500 MHz ^{1}H-NMR study of the interaction of daunomycin with B and Z helices of d(CGm^{5}CGCG)

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The interaction of daunomycin with B and Z helices of a self-complementary DNA fragment d(CGm^{5}CGCG) in solution was studied by ^{1}H-NMR spectroscopy at 500 MHz. The results show that the B-Z transition kinetics is not affected by addition of daunomycin. Daunomycin binds exclusively to the B form of d(CGm^{5}CGCG). Z exchanges with B while the latter also exchanges with the B duplex-daunomycin complexes.

^{1}H-NMR Daunomycin B-DNA Z-DNA Drug-DNA interaction

1. INTRODUCTION

The interaction of a large number of intercalated drugs (daunomycin, adriamycin, etc.) with DNA has been extensively studied [1–10]. It is now well established that daunomycin binds non-covalently to B-DNA helices by intercalating its anthraquinone ring between the DNA base pairs. Recently, in the case of poly d(GC), Chaires [11] showed that daunomycin can under some conditions convert the Z form back to the B form. The Z duplex formation was interrupted by addition of daunomycin. Similar results were obtained by Van Helden [12], and Chen et al. [13] who studied the effect of adriamycin on the B-Z transition of poly d(GC) and poly d(Gm^{5}C). However, the mechanism by which intercalators inhibit the B-Z transition remains poorly understood. In very recent studies on short methylated DNA fragments in solution [14–17], we showed that the B and Z forms can be observed distinctly and that several daunomycin-B duplex complexes are in slow exchange with the drug-free B duplexes. This allows the different intercalation sites and their occupation probability in the B form to be determined. We were prompted by these results to study the intercalation of daunomycin with the B and Z forms simultaneously present in solution.

Here, the interaction of daunomycin with the B and Z helices of a short DNA fragment, d(CGm^{5}CGCG), was investigated by ^{1}H-NMR spectroscopy at 500 MHz.

2. MATERIALS AND METHODS

d(CGm^{5}CGCG) was synthesized in solution from dimers according to a standard procedure [15,16]. 5-Methylcytidine was prepared from thymidine according to Sung [18]. The hexamer was dissolved in ^2H_2O containing 1.8 M NaCl + 5 mM PO_4^{2-} and was freed of possible divalent ions by addition of EDTA (~0.1 mM). The pH was adjusted to 7–8 by the addition of small amounts of NaOH. The sample was then lyophilized twice in ^2H_2O and redissolved in ^2H_2O to a final concentration of 2 mM. Daunomycin was purchased from Sigma, lyophilized twice in ^2H_2O and then redissolved to a final concentration of 10 mM. Variable amounts of this solution were added to the oligonucleotide solution. ^{1}H-NMR 500 MHz nonexchangeable proton spectra were recorded on a Bruker WM 500 instrument and referenced

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relative to internal 3-(trimethylsilyl)-$^2H_4$-propionic acid (TMP). The exchangeable imino proton spectra in 80% ^1H_2O–20% ^2H_2O were obtained by a two-pulse sequence [19,20].

3. RESULTS

All the experiments described below were performed in the temperature range 35–55°C with 1.8 M NaCl solutions. Under these conditions the B and Z double helical structures of d(CGm^{5}CGCG) are simultaneously present in slow exchange (on the NMR time scale) with equivalent proportions at 35°C [16].

3.1. *Nonexchangeable protons*

Fig.1a and b shows how the addition of increasing amounts of daunomycin to a d(CGm^{5}CGCG) solution (35°C, 1.8 M NaCl) affects the base proton spectra. Let us consider the less crowded region, i.e., the high-field region (fig.1b). In the absence of daunomycin two major signals with practically equivalent intensities are observed and correspond to the d(m^{5}C) methyl protons in the B and Z duplexes [16]. As the total daunomycin/duplex concentration ratio R increases the B signal broadens and splits into exactly 3 peaks (i.e., with identical chemical shifts) as in the case of 0.1 M NaCl solutions [17] where only the B form is present. In [17] we showed that the broadening and splitting of the methyl protons signal are due to the formation of several B duplex-daunomycin complexes in slow exchange with the free B duplex, corresponding to the various intercalation possibilities. Simultaneously the intensity of the Z signal decreases but its linewidth and chemical shift are unchanged. In the low-field region (fig.1a), the same phenomenon is observed: the H8

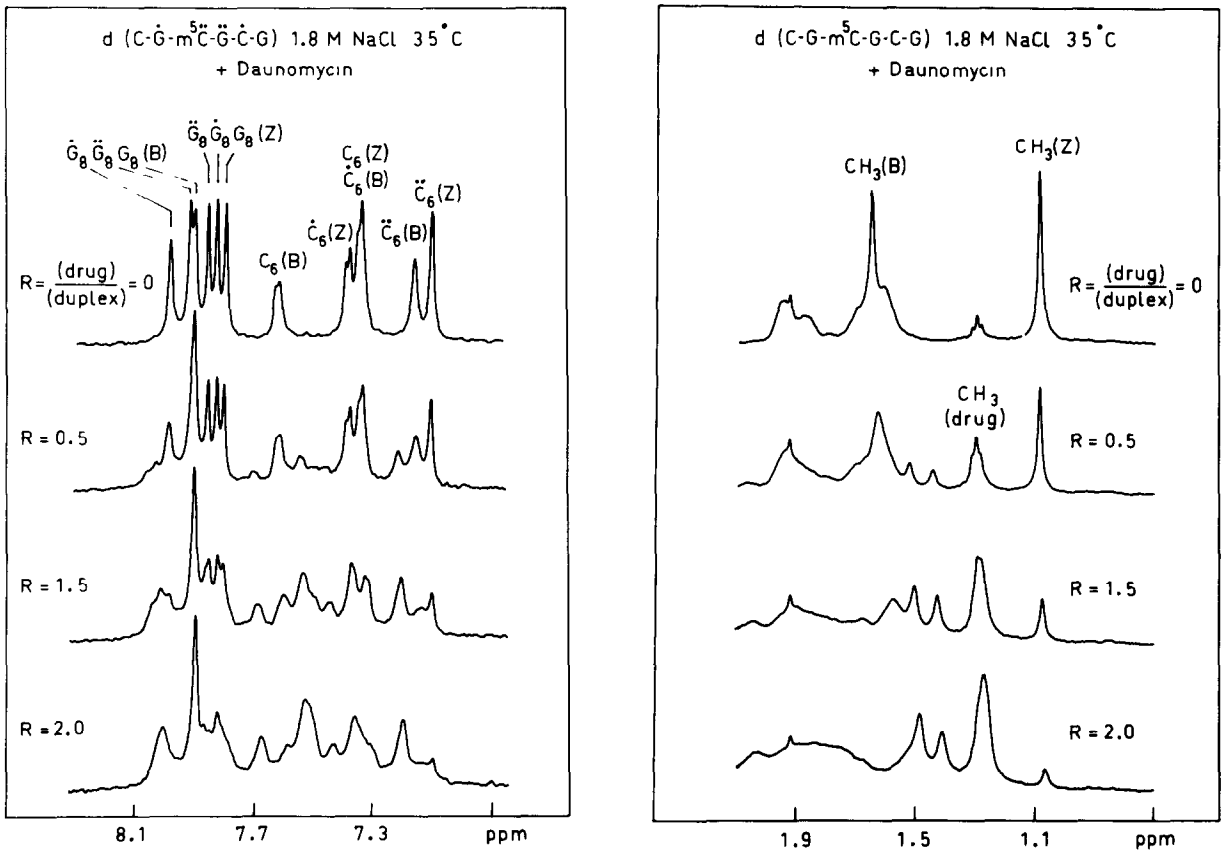

Fig.1. 500 MHz ^{1}H-NMR nonexchangeable proton spectra of d(CGm^{5}CGCG) in neutral aqueous solution (1 mM helix concentration, 1.8 M NaCl, 35°C) with addition of variable amounts of daunomycin.

and H6 proton signals of the B form broaden, split into several peaks and display a spectrum which is similar to that observed in 0.1 M NaCl solution [17] whereas only the intensity of the H8 and H6 proton signals of the Z form is affected. This is particularly clear in the case of the d(m^5C) H6 proton resonance (7.1 ppm) but less observable in the case of the other Z resonances which are overlapped by the H1, H2 and H3 daunomycin signals ($R > 1$).

At various R values, the relative proportion of Z duplex, z ($= [Z]/c_o$ where [Z] and c_o are the Z duplex and total duplex concentrations) can be determined from integration of the d(m^5C) H6 and CH_3 Z proton signals; c_o is obtained directly from integration of the corresponding B and Z signals when R is zero. R values are determined from integration of the daunomycin CH_3 protons at 1.3 ppm. z is plotted vs R in fig.2; when R becomes greater than 2, the z fraction is less than 10%.

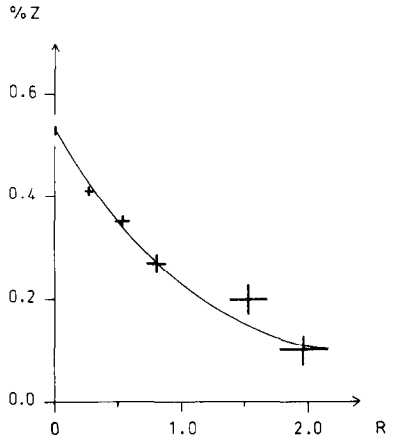

Fig.2. Variations of the Z duplex proportion vs the total daunomycin/duplex concentration ratio R. The solid line is computed from eqn 6 (see text).

3.2. *Exchangeable protons*

To obtain additional information on the interac-

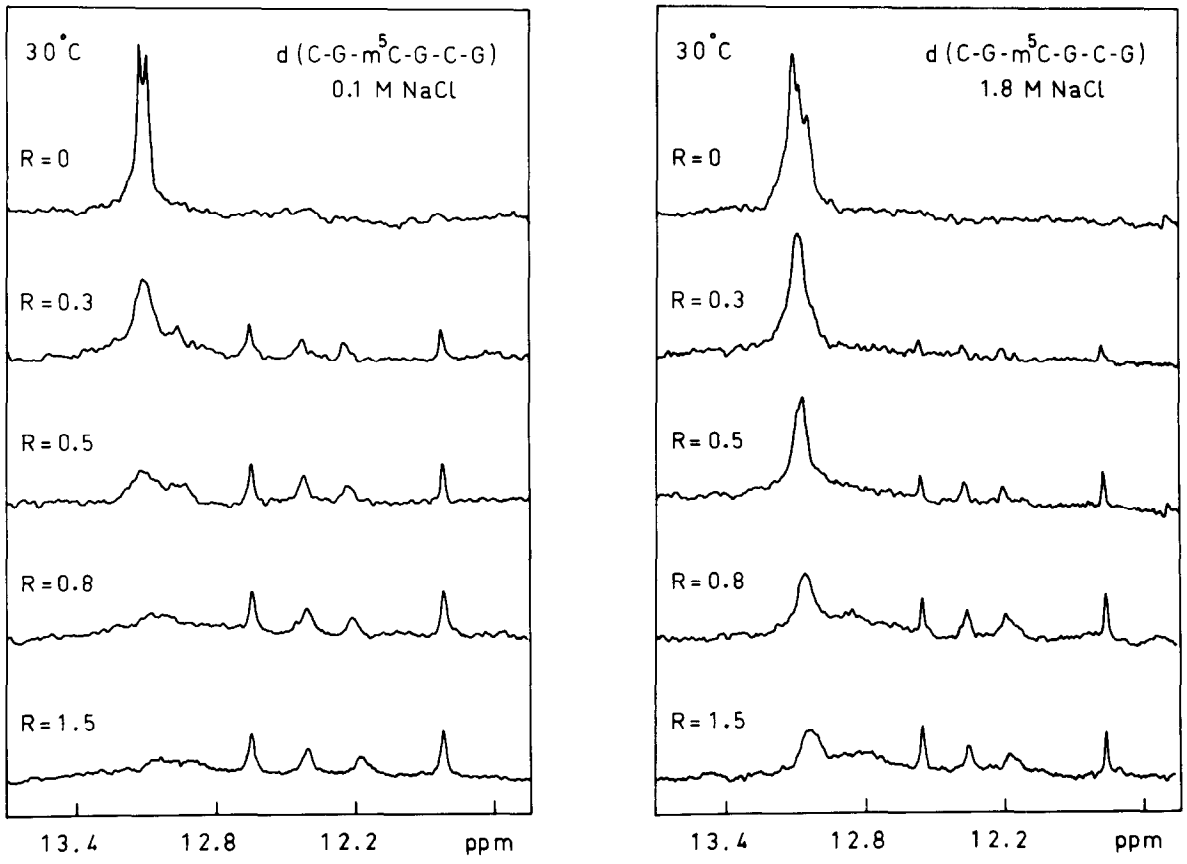

Fig.3. 500 MHz ^{1}H-NMR exchangeable imino proton spectra of d(CGm^5CGCG) in neutral aqueous solution (1 mM helix concentration, 30°C) with addition of variable amounts of daunomycin: (a) 1.8 M NaCl, (b) 0.1 M NaCl.

tion of daunomycin with B and Z helices of d(CGm^{5}CGCG), exchangeable imino proton spectra were also recorded in the absence and presence of various amounts of daunomycin. Fig.3a shows the 500 MHz spectra of exchangeable imino protons of d(CGm^{5}CGCG) (30°C, 1.8 M NaCl) for different R values. These spectra are compared to those of a 0.1 M NaCl solution under the same conditions (fig.3b).

In 0.1 M NaCl solution, as R increases the B duplex imino proton signal (13.1 ppm) broadens dramatically whereas 4 additional resonances appear at higher field (12.7, 12.45, 12.3 and 11.85 ppm). Saturation transfer experiments have shown that, among these new resonances, the two peaks in the middle (12.45 and 12.3 ppm) are in chemical exchange with the imino proton signal at 13.1 ppm and correspond to the imino proton in the B duplex-daunomycin complex; the two other resonances are a priori assigned to the OH protons of the complexed daunomycin on the basis of the work of Patel et al. [3].

In 1.8 M NaCl solution, the B and Z imino proton signals are close together (δ = 13.1 ± 0.08 ppm). As R increases, the 4 resonances of the B duplex daunomycin complex are observed. However, the intensity of these peaks increases more slowly than in 0.1 M NaCl solution and when R = 1.5, a residual peak is still observed at 13.0 ppm while in 0.1 M NaCl solution, the signal in the same region is broadened upon detection.

4. DISCUSSION

The above results relative to the nonexchangeable and exchangeable proton spectra of d(CGm^{5}CGCG) in 1.8 M NaCl solution show that, as the daunomycin concentration increases: (i) the proportion of both B and Z free duplexes decreases; (ii) only the B duplex-daunomycin complex is detected whereas no signal corresponding to a Z duplex-daunomycin complex is observed. Moreover the linewidth of the Z proton signals is directly related to the exchange time τ_{ZB} (Z⟶B). Below 55°C, τ_{ZB} can be determined from the observed linewidth of Z signals [16]:

$$\pi\Delta\nu_{obs} = 1/T_2 + 1/\tau_{ZB}$$

Linewidth measurements at various temperatures (between 35 and 55°C) show that addition of daunomycin has no significant effect on the exchange time.

These results show that daunomycin only interacts with the B form of d(CGm^{5}CGCG). This conclusion is supported by the fact that, as mentioned by Chaires [11], the structure of the daunomycin-B helix complex which was determined from an X-ray study [9] is not possible for a left-handed Z helix.

Consequently, in a first approximation, we can consider the two following reactions:

$$B \longleftrightarrow Z \text{ and } B + \text{drug} \longleftrightarrow B^+$$

Let b, b^+ and d be the relative proportions of the free B duplex, B duplex-daunomycin complex and free daunomycin. The equilibrium constants are given by:

$$k = z/b \quad (1)$$

and

$$c_o k' = b^+/bd \quad (2)$$

with

$$z + b + b^+ = 1 \quad (3)$$

and

$$b^+ + d = R \quad (4)$$

Combination of eqns 1 and 3 gives:

$$z(1 + 1/k) + b^+ = 1 \quad (5)$$

Consequently, as R increases, b^+ increases and z decreases. k can be determined from integration of B and Z signals when R = 0. We find k = 1.12 ± 0.05. b and b^+ fractions can be deduced directly from the experimental values of z (fig.2) according to eqns 3 and 5. In particular, when R = 1, z is about 20% then b = 18% and $b^+/(b + b^+)$ = 78%. This result confirms the fact that as in 0.1 M NaCl solution [17] in complexed form, a single daunomycin molecule is associated with one B duplex.

From the values of b, b^+ and R and according to eqns 2 and 4 the constant k' can be determined for various R values (extreme values excepted – 0.2 and 2.0 – since d in the first case and b^+ in the second one are less than 10%, i.e., the experimental error). We find $k' = 5.5 \pm 1 \times 10^3$ M^{-1} (with c_o = 1 mM). This value is in agreement with those previously estimated [10,17].

Combination of eqns 1–4 leads to the relationship between z and R:

$$z^2(c_o k'(k+1)) + z(c_o k' k(R-1) + 1 + 1/k) - 1 = 0 \quad (6)$$

The computed curve $z = f(R)$ obtained for $k = 1.12$ and $c_o k' = 5.5$ fits the experimental data (fig.2).

Part of our results are in agreement with those of Chaires [11] and Van Helden [12] relative to poly d(GC). The addition of daunomycin or adriamycin to poly d(GC) (4 M NaCl solution) shifts the B-Z equilibrium to the left. However, in the case of poly d(GC), the kinetics of the B-Z transition is affected upon addition of daunomycin while the exchange time τ_{ZB} between B and Z forms of d(CGm^{5}CGCG) is unchanged. Chaires has observed that drug binding to the B form of poly d(GC) is strongly favored. This work shows that daunomycin binds exclusively to the B form of d(CGm^{5}CGCG).

The present study shows the power of ^{1}H-NMR as a method of investigating the drug-DNA fragment interaction in solution. With a judicious choice of DNA fragments, the slow exchange conditions are reached and each of the free or complexed double helical structures gives rise to separate signals; thermodynamic and kinetic aspects of the various equilibria can easily be studied.

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