Binding Affinity and Site Selectivity of Daunomycin Analogues†

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ABSTRACT: We have tested a series of daunomycin analogues for binding affinity to a group of oligonucleotides that contain binding sites specific for daunomycin and that were previously screened for relative binding affinity for daunomycin. The series of drugs differed from daunomycin in the sugar moiety, including substitution of a hydroxyl group for the charged amino group and replacement of the 2′-OH by an iodo substituent. Data were analyzed by Scatchard plots and association constants were estimated from the y-intercept at saturating levels of oligonucleotide. Because of the solubility problems associated with these compounds, Scatchard plots could not be extended to high levels of binding. A second method of analysis of the fluorescence data confirmed the semiquantitative association constants obtained from the intercepts of the Scatchard plots. The association constants were in the range of 10^5–10^7 M^{-1}. When compared with daunomycin, the compounds with hydroxyl substituted for the amino group in the sugar ring generally bound less well to the oligonucleotides, by factors of up to several hundred. Much of the binding lost upon removal of the charged amino group was restored, however, with compounds containing an iodo substituent on the sugar ring. Changing the iodo-substituted sugar from the natural L-form to the D-form diminished binding by 6–50-fold, depending on sequence. This result implies a stereospecific interaction of the natural sugar with the DNA chain. A positively cooperative curve was observed in the Scatchard plot for the D-form sugar. Binding constants for a given analogue to a set of sequences varied by roughly 5-fold in all cases, but there was no sequence that was consistently preferred from one analogue to another.

Anthracyclines have been extensively used as antineoplastic agents against certain cell lines (Maniar et al., 1988). Since the discovery of adriamycin and daunomycin, two of the principal anthracyclines used as chemotherapeutic agents, the synthesis of many analogues has been vigorously pursued. One objective is to overcome the resistance that certain cell lines develop against these antibiotics (Maniar et al., 1988; Schneider et al., 1989), and a second is to avoid the side effects associated with both adriamycin and daunomycin (Arcamone, 1981; Lown, 1989; Myers et al., 1986).

Since the cytotoxic mechanism by which these drugs act is unclear, the influence of structural alteration on activity remains a question to be resolved empirically. For example, the influence may be on transport, or on physical interaction with DNA, or on interactions with enzymes that act on DNA, such as the topoisomerases. The effectiveness of individual compounds is clearly modulated by structural detail. Adriamycin, for example, is more effective against most tumors than is daunomycin, and it exhibits a higher binding constant to calf thymus DNA (Graves & Krugh, 1983; Chaires, 1990). Yet the two compounds differ by only a hydroxyl group. This seemingly minor structural difference affects the pharmacotherapeutic properties of the two drugs (Arcamone, 1981).

The class of antibiotics used in this study are composed of two main structural constituents, a planar anthraquinone ring and a sugar. The planar ring is presumably capable of intercalating between the base pairs, and the sugar ring sits in the minor groove (Wang et al., 1987), where it may make contacts with the bases or the backbone chain, thereby helping to anchor the drug. Substitutions, or the removal of functional groups on the aglycon ring, have also led to differences in affinity (Hammer et al., 1989). Compounds with alkyl and halogen substitutions on the sugar ring have been synthesized and tested for biological activity, which varies considerably with structure [Arcamone, 1981; Lown, 1989; Hammer et al., 1990; Horton et al. (1984) and references therein].

When DNA binding compounds are developed that are potentially effective therapeutic agents, the binding characteristics and selectivity of the drugs become important issues. Daunomycin, the parent compound in this study, was tested earlier for site selectivity and affinity using a system of oligonucleotides designed to contain enhanced binding sites for the drug (Roche et al., 1994). The study described here compares the binding characteristics of a series of anthracyclines with sugar moieties that have substituents different from those of daunomycin. All of the compounds tested (Figure 1) lack the amino group characteristic of the daunomycin sugar daunosamine, and therefore lack a positive charge at neutral pH. The affinity and site selectivity of these compounds was measured using the series of oligonucleotides of varying sequence previously tested for binding to daunomycin (Roche et al., 1994). The sequences of the oligonucleotides were selected because they were reported to be strong binding sites for daunomycin. The accuracy of the binding data is limited by the poor solubility of the compounds under study. Accordingly, factors of 1.5 or less between binding constants should not be considered significant.

MATERIALS AND METHODS

Oligonucleotides. The sequences used are listed in Table 1. The synthesis and characterization were reported elsewhere (Roche et al., 1994).

Antibiotics. The anthracyclines used were synthesized by G. A. Sulikowski (Suzuki et al., 1990) and D. B. Berkowitz (Berkowitz et al., 1992).

Binding Studies. The series of compounds with altered sugar rings were studied primarily by fluorescence binding assays, as described previously (Chaires et al., 1982b; Blake

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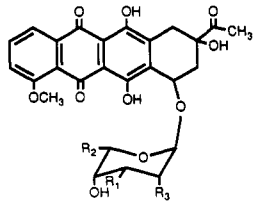

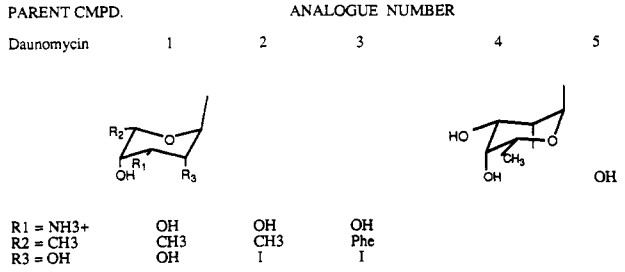

FIGURE 1: Structures of the compounds used in this study. In the first three compounds the sugar ring is as shown. The substitutions are as depicted in the figure. In the last compound the sugar ring is replaced with the ring in the D-form. The aglycon has a hydroxyl in place of the sugar ring.

Table 1

drug	oligonucleotide	K_1[a]	K_1[b]
daunomycin	CG	9.8×10^7	
	AG	4.0×10^7	
	TC	4.0×10^7	
	CS	5.0×10^7	
	TA	5.0×10^7	
	RA	3.0×10^7	
1	CG	2.0×10^6	1.8×10^6
	AG	2.0×10^6	1.3×10^6
	TC	8.0×10^5	5.1×10^5
	CS	2.0×10^6	1.8×10^6
	TA	5.0×10^5	1.0×10^6
	RA		
2	CG	1.2×10^7	7.8×10^6
	AG	2.0×10^7	2.1×10^7
	TC	7.0×10^6	4.6×10^6
	CS	1.0×10^7	3.9×10^6
	TA	3.0×10^7	
	RA	4.0×10^7	
3	CG	4.0×10^7	3.1×10^7
	AG	8.0×10^6	1.8×10^7
	TC	3.0×10^7	7.2×10^6
	CS	2.5×10^7	3.0×10^7
	TA	6.0×10^7	8.0×10^7
	RA	8.0×10^7	1.0×10^8
4	CG	5.0×10^5	4.5×10^5
	AG	4.5×10^5	5.3×10^5
	TC	4.7×10^5	1.1×10^6
	CS	1.2×10^6	9.4×10^5
	TA	7.0×10^5	2.0×10^6
	RA		2.0×10^6
5	CG		3.5×10^5
	AG		4.9×10^5
	TC		1.1×10^5
	CS		3.0×10^5

[a] All constants (K_1) reported are taken as the y-intercept of a Scatchard graph. [b] The second set of constants, K_1, are taken from the slope of the graph using eq 1, with $n = 1$, as explained in Materials and Methods. All measurements were performed in sodium phosphate buffer, 0.1 M Na^+, pH = 7.3, and 0.001 M Na_2EDTA at 20 °C.

& Peacocke, 1968; Bloomfield et al., 1974; Roche et al., 1994). This method seemed most appropriate primarily due to the decrease in solubility associated with the removal of the positive charge on the daunosamine sugar. The solubility limits of each compound were tested in the reaction buffer prior to their use, using the Beer's law relationship. All solutions of drug were kept within the solubility limits, and the range of linearity of Beer's law. Drug solutions in the concentration range of (2.0–3.5) × 10^{-7} M were titrated with increasing oligonucleotide concentration. The photochemical degradation of each compound was also measured independently by exciting an aliquot of drug continuously for more than 1 h and recording the emission as a function of time. Absorbance studies were conducted in a long path-length cell, from 2 to 5 cm, because the compounds were not soluble to an appreciable extent in the reaction buffer.

Fluorescence assays were conducted according to the method described previously (Roche et al., 1994). Briefly, an aliquot of drug was dissolved in the reaction buffer and the change in fluorescence intensity was measured as a function of added oligonucleotide. All measurements were recorded relative to an internal rhodamine reference, which accounted for any fluctuation in the lamp intensity.

Mathematical Treatment. The data were collected in the same manner as described previously (Chaires et al., 1982b; Blake & Peacocke, 1968; Roche et al., 1994) for the fluorescence binding assays and were normalized as previously. Specifically, i is the fluorescence intensity, in arbitrary units, divided by the drug concentration. A 1-mL sample of drug was aliquotted into a cuvette and oligonucleotide was added until the fluorescence or absorbance no longer changed. The signal intensity, i_{ap}, is plotted as a function of the total number of free sites, $(nC_N - C_T)$, where n represents the number of sites on the oligonucleotide, C_N the total nucleotide concentration, and C_T the total drug concentration, according to the following linear equation:

$$i_{ap} = i_B + \frac{i_F - i_{ap}}{K(nC_N - C_T)} \quad (1)$$

The limiting value of the fluorescence intensity, i_B, is extrapolated at high oligonucleotide concentration and used to calculate the free concentration of drug for each data point [Chaires et al., 1982b; Blake & Peacocke, 1968; for a derivation of the above equation see Bloomfield et al. (1974) and Roche et al. (1994)]. The limiting slope of this plot is K^{-1}. In cases such as the present study, in which the number of sites, n, cannot be reliably determined, the plot can be used to determine the value of the product of n and K.

The results of multiple runs were plotted in the form of Scatchard-type graphs (Scatchard, 1949). The intercept of the Scatchard curve with the y-axis, formally equal to the product nK, was taken as the total binding constant assuming $n = 1$, and it is this value that is tabulated for each antibiotic tested. The values for the binding constant K, obtained by Scatchard analysis, were compared with the values of K obtained from the slope defined in eq 1, again assuming $n = 1$.

In some cases, the data were scattered at low r, such that a definitive intercept was difficult to ascertain. If this was observed, a line was drawn at r equal to 1, where the data converged, and this value was taken as the binding constant. Limited drug solubility prevented use of higher drug concentrations as would normally be appropriate in cases of weak binding. Because the oligonucleotide concentration far exceeded the drug concentration in every case, much of the drug was bound. Thus, in the limit of low r the solubility limits are not exceeded. However, it is the values at high r that are difficult to measure due to the solubility limit imposed on the total concentration of drug, and without data at high r the number of binding sites cannot be determined. We emphasize that the results we report here are semiquantitative and are

useful primarily for comparison of one drug with another, the binding constants for which vary by 1–2 orders of magnitude. Variations of less than a factor of 1.5 should not be considered significant.

To compare the strength of binding and to estimate the sites per molecule of oligonucleotide, the raw fluorescence data were normalized as above, which takes into account the total drug concentration. Since the fluorescence of these compounds is linear in concentration of drug within the range of concentrations used in this study, normalizing the fluorescence this way yields the fluorescence per mole of drug. This parameter is plotted as a function of the ratio of added nucleotide to drug (Karpel et al., 1990). If the binding is strong, then this plot would be linear with a break in the slope at the ratio equivalent to the number of binding sites per oligonucleotide. If there are multiple sites of the same relative affinity, then a curve would result. The change in the slope of the curve as a function of r gives some indication of how many sites are present and how strong the sites are. When the drug approaches saturation of the binding sites on the oligonucleotide, the graph levels off. Plotting the data in this manner allows one to compare the binding of the drug to different oligonucleotides, and the scatter in the data, that appears in the Scatchard analysis, does not appear here. From this plot, the difference in the residual fluorescence is also evident.

RESULTS

The oligonucleotides used in this study are a subset of those listed in Table 1 in the accompanying paper (Roche et al., 1994). Figure 1 indicates the changes made in the daunosamine sugar.

General Observations. The sugar ring found in daunomycin was modified in several ways in the synthesis of the compounds examined in this study. The aglycon segment was held constant throughout the study as the carbohydrate segment was altered (Figure 1, compounds **1**–**4**). In one instance the aglycon lacking any sugar at C_1 (i.e., daunomycinone, compound **5**) was examined. For each compound tested, the results were analyzed according to two criteria—the change in affinity relative to daunomycin and the sequence selectivity. The results are presented in Figures 2–5 and Table 1 and are summarized below for each compound.

In general we find that changing the nature or handedness of the sugar ring on the daunomycin chromophore had a major effect in the strength of binding relative to daunomycin. A major source of the reduction in affinity appears to be removal of the charge on the sugar ring, resulting in loss of affinity by 20–60-fold, depending on sequence. For two of the compounds, **2** and **3**, which contain iodo-substituted sugars, the affinity was similar to that of daunomycin, within a factor of 2. Figure 2A shows a typical Scatchard plot, with characteristic scatter of the data in the limit as r approaches zero. For two of the compounds, **1** and **4**, there was evidently a positive cooperativity in the Scatchard graph (for example, see Figure 2B). Of particular interest is the reduction in binding affinity, by about an order of magnitude, when the handedness of an iodo derivative of the natural L-form sugar (compound **2**) was changed to the D-form (compound **4**). This result implies a stereospecific interaction of the sugar with the DNA chain.

By contrast, these changes in the sugar had relatively little influence on the extent of sequence *selectivity* of the compounds, meaning the range of binding constants (approximately 5-fold) for the different sequences. However, the

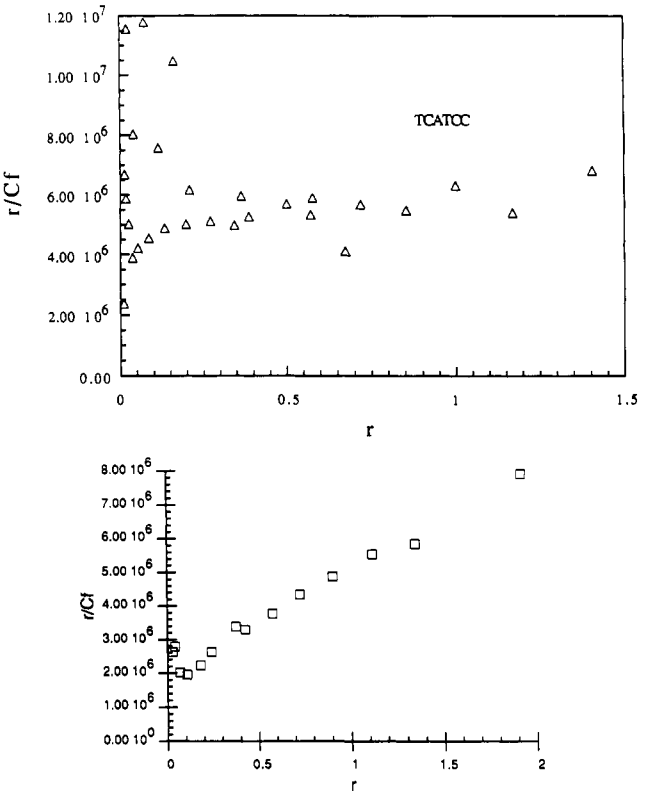

FIGURE 2: (A, top panel) Scatchard graph of iodomethyl-L and the TC oligonucleotide. Initial concentration of drug is 2.5×10^{-7} M; final concentration of oligonucleotide is 10^{-5} M. The data reflect three titrations. At $r = 1$ the data converge, and the y-intercept is taken at this point. (B, bottom panel) Scatchard graph of iodomethyl-D and the TC oligonucleotide. Initial concentration of drug is 3.30×10^{-7} M; final concentration of oligonucleotide is 4.0×10^{-4} M. Positive cooperativity is contrasted to the top panel, where the curve is normal.

different analogues did not generally exhibit the same sequence *preference*, meaning which sequences bind best and which worst.

The residual fluorescence at the end of the titration varied with each of the compounds tested here and in general was higher than the final values obtained with daunomycin in almost every case, except for the alternating TA and nonalternating A_{20}·T_{20} oligonucleotides. In both of these sequences the residual fluorescence is about 50% in almost every case, regardless of the drug.

Specific Observations: *Compound* **1**. A major alteration on the sugar ring was removal of the positive charge, by substituting a hydroxyl for the amine (compound **1**), leading to 20–60-fold reduced DNA affinity and a substantial loss of solubility. The loss of binding affinity is not entirely unexpected and can be explained generally on thermodynamic grounds, because the advantage of binding a positively charged ligand to a negatively charged helix is lost.

In addition, there appears to be a cooperative binding effect, indicated by the curve in the Scatchard graph, similar to that observed for compound **4** as illustrated in Figure 2B. Curvature similar to this had been observed for other anthracyclines (Rosenberg et al., 1986; Graves & Krugh, 1978; Chaires et al., 1982b). The effect is apparently independent of the solubility and seems to be associated with the overall conformation of the helix.

The sequence selectivity of compound **1** is about the same as that of daunomycin. The Scatchard analysis shows about

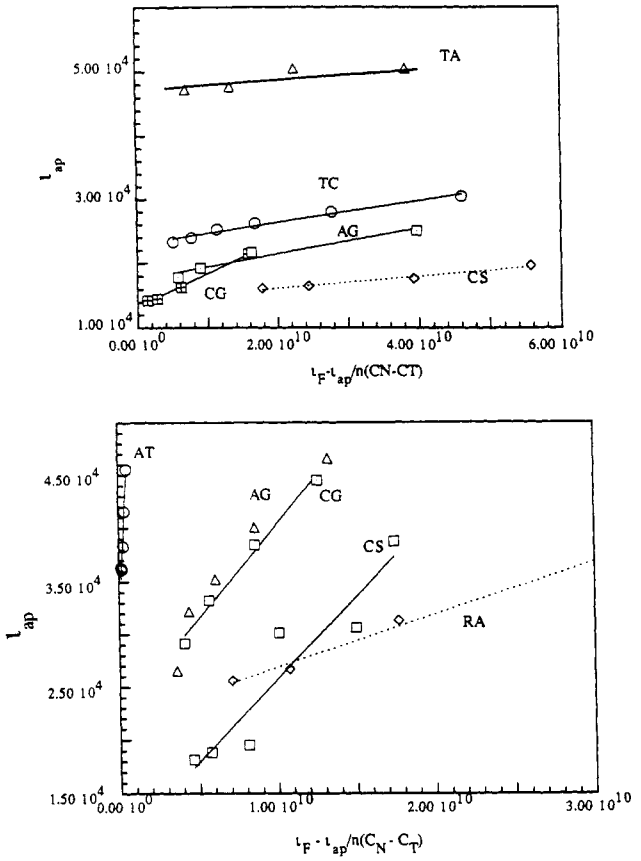

FIGURE 3: (A, top panel) Graph of the limiting slope for a series of oligonucleotides with the methyl-L sugar, according to eq 1. The slope is the reciprocal of the association constant as described in Materials and Methods. Initial drug concentration is 2.5×10^{-7} M in all cases. Final concentration of oligonucleotide varies. (B, bottom panel) Same graph for the iodomethyl-D sugar. Initial drug concentration is 2.5×10^{-7} M.

4-fold differences in the y-intercept obtained for this compound (Table 1), and plots of the data according to eq 1 indicate the same result (see the slopes in Figure 3). As can be seen, the limiting slopes in the presence of saturating levels of oligonucleotides are similar, differing by a factor of about 3. Where small changes in preference can be determined, the statistical significance of these is marginal. Certainly there is no major trend toward one sequence over another.

The residual fluorescence in this case was higher than in any of the oligonucleotides tested with daunomycin, for which the value in calf thymus DNA and in the CG oligonucleotide was about 2%. However, in compound **1**, and in the other compounds tested here, the residual fluorescence was as high as 30% in many of the sequences (see plots in Figure 4). This implies that the chromophore in these drugs is binding in a different mode from that of daunomycin. Since fluorescence is sensitive to the environment of the chromophore, this need not mean that the drug is binding to a different site; possibly the orientation of the drug in the site might be different such that quenching is reduced.

With compound **1**, it was also observed that the absorption spectrum in the presence of saturating concentrations of oligonucleotide were not accompanied by any major drop in extinction or shift in the spectrum (data not shown). This supports the observation in the fluorescence assays that the binding mode is different than that was observed with daunomycin. However, due to the solubility limits, the drug concentration may be below that necessary to observe small changes.

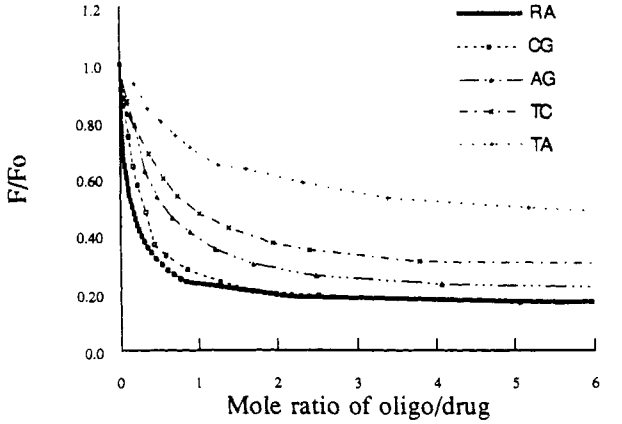

FIGURE 4: Plot of the adjusted fluorescence as a function of the ratio of drug to oligonucleotide, for the iodophenyl-L compound, **3**, expressed as moles of double helix. Initial concentration of drug is 2.0×10^{-7} M, and final concentration of oligonucleotide varies with sequence.

Compounds **2** *and* **3**. In these cases the C2′-hydroxyl group of the daunosamine sugar was replaced with an iodo group. Again here, the 3′-amino group was deleted and replaced with a hydroxyl. Two sugars of this type were synthesized. In one (analogue **2**), the methyl substituent on the sugar ring was retained, as in daunomycin. In the other, that methyl group was replaced with a phenyl (compound **3**).

For the L-handed isomer of both of these compounds, binding activity increased over compound **1**, where only the amino group was removed. The iodo group on position 2′ of the sugar ring enhances binding by a factor of at least 5–10 over the uncharged sugar ring; the reason for this is not obvious. Iodo substituents in general are bulky, but they are no more electronegative than carbon. There may be some subtle interaction in the minor groove between the iodo group and one of the bases, due to the polarizability of the iodo group, or because of its sheer bulk the iodo group may force the sugar into a conformation that places the methyl group in a convenient location to interact with the bases (Hawley et al., 1990).

Some of the sequences exhibit cooperativity in the Scatchard analysis when tested with compound **2**, but with other sequences this was not observed. The Scatchard plots were noisy at sufficiently low r values, as illustrated in Figure 2A. As described in Materials and Methods, the intercept taken as the total binding constant was extrapolated from the point where the data converge, which was usually at an r value of 1 or less. The sequence preference of compound **2** appears to be altered, with the sequences containing As (TA) being slightly favored over those with Gs (CG) (Table 1). This could be due to the presence of the iodo group which serves to enhance hydrophobic interactions.

The phenyl compound (**3**) exhibits the same increase in affinity relative to compound **1**, presumably due to the iodo group, but the preferences for the series of oligonucleotides is again different from that of daunomycin. The sequence that seems to bind the best is the one chosen at random (RA), with the other sequences being fairly similar (Figure 4 and Table 1), whereas the RA sequence was the weakest binder for daunomycin. For compound **3**, as with the methylated sugar, it is conceivable that the phenyl residue may somehow increase the hydrophobicity, or perhaps affect local polarizability, and thereby increase the interaction with the bases. Alternatively, the iodo group may serve to enhance the interaction by forcing the sugar into the minor groove in a conformation that favors this interaction. The Scatchard

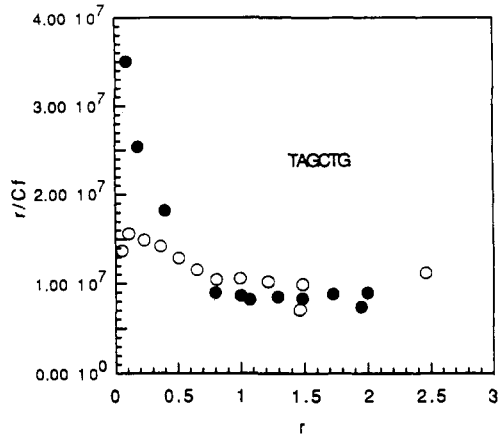

FIGURE 5: Scatchard graph of the iodophenyl-L compound with the AG oligonucleotide. Initial concentration of drug is 2.0×10^{-7} M.

treatment gave good results for $r \geq 1$ with the phenyl compound; for example, note the data for the AG sequence in Figure 5. In both compounds containing the iodo substituent, the sequence that was selected at random seems to bind well.

Compound **4**. The last substituent change inverts the iodomethyl sugar from the natural L-form (compound **2**) to the D-form (compound **4**). Overall the relationship of **2** and **4** is one of diastereomerism since the configuration in the aglycon sector is identical. The compound with a methyl substituent and D-form sugar binds very poorly, having affinities on the same order as compound **1**, which has the charge removed from the sugar and no compensating iodo group. The ratio of binding constants of compounds **2** and **4** vary from a high of about 50 for the AG sequence to a low of about 6 for the CS sequence. (The geometric means of binding constants determined by the two different methods were used for this comparison.) Typically the D-form sugar seems to bind in a cooperative manner, exhibiting positive Scatchard curves in almost every case (Figure 2B).

Compound **5**. The aglycon, the daunomycin chromophore without any sugar ring, was also tested in this series of drugs (Figure 1, compound **5**). The compound was not very soluble and binds poorly to the oligonucleotides, having binding constants on average about 4–5-fold lower than compound **1**. There was no more selectivity observed with this compound than with the others. We note that the compound is relatively unhindered in its approach to the oligonucleotide, not being confined by the sugar ring. The similarity of binding constants for the aglycon and compound **4** (excepting the case of the TC sequence) supports the view that few productive interactions with DNA are formed by the D-sugar. We note that the L-sugar configuration pertains in all anthracycline drugs.

The results with the oligonucleotide containing only tracts of As showed no real preference for any of the drugs. In general, all of the drugs bind poorly to this molecule, having association constants at least 10 times less than the other sequences. It has already been shown that the solution structure of the molecule resembles that of the nonalternating polymer poly(dA·dT) (Nadeau & Crothers, 1989).

DISCUSSION

The issue of selectivity in this study is one of relative binding affinities among the compounds tested. The electrostatic interaction of daunomycin with the oligonucleotides cannot be ignored. As a charged molecule, its interaction with the backbone contributes to the binding, which is also modulated by the overall shape of the drug molecule in solution. The shape of the L-form sugar lends itself to binding to a B-form helix. The aglycon portion intercalates such that the sugar moiety nestles in the minor groove and H-bonds are made with the bases [see Wang et al. (1987) for a review of the crystal structure of the drug bound to oligonucleotides]. Daunomycin has been shown to bind specifically to B-form helices, and is capable of flipping a Z-form helix to B-form (Chaires, 1985).

The DNA template to which these drugs bind is not conformationally isotropic. Since the microstructure of an oligonucleotide is determined by the base sequence at the binding site, the sequence of the bases determines the overall shape of the oligonucleotide. Because the sequence differs from molecule to molecule, the structure of each differs also. The A-tracts at the ends of the molecule may have an effect on the shape of the molecule, and have at the very minimum introduced a junction between the core and the A-tracts. This difference in structure may manifest itself in a change in the orientation of the sugar ring in the minor groove. Crystal structures of various oligonucleotides with daunomycin indicate changes in the orientation and conformation of the sugar from one oligomer to another (Nunn et al., 1991).

Daunomycin has a high affinity for each of the oligonucleotides tested relative to the compounds examined here, except for those containing an iodo substituent in the L-sugar (**2** and **3**). Removal of the charge limits the binding of our set of molecules, as illustrated by the data for compound **1**. The selectivity, as judged by the ratio of the strongest to the weakest binding constant for a set of sequences, is comparable for all these compounds. While there may be some common and some general trends among the different drugs, there is no sequence that stands out as preferred by all the drugs.

The drop in affinity of compound **1**, presumably due to loss of the positive charge, is analogous to data for steffimycin, an anthracycline that is not charged at neutral pH, whose binding constant is reduced relative to a charged ligand such as adriamycin (Sriram et al., 1991). A comparison with the drugs tested here cannot be taken too far because the functional groups around the ring on steffimycin are also different from those on the aglycon ring in daunomycin. This affects site binding since the contacts made with the bases in the intercalation site are changed. Substitutions such as alkylation of the oxygens alter the charge distribution and the potential for forming H-bonds with the bases (Hammer et al., 1990).

Both the iodomethyl-L and the iodophenyl-L bind with affinities of approximately the same order of magnitude as daunomycin. This could be due to the polarizability of the iodo group as was suggested in an earlier study (Hawley et al., 1989). Both seem to bind to the randomly chosen sequence and the alternating TA sequence better than the other sequences, Table 1, but only by a factor of 2.

Removal of the sugar ring entirely seems to limit binding the most and has the largest effect of the different substituents studied here. The aglycon does not bind well at all, several times worse even than compound **1**. The environment of the oligonucleotide is more hydrophobic than the environment of the bulk solvent, encouraging the relatively insoluble aglycon to associate with the DNA. The difference in affinity for the oligonucleotides tested here is not large, varying within the range of $(1–5) \times 10^5$ M^{-1}. This 5-fold variation of binding constant is comparable to that observed for the other compounds studied.

The absolute configuration of the sugar ring strongly affects association, the D-conformation having substantially lower K

values than the respective L-conformation. This result, along with the much enhanced binding of **2** relative to the aglycon **5**, shows clearly that there is a stereospecific interaction between the natural L-sugar and the DNA host in the complex. There may be some modest perturbation of sequence preference in the D-form sugar. For example, the CS sequence is highest in affinity for compound **4** and next to worst for compound **2**. Thus the sugar may contribute to sequence preference, possibly by an indirect mechanism such as altering chromophore position, but we stress again that the differences are quite small. Stereospecific interaction of the daunomycin sugar ring with DNA reinforces earlier conclusions (Nicolaou & Dai, 1991; Drak et al., 1991; Aiyar et al., 1992) that the carbohydrate moiety of DNA binding drugs such as calicheamicin can be responsible for both affinity and specificity in the DNA binding reaction.

The influence of the sugar component of anthracyclines was also apparent with an analogue that had a trisaccharide instead of the monosaccharide found in the compounds studied here. In that case, interaction of the sugar with the minor groove changed the kinetics of binding from milliseconds to minutes (unpublished results).

The D-form sugar exhibits a positive Scatchard curve in almost every case. It is not clear why this diastereomer should behave differently in this regard than the compounds with the L-sugar. It is possible that the solution structure of the drug is altered, such that the sugar does not fit well in the minor groove. It is also possible that this compound, as well as some of the others, is not intercalating totally but binding instead to the grooves where they can interact to yield cooperative binding. The phenyl compound with the D-form sugar may assume a conformation in solution in which the aglycon ring is stacked about the phenyl ring, as is observed for the crystal structure of the compound (Berkowitz et al., 1992). The optical spectra seemed to indicate some stacking interaction, but this is not definitive (data not shown). It did not seem that solubility was a problem, although this is a consideration that cannot be ignored.

CONCLUSIONS

Changing the sugar ring on daunomycin alters the affinity of the drug. With a few exceptions, the affinity of these compounds is lower than daunomycin, by factor of up to several hundred. Part of this is due to the lack of a charge on the sugar. The range of selectivity of binding to different sequences exhibited by the various compounds is comparable to that observed for daunomycin. Adding an iodo substituent restores some of the binding but both iodo-substituted compounds seem to prefer oligonucleotides with As, possibly due to the bulk of the iodo group or to hydrophobic interactions with the sequence.

In general, the L-form of the sugar elicits a more favorable interaction than the D-form and is the naturally occurring isomer. The D-form sugar may not fit well in the minor groove, permitting few favorable contacts to be made.

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