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Effects of solute conditions on the relative affinities of the oligonucleotides d(G-C)$_5$ and d(A-T)$_5$ for the anthracycline drug 2-fluoro-4-demethoxydaunomycin

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^{19}F NMR has been used to show that changes in NaCl concentration, as well as the presence of lysine or arginine, affect the equilibrium distribution of the synthetic anthracycline 2-fluoro-4-demethoxydaunomycin (2FD) between binding sites on d(G-C)$_5$ and d(A-T)$_5$ in a 1 : 1 : 1 molar aqueous system: 2FD/d(G-C)$_5$/d(A-T)$_5$. Varying the pH between 6.2 and 7.7 had no effect. NaCl concentrations below 0.1 M led to a d(G-C)$_5$ preference while above 0.1 M a preference for d(A-T)$_5$ was observed. At comparable solute concentrations, use of either lysine or arginine resulted in a significant drug preference for d(G-C)$_5$ compared to systems containing only NaCl.

NMR, ^{19}F-; Daunomycin; Adriamycin; Sequence specificity; DNA; Oligonucleotide

1. INTRODUCTION

Within the last 15 years, many attempts have been made to establish and determine DNA base pair specificity for the clinically important intercalating antibiotics, daunomycin and adriamycin [1–7]. Most studies have focused upon comparing relative drug affinity for either GC or AT-rich DNA sequences, and on comparison of poly(G-C) vs poly(A-T) affinity [5]. As has been pointed out [5,8], the literature in this area contains many contradictory results and thus far no clear-cut proof of the existence or otherwise of any form of poly(G-C) vs poly(A-T) specificity has been demonstrated [8].

Recent experiments in this laboratory, using ^{19}F NMR to estimate the distribution of the daunomycin analog 2-fluoro-4-demethoxydaunomycin (2FD) (fig.1) between the oligonucleotides d(G-C)$_5$ and d(A-T)$_5$, have demonstrated that under typical conditions (i.e. 0.1 M NaCl, 10 mM phosphate, pH 7.0), daunomycin and adriamycin are unlikely to exhibit any binding preference for either poly(G-C) or poly(A-T) [9]. Here we report the results of an extension of this work which shows that the nature and concentration of solute species other than drug and oligonucleotide species can have a pronounced effect on the equilibrium distri-

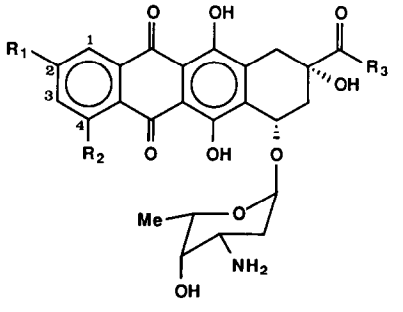

Fig.1. Structures of relevant anthracyclines: $R_1 = F$, $R_2 = H$, $R_3 = CH_3$, 2-fluoro-4-demethoxydaunomycin; $R_1 = H$, $R_2 = OCH_3$, $R_3 = CH_3$, daunomycin; $R_1 = H$, $R_2 = OCH_3$, $R_3 = CH_2OH$, adriamycin.

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bution of 2FD between d(G-C)$_5$ and d(A-T)$_5$. To this extent, we have studied the effects of varying NaCl, lysine and arginine concentrations on this distribution.

While the results presented show that variation in NaCl concentration can help explain some of the conflicting reports found in the literature, the results obtained using lysine and arginine are of special pharmacological interest, since the balance of DNA present in eukaryotic cells is electrostatically associated with lysine and arginine side chains which lie on the surface of nucleosomal proteins [10,11]. This being the case, the variation in base pair preference observed with changing lysine and arginine concentrations reported here suggests that anthracycline binding specificities may be strongly influenced by DNA association with proteins in the cell nucleus.

2. EXPERIMENTAL

2.1. *Reagents*

2-Fluoro-4-demethoxydaunomycin hydrochloride (2FD) was prepared as described by Irvine et al. [12]. 2FD denotes the hydrochloride when referring to the solid, and to the cation when referring to the aqueous solute.

Oligonucleotides, d(A-T)$_5$ and d(G-C)$_5$, as well as Sephadex G-15 were obtained from Pharmacia P-L Biochemicals. Amino acids were obtained from BDH. All other reagents were of analytical grade.

2.2. *Sample preparation for NMR*

The lyophilised, desalted 1:1:1 mixture of 2FD, d(AT)$_5$ and d(GC)$_5$ used as starting material in these studies was prepared as in [9] and all solutions were ≈1.5 mM with respect to these components. For the series of experiments employing a range of NaCl concentrations, the starting material was dissolved in 0.7 ml of 3 mM phosphate buffer at pH 7.0 in ^2H_2O, and solid NaCl added to obtain the required concentrations. For the experiments at pH 6.2 or 7.7, the same procedure was followed to obtain an NaCl concentration of 0.13 M, and the pH was then adjusted using 2 M HCl or 2 M NaOH. For experiments using lysine or arginine, the same procedure was followed and the pH was adjusted to 7.0.

2.3. *NMR spectroscopy*

NMR spectra were recorded on a Varian XL-300 spectrometer operating at 282.2 MHz for the ^{19}F nucleus. The spectra were accumulated with a 3.5 s recycle time and a 60° pulse. No proton decoupling could be carried out. Unless otherwise stated, all spectra were obtained at 20°C. Relative drug preference for d(A-T)$_5$ vs d(G-C)$_5$ was estimated by cutting and weighing ^{19}F peaks assigned to drug associated with respective oligonucleotides, and then taking the ratio, R, of these weights by dividing the area value for d(A-T)$_5$ associated drug with that for d(G-C)$_5$ associated drug.

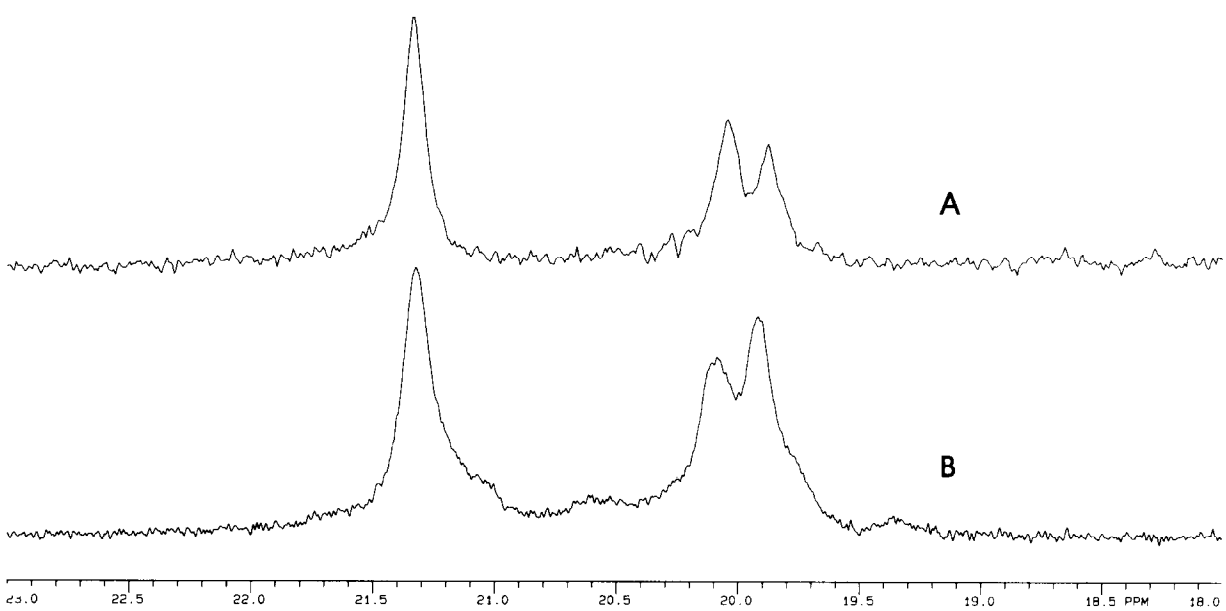

Fig.2. (A) ^{19}F NMR spectrum of a 1:1:1 molar mixture of 2-fluoro-4-demethoxydaunomycin (2FD), d(G-C)$_5$ and d(A-T)$_5$, each at 1.5 mM, 25°C, in 100 mM NaCl and 3 mM phosphate (pH 7.0). (B) ^{19}F NMR spectrum of the same system in 7 mM NaCl and 3 mM phosphate (pH 7.0).

3. RESULTS

A typical ^{19}F NMR spectrum of a 1:1:1 molar mixture of 2FD, d(G-C)$_5$ and d(A-T)$_5$ is shown in fig.2. We have previously assigned [9] the single broad peak at 21.5 ppm to 2FD associated with d(A-T)$_5$, and the two peaks at 20.0 and 20.2 ppm to 2FD associated with d(G-C)$_5$. Fig.2 also shows an example of the type of result obtained for the same system when alternative solvent conditions were employed.

The effects of increasing NaCl concentration on the equilibrium distribution of 2FD between binding sites on d(A-T)$_5$ and d(G-C)$_5$ are given in fig.3. The effects of changing pH are also demonstrated.

The results in fig.3 demonstrate that NaCl concentration has a significant effect on the equilibrium distribution of 2FD between d(A-T)$_5$ and d(G-C)$_5$. Above 0.1 M NaCl the drug shows a preference for binding sites on d(A-T)$_5$ while at lower concentrations there is a preference for sites on d(G-C)$_5$. In the absence of NaCl and buffer, the preference for sites on d(G-C)$_5$ is very pronounced. Changing the pH of a solution at 0.13 M NaCl gave *R* values very close to those predicted by interpolation of the data obtained at pH 7.0 indicating that pH had little effect on the distribution of drug. It was also found that variation in the concentration of phosphate had no significant effect on drug distribution.

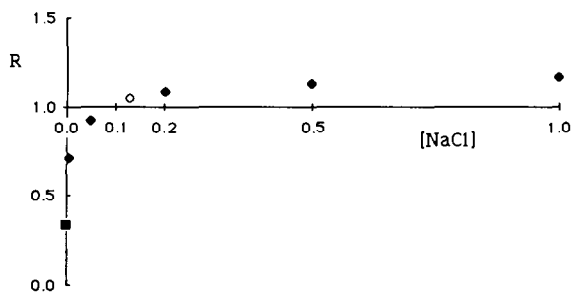

Fig.3. Effect of increasing NaCl concentration on the equilibrium distribution of 2-fluoro-4-demethoxydaunomycin (2FD) in a 1:1:1 molar mixture of 2FD, d(G-C)$_5$ and d(A-T)$_5$, each at 1.5 mM and at 20°C. *R* is the ratio of ^{19}F NMR peak area due to 2FD bound to d(A-T)$_5$ over that due to 2FD bound to d(G-C)$_5$. [NaCl] is molar. (♦) pH 7.0; (◇) values obtained with the pH adjusted to either 6.2 or 7.7 (i.e. *R* was the same regardless of pH); (■) system in the absence of NaCl and phosphate (pH 6.2) – with the exception of this last point, all of the systems studied were in 3 mM phosphate.

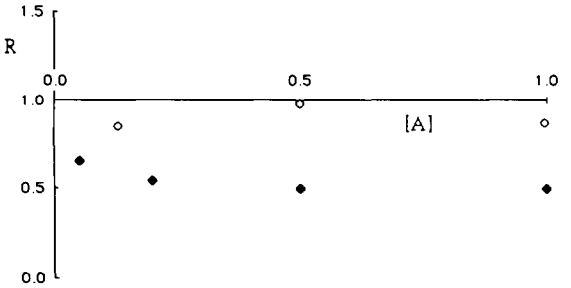

Fig.4. The effect of various concentrations of lysine (◇) and arginine (♦) on the equilibrium distribution of 2-fluoro-4-demethoxydaunomycin (2FD) in a 1.5 mM, 1:1:1 molar mixture of 2FD, d(G-C)$_5$ and d(A-T)$_5$, at 20°C, pH 7.0, in 3 mM phosphate. *R* is the ratio of ^{19}F NMR peak area due to 2FD bound to d(A-T)$_5$ over that due to 2FD bound to d(G-C)$_5$. [A] is the molar concentration of either lysine or arginine.

The effects of different lysine and arginine concentrations on the equilibrium distribution of 2FD between binding sites on d(A-T)$_5$ and d(G-C)$_5$ are given in fig.4.

Fig.4 shows that changing concentrations of lysine and arginine affect the equilibrium distribution of 2FD between binding sites on d(A-T)$_5$ and d(G-C)$_5$, and that these effects are unlike those observed for NaCl. Relative to systems containing NaCl, both lysine and arginine promote drug binding to sites on d(G-C)$_5$, with arginine inducing the most pronounced bias.

4. DISCUSSION

4.1. *Implications for previous studies*

It has been known for some time that NaCl concentration affects the interaction of anthracycline drugs with DNA. In 1982 Chaires et al. [13] reported that the binding constant for association between daunomycin and calf thymus DNA was reduced by a factor of three as the NaCl concentration was increased from 0.2 M to 1 M. In a later study, Chaires [14] also showed that both the enthalpy and entropy of daunomycin binding to calf thymus DNA were affected by changes in NaCl concentration in the region 0.05 to 1 M.

Despite these findings, past investigations into the DNA base pair binding site specificity of daunomycin and other anthracycline drugs have used a variety of NaCl concentrations. Phillips et al. [2] found that daunomycin bound with equal affinity to poly(G-C) and poly(A-T) at 20°C in a

system containing 0.15 M NaCl; Chaires [5] found that at 20°C and 0.19 M NaCl daunomycin showed a preference for poly(A-T) over poly(G-C), and DuVernay et al. [3] observed that adriamycin showed a very strong preference for poly(G-C) over poly(A-T) at 25°C and 0.05 M NaCl. Though attenuated, this same order of preference is reflected in the results given in fig.3, a finding which suggests that variation in NaCl concentrations may have led to the apparent inconsistency in earlier reports, and that future investigations of anthracycline base pair specificity should be conducted using at least two different NaCl concentrations.

As a final point, we note that Tsou and Yip [1] have observed an adriamycin preference for poly(G-C) over poly(A-T) at 30°C and 0.15 M NaCl. While this result may appear to be inconsistent with that of Phillips et al. [2], as well as with the general hypothesis presented here, the higher temperature employed by these workers may have led to a bias in drug binding towards sites on the more thermally stable poly(G-C) [9,15].

4.2. *Effects of lysine and arginine*

The bulk of DNA present in eukaryotic cells is found in chromatin, where it is intimately associated with histones [10,11]; these are basic proteins which have an unusually high content of lysine and arginine residues. DNA is anionic, and under normal cellular conditions the positively charged side chains on these residues bind a large fraction of total DNA by electrostatic attraction [11].

When used clinically, it is likely that daunomycin and adriamycin act on DNA in the form of its chromatin complex [16]. For this reason a study of the effects of free lysine and arginine on 2FD binding preference between d(G-C)$_5$ and d(A-T)$_5$ was undertaken, the aim having been to establish whether anthracycline binding site selectivity might be affected by DNA-histone association. As the results show, relative to the NaCl systems studied both amino acids enhance 2FD preference for d(G-C)$_5$ over d(A-T)$_5$, with arginine having a pronounced effect. Although it is not possible to extrapolate directly from our model system to the drug chromatin system likely to exist clinically, the result obtained here serves to demonstrate the potential for error inherent in studies of anthracycline-DNA binding site preference which do not take cellular conditions into account.

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