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Studies on the ability of minor groove binders to induce supercoiling in DNA

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The effect of various non-intercalating minor groove binders on closed circular DNA in the presence of topoisomerase I has been studied by means of agarose gel electrophoresis. Analogues of the netropsin series (lexitropsins) and SN-6999 can effectively produce positive supercoils, as indicated by analysis of the topoisomers in the presence of chloroquine and the evaluated linking number changes. Analogues of the distamycin series are less effective, and bisquaternary ammonium heterocycles, as well as DAPI and pentamidine, were found to be ineffective ligands. The large differences observed in the ability of minor groove binders to induce positive supercoils are discussed.

Minor groove binder; Plasmid DNA; Supercoiling

1. INTRODUCTION

Variations in DNA supercoiling mediated by external ligands, such as proteins and small organic molecules, are of considerable interest as their effects could give information about the determinants of topological changes involved in transcription, replication, recombination or DNA repair. Drug-induced effects on DNA play a role with respect to their interference with enzymes of DNA metabolism, such as topoisomerases [1–3]. Binding of small intercalative ligands, such as EB or CQ, to a topoisomer unwinds DNA as a consequence of intercalation and, upon increasing ligand concentration, alter the superhelix density from negatively supercoiled, via the relaxed, to the positive supercoiled state. Binding of the non-intercalative drug, Nt, to closed circular DNA induces negative supercoiling. However, Nt is known to alter the superhelix density, producing positive supercoils when it binds to DNA in the presence of topoisomerase I, and it is subsequently removed by phenol treatment [4]. Since minor groove binders may differ in their binding ability to DNA [5], it was of interest to investigate whether non-intercalators of different structure will induce the same effect with variable efficiency. Using gel electrophoretic analysis in a comparative study we found that the potency of non-intercalators to modulate supercoiling of DNA strongly depends on the ligand structure and their differential binding behaviour.

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Abbreviations: EB, ethidium bromide; CQ, chloroquine; Nt, netropsin; Nt-Im$_x$, netropsin analogues (lexitropsins) containing two (x = 2) or three (x = 3) *N*-methylimidazole residues; bis-Nt-x (x = 5,6,8) bifunctional netropsins linked by x methylene residues; Dst-3, distamycin A; Dst-2, analogue of distamycin containing two *N*-methylpyrrole groups; Dst-Im$_2$, distamycin containing two *N*-methylimidazole groups; DAPI, 4′,6-diamino-phenylindole; SN-6999, SN-16841, SN-13282, SN-6131, SN-6132, SN-18071, bisquaternary ammonium heterocycles.

2. MATERIALS AND METHODS

2.1. *Drugs*

EB, CQ and DAPI were from Serva (Heidelberg); pentamidine was a gift from D. Schweizer (Vienna); Hoechst 33258 and Dst-3 were purchased from Sigma. Nt was a purified product isolated from *Streptomyces netropsis* as described previously [6]. Analogues of Nt and distamycin, including imidazole-containing lexitropsins, as well as bis-netropsins (bis-Nt-x), were synthesized in the laboratory of J.W. Lown. Bisquaternary ammonium heterocycles (SN-6999 and related bisquat's) were gifts of B. Baguley (Auckland, New Zealand). Structures of lexitropsins are given in [5,7,8], bis-Nt-x in [9], and bisquat's can be found in [5,10]. Chemical structures of representatives of different classes of non-intercalators used are presented in Fig. 1.

2.2. *DNA*

Plasmid DNAs were purified from *E. coli* strains harbouring the two derivatives of pUC18: pUC18-1.5 and pUC18-3.4, the clones were gifts of D. Schweizer (Vienna) [11,12]. pBR322 DNA was isolated from *E. coli* according to standard procedures by density gradient centrifugation.

2.3. *Enzymes*

Topoisomerase I was isolated from chicken erythrocytes according to the method described by Trask and Muller [13].

2.4. *Topoisomerase I-mediated assay of drug-plasmid DNA complexes*

Supercoiled plasmid DNA was equilibrated with added ligands for 10 min prior to topoisomerase assay, then 0.5 μg DNA was relaxed with 1 U topoisomerase I in 50 mM Tris-HCl (pH 8.0), 100 mM NaCl,

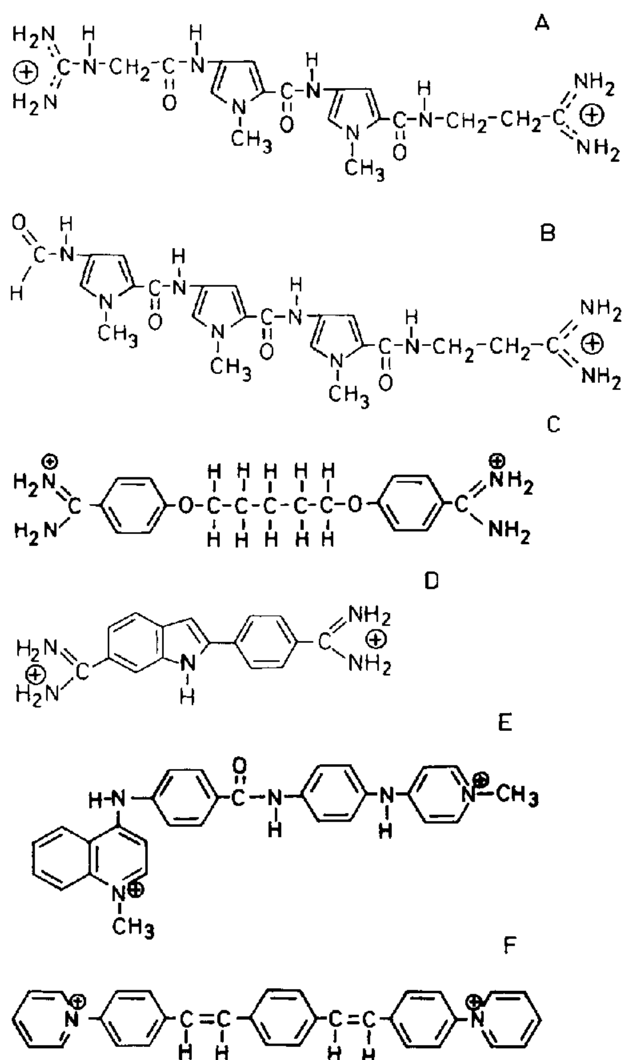

Fig. 1. Chemical structures of some representative members of different classes of minor groove binders: netropsin (A), distamycin A (B), pentamidine (C), DAPI (E), SN-6999 (F) and SN-18071 (G).

and 1 mM EDTA (total reaction volume 20 μl) at 37°C for 2 h. DNA was then extracted 3 times with phenol equilibrated with 50 mM Tris-HCl (pH 8.0). The aqueous phases were treated 5 times with ether to remove phenol, then DNA was precipitated with ethanol and dissolved in TE buffer (10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA). Samples were electrophoresed in 1% agarose gels in 50 mM Tris-acetate (pH 8.0), 20 mM sodium acetate, 2 mM EDTA, 18 mM NaCl at 30 V/cm for 18 h. Staining of the DNA in EB (0.5 μg/ml), photography and scanning of negatives have been described previously [3]. Gels with CQ contained CQ at concentrations of 1.5 μg/ml or 3 μg/ml in both the gel and the electrophoresis buffer.

3. RESULTS AND DISCUSSION

To investigate the effect of various minor groove binders on the torsional tension of closed circular duplex DNA, two plasmid derivatives of pUC18, containing two different inserts with a nucleotide length of 309 (pUC18-3.4) and 313 (pUC18-1.5) have been used [11]. The inserts have an AT content of 54.1 and 42.1 mol%, respectively [12]. Fig. 2 shows the results of the relaxation assay of pUC18-3.4 in the presence of increasing amounts of Nt-Im$_3$ (panel A) and SN-6999 (panel B). At a total ratio $r' = 0.05$ to $r' = 0.2$ (drug per nucleotide) both drugs induce changes in the superhelical density of the DNA, as indicated by the faster migration of the topoisomers. To determine the sign of the drug-induced supercoiling of topoisomers, samples were electrophoresed in gels containing CQ. CQ intercalates into DNA and will shift negatively supercoiled DNA upwards on the gel under our experimental conditions, while positively supercoiled or relaxed DNA is shifted downwards [14]. Fig. 2 demonstrates that in the presence of 1.5 μg/ml CQ, Nt-Im$_3$ and SN-6999 produce downward shifts (CQ in panels A and B, lanes 7–10). Therefore both drugs have induced positively supercoiled topoisomers at $r' = 0.05$ ligand per nucleotide. Some positively supercoiled topoisomers appear already at $r' = 0.025$ as indicated in presence of CQ (Fig. 2, lane 7). Nt was found to produce the same effect in

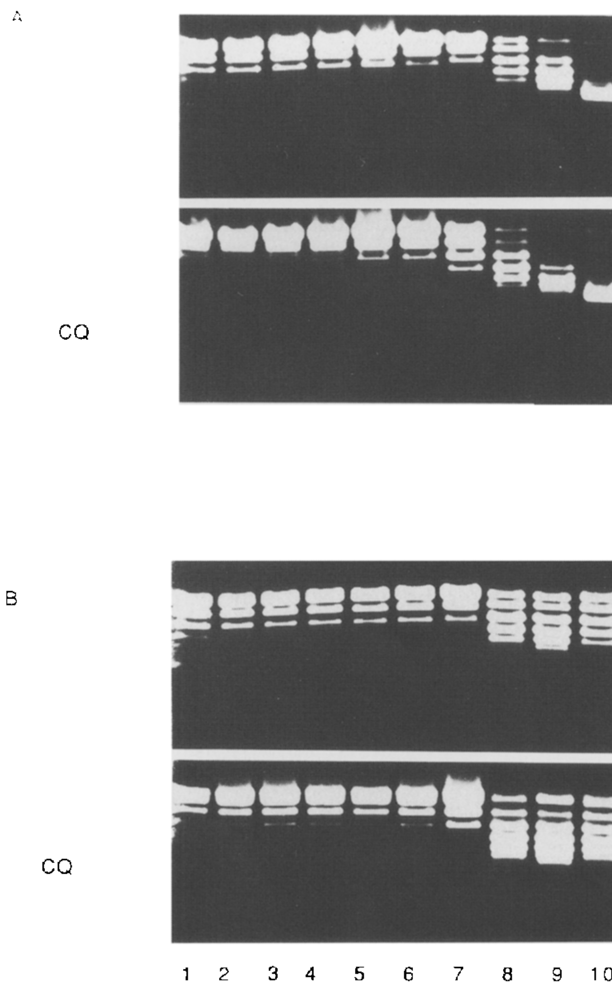

Fig. 2. Effect of Nt-Im$_3$ (A) and SN-6999 (B) on pUC18-3.4 plasmid DNA in the topoisomerase I-mediated relaxation assay (see section 2). CQ means electrophoresis was performed in the presence of 1.5 μg/ml CQ. Lane 1, DNA alone; lanes 2–10, total ratio $r' = 0.001$, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.1 and 0.2, respectively.

pUC18-3.4 DNA (not shown), which is similar to that reported by Snounou and Malcolm for pAT153 DNA [4]. We also compared the effect of Nt and SN-6999 on pBR322 DNA, which basically was the same, but supercoiled topoisomers appeared already at lower levels of the drugs; $r' = 0.02$ (not shown). Surprisingly, bifunctional analogues in the Nt series, which all bind AT-specifically to DNA, did not induce positive supercoiling with comparable or higher efficiency, as observed for the monomers, Nt and Nt-Im_3 (gel data not shown; cf. Table I). Thus linkage of two monomeric Nt units does not increase, and even lowers, the potency of these ligands to induce supercoiling in DNA. The result for two examples of the distamycin series is shown in Fig. 3. It is evident that both the AT-specific drug, Dst-3, and the analogue, Dst-Im_2, which permits binding to GC pairs [15], only induce weak downward shifts, as can be seen at rather high concentrations, $r' = 0.1$ and $r' = 0.3$, respectively (panel A, lanes 5,6; panel B, lanes 6,7). For Dst-3, inhibition of the enzyme occurs at $r' = 0.5$, and therefore negatively supercoiled DNA persists (panel A, lane 7). Our results show that the potency of non-intercalative minor groove binders to induce changes in DNA supercoiling differs significantly among various types of the ligands. Since most of these non-intercalators prefer AT base pair sequences of B-DNA [5], it was of interest to compare the induced effect of various drugs between the two plasmid derivatives of pUC18, the inserts of which differ in their AT content and AT clusters [12]. However, pUC18-3.4 DNA did not show significant differences compared with pUC18-1.5 DNA for various ligands (data not shown). Obviously the AT sequence preference of non-intercalators is not a determining factor in the potency of the drugs to induce positive supercoiling in the topoisomerase I assay. This conclusion is also supported by the observed effect of Nt-Im_3 (Fig. 2) which, in contrast to Nt, can accept GC pairs on interaction with DNA [7,8,15–18]. Moreover, the AT-specific ligands, DAPI and pentamidine (Fig. 1), are ineffective in producing positive supercoils in DNA. Pentamidine starts to induce positively supercoiled topoisomers first at $r' = 0.3$, and a further increase to $r' = 1.0$ produces only a slight enhancement of topoisomers (not shown), whereas no effect appears at low ligand concentration (Table I). For

Table I

Change in linking number, ΔLk, induced upon binding of minor groove binders to plasmid DNA of pUC18-3.4 and pUC18-1.5

Ligand	ΔLk	
	$r' = 0.05$	$r' = 0.2$
Nt	+2	+4
Nt-Im_2	+2	+4
Nt-Im_3	+2	+5
SN-6999	+2	+2
bis-Nt-6	0	+2[a]
bis-Nt-8	0	+1[a]
bis-Nt-5	0	0
Dst-3	0	+1[b]
Dst-2	0	+1[b]
Dst-Im_2	0	+1[b]
SN-16841	0	0
SN-13282	0	0
SN-6131	0	0
SN-6132	0	0
pentamidine	0	0
DAPI	0	−2
Hoechst 33258[c]	–	–
Chromomycin A_3[c]	–	–
SN-18071[c]	–	–

Positive ΔLk results after removal of drugs in the topoisomerase I assay (see section 2).
[a] At $r' = 0.075$ and $r' = 0.1$
[b] At $r' = 0.3$
[c] Inhibition of topoisomerase I

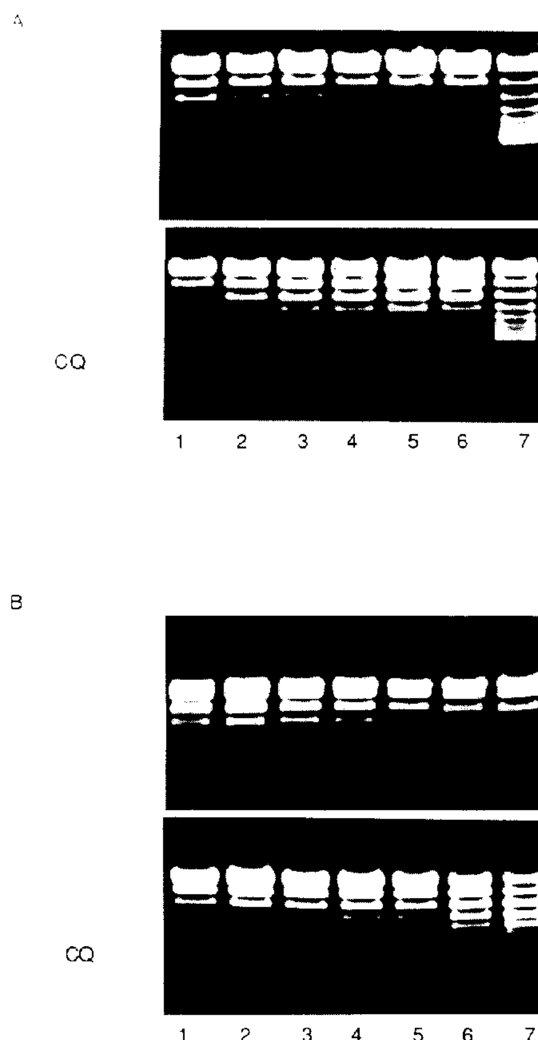

Fig. 3. Effect of Dst-3 (A) and Dst-Im_2 (B) on pUC18-3.4 plasmid DNA in the topoisomerase I-mediated relaxation assay. CQ means electrophoresis was performed in the presence of 3 μg/ml CQ. Lane 1, DNA alone; lanes 2–7, $r' = 0.02, 0.05, 0.075, 0.1, 0.3, 0.5$, respectively.

DAPI no effect is found up to $r' = 0.1$ (not shown), whereas at high ligand concentrations, $r' = 0.2$ to $r' = 0.5$ negatively supercoiled topoisomers occur (Fig. 4, lanes 4–6); for comparison Nt causes strong positive supercoiling, as shown in lane 7. Inhibition of topoisomerase I by DAPI can be excluded, since enhancement of the drug concentration did not change the result. The DAPI-induced negative supercoils can be explained by an intercalation which may occur only at the higher concentration levels, as found by Kubista et al. [19]. Obviously, non-intercalative binding of DAPI occurring at lower concentrations does not produce positive supercoils. From the gel electrophoretic data, the change in linking number, ΔLk, induced by various non-intercalators, has been obtained, and which can be used as a measure of the ability of the ligands to produce positive supercoils in the topoisomerase assay. For supercoiled plasmid DNA the linking number $Lk = Lk_0 + \Delta Lk$, where Lk and Lk_0 are the linking numbers of a supercoiled topoisomer and the relaxed form of the plasmid. The superhelical density (specific linking difference) of a topoisomer is given by the equation $\sigma = \Delta Lk/\Delta Lk_0$ [20]. Table I summarizes the linking number change induced by all types of minor groove binders investigated. An attempt was made to calculate the average helical twist of the DNA increase for different ligands in the bound state, which requires knowledge of the amount of bound ligand molecules. However, by using literature data available for various ligand–DNA complexes, a high uncertainty appeared in the results caused by a wide variation of binding constants of individual ligands due to different DNA's and a variety of methods used and applied under different conditions. Thus totally unrealistic data are obtained for a correlation to our ligand–pUC18-3.4 complex, so that we prefer to compare the ΔLk values at a given total ratio, r', ligand per nucleotide, which can be taken as a measure of the ability of various non-intercalators to induce supercoiling in DNA. As indicated in Table I the potency of minor groove binders to induce positive supercoiling (+ΔLk) is the highest for Nt and its analogues, including SN-6999, and it decreases for bis-Nt-6, whereas distamycins only produce a weak effect. If should be mentioned that in the topoisomerase assay, positive supercoiling results from the Nt-induced changes after removal of Nt; on binding to closed circular DNA, however, Nt induces negative supercoiles [21]. The bisquaternary ammonium heterocycles (SN compounds), pentamidine, as well as DAPI, are ineffective agents. In general, this differential behaviour of non-intercalators is supported by recent findings of a variation of ligand-induced winding angle changes derived from sedimentation velocity studies [21]. For example total changes in the average DNA winding angle produced at concentrations of drug molecules added per DNA molecule have been found for Nt and bis-Nt-6 of 7.4° and 5.4°, respectively, whereas for the weakly effective drug, bis-Nt-5, a distinctly lower value of 2.2° was calculated [21]. Some deviations found in this latter report, e.g. a lower efficiency of Nt-Im_3 and a higher efficiency of bis-Nt-6, may be related to the different methods used. For Hoechst 33258, chromomycin A_3 and SN-18071, we were unable to detect ΔLk since these ligands clearly inhibited topoisomerase I (Table I). From sedimentation analysis, however, Hoechst 33258 was found to be weakly effective (with a winding angle change of 2.5°) and SN-18071 ineffective in inducing changes in supercoiling [21].

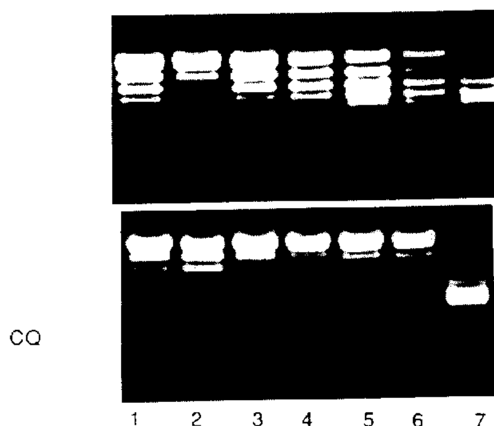

Fig. 4. Effect of DAPI on pUC18-3.4 plasmid DNA in the topoisomerase I-mediated relaxation assay. CQ means electrophoresis was performed in the presence of 3 μg/ml CQ. Lane 1, DNA alone; lanes 2–6, $r' = 0.1$, 0.2, 0.3, 0.4, 0.5, respectively; lane 7, $r' = 0.1$ Nt.

The capability of some minor groove binders to induce supercoiling in DNA cannot be related simply to a single binding property of the ligand. Several factors have to be considered which can determine the highest local fit in the minor groove depending on the ligand structure, and this may be associated with geometrical distortions leading to changes in supercoiling. Besides the geometry and flexibility of the ligand, its capacity to form hydrogen bonds to bases within the minor groove, together with electrostatic interactions and van der Waals contacts, may determine the efficacy of the non-intercalator to induce positive supercoiling of DNA in the topoisomerase assay. Although most of the groove binders (Fig. 1, Table I) prefer AT sequences, individual differences in their DNA binding effects have been documented by various methods (for reviews see [5,22,23]). Studies on X-ray crystal analysis of a Nt–dodecamer complex have revealed that Nt, upon binding to a homologous AT center, bends the helix axis by about 8° backward from the binding site and causes a widening of the minor groove [22,24]. The isohelical alignment within the groove is facilitated by the flexibility of the Nt molecule and by the formation of a maximal number of hydrogen bonds (displacing the spine of hydration on AT sequences), by electrostatic attraction (due to the deepest molecular electrostatic potential in

the AT minor groove [23]), as well as by contacts with the wall of the groove [24]. These factors may determine local structural perturbations and will depend on the chemical nature of the ligand. In a closed circular plasmid the linking number is defined as Lk = Wr + Tw, where Tw is the twist and Wr is the writhe [20,24]. Helix axis bending could occur in several AT-rich Nt-binding regions on the plasmid, leading to a change of writhe, and hence it would alter the helical twist, which in turn results in a change of the linking number. In our case the increase in the linking number (Table I) also reflects an increase in the helical twist. In contrast to Nt, DAPI causes a much lower bending of the helix axis, as determined by X-ray crystal analysis [25]; the lower effect was ascribed to the shorter DAPI molecule which is unable to produce a strong leverage on the helix axis [25]. This might explain the absence of any effect of DAPI on inducing positive supercoils, while the weak unwinding effect observed in our results (Fig. 4, Table I) can be ascribed to an intercalation at GC sites at high concentrations of DAPI [19]. The Nt analogues, Nt-Im_2 and Nt-Im_3, are also dicationic ligands and could cause a similar effect to Nt, but they accept GC pairs [16–18] and can therefore act on mixed AT/GC sequences. Bis-Nt-6 is less effective at increasing the linking number (Table I). That the result differs to some extent from the data obtained from a recent sedimentation analysis [21] may be due to the different experimental methods used. The differential effect on plasmid DNA of bis-Nt-6 and bis-Nt-8 on the one hand and of bis-Nt-5 on the other (Table I) can be ascribed to a bidentate binding mode of the first two ligands and a monodendate binding of the latter [26,27]. Evidently, the whole molecule of bis-Nt-5 is not properly located within the minor groove (base interaction becomes out of phase) and therefore it is less effective than the monomer Nt molecule. Bis-netropsins would require AT-rich sequences at proper distances for adaption of their monomeric units, which alternatively could explain their lower efficiency of inducing positive supercoiling.

The very weak or almost no effect of distamycins on the production of positive supercoiling in DNA (Fig. 3, Table I) could be related to their monocationic structure, although this is not proven at present. However, it is interesting to note that in contrast to Nt, X-ray studies have indicated that no distamycin-induced bending of the helix axis occurred for its complexing with the central part -AAATTT- of a dodecamer [28].

The ineffectiveness of most of the SN ligands (Table I) can be understood in terms of the relative rigidity of these compounds [29], which disfavours an optimal isohelical alignment along the minor groove so that a deep penetration of the whole molecule cannot occur. As an exception SN-6999 permits a better fit within the groove and therefore it behaves like Nt (Fig. 2, Table I). A significantly different change observed in viscometric titrations between SN-6999 [30] and SN-18071 (Reinert, K.E., unpublished data) supports this interpretation. Pentamidine, which is an ineffective ligand, too, can form two hydrogen bonds only as well as van der Waals contacts and induces a narrowing [31] instead of a widening of the minor groove. This binding mode probably implies that pentamidine cannot effectively replace the spine of hydration in the minor groove and consequently perturbs the helix to a much lower extent compared to the effect of Nt.

The results imply that the ability of non-intercalating groove binders to produce supercoils in DNA strongly depends on structural features of the ligands. The supercoiling potency of these drugs may also influence their inhibitory effects on DNA topoisomerases and other enzymes of DNA metabolism.

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REFERENCES

[1] Drlica, K. and Franco, R.J. (1988) Biochemistry 27, 2253–2259.
[2] Woynarowski, J.M., Sigmund, R.D. and Beerman, T.A. (1989) Mol. Pharmacol. 35, 177–182.
[3] Störl, K., Störl, J., Zimmer, Ch. and Lown, J.W. (1993) FEBS Lett. 317, 157–162.
[4] Snounou, G. and Malcolm, A.D.B. (1983) J. Mol. Biol. 167, 211–216.
[5] Zimmer, Ch. and Wähnert, U. (1986) Prog. Biophys. Mol. Biol. 47, 31–112.
[6] Zimmer, Ch., Reinert, K.E., Luck, G., Wähnert, U., Löber, G. and Thrum, H. (1971) J. Mol. Biol. 72, 329–348.
[7] Lown, J.W., Krowicki, K., Bhat, G.U., Skorobogaty, A., Ward, B. and Dabrowiak, J.C. (1986) Biochemistry 25, 7408–7416.
[8] Burckhardt, G., Luck, G., Zimmer, C., Störl, J., Krowicki, K. and Lown, J.W. (1989) Biochim. Biophys. Acta 1009, 11–18.
[9] Lown, J.W., Krowicki, K., Balzarini, J., Newman, R.A. and DeClercq, E. (1989) J. Med. Chem. 32, 2368–2375.
[10] Baguley, B. (1982) Mol. Cell. Biochem. 43, 167–181.
[11] Burckhardt, G., Votavova, H., Jantsch, M., Zimmer, C. and Lown, J.W. and Schweizer, D. (1993) Cytogenet. Cell Genet. 62, 19–25.
[12] Jantsch, M., Hamilton, B., Mayr, B. and Schweizer, D. (1990) Chromosome 99, 330–335.
[13] Trask, D.K. and Muller, M.T. (1983) Nucleic Acids Res. 11, 2779–2800.
[14] Shure, M., Pulleyblank, D.E. and Vinograd, J. (1977) Nucleic Acids Res. 4, 1183 1206.
[15] Zimmer, Ch., Luck, G., Burckhardt, G., Krowicki, K. and Lown, J.W. (1987) in: Structure and Expression, vol. 2, DNA and its Drug Complexes (R.H. Sarma and M.H. Sarma eds.) pp. 291–303. Adenine Press, Schenectady, NY.
[16] Lown, J.W. (1988) Anti-cancer Drug Design 3, 25–40.
[17] Lee, M., Krowicki, K., Hartley, J.A., Pon, R.T. and Lown, J.W. (1988) J. Am. Chem. Soc. 110, 3641–3643.
[18] Rao, K.E., Shea, R.G., Yadagiri, B. and Lown, J.W. (1990) Anti-cancer Drug Design 5, 3 20.
[19] Kubista, M., Akerman, B. and Norden, B. (1987) Biochemistry 26, 4545–4553.
[20] Bauer, W. (1990) Landold-Börnstein New Series Group VII, vol. 1, Nucleic Acids, Subvol. d'Physical Data, Springer-Verl., Berlin.

[21] Triebel, H., Bär, H., Walter, A., Burckhardt, G. and Zimmer, Ch. (1993) submitted.
[22] Kopka, M.L. and Larsen, T.A. (1992) in: Nucleic Acid Targeted Drug Design (C.L. Propst and T.J. Perun eds.) pp. 303–374, Marcel Dekker, New York.
[23] Pullman, B. (1989) Advances in Drug Research, Academic Press, vol. 18, pp. 1–113.
[24] Kopka, M.L., Yoon, C., Geodsell, D., Pjura, P. and Dickerson, R.E. (1985) J. Mol. Biol. 183, 553–556.
[25] Larsen, T.A., Geodsell, D.S., Cascio, P., Grzeskowiak, K. and Dickerson, R.E. (1989) J. Biomol. Struct. Dyn. 7, 477–491.
[26] Kissinger, K.L., Dabrowiak, J.C. and Lown, J.W. (1990) Chem. Res. Toxicol. 30, 162–168.
[27] Beerman, T.A., Woynarowski, J.M., Sigmund, R.D., Gawron, L.S., Rao, K.E. and Lown, J.W. (1991) Biochim. Biophys. Acta 1090, 52–60.
[28] Coll, M., Frederick, C.A., Wang, A.H.-J. and Rich, A. (1987) Proc. Natl. Acad. Sci. USA 84, 8385–8389.
[29] Lavery, R., Zakrzewska, K. and Pullman, B. (1986) J. Biomol. Struct. Dyn. 3, 1155 1170.
[30] Luck, G., Reinert, K.E., Baguley, B. and Zimmer, Ch. (1987) J. Biomolec. Struct. Dyn. 4, 1079–1094.
[31] Edwards, K.J., Jenkins, T.C. and Neidle, S. (1992) Biochemistry 31, 7104–7109.