Trisintercalation in DNA by *N*-[3-(9-Acridinylamino)propyl]-*N*,*N*-bis[6-(9-acridinylamino)hexyl]amine

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Viscometry, u.v. spectroscopy, fluorescence, and flow linear dichroism of *N*-[3-(9-acridinylamino)propyl]-*N*,*N*-bis[6-(9-acridinylamino)hexyl]amine (**1**) in the presence of DNA indicate that it is trisintercalating, and that it slowly 'crawls' along the DNA to find its optimal binding site.

DNA-affinity, particularly intercalative association, is common for a large number of biologically active polycyclic aromatic compounds,[1] and it is widely believed that their cytostatic, mutagenic, and carcinogenic properties are intimately related to their *in vivo* DNA-interaction.[1—3] However, other studies have indicated that the cytostatic action of at least some DNA-intercalating compounds may be mediated through the cellular membrane.[4,5] This has resulted in an intense effort to construct polyintercalating molecules in order to study their biological activity and interaction with DNA,[1,6] and we have recently reported the synthesis of potentially trisintercalating[7,8] and tetraintercalating[8] 9-acridinylamines. Simultaneously, Atwell *et al.*,[9] reported the synthesis of a related tri-9-acridinylamine, and on the basis of u.v. absorption and emission spectroscopy, and DNA unwinding studies, found reliable evidence in favour of trisintercalation.

We now report that *N*-[3-(9-acridinylamino)propyl]-*N*,*N*-bis[6-(9-acridinylamino)hexyl]amine (**1**)[7] appears to bind to DNA by trisintercalation in a step-wise process. Furthermore, we present data which indicate that the biological activities of related mono-, bis-, or tris-intercalating 9-aminoacridinyl derivatives are of the same order of magnitude.

The intercalation of compound (**1**) was examined by (i) its influence on the viscosity of sonicated calf thymus DNA,[10] (ii) by u.v. and fluorescence measurements,[2] and (iii) by flow linear dichroism.[11]

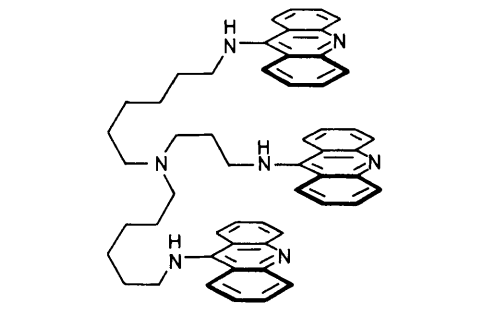
The viscosity increase of sonicated calf thymus DNA as a function of concentration of (**1**)—(**3**) was measured in order to compare the effect of (**1**) with those of the established mono- and bis-intercalators, (**3**) and (**2**).[6,12] These measurements can be expressed in terms of the viscosity index (the slope of L/L_0 as a function of [reagent]/[base-pair]) which reflects the degree of intercalation[2,6,10] (Table 1).

The increase in viscosity index from 1.4 for a monointercalator to 4.3 for compound (**1**) is consistent with trisintercalation for the latter. The indices of (**1**)—(**3**) are lower than the theoretical values.[1] However, this has previously been found for other similar 9-aminoacridine derivatives.[6,12]

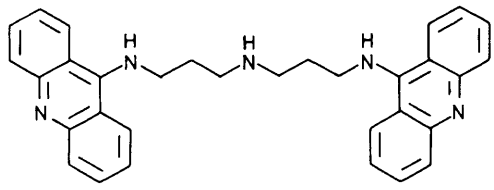
The long-wavelength u.v. absorptions of compounds (**1**)—(**3**) in aqueous solutions of calf thymus DNA exhibit a hypochromic effect and show bathochromic shifts. The molecular extinction coefficients ($\lambda > 400$ nm) per chromophore of (**1**)—(**3**), bound to DNA, are almost equal (±8%) indicating similar binding. Furthermore, the fluorescence quenching of (**1**)—(**3**) as a function of DNA (base-pair/acridine nucleus) is identical.

The flow linear dichroism (*LD*) spectra of (**1**), (**2**), and 9-aminoacridine are very similar (Figure 1), all showing a strong negative *LD* in the 400 nm absorption band (corresponding to a short-axis polarized transition in 9-aminoacridine).[13] The reduced dichroism $LD_{red.}$ (= LD/A) at 430 nm is approximately equal to that of the intrinsic DNA absorption at 260 nm, as expected for intercalation.[11]

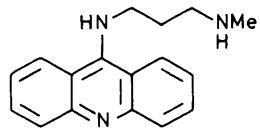
The binding constants of compounds (**1**)—(**3**) were estimated in a preliminary study from equilibrium analysis in a two-phase system consisting of aqueous dextran–polyethylene



(**1**)



(**2**)



(**3**)

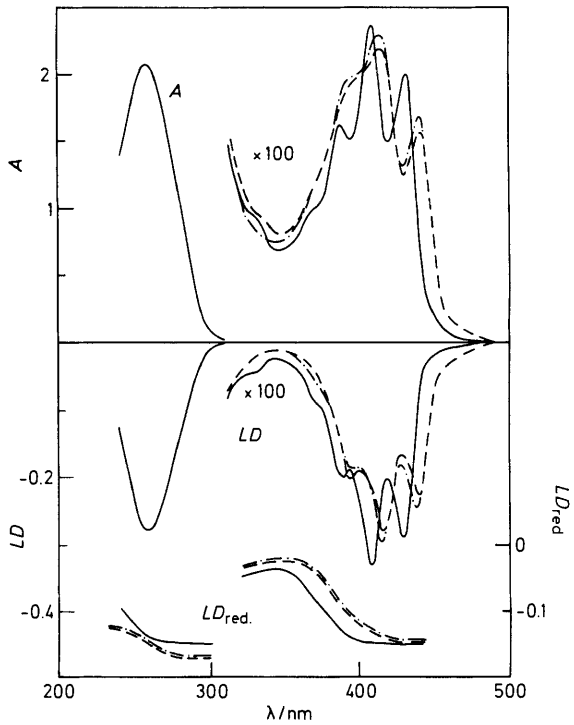


Figure 1. Absorbance (*A*), linear dichroism (*LD*), and reduced dichroism ($LD_{red.}$ = LD/A) spectra for the DNA complexes with 9-aminoacridine (——), compound (**1**) (— · — ·), and compound (**2**) (– – –). DNA concentration was 0.294 mM phosphate, ionic medium 1 mM NaCl and 1 mM Na_2EDTA. All spectra refer to the same binding ratio r of acridinyl residue to base: r = 0.015. All spectra are normalized to 1 cm optical path-length. The *LD* spectra were run at a flow gradient $g = 2000\ s^{-1}$.

Table 1

		U.v. absorption[b]				Cytostatic properties[c]				
	Viscosity	Free drug		Bound drug		Cell	DNA	RNA	Protein	Cellular
Compound	index[a]	λ/nm	ε/dm³ mol⁻¹ cm⁻¹	λ/nm	ε/dm³ mol⁻¹ cm⁻¹	viability	synthesis	synthesis	synthesis	uptake[d]
(**1**)	4.3	410	27300	415	20030	7.3	31	6.2	10	80%
(**2**)	3.0	406	18000	408	11470	(4.1)[e]	(65)[e]	(20)[e]	(24)[e]	60%
(**3**)	1.4	409	10900	416	6250	4.7	16	25	249	20%

[a] Mean value of at least two determinations, ±5%. [b] In 10 mM Tris, 10 μM EDTA (ethylenediamine tetra-acetic acid), pH 7.0; [drug]/[DNA] (base-pair) were: (**1**) 0.011, (**2** 0.017, (**3**) 0.034. [c] Determined as the concentration of drug in μM giving 50% inhibition when compared with untreated references. [d] Measured by fluorescence as percentage of added drug (1 μg/ml) associated with the cells, isolated by centrifugation. [e] These measurements were undertaken with the spermidine derivatives, *i.e.*, *N*-[3-(9-acridinylamino)propyl]-*N*-[4-(9-acridinylamino)butyl]amine.

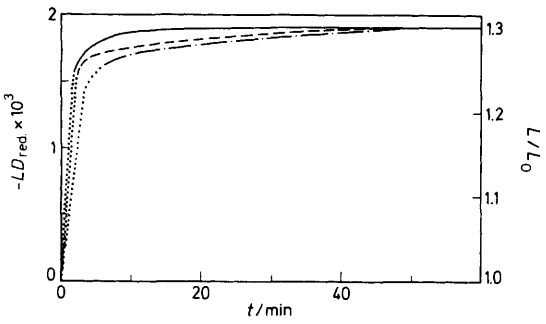


Figure 2. Time dependency of the flow linear dichroism signal ($LD_{red.}$) and the viscosity (L/L_0) of calf thymus DNA after addition of (**1**). —— ($LD_{red.}$), equal volumes mixed; [DNA] = 0.3 mM, [**1**] = 0.0015 mM. - - - - (L/L_0), high local concentration of (**1**). - · - · - (L/L_0), equal volumes mixed; [DNA] = 0.3 mM, [**1**] = 0.0015 mM.

glycol.[14] This showed that all the binding constants were $>10^6$ mol^{-1} l, and that all reagent added was >99% bound under the various experimental conditions.

The DNA-binding kinetics of (**1**) compared to those of (**2**) and 9-aminoacridine are interesting. Wheres the *LD* of 9-aminoacridine and compound (**2**) developed immediately upon mixing with DNA (within one minute), the binding of compound (**1**) showed a time dependency, the signal developing during 10—60 min depending on the mixing conditions (10 min for equal volumes of 0.3 mM DNA and 0.0015 mM reagent and longer times with high local reagent concentrations). A corresponding time dependency was also observed in the viscosity after mixing DNA and compound (**1**) (Figure 2).

The exceptionally long equilibration times for compound (**1**) indicate that upon mixing with DNA it binds locally with intercalation of only one or two of its 9-aminoacridinyl groups. Subsequently, a redistribution of the intercalating groups takes place until the preferred site is reached. It is very likely that this 'search' along the DNA involves 'crawling,' whereby the compound does not leave the DNA after the first association has taken place. This would resemble the way DNA sequence specific proteins, such as repressors and restriction enzymes, *etc.*, are believed to find their binding sites.[15]

The effects of (**1**), (**2**), and (**3**) on the DNA, RNA, and protein synthesis in L 1210 mouse leukemia cells, their effect on the growth of these cells, as well as their cellular uptake were of the same order of magnitude (Table 1). This suggests that the cytostatic action is not dependent on the number of DNA intercalation ligands per molecule in this series (*cf.*[1]).

This work was supported by the Danish Medical Science Research Council, with a grant to J. B. H.

Received, 15th November 1983; Com. 1497

References

1 For a recent review see: M. J. Waring, in 'The Molecular Basis of Antibiotic Action,' 2nd edition, eds. E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring, Wiley, New York, 1981, pp. 273—341.
2 For a recent review, see: W. D. Wilson and R. L. Jones, in 'Intercalation Chemistry,' eds. M. S. Whittingham and A. J. Jacobson, Academic Press, New York, 1981.
3 W. A. Denny, G. J. Atwell, and B. C. Baguley, *J. Med. Chem.*, 1983, **26**, 1619; 1625.
4 R. M. Fico, T. K. Chen, and E. S. Canellakis, *Science*, 1977, **198**, 53.
5 T. R. Tritton and G. Lee, *Science*, 1982, **217**, 248.
6 J. B. Hansen, P. E. Nielsen, T. Koch, O. Buchardt, M. Wirth, and B. Nordén, *Biochemistry*, 1983, **22**, 4878.
7 J. B. Hansen and O. Buchardt, *J. Chem. Soc., Chem. Commun.*, 1983, 162.
8 J. B. Hansen and O. Buchardt, *J. Chem. Soc., Chem. Commun.*, 1983, 1015.
9 G. J. Atwell, W. Leupin, S. J. Twigden, and W. A. Denny, *J. Am. Chem. Soc.*, 1983, **105**, 2913.
10 L. P. G. Wakelin, M. Romanos, T. K. Chen, D. Glaubiger, E. S. Canellakis, and M. J. Waring, *Biochemistry*, 1978, **17**, 5057.
11 B. Nordén and F. Tjerneld, *Biopolymers*, 1982, **21**, 1713.
12 R. G. McR. Wright, L. P. G. Wakelin, A. Fieldes, R. M. Acheson, and M. J. Waring, *Biochemistry*, 1980, **19**, 5825.
13 Y. Matsuoka and B. Nordén, *Chem. Phys. Lett.*, 1982, **85**, 302.
14 B. Nordén, F. Tjerneld, and E. Palm, *Biophys. Chem.*, 1978, **8**, 1.
15 O. G. Berg, R. B. Winter, and P. H. von Hippel, *Trends Biochem. Sci.*, 1982, **7**, 52.