Complex Formation between Naphthothiopheneethanolamines and Deoxyribonucleic Acids†

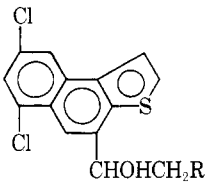
Edward J. Olmstead, James W. Panter, David W. Boykin, Jr., and W. David Wilson*

ABSTRACT: Reduced viscosities, viscometric titrations, fluorescence, and flow dichroism measurements indicate that the aromatic ring system of naphthothiopheneethanolamines (NA) is intercalated on binding to DNA. No marked change in binding was found on substituting a dibutylamino group for a piperidyl ring on the side chain. Ionic strength studies, however, did indicate that the positive charge on the side chain is quite important for binding. Both the binding equilibrium constant and the number of binding sites, as determined from a Scatchard plot of spectrophotometric results, decreased on increasing the sodium chloride concentration. The increase in T_m for several DNA samples, caused by adding NA to DNA at a fixed ratio, showed a marked dependence on the percentage of AT base pairs. The conclusion drawn from these data is that the DNA-NA complex involves high specificity of the NA for AT base pairs, intercalation of the aromatic ring system, and binding of the NA side chain in the DNA minor groove with strong interaction between the positively charged side chain and the DNA phosphate groups.

Developing an understanding of the complex formed between a drug and its cellular receptor is of obvious importance in studying the related disease, the mechanism of action of the drug, and aids in design of new and more efficacious compounds. For most drugs, the cellular receptor is unknown and even in cases where it is known, isolation and purification of it for *in vitro* studies often proves impossible. Since DNA can be obtained in a highly purified form, drugs which involve complex formation with DNA as part of the postulated mode of action have received much attention (*cf.* Hahn, 1971). In particular the intercalation theory of Lerman (1963, 1964) has provided a model which classifies compounds into those which intercalate and those that bind outside of the DNA helix. Many investigations have had as one of the primary goals, differentiation between these two modes of binding.

Hahn *et al.* (1966) has shown that the probable mode of action for the antimalarial drug chloroquine involves formation of a complex with DNA through intercalation of the chloroquine aromatic ring system and interaction of the positively charged side chain with the negatively charged DNA phosphate groups. The methods used to demonstrate intercalation of chloroquine included ultraviolet spectral changes, T_m measurements, intrinsic viscosities, and flow dichroism. These results have been substantiated by the finding that chloroquine causes unwinding followed by reverse coiling of closed circular supercoiled DNA, as is typical for intercalating compounds (Waring, 1970).

We have been studying a group of naphthothiopheneethanolamines[1] which show significant antimalarial activity (Das *et al.*, 1972). DNA binding studies with these compounds indicated that although factors such as membrane permeability are important, a group of NAs active against malaria gave DNA binding constants in the same order as their activity against the parasite (Panter *et al.*, 1973). These initial studies also suggested possible intercalation of the aromatic portion of the NA and interaction of the side chain with the DNA phosphate groups. Because of the possible importance of DNA interactions in the mode of antimalarial action of these compounds, and also because of the need for more detailed binding data on compounds which have both a planar aromatic ring system which can intercalate and a side chain which binds outside the DNA helix, we have investigated the binding of a dibutylamino NA with significant antimalarial activity, **1** (Das *et al.*, 1972), and its analog in the piperidyl series, **2**, which has



1, R = N(*n*-Bu)$_2$·HCl

2, R =  ·HCl

the highest affinity for DNA found for the NAs studied to this time (Panter *et al.*, 1973). The goals of this work were to obtain results, using viscosity and flow dichroism measurements, pertaining directly to the mode of binding of the aromatic portion of the NA, to determine whether there is a preference for the NA to bind to AT or GC base pairs, to determine what part the positively charged side chain plays in binding of the NA to DNA.

Experimental Section

Materials

NA (Das *et al.*, 1972) stock solutions were prepared in absolute ethanol at concentrations of approximately 10^{-3} M (Panter *et al.*, 1973). A low molecular weight ($[\eta]$ = 35.2 dl/g at I = 0.012) calf thymus DNA (Miles Laboratores, Inc., Lot 36-155) was used for viscosity, binding, and thermal denaturation experiments. A higher range molecular weight DNA (Worthington Biochemical Corp., Lot 33H

† From the Department of Chemistry, Georgia State University, University Plaza, Atlanta, Georgia 30303. *Received September 9, 1974.*

[1] Abbreviations used are: NA, naphthothiopheneethanolamine; DNA-P, DNA phosphorus.

742) was used for flow dichroism experiments. Other DNA samples used in denaturation experiments were: *Micrococcus lysodeikticus* (Miles Laboratories, Inc., Lot 12); *Escherichia coli* (Worthington Biochemical Corp., Lot 43H 027); and poly(dAT-dAT) (Miles Lot 11-34-317). For most experiments DNA stock solutions in 7.5×10^{-3} M sodium phosphate-1×10^{-3} M EDTA buffer (pH 6.0. $I = 0.012$) were used.

1,5-Diaminopentane (Aldrich Chemical Company, Inc.) was vacuum distilled, the HCl salt was prepared (dry HCl gas was bubbled through a 10% v/v solution of the amine in ethanol), and the resulting precipitate was collected, recrystallized twice from ethanol, and dried. The salt had a melting point of 255–256° compared to a literature value of 255° (Braun, 1904) and elemental analysis for carbon and hydrogen agreed with calculated values. For viscometric titrations a 0.02 M solution of the salt was prepared in 7.5×10^{-3} M sodium phosphate, 1×10^{-3} M EDTA buffer (pH 6.0, $I = 0.012$) containing 5% ethanol by volume (now called standard buffer). Because of solubility limitations on the NA, it was necessary to add them to DNA from stock solutions in ethanol (Panter *et al.*, 1973) and, for consistency, a concentration of 5% ethanol was used in all experiments.

Other chemicals were of the highest purity commercially available. Water was glass redistilled.

Methods

Viscosity Studies. The viscometer used was a Cannon-Ubbelohde four-bulb dilution viscometer (Cannon Instrument Co., No. 75s-177). For measurement of specific viscosity solutions of NA with DNA at a ratio of 1 mol of NA: 20 mol of DNA-P were prepared in standard buffer. From the DNA concentrations in g/dl the reduced viscosities were calculated and extrapolated to zero shear rate. A plot of the square root of the shear rate *vs.* the log of the reduced viscosity for each viscometer bulb (Lerman, 1964) gave a linear relationship for shear rates of less than 500 when using low molecular weight DNA.

For viscometric titrations a measured volume of DNA (2×10^{-4} M DNA-P in standard buffer) was titrated with a concentrated solution of NA in absolute ethanol. After each addition of NA the solution in the viscometer was mixed for 30 min by gentle airflow. No change in viscosity with time could be detected after 30 min. The NA was added with a calibrated microliter syringe and the total volume change at the end of any titration never exceeded 2%. The effect of adding just ethanol to the DNA solution was also determined and subtracted from the change caused by the NA in ethanol.

Flow Dichroism. DNA and DNA–NA solutions were driven from a 50-ml syringe through a narrow path cuvet (special purchase from Precision Cells, Inc., 0.25-mm light path) using a variable speed synchronous motor driven pump (Sage Instruments, Model 255-1) (Cavalieri *et al.*, 1956; Lerman, 1963). Polarized light was obtained from a polarizing filter (Polacoat Co., U.V. 105) in a rotatable mount. The filter and cell were mounted in the sample compartment of a Beckman Acta V spectrophotometer. Temperature was maintained at 25° using a circulating water bath with coils around the sample syringe and in the spectrophotometer sample compartment. Flow rates in the range of 20 ml/min were typically used.

Thermal Denaturations. The procedure was essentially the same as described previously (Panter *et al.*, 1973) except that the NA to DNA ratio was 0.31 rather than 1.0.

Table I: Reduced Viscosities for DNA and Complexes of **1** and **2** with DNA.[a]

DNA Concn ($g/dl \times 10^{-3}$)	Reduced Viscosities (dl/g)		
	DNA	DNA–1	DNA–2
9.25	37.1	48.1	48.4
7.40	36.7	47.0	47.4
6.20	36.4	46.6	47.2

[a] The ratio of **1** and **2** to DNA-P was 1:20 in all experiments with these compounds.

Spectrophotometric Binding Experiments. Solutions of NA were prepared in standard buffer and solid sodium chloride was added for studies at different sodium ion concentrations and different ionic strengths. The NA concentration was always near 2.5×10^{-5} M and a 5-cm spectrophotometer cell was used so that initial absorbances at 310 nm were near one. The total decrease in absorbance on adding DNA was usually around 50%. Aliquots of a DNA stock solution (usually around 3×10^{-3} M DNA-P in standard buffer) were added to the NA solution using a calibrated microliter syringe. The DNA was added to the NA because concentrated solutions of NA could not be prepared in standard buffer. The DNA additions were monitored by adding DNA to a cuvet containing only buffer and measuring the increase in DNA absorbance. This ensured that the DNA was actually passing through the syringe in the expected amounts. After adding the DNA, the solutions were gently mixed for 30 sec using a small magnetic stirring bar in the cuvet. Longer stirring times gave no additional decrease in NA absorbance. The measured absorbance was corrected for the small volume increase caused by the DNA addition and for any DNA absorbance or light scattering as determined from a blank titration. Absorbances were measured using a Beckman Acta V spectrophotometer with temperature maintained at 25° as above.

Fluorescence. The effect of DNA on the fluorescence emission spectrum of NA in standard buffer was determined using the Spectral Fluorescence Accessory (Model 569384) to the Acta V spectrophotometer. The exciting wavelength was selected using a Corning narrow pass filter No. 7-60 (maximum transmission near 350 mn) and the spectrum of the radiation emitted at 90° to the incident light beam was determined with the Acta V monochromator.

Results

Viscosity Studies. The zero shear rate, reduced viscosities of DNA alone, and complexes of DNA with **1** and **2** at different DNA concentrations are given in Table I. The molar ratio of NA to DNA-P was maintained at 1:20 for each concentration. It can be seen from Table I that when **1** and **2** are added to DNA at the same ratio, they have essentially identical effects on the DNA viscosity. Intrinsic viscosities were not calculated due to the difficulty of maintaining a constant amount of bound NA as the DNA concentration is reduced. This is especially true for compound **1** for which accurate equilibrium data could not be determined owing to solubility limitations. Although these viscosity increases are indicative of intercalation, it is felt that viscometric titrations give more information about the

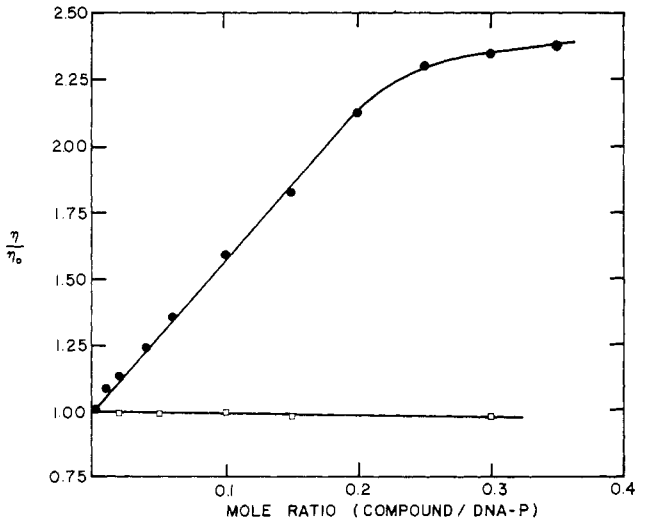


FIGURE 1: Viscometric titration of DNA with NA (●) and 1,5-diaminopentane (□). The ratio (η/η_0) of the viscosity of DNA with added compound (η) to DNA alone (η_0) is plotted as a function of the molar ratio of compound to DNA.

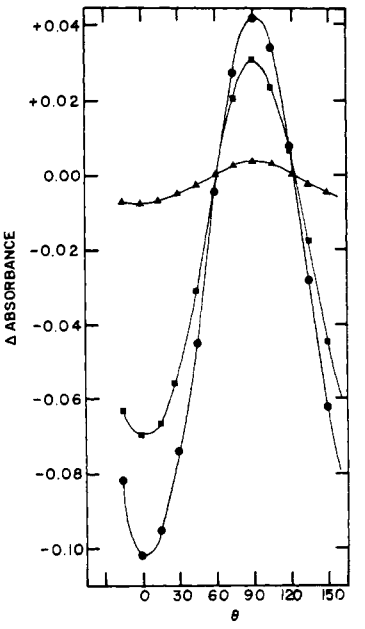


FIGURE 2: Flow dichroism measurements for calf thymus DNA at 260 nm (■, 3.1 × 10^{-3} M DNA-P) for DNA-NA at 260 nm (● 2.88 × 10^{-3} M DNA-P, 4.97 × 10^{-4} M NA) and for DNA-NA at 310 nm (▲, 2.88 × 10^{-3} M DNA-P, 4.97 × 10^{-4} M NA).

mode of binding of compounds to DNA. In Figure 1 are curves for the titration of DNA with **2** and with 1,5-diaminopentane which is known to bind externally to the DNA double helix (Mahler and Mehrotra, 1963). As can be seen, the DNA-NA complex exhibits a large rise and then a leveling off in reduced viscosity with increasing NA while the DNA-diaminopentane complex continues a much lower decreasing change in viscosity as a function of added compound.

Flow Dichroism. Another method for studying the mode of binding of planar aromatic compounds to DNA is by measuring the orientation of their ring system with respect to the DNA bases. This can be investigated by flow aligning the asymmetric DNA molecule in a narrow path spectrophotometer cuvet and measuring the change in absorbance of polarized light caused by the alignment for both the DNA and DNA small molecule complex. A plot of the change in absorbance as a function of the angle of the entering polarized light (zero degrees is parallel to the direction of flow) is given in Figure 2. If the NA ring system is intercalated, the fractional change (absorbance change divided by the total absorbance) should be the same for DNA and the DNA-NA complex. From Figure 2 the calculated fractional changes for DNA at 260 nm are −0.136 at 0° and +0.060 at 90°, and for the DNA-NA complex are −0.138 at 0° and +0.057 at 90°. This indicates that the DNA is maintained in the double helical conformation when the NA is bound and is consistent with parallel alignment of the NA ring system to the DNA base pairs; 36% of the absorbance at 260 nm for the complex is due to the NA. The absorbance change at 310 nm, which is due almost entirely to the NA, is also shown in Figure 2. Although the magnitude of the change is too small for accurate calculations, because of the limitations of the spectrophotometer, it can be seen that the changes are in the expected directions, the relative changes at 0 and 90° are as expected, and the approximate fractional changes are quite close to those given above for DNA.

Spectrophotometric Binding Studies. In addition to the mode of NA binding to DNA, the number of binding sites, Nap, and binding equilibrium constant, Kap, are important in understanding the DNA-NA complex. Using the change

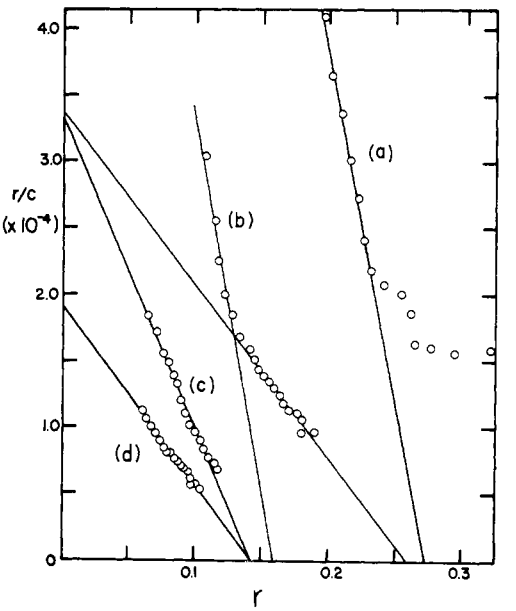


FIGURE 3: Scatchard binding curves at varying ionic strengths where r is the number of moles of NA bound per DNA-P and c is the molar concentration of free NA: (a) NA concentration of 2.72 × 10^{-5} M, titrated with 3.41 × 10^{-3} M DNA-P, ionic strength 0.012; (b) NA concentration 2.62 × 10^{-5} M titrated with 3.41 × 10^{-3} M DNA-P ionic strength 0.062; (c) NA concentration of 2.29 × 10^{-5} M titrated with 3.4 × 10^{-3} M DNA-P, ionic strength 0.087; (d) NA concentration 2.95 × 10^{-5} M titrated with 3.4 × 10^{-3} M DNA-P, ionic strength 0.112.

in NA extinction coefficient on binding to DNA (Panter *et al.*, 1973), these constants can be determined by the Scatchard plotting method (Scatchard, 1949). In Figure 3 the results of four spectrophotometric binding experiments at different ionic strengths are plotted by the Scatchard method. The binding constants are large enough so that the absorbance of the completely bound form can be determined by adding excess DNA. The data from binding experiments of the type are most accurate in the range from 20 to 80% of the added NA bound to DNA (Deranleau, 1969) and all

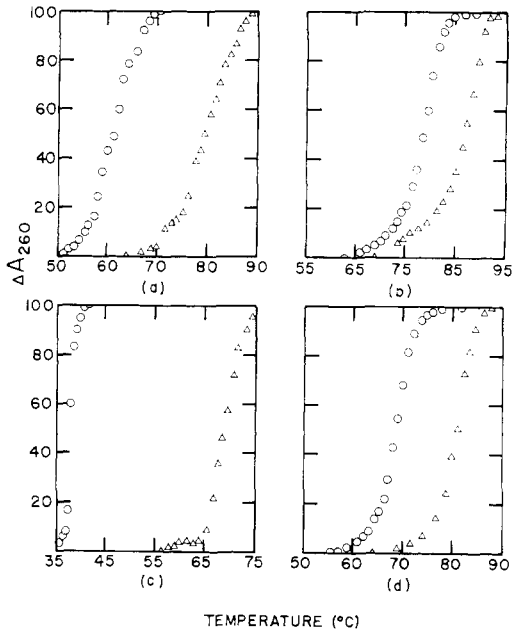


FIGURE 4: Thermal denaturation curves for DNA samples of varying A-T content without NA (●) and with NA (▲) at a molar ratio of 0.31 NA/DNA-P: (a) Calf thymus DNA; (b) *M. lysodeikticus;* (c) poly(dAT-dAT); and (d) *E. coli* DNA. The per cent change in absorbance at 260 nm (ΔA_{260}) is plotted as a function of centigrade temperature.

of the experimental points in this range are included in Figure 3. The points in Figure 3 are experimental and the straight lines are computer generated from a least-squares program. In curves a and b of Figure 3 two classes of binding sites are indicated by the curvature of the experimental points. The points are visually divided into two groups before computer evaluation. The large difference in equilibrium constants for the binding sites being studied in these two cases makes this separation reasonably straightforward. The number of binding sites and the binding equilibrium constants determined from the data in Figure 3 are collected in Table II. All binding constants are reduced on increasing ionic strength and the number of apparent binding sites is also reduced in each case.

It should be mentioned here that the Kap value reported by Panter *et al.* (1973) for strong binding of these NA at ionic strength 0.012 was approximately twice that reported here, while the values for Nap are essentially the same (0.25 and 0.27). There are two primary reasons for this difference in Kap. First, because of the low solubility of the NA, the binding constant could not be determined using spectrophotometric changes on adding NA to DNA solutions. The procedure used by Panter *et al.* (1973) involved preparing separate solutions containing a constant concentration of NA but varying amounts of DNA. Slight errors in pipetting NA can give large errors in these measurements. In this report the DNA and NA solutions were made up in the same buffer (containing ethanol) and the DNA was titrated into the NA solution in a 5-cm cuvet fixed in position in the spectrophotometer. This procedure greatly increases the reproducibility of the measurements, allowing a determination of more points, which facilitates the determination of both Kap and Nap. A second possible source of error in these experiments is the curvature of the Scatchard plot and extended extrapolation to the *r/c* axis necessary at low ionic strength (Kap can be determined from the slope of the plot or from the *r/c* intercept.) As can be seen from Figure 3, a deviation in the slope of the plot would cause a greater change in the *r/c* intercept than in the *r* axis intercept, giving more error in the determination of Kap than Nap. The best solution to these problems seems to be to use higher ionic strength when doing comparisons of different compounds. The curvature of the Scatchard plots in Figure 3 decreases significantly at higher ionic strength (greater than 0.05) and both Kap and Nap can be more unequivocally determined. The values at low ionic strength should not be ignored, however, since they determine whether any outside binding is possible and also allow a determination of the maximum possible number of intercalation sites.

Table II: Binding Data for the DNA-2 Complex at Different Ionic Strengths.

Ionic Strength	Na^+ Molarity	Kap × 10^{-5}	Nap
0.012	0.010	5.34	0.27
0.062	0.060	5.63	0.16
0.062	0.060	1.30	0.26
0.087	0.085	2.33	0.14
0.112	0.100	1.34	0.14

Thermal Denaturation. To determine whther there is any base pair specificity for NA binding, thermal denaturation experiments for DNA–NA complexes varying from 100% A-T to 28% A-T were performed. For denaturation curves, which are shown in Figure 4, the per cent change in absorbance is plotted as a function of temperature. For calculating the per cent change in absorbance, 0% was arbitrarily taken at 15° below the T_m and 100% change at 10° above T_m (T_m is the temperature at 50% change in absorbance). For the above calculations the T_m was first approximately determined by plotting absorbance *vs.* temperature for each denaturation experiment. Shifting the above limits farther from the T_m (for example 20° below and 15° above T_m) makes no more than a 0.5° change in T_m for any of the curves. In Figure 5 the change in T_m caused by the addition of NA to the different DNA samples (ΔT_m) is plotted as a function of the per cent AT for each denaturation experiment. The points are calculated from the curves in Figure 4 and the line was computer generated assuming linear behavior. Although there is at present no theoretical reason to assume a linear change in ΔT_m with per cent AT, any curvature in the plot of the data would be slight and would not change the general interpretation of the results.

Fluorescence. The fluorescence emission spectrum of **2** gives two peaks of approximately equal magnitude at 348 and 364 nm. The fluorescence quantum yield of both peaks decreases by at least a factor of ten on binding to calf thymus DNA. The fluorescence quantum yields for this compound and other NAs studied were too low for use in obtaining quantitative binding data.

Discussion

In a previous study (Panter *et al.*, 1973) we have found that although NAs with dibutylamino side chains bind to DNA and are good antimalarial agents, NAs with piperidyl side chains bind to DNA with similar binding constants but show no antimalarial activity. One possible explanation for

this is a different mechanism for binding of the two types of NA to DNA. Reduced viscosity data presented here in Table I for the two NAs differing only in side chain indicate that they have essentially the same effect on DNA, and the increase in reduced viscosity for both points to intercalation as the mode of binding. It seems then that both the binding constant and mechanism of interaction of these compounds with DNA are very similar and cannot account for the large differences in their antimalarial activity. The most striking difference between the two classes of NAs is in solubility (Panter *et al.*, 1973) which could explain the differences in activity through unequal rates of excretion or transport through membranes. Obviously other experiments will be necessary before a molecular understanding of the activity differences of these compounds is reached.

Since the compounds seem to interact with DNA in a similar manner, their mechanism of interaction with DNA was investigated employing the piperidyl compound **2** because of its greater solubility. The absorbance decreases (Panter *et al.*, 1973) and large fluorescence change on binding of **2** to DNA indicate a strong interaction of the NA aromatic ring system with the DNA double helix. This supports intercalation as the mode of binding, but is not conclusive proof since hydroxystilbamidine (Margo *et al.*, 1973; Festy and Daune, 1973) which does not intercalate, shows absorbance and fluorescence changes of similar magnitude. The reduced viscosities and particularly the viscometric titration which show large increases on addition of NA to DNA are more direct proof of intercalation. Some compounds such as netropsin (Zimmer *et al.*, 1971) or hydroxystilbamidine (Margo *et al.*, 1973; Festy and Daune, 1973), which bind outside of the DNA helix, cause slight increases in viscosity at low ratios of compound to DNA, but these are small compared to the change caused by the NA. Also in the NA viscometric titrations, the viscosity levels off at a high value while the viscosity of nonintercalating compounds either decreases continuously or shows a slight increase followed by a decline as the ratio of compound to DNA is increased (*cf.* a diamine in Figure 1). These viscosity increases coupled with the fact that the flow dichroism results for the DNA–**2** complex shown in Figure 2 are of the same sign and magnitude as those obtained for DNA alone provide strong evidence for intercalation of the NA aromatic ring system.

In binding studies the manner in which modifications of the macromolecular receptor affects interactions can frequently provide as much information as modification of the small molecule. With DNA the base pair composition can be varied by using samples from different species or by using synthetic DNA. Data are presented in Figures 4 and 5 showing how varying the AT composition affects the T_m increase on adding a fixed ratio of NA to each DNA. As can be seen there is a striking dependence of ΔT_m on the per cent AT in a DNA sample. The question of whether this base pair specificity originates with the NA aromatic ring system or the side chain or some combination of the two cannot be determined from these data. Ethidium bromide, for example, which intercalates but has no charged side chain shows no AT specificity (Le Pecq and Paoletti, 1967). The specificity of proflavine, which has no side chain, is in question since Kleinwachter *et al.* (1969) have reported some AT specificity for this compound while Ellerton and Isenberg (1969) found that the binding constant of proflavine did not depend on the AT composition of the DNA being studied. Many compounds which bind outside of the

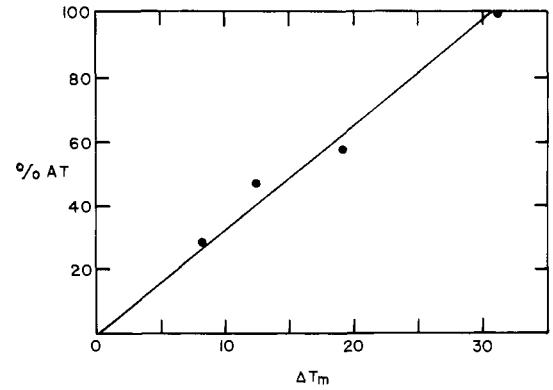


FIGURE 5: Changes in DNA T_m values due to addition of NA (ΔT_m) were determined from Figure 4 and are plotted as a function of per cent AT for each DNA. See Figure 4 for the DNA samples used.

DNA double helix, on the other hand, such as distamycin and netropsin (Zimmer *et al.*, 1971), some amines (Von Hipple, 1973), and diamines (Mahler and Mehrotra, 1963) show strong AT specificity. Actinomycin which has an intercalating ring system and a side chain which binds outside shows strong GC specificity which seems to be due primarily to the interactions of the side chain with guanine (Sobell *et al.*, 1971) although some contribution from the aromatic ring system apparently also occurs with this compound (Müller and Crothers, 1968). Chloroquine which also has both an intercalating aromatic ring system and a side chain seems to show some slight specificity for guanine (Hahn *et al.*, 1966). Therefore, since many compounds which show base specificity in DNA binding either bind outside or have side chains which bind outside of the double helix, it seems probable that at least part of the base specificity of the NA is due to its side chain. To test the point we are now synthesizing NAs with altered side chains to measure binding specificity.

The reduced viscosity results indicate that NA binding is not greatly changed when either a dibutylamino or a piperidyl side chain is present. This does not, however, answer the question of the importance of the positive charge on the side chain to binding. The large decrease in binding shown in Figure 3 on increasing ionic strength indicates that the interaction of the positive charge with the DNA phosphate groups is an important contribution to binding. Both the binding equilibrium constant and the number of binding sites decrease with increasing concentrations of NaCl. Gabbay and coworkers (Sanford *et al.*, 1975) have recently suggested that a decrease in binding of the compound on increasing the concentration of sodium ion indicates binding of the compound or its side chain in the minor groove. This is based on the X-ray data of Rosenberg *et al.*, (1973) which shows a strong binding site for sodium ions in the minor groove of the double helix. According to this theory the side chain of the NA would project into the minor groove. In general, investigations of small molecule binding to DNA have not been able to determine in which groove of DNA the molecule is bound or even whether there was a preference of one groove over another. Gabbay's suggestion then is most important and tests of sodium binding to AT sequences especially in solution (in contrast to crystal binding) should prove most helpful in characterizing binding interactions.

Another possible source of both the curvature of the Scatchard plots and of the decrease in Nap at higher ionic

strengths[2] is the fact that there are ten different intercalation sites in the double helical DNA molecule (Gabbay *et al.*, 1972) which could interact quite differently with the NA. From Figure 3 it can be seen that on increasing ionic strength, Nap decreases to a value near 0.14 and shows only a slight decrease after this point. This could indicate that some NA intercalation sites are more strongly affected by ionic strength (or Na^+) than others and are essentially eliminated at an ionic strength of 0.10. This results in a lower Nap value and a Scatchard plot of much less curvature since the intercalation sites are now more homogeneous. It should be mentioned that this is consistent with Gabbay's work since Rosenberg *et al.* (1973) found specific binding of Na^+ ion to double helical AU and not to GC. From the X-ray work it seems that any specific Na^+ binding in the minor groove of a double helix composed of mixed AT and GC base pairs would be very weak and would not interfere with NA binding.

The most probable mode of binding of NA is by intercalation of the aromatic ring system between DNA bases with a high specificity for AT base pairs. The side chain probably lies in the minor groove of the double helix and exchanging a dibutylamino for a piperidyl side chain causes little difference in the binding. The interaction of the positive charge on the side chain with the DNA phosphate groups provides a significant portion of the binding energy at low ionic strength. It seems probable the side chain accounts for part of the AT specificity of the NA, but the reason for this base selection is not clear at this time. We have found using Pauling-Cory-Koltun molecular models and the above constraints on the NA-DNA interaction that a hydrogen bond could form between the hydroxyl group on the NA side chain and the thymine carbonyl oxygen in the DNA minor groove. This would help account for the AT specificity and the location of the NA side chain in the minor groove. This is the same interaction that Henry (1972) has proposed for aryl amino alcohols as a result of model building studies. Further studies on NAs with varied structure should allow determination of a detailed binding model.

Acknowledgments

We would like to thank Professor E. J. Gabbay for allowing us to read a prepublication manuscript of his work. We would also like to express appreciation to Dr. Fred Hahn for helpful suggestions about design of flow dichroism experiments. Discussions of this research with Dr. B. P. Das and M. W. Davidson were most helpful and the assistace of Miss Koni McCravy in preparing this manuscript is acknowledged.

[2] At low ionic strength (curve a in Figure 3) curvature of the Scatchard plot leads to two Kap and two Nap values. The highest Kap value (with Nap of 0.27) is presumably due to intercalation, while the lower Kap and higher Nap values are probably associated with outside electrostatic binding of the NA to the DNA phosphate groups (Panter *et al.*, 1973).

References

Braun, J. V. (1904), *Chem. Ber. 37,* 3583.
Cavalieri, L. F. Rosenberg, B. H., and Rosoff, M. (1956), *J. Amer. Chem. Soc. 78,* 5235.
Das, B. P., Campbell, J. A., Samples, F. B., Wallace, R. A., Whisenant L. K., Woodard, R. W., and Boykin, D. W. (1972), *J. Med. Chem. 15,* 370.
Ellerton, N. F., and Isenberg, I. (1969), *Biopolymers 8,* 767.
Festy, B., and Daune, M. (1973), *Biochemistry 12,* 4827.
Gabbay, E. J., De Stefano, R., and Sanford, K. (1972), *Biochem. Biophys. Res. Commun. 46,* 155.
Hahn, F. E. (1971), *Progr. Mol. Subcell, Biol. 2,* 1.
Hahn, F. E., O'Brien R. L., Ciak, J., Allison, J. L., and Olenick, J. G. (1966), *Mil. Med. 131,* 1071.
Henry, D. W. (1972), *Nat. Med. Chem. Symp. Amer. Chem. Soc. Proc. 13,* 41.
Kleinwachter, V., Balcarona, Z., and Bohacetz, J. (1969), *Biochim. Biophys. Acta 174,* 188.
LePecq, J. B., and Paoletti, C. (1967), *J. Mol. Biol. 27,* 87.
Lerman, L. S. (1963), *Proc. Nat. Acad. Sci. U. S. 49,* 94.
Lerman, L. S. (1964), *J. Cell. Comp. Physiol. 64, Suppl 1,* 1.
Mahler, H. R., and Mehrotra, B. D. (1963), *Biochim. Biophys. Acta 68,* 211.
Margo, S. L., Boykin, D. W., and Wilson, W. D. (1973), Abstracts of the Southeastern Regional Meeting of the American Chemical Society, p 71.
Müller, W., and Crothers, D. M. (1968), *J. Mol. Biol. 35,* 251.
Panter, J. W., Boykin, D. W., and Wilson, W. D. (1973), *J. Med. Chem. 16,* 1366.
Rosenberg, J. M., Seeman, N. C., Kim, J. J. P., Suddath, F. L., Nicholas H. B., and Rich, A. (1973), *Nature (London) 243,* 150.
Sanford, K., Scofield, R. E., Adawadkar, P. D., and Gabbay, E. J. (1975), *Arch. Biochem. Biophys.* (submitted).
Scatchard, G. (1949), *Ann. N. Y. Acad. Sci. 51,* 660.
Sobell, H. M., Jain, S. C., Sakore, T. D., and Nordman, C. E. (1971), *Nature (London) 231,* 200.
Waring, M. (1970), *J. Mol. Biol. 54,* 247.
Zimmer, C., Reinert, K. E., Luctz, G., Wahnert, U., Löber, G., and Thrum, H. (1971), *J. Mol. Biol. 58,* 329.