13		
C5	f-DNA	7.30	5.41	5.67	1.85	2.30	4.82	4.16		
	b-DNA	7.06	6.01	5.82	2.14	2.00	4.84	4.14	4.03	4.03
	Δ	0.24	-0.60	-0.15	-0.29	0.30	-0.02	0.02		
G6	f-DNA	7.93		6.15	2.60	2.35	4.66	4.18		
	b-DNA	7.53		5.93	2.57	2.33	4.60	4.07	3.88	4.03
	Δ	0.02		0.22	0.03	0.02	0.06	0.11		

Nonexchangeable Proton Chemical shifts of Free and Bound SM-5887

	H1,2,3,4	7	8,14	10ax	10eq	1'	2'ax	2'eq	3'	4'	5'
f-drug	7.85	5.13	2.57	3.24	3.14	5.42	1.99	1.81	4.07	3.91	3.97
b-drug	7.70	5.11	2.44	3.31	3.11	5.65	1.98	1.82	3.51	3.58	3.98
Δ	0.15	0.02	0.13	-0.07	0.03	-0.23	0.01	-0.01	0.56	0.33	-0.01

Table 2: Intermolecular NOEs in the SM-5887-DNA complex

SM-5887	DNA	intensity
1,2,3,4	C1H5, C1H6	s
	C5H5, C5H6	w
7	G6H1'	m
10	C1H2", G2H4'	m
	G2H5', T3H5'	w
	G2H1'	s
14	T3H5'	w
1'	G6H4	m
	G6H1'	s
2'	G6H5', G6H4'	m
2"	G6H5', G6H4'	m
3'	T3H1'	m
4'	A4H4', A4H5'	m
5'	T3H4', T3H1'	m

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