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Anthracycline-binding induced DNA stiffening, bending and elongation; stereochemical implications from viscometric investigations[1]

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ABSTRACT

Upon interaction of the three anthracycline antibiotics daunomycin, adriamycin, and aclacinomycin A with calf thymus DNA the relative changes of both DNA contour length, $\Delta L/L^o$, and persistence length, $\Delta a/a^o$, have been determined as a function of r, the ratio of bound ligand molecules per DNA mononucleotide. From the r dependence of $\Delta a/a^o$ a measure for the stiffening effect and also the angle γ of ligand-induced DNA bending could be derived[9]. Experimental basis are titration viscometric measurements upon both low and high molecular weight DNA.

It was found that the DNA contour length increases linearly with r by approximately 0.34 nm per bound drug molecule. The comparatively very high DNA stiffening effect measured in solution is understandable as a result of helix clamping by at least two anthracycline groups of sufficient long distance. The variation of γ on DNA interaction with different anthracycline derivatives find their explanation in terms of different values of the mismatch to in-register binding prior to complex formation.

From an analogous interpretation of viscosity measurements by Arcamone and coworkers upon high molecular weight DNA with many anthracycline derivatives[18-20] it can be concluded that DNA interaction by both amino sugar and 9-acetyl group are responsible for the generation of strong anthracycline binding mediated DNA stiffening effects in solution.

(A combined analysis of the viscosity measurements by Cohen & Eisenberg[44] and Armstrong et al.[43] upon DNA interaction with proflavine indicates a very small DNA stiffening effect, $\gamma = 6.7^o$ and a helix elongation by 0.35 nm per bound ligand molecule.)

INTRODUCTION

The efficacy of many antibiotics is assumed to be a result of their interaction with DNA[2]. This, above all, concerns ligands intercalating into the DNA double helix. Two of the most important anticancer drugs are the anthracycline derivatives daunomycin (≡ daunorubicin: Dau) and adriamycin (≡ doxorubicin: Adr). Their structures differ only in the 14-H and 14-OH group, respec-

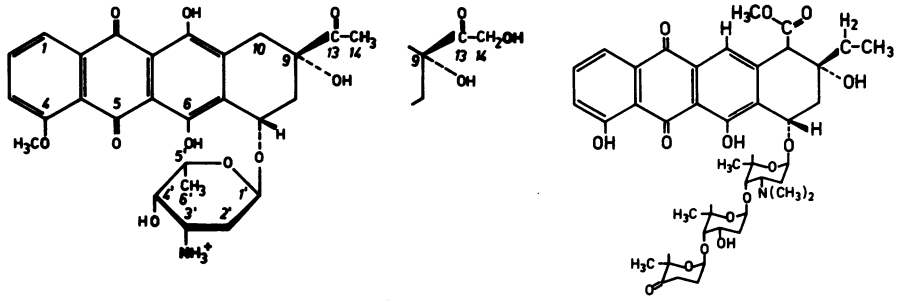

Fig. 1 Structure of daunomycin and adriamycin (OH in position 14)
Fig. 2 Structure of Aclacinomycin A

tively (Fig. 1). Adriamycin particularly exhibits high activity against various kinds of tumours [3]. Recent findings suggest membrane mechanisms to be involved in the cytotoxic effects of adriamycin without excluding the target role of DNA interaction [4]. Relatively few details are known about the properties of the DNA-anthracycline complexes.

This paper first deals with the measurement of the relative change of both DNA contour length, $\Delta L/L^{o}$, and persistence length, $\Delta a/a^{o}$, upon DNA interaction with daunomycin and adriamycin and also with the therapeutically very efficient anthracycline aclacinomycin A [5] (Acl, Fig. 2) as a function of r, the ratio of bound drug molecules per DNA phosphate. These two quantities can be derived simultaneously from the relative change of DNA viscosity of both a low and a high molecular weight DNA sample [6-8].

A symmetric parabola-like increment of the persistence length change is typical of kinking or local bending of the helical DNA molecule upon binding of small ligands [9]. It has been observed for all systems investigated. Its experimental determination, i. e. its separation from the stiffening effect, permits to evaluate the bending angle γ together with a measure for the stiffening effect, ΔA_{llig}. This quantity represents the length of a chain element, the total stiffening of which is hydrodynamically equivalent to the real effect. We have reason for avoiding the extended use of the expression "kinking" in this paper in order to prevent confusion with with special kink models [10,11].

The peculiarities in the experimental derived quantities γ

and ΔA_{1lig} will be interpreted in terms of stereochemically determined constraints or clamps between ligand and DNA helix, mediated mainly by hydrogen bonds and electrostatic interactions (refs. 8,9,12-14).

In particular, it will be suggested that high ΔA_{1lig} values correlate with great distances between the clamping points of the DNA-ligand complex taken parallel to the helix axis. The bending angle γ is proposed to be a measure for a mismatch $\Delta\lambda$ between complementary groups of DNA helix and ligand prior to complex formation. It is a behaviour similar to that recently analyzed for DNA interaction with different derivatives of the oligopeptide antibiotic distamycin [9] (in the range of non-cooperative binding[15]), with actinomycin D [9,16] and the anthracycline violamycin BI [17].

Viscosity changes being typical for local DNA bending on binding of different anthracycline antibiotics can also be recognized in almost all experimental curves, obtained by Arcamone and coworkers, on interaction of high molecular weight DNA with more than twenty daunomycin and adriamycin derivatives [18-20]. For other relevant communications see some reviews [21,22,2] and papers [23-25]. Many of these compounds varying only in one group or being stereoisomers considerably differ in their ability of stiffening and/or bending the DNA double helix. Already a qualitative or semiquantitative discussion of the DNA viscosity measurements for some of these systems demonstrates the influence of different stereochemical factors on the interaction mechanism. Acetylation and epimerization of the 3'-amino sugar or deacetylation in position 9, for example, reduce the high anthracycline-induced DNA stiffening to a negligible amount.

MODELS OF LIGAND-INDUCED DNA STIFFENING AND BENDING

Stiffening

The anthracycline antibiotics daunomycin and violamycin BI induce a comparatively very high local stiffening of the DNA double helix in solution[23,17]. A reasonable quantitative interpretation of such a strong effect by means of eq.(6) below demands a clamping of the double helix by ligand groups in a mutual distance being comparable with the length of the binding

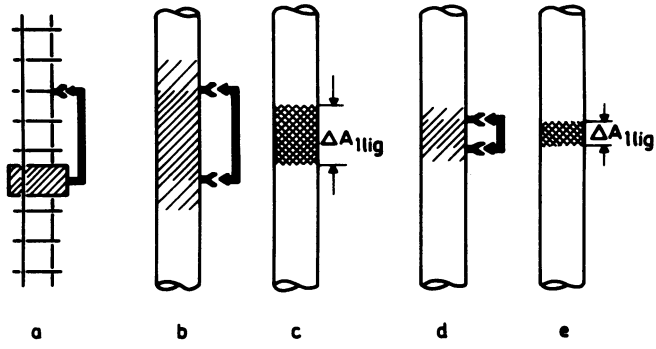

Fig. 3 Clamping of a DNA double helix segment by a ligand molecule is suggested to induce a stiffening effect, quantitatively described by ΔA_{1lig}, the hydrodynamically equivalent length of a totally stiffened worm-like chain segment. See also the text.

site. Therefore, the DNA interaction of the amino sugars[26] of these compounds is necessary to generate this effect in solution, and there should be another situation compared to that in related systems crystallized by means of tetravalent cations,[41] competing with the amino sugar for phosphate interaction. Acridines (this paper) and other intercalating drugs without comparable side groups[15,51,52] as well as special anthracyclines with modified amino sugar (see below) exhibit an almost negligible stiffening effect. Fig. 3a represents a simplified situation and Fig. 3b demonstrates DNA clamping by a bifunctional ligand in a more generalized scheme. The internal mobility of the DNA segment between the two interacting groups is partially reduced, perhaps even beyond them. From the change of the persistence length with r the length ΔA_{1lig} of a totally stiffened, hydrodynamically equivalent chain segment can be calculated (Fig. 3c; eq.(6), below)[6,8,9]. This means that such a hypothetical segment would produce the same alteration of the polymer dimensions as the real partially stiffened one. DNA ligands interacting via two groups of a smaller clamping-site distance (Fig. 3d) should induce a smaller stiffening effect ΔA_{1lig} (Fig. 3e).

Local Bending

Let us assume that the side chain of an (anchored) intercalated ligand in Figs. 4 is not able, for stereochemical reasons, to

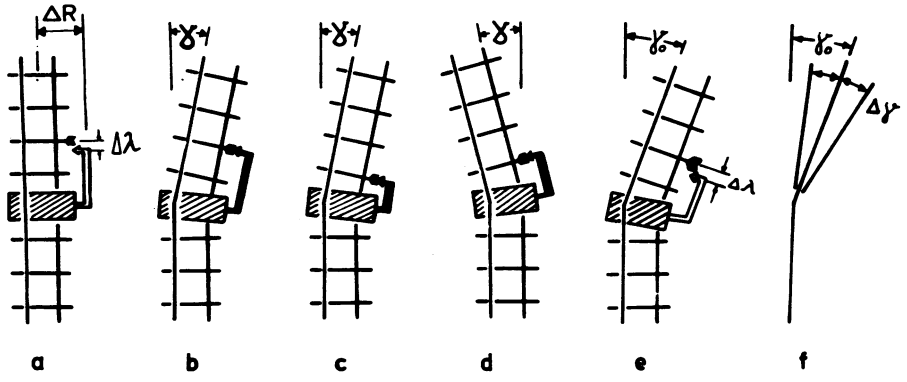

Fig. 4 DNA clamping ligands with two binding groups generally are able to cause DNA bends of different bending angles γ and different DNA stiffening effects. For details see the text.

form a hydrogen bond to the non-deformed double helix. The respective distance $\Delta\lambda$ between donor and acceptor has been defined as the mismatch of in-register fitting [9]. Bending of the DNA helix (e.g. by random fluctuations) permits to overcome this mismatch if the distance ΔR between ligand and "neutral fiber" of the elastic polymer (Fig. 4a) differs from zero (Figs. 4a,b). For an elementary rough model the resulting bending angle γ only depends on ΔR, $\Delta\lambda$ and, for the three-dimensional case, on the angle δ between the vector $\overrightarrow{\Delta\lambda}$ and the helix axis [9]:

$$\gamma = \frac{\Delta\lambda \sin\delta}{\Delta R} \qquad (1)$$

(if we assume ΔR to be the same for the different clamping groups). The special models of Figs. 4b and 4c, with different lengths of the chains, shall be characterized by the same values for $\Delta\lambda$ and γ. This assumption implies that the bending angle γ may be the same for very different lengths of the side group. Such systems, however, can be discriminated by their different DNA stiffening properties ΔA_{11ig} (Figs. 3b-e).

In Figs. 4c and 4d the models differ in the sign of γ and, consequently, in that of $\Delta\lambda$. By means of hydrodynamic methods no direct discrimination between both cases is possible. Between positive and negative $\Delta\lambda$ values, however, a distinction would then be conceivable in certain limits, if the special situation of Fig. 4e was realized, i.e. if intercalation of the chromophore

alone would produce a finite bending or kinking angle γ_o. Such a situation, if realized for any system, would implicate a modified relation substituting eq.(1) with $\Delta\lambda$ being proportional to $(\gamma-\gamma_o)$ instead of γ. Positive and negative $\Delta\lambda$ values would result in $\gamma<\gamma_o$ and $\gamma>\gamma_o$, respectively ($\Delta\gamma \lessgtr 0$, Figs.4e,f).

MATERIALS AND EXPERIMENTAL METHODS

Low and high molecular weight samples from calf thymus DNA, prepared by D.C. Eva Sarfert [27], Dept. of Molecular Biochemistry, were applied and obtained as described elsewhere [28]. Their respective molecular weights were $M_l = 0.5 \times 10^6$ and $M_h = 10 \times 10^6$.

Adriamycin and daunomycin were gifts of Prof. F. Arcamone, aclacinomycin A was donated by Prof. H. Umezawa.

The solvent was SSC buffer (0.195 M Na^+) at pH 6. (pK values of 8 to 8.5 are expected for the anthracyclines [29].)

The titration viscometric measurements were performed at 25°C by means of a Zimm-Crothers viscometer modified for titration experiments and equipped with an electro-optical time measuring device and with a sensitive temperature control for the DNA solution and for the driving magnet [30,8]. The use of filtered red light was necessary in order to prevent photodegradation of the drugs. The intrinsic viscosity $[\eta]$ was approximated by $\ln\eta_{rel}/c$ (η_{rel}: relative viscosity, c: DNA concentration) with $(\eta_{rel}-1)$ values for the pure DNA preparations of only 0.050 for M_l and of 0.058 and 0.117 for M_h.

Binding corrections were derived from the binding isotherms $r(c_f)$ (c_f: concentration of the free monomeric ligand), spectroscopically determined at 25.0 °C by Drs. F.A. Gollmick, U. Katenkamp, H. Schütz, E. Stutter and I.Petri from this department [31-34]. The accessible r-range was limited by interferences indicating the beginning of surface film formation in the viscometer.

THEORETICAL BASIS

Change of DNA Persistence Length and Contour Length

The theoretical dependence of the relative change of DNA viscosity $\Delta y = \Delta[\eta]/[\eta]^o$ on the relative change of DNA persistence length, $\Delta a/a^o$, and contour length, $\Delta L/L^o$, has been described by

Table 1 Intrinsic DNA viscosity and parameters[7] of eq.(2) for the DNA samples investigated (l,h) or discussed in this paper

DNA	$[\eta]^{\circ}$/dl g^{-1}	K_a	$(a_\eta+1)$	Q_{aa}	Q_{LL}	Q_{aL}
l	4.2	0.58	2.23	-0.27	1.22	1.59
h	53.	1.17	1.74	+0.03	0.63	2.15
ref.44	2.96	0.50	2.31	-0.28	1.38	1.46
ref.43	31.5	1.07	1.78	-0.04	0.66	2.05

a second order Taylor-series approximation[7]

$$\Delta y = K_a \cdot (\Delta a/a^{\circ}) + (a_\eta+1) \cdot (\Delta L/L^{\circ}) + Q_{aa}(\Delta a/a^{\circ})^2 + Q_{LL}(\Delta L/L^{\circ})^2 + Q_{aL}(\Delta a/a^{\circ})(\Delta L/L^{\circ}). \quad (2)$$

Superscript $^{\circ}$ denotes the properties of the uncomplexed DNA molecules. The numerical values for the coefficients are listed in Table 1. They depend on M and $[\eta]^{\circ}$, respectively, the first order coefficients K_a and $(a_\eta+1)$ in an opposite manner[6-8]. $\Delta a/a^{\circ}$ and $\Delta L/L^{\circ}$, consequently, can be determined by viscosity measurements upon both a high and a low molecular weight DNA preparation. See also a graphic representation of this procedure in paper 8. Helix diameter changes[7,6] may be neglected for the systems treated.

Bending and Stiffening Increments of Persistence Length Change

Both stiffening and local bending (or kinking) may contribute to an experimental $\Delta a/a^{\circ}$ value. In first approximation[15] we write[9]

$$(\Delta a/a^{\circ})_{exp} = (\Delta a/a^{\circ})_{st} + (\Delta a/a^{\circ})_{kn} \quad (3)$$

or

$$\frac{d}{dr}(\Delta a/a^{\circ})_{exp} = \frac{d}{dr}(\Delta a/a^{\circ})_{st} + \frac{d}{dr}(\Delta a/a^{\circ})_{kn} \quad (4)$$

To separate both increments a theoretical criterion is available, derived, for non-cooperative binding, from elementary symmetry considerations. Fig. 5 demonstrates the character of the r-dependence for the kinking or local-bending increment of the relative change of persistence length, $f(r) = (\Delta a/a^{\circ})_{kn}$, from graphical reasons drawn for the special case of a five base pairs binding site. For details see Fig. 5 and refs. 9,54.

With a ligand which both stiffens and bends the DNA double helix we expect an experimental $\Delta a/a^{\circ}$ vs. r curve with an approx-

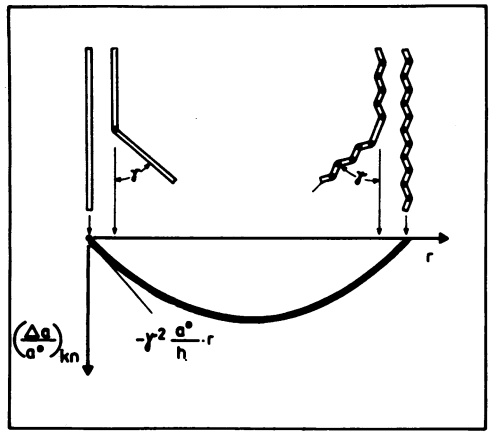

Fig. 5 Characteristic relative change of the kinking or bending increment of DNA persistence length $(\Delta a/a^o)_{kn}$ with r on interaction of the helical molecule with a helix-bending ligand. For small kinking angles γ the linear DNA molecule and the one with a hypothetically utmost number m_{max} of kinks or local bends are hydrodynamically almost equivalent. This also holds true for corresponding couples with $(m_{max} - \nu)$ and ν bends ($\nu = 1,2,..$). The resulting symmetry of the curve in Fig.5 is a consequence of the DNA helix character[9,54]. An argumentation on the basis of helix models with linear chain elements is justified[9].

imately linear contribution from DNA stiffening being superimposed by a parabola-like increment of the type presented in Fig. 5. Vice versa, with a corresponding interpretation of experimental curves we are able to separate both increments. A linear increase of the stiffening increment of persistence length, $(\Delta a/a^o)_{st}$, with r is represented by the secant of the experimental $(\Delta a/a^o)$ vs. r curve, if no base sequence dependence of γ exists. The difference between both lines is an approximation for $(\Delta a/a^o)_{kn} = f(r)$. From its initial slope $\frac{d}{dr}(\Delta a/a^o)_{kn/r\to o}$ we derive the bending angle γ (see Fig. 5)[9]

$$\frac{d}{dr}(\Delta a/a^o)_{kn/r\to o} = -\frac{\gamma^2}{h/a^o} \qquad (\gamma^2 \ll 1) \qquad (5)$$

(h: translation per base pair, 0.34 nm; see also ref. 56).

The "hydrodynamically equivalent DNA segment length of total stiffening" per bound ligand molecule follows from the slope of the $(\Delta a/a^o)_{st}$ dependence on r [8];

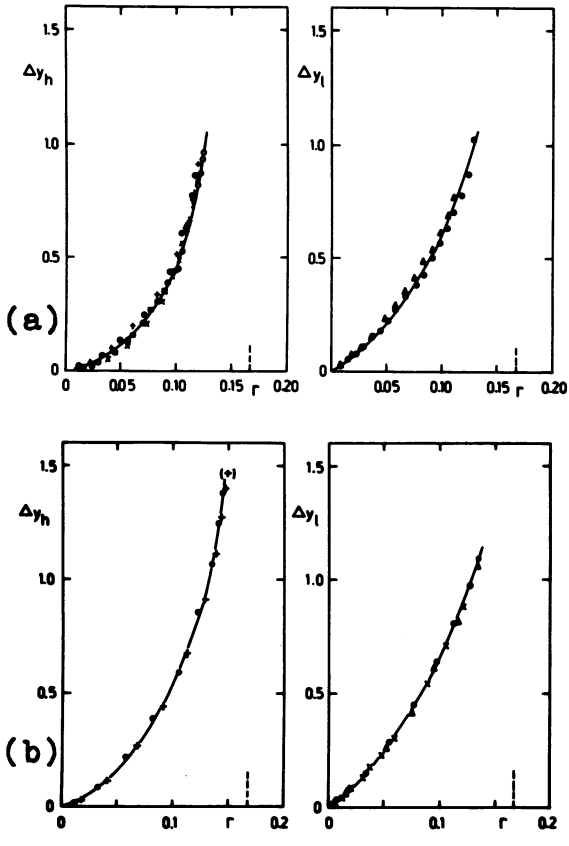

Fig. 6 Dependence of $\Delta y = \Delta[\eta]/[\eta]^o$ on r for a high and a low molecular weight calf thymus DNA sample (see Mat. and Meth.). The bound drug is (a) daunomycin and (b) adriamycin, respectively. The saturation values of r are marked.

$$\Delta A_{1lig} = \frac{h}{2} \frac{d}{dr}(\Delta a/a^o)_{st} \qquad (6)$$

and, analogously, the increase of DNA contour length per bound ligand molecule is [8]

$$\Delta L_{1lig} = \frac{h}{2} \frac{d}{dr}(\Delta L/L^o) \qquad (7)$$

The individual influence of DNA polymolecularity of a special system upon quantitative results was shown to be negligible [8].

RESULTS AND DISCUSSION

DNA Interaction with Daunomycin and Adriamycin

Figs. 6a,b present the relative changes $\Delta y \equiv \Delta[\eta]/[\eta]^o$ of DNA viscosity with r on interaction with daunomycin and adriamycin, respectively, for high (Δy_h) and low (Δy_l) molecular weight DNA. The dependences of $\Delta L/L^o$ and $\Delta a/a^o$ on r are plotted in Figs.

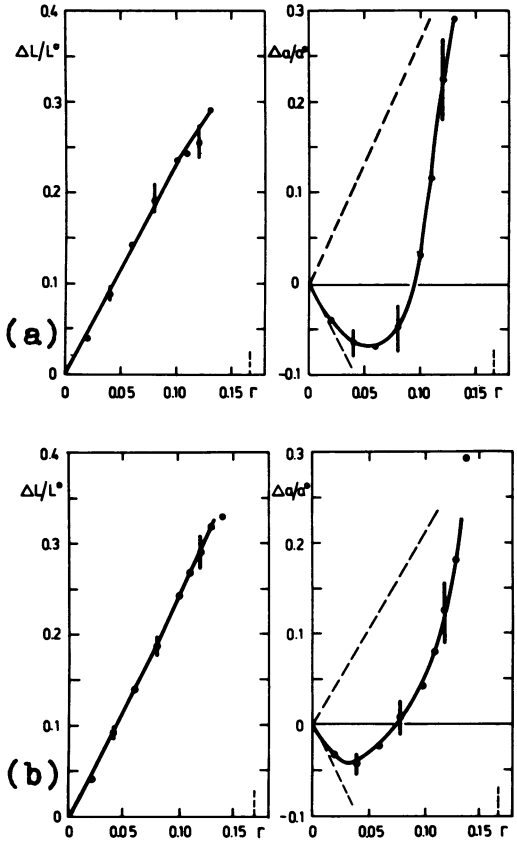

Fig. 7 Dependence of $\Delta L/L^{o}$ and $\Delta a/a^{o}$ on r for DNA interaction with (a) daunomycin and (b) adriamycin as derived from Figs. 6a and 6b. The changes of DNA persistence length are suggested to involve linear stiffening increments (dashed lines) and, consequently, convex contributions from bending.

7a,b. These data are the roots of two equations (2) with interpolated Δy_h and Δy_l for a series of r values (coefficients listed in Table 1). The bars mark the interval following from extreme (Δy_h, Δy_l)- combinations within the limits of experimental error.

With eq. (7) we get an increase of DNA contour length per bound ligand molecule of $\Delta L_{1Dau} = 0.39$ nm and $\Delta L_{1Adr} = 0.40$ nm, respectively (Table 2). The ΔL_{1lig} values are near to 0.34 nm, theoretically expected for an intercalating drug. Also the intercalation-induced DNA unwinding angle is the same within the limits of error [35,36]. It is conceivable that potential additional elongation increments are due to multipoint DNA-ligand constraints[37] since we learned that a linear non-intercalating helix-clamping ligand like netropsin is unambigously able to induce DNA elongation effects $\Delta L_{1Nt} > 0$ [6,8,28,38]. A neglect of the

Table 2 Change of DNA contour length, $\Delta L/L^o$, kinking angle γ, and stiffening effect, ΔA_{11ig}, derived by means of eqs.(7),(5), and (6), respectively, from the quantities listed in the other columns and valid for small r values

	$\frac{d}{dr}(\frac{\Delta L}{L^o})$	$\frac{d}{dr}(\frac{\Delta a}{a^o})_{exp}$	$\frac{d}{dr}(\frac{\Delta a}{a^o})_{st}$	$\frac{d}{dr}(\frac{\Delta a}{a^o})_{kn}$	$\Delta L_{11ig}^{+)\#)}$ nm	$\gamma^{\#\#)}$	$\Delta A_{11ig}^{++)}$ nm
Adr	2.35	-2.4	2.0	-4.4	0.40	10.5°	0.34
Dau	2.30	-2.5	2.7	-5.2	0.39	11.4°	0.46
Aol	2.1	-2.2	1.7	-3.9	0.36	9.9°	0.29
PF[#+)]	2.07	-1.3	0.5	-1.8	0.35	6.7	0.08

+) maximum absolute errors approximately 10 %
#) For apparent ΔL_{11ig} values obtained on neglection of the $\Delta a/a^o$ changes of sonicated DNA see the text
##) maximum errors approximately 15 %
++) maximum errors approximately 20 %
#+) Δy_h values by Armstrong et al.[43], Δy_l values by Cohen & Eisenberg[44]

change of persistence length on interpretation of the viscosity changes of the sonicated DNA samples would provide ΔL_{11ig} values being considerable smaller[58]. ΔL_{11ig} values derived for daunomycin and also aclacinomycin A at 0.0025 M Na^+ from electrodichroitic measurements[39,40] represent 78 % and 45 %, respectively, of those given in Table 2.

For both systems the DNA persistence length increases by more than 30 % (Figs. 7a,b). The secant of the experimental curve is correlated to DNA stiffening (see preceding section), whereas the difference between both lines, the parabola-like increment, is of the type expected for bending of double-helical DNA on random ligand binding. In Table 2 the different slopes for $r \to 0$ are listed as taken from Figs. 7a,b in order to calculate those quantities, compiled in the three right-hand columns, by means of eqs. (5,6;7).

On calf thymus DNA interaction with daunomycin and adriamycin, the equivalent length of total stiffening, $\Delta A_{11ig} = 0.46$ nm and 0.34 nm, respectively, are unusually high, i.e. they are comparable with the length of the binding site. This fact demands the existence of a multi-point clamping mechanism with a contribution by the amino sugar in the minor[41,42] groove. The sec-

ond strong ligand-anchoring seems to be realized by the 9-acetyl chromophore substituent. This group is reportet to exhibit a natural fit into the small groove of the right-handed double helix[41]. Its modification causes, besides a considerable effect in biological activity[41,21], also a drastic one in ΔA_{11ig}, as to be demonstrated below. For adriamycin-DNA interaction Quigley et al. discuss a 14-hydroxy hydrogen bond to O3 of the phosphate group[41]. This suggestion may explain the potential variation in ΔA_{11ig} compared to DNA-daunomycin interaction.

The values for the bending angle γ, 11.4° and 10.5°, indicate a remarkable deformation of the double helix, even if the intercalation of the chromophore alone would produce a finite angle γ_o. The difference of the two γ values is within the limits of experimental error.

By crystallographic X-ray analysis of the complex between daunomycin and the d(CpGpTpApCpG) duplex no bonding between oligonucleotide and amino sugar and no helix bending had been observed[41]. Crystallization in these experiments was achieved by addition of considerable amounts of fourfold positively charged spermine molecules. These oligocations obviously are able to compete successfully with the amino sugar for the DNA phosphate groups. Hence, the failure of the contact between amino sugar and DNA phosphate group in the crystallized state does not argue against its presence in solution. For our systems, evidence in favour of the existence of a bonding is given by the pronounced stiffening described and, furthermore, in a supplementary discussion following below. With the clamping model also the absence of helix bending in the crystallized complex[41] seems not to be an unreasonable result.

DNA-Aclacinomycin A Interaction

In analogy to the results of the preceding chapter Fig. 8a represents the experimental curves for the viscosity changes of DNA upon interaction with aclacinomycin A and Fig. 8b the underlying relative changes of contour length and persistence length. The different effects produced by the binding of one ligand molecule are listed also in Table 2. The elongation of 0.36 nm is almost that of 0.34 nm expected for intercalation and the DNA bending angle γ is similar to that induced by adriamycin binding,

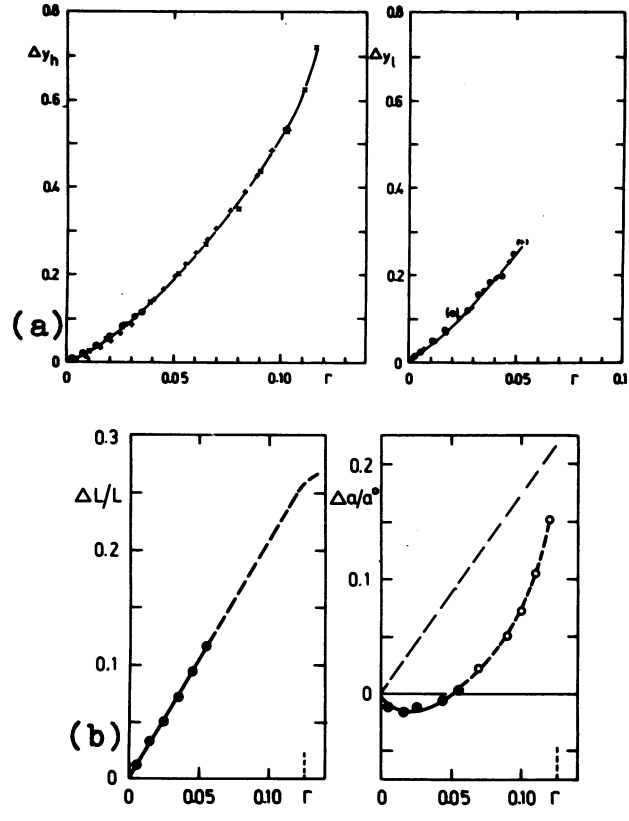

Fig. 8 Dependences on r analogous to Figs. 6 and 7 for aclacinomycin A interaction with calf thymus DNA; (a) Δy_h, Δy_l, (b) $\Delta L/L^o$, $\Delta a/a^o$. (Since Δy_l data for higher r values were not available as mentioned in Mat. and Meth., the linear $\Delta L/L^o$ dependence on r has been extrapolated in order to calculate the last $\Delta a/a^o$ values from Δy_h by means of one equation (2).)

but the stiffening effect ΔA_{1Acl}, compared to that by daunomycin, is significantly smaller.

Since, compared to the other two drugs treated, several groups are modified also several other interactions could be discussed and, consequently, several tentative explanations for the variation in ΔA_{1lig}. Considering the comparatively longer sugar chain of aclacinomycin A (Fig. 2, with the highest DNA association constant[34]) we expected a higher ΔA_{1Acl} value. To understand the experimental result we could assume the absence of any

hydrogen bond formation between the DNA helix and the two external sugar moieties. Also the methylation of the amino group may weaken the DNA contact but also, as discussed below, the modifications in position 9 and 10 are conceivable to change the anchoring of the ligand to the DNA double helix. A unique decision should be possible by experiments upon different derivatives with stepwise alteration of one group only.

Weak DNA Helix-Stiffening by Proflavine Interaction

Proflavine (PF) is an aminoacridine which, consequently, has no sugar side chain. It intercalates into the DNA double helix without being able to form a second bond to DNA in some distance from the chromophore. Hence, its DNA stiffening effect is expected to be small (Fig. 3). Thorough relevant viscosity measurements have been published by Cohen and Eisenberg for low molecular weight DNA[44] and by Armstrong et al. for high molecular weight DNA of the same base composition[43]. Twofold application of eq. (2) (coefficients in Table 1) to interpolated values of the corresponding Δy_l and Δy_h data delivers the r-dependence of $\Delta a/a^o$ and $\Delta L/L^o$ (Fig. 9). The $\Delta L/L^o$ vs. r plot represents a typical intercalation mechanism with $\Delta L_{1\,PF}$ near 0.34 nm (eq. (7); Table 2; see also ref.59). The comparison of the $\Delta a/a^o$ dependence on r with Fig.5 suggests the existence of a proflavine induced DNA bending effect. With a saturation value of r 0.2 •• 0.25 the stiffening and bending increments of the relative change of persistence length at low r-values were estimated (Fig. 9) as listed in Table 2. The bending angle is 6.7^o and the hydrodynamically equivalent DNA segment length of total stiffening, $\Delta A_{1\,PF} = 0.085$ nm, is very small compared to those for DNA interaction with the anthracyclines of Table 2.

The bending characteristics of Fig. 5 are distinctly involved in the curved shape of the experimental r-dependence of viscosity for the high molecular weight DNA[9,7]. Hence, from the similar form of the curve for the DNA complex with acridine orange[43] also a similar γ value has to be expected. At 0.0025 M Na^+ local bending or kinking of DNA could not be observed by means of electrodichroitic measurements on interaction with several intercalators[45]. (In this context it may be mentioned that also some discrepancies exist about the existence[46-48] or non-existence[49],

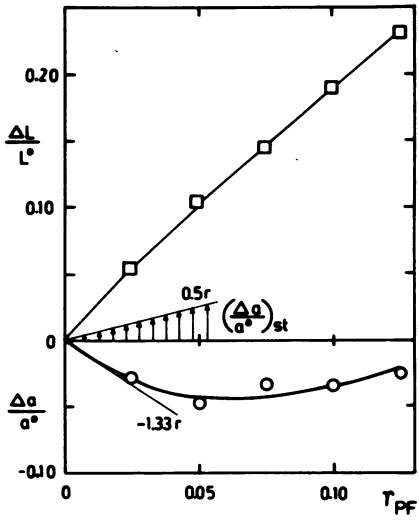

Fig. 9 $\Delta L/L^{\circ}$ and $\Delta a/a^{\circ}$ vs. r for DNA interaction with proflavine as derived from viscosity for low[44] and high[43] molecular weight DNA (58 % A·T)). Symbols refer to interpolated Δy values.

[50] of peculiarities in DNA helix conformation at very low ionic strength.)

DNA Interaction with other Anthracycline Derivatives

Arcamone and coworkers have measured the relative change of viscosity for high molecular weight calf thymus DNA (Δy_h) upon interaction with more than twenty cancerostatic anthracycline derivatives [18-20]. With reasonable assumptions about intercalation-induced $\Delta L/L^{\circ}$ effects qualitative conclusions may be drawn about the structure of the complexes without any calculation (cf. also Fig.1 of paper 8).

With a binding site size of two, three or four base pairs per bound ligand molecule intercalation with $\Delta L_{1lig} = 0.34$ nm induces a viscosity increase Δy_h for high molecular weight DNA of about 100 %, 64 %, or 47 %, respectively (eq.(2)). The excess up to saturation must result from DNA stiffening. DNA bending by ligand binding may be qualitatively estimated from the curvature of the $\Delta y_h(r)$ dependence.

Some examples will be presented in this chapter. Structural variations of some of these anthracyclines will be correlated

(i) to a drop of ΔA_{1lig} to an almost vanishing value, suggesting a reduction of the number of clamping points and/or (ii) to considerable changes of the angle γ of local DNA bending indicating other variations in specific interaction.

For the N-acetylated derivatives of daunomycin and adriamycin the sugar moiety is uncharged and, therefore, expexted not to be involved in a strong bond to the DNA double helix. This assumption is supported by the low apparent association constant and, for this first example, by the absence of a significant stiffening effect [19,20]. The DNA bending angles for these two systems must be of considerable magnitude. The question arises, as in the case of DNA-proflavine interaction, whether or not DNA bending for these examples is a result of chromophore interaction alone.

A second example with negligible ligand-induced stiffening effect is DNA interaction with 3'-epi-daunomycin. For this drug a direct contact between DNA-phosphate and the 3'-amino group seems to be prevented.

This also has to be assumed for the β-anomer of adriamycin. The viscometric DNA response reported for high molecular weight DNA [19] exhibits the smallest rise among the many systems reported. Hence, stiffening and intercalation exclude each other. Since, however, the α- and the β-anomer both unwind superhelical DNA in a similar and high degree[24], preference has to be given to intercalation and not to stiffening.

Paper 18 reports on DNA interaction with the two epimers of 9-deacetyl daunomycin, two antibiotics of different anticancer efficacy. (H,OH) and (OH,H), respectively, are the only side groups of C9. The observed maximum Δy_h value of only 0.4 is also very small compared to that measured on DNA-daunomycin interaction (Fig.6a). If intercalation is reasonable assumed the stiffening effect inevitably must be negligible, and this suggestion simultaneously rules out the existence of two clamping points between ligand and DNA double helix in greater distance. For daunomycin and these derivatives the amino sugar is the same, what is expected also for its DNA anchoring abilities. The discussed results are, therefore, understood by assuming, for the 9-acetyl group of daunomycin itself, an anchoring to DNA (in

accord with the suggestions by Quigley et al.[41]), whereas this second DNA clamping is obviously released for the two epimers treated.

The 4'-O-methyl derivatives of daunomycin and adriamycin are very efficient antitumour drugs. They induce very different DNA bending as reflected by very different curvatures in the plots for the viscometric DNA response $\Delta y_h(r)$ [20]. The bending angle seem to differ by more than a factor of two [60]. This finding implicates that the replacement of the 4'-OH group of the sugar by an OCH_3 substituent should be followed, at least for the daunomycin derivative, by a rearrangement of the groups involved in DNA clamping.

A remarkable antitumour activity is also characteristic of 9-deoxy-adriamycin [20]. The DNA stiffening effect must be similar to that produced by adriamycin, but the small curvature in the viscometric response seems to reflect a smaller bending angle and, consequently, another arrangement between DNA helix and the substituents in position 9. This again illustrates the role of the C9 side group in the constraints between DNA helix and chromophore.

In the toxic 9,10-monoanhydro-daunomycin [20] the 9-OH group is removed together with the hydrogen in position 10 and a double bond is formed between C9 and C10. The changes in bending angle and stiffening must be substantial. An almost linear viscosity rise indicates a very low bending angle, and the small stiffening effect seems to be comparable to that induced by acridines, if again the existence of intercalation is assumed.

Arcamone et al. found an approximately linear correlation between maximum DNA viscosity increase (Δy_h), logarithm of DNA-association constant and anti-tumour efficacy for numerous daunomycin and adriamycin derivatives [61]. According to the scope of this paper high Δy_h maximum means multipoint clamping in reasonable coincidence with the higher gain in free energy, and the concomitant higher biological efficiency seems to be coupled with stronger constraints between DNA helix and drug. On the other hand, low efficiency means low maximum Δy_h or non-multipoint clamping and low gain of free energy (association constant). The quantitative and qualitative results reported in this paper

have enabled us to gain some details of stereochemical relevance for DNA interaction with different anthracycline derivatives. Quantitative data could be obtained for all anthracyclines in the manner described, also with DNA of different base composition. In a following step model building would be advantageous. Even if future research would ever find that anthracycline interaction to DNA is not the only effect on the mechanism of their cancerostatic activity, the investigations presented would describe a novel approach for investigating stereochemical details of DNA ligand interaction. More detailed quantitative studies with several suitable derivatives should help to correlate structure and function of biological effectors.

The occurrence of DNA bending by drug interaction seems to be a fairly general feature in DNA ligand interaction. Other intercalating drugs with this ability are, e. g., actinomycin [9, 16], aposafranine[51], and most of the different tilorone derivatives, the DNA interaction of which has been studied in this laboratory[52]. Viscometric titration experiments with sonicated DNA on interaction with a bisintercalator exhibit features[53] which correspond to those expected on ligand-induced local bending. This seems to be reasonable since intercalation of the two coupled chromophores without any significant mismatch before complex formation is scarcely to be expected.

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59. Neglecting the influence of changes of persistence length a preceding theoretical formalism[44] delivers, from the same viscosity experiments with sonicated DNA, $\Delta L_{1PF} = 0.27$ nm. A deficiency to 0.34 nm has occasionally been interpreted as a result of a second non-intercalative binding.
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