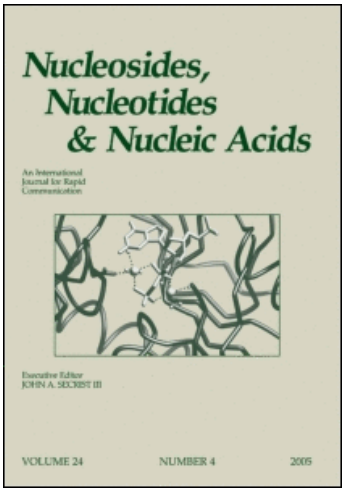


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# DNA Intercalation and Photoinduced Cleavage by 4-Nitro(N-Hexylamine)1,8-Naphthalimide

Jyoti Mehrotra[a]; Krishna Misra[a]; Rakesh K. Mishra[b]
[a] Department of Chemistry, University of Allahabad, Allahabad, India [b] Centre for Cellular and Molecular Biology, Hyderabad, India

**To cite this Article** Mehrotra, Jyoti , Misra, Krishna and Mishra, Rakesh K.(1994) 'DNA Intercalation and Photoinduced Cleavage by 4-Nitro(N-Hexylamine)1,8-Naphthalimide', Nucleosides, Nucleotides and Nucleic Acids, 13: 4, 963 — 978
**To link to this Article: DOI:** 10.1080/15257779408011870
**URL:** http://dx.doi.org/10.1080/15257779408011870

NUCLEOSIDES & NUCLEOTIDES, 13(4), 963-978 (1994)

# DNA INTERCALATION AND PHOTOINDUCED CLEAVAGE BY 4-NITRO(N-HEXYLAMINE)1,8-NAPHTHALIMIDE

Jyoti Mehrotra[1], Krishna Misra[1] and Rakesh K Mishra[2]*

1. Department of Chemistry, University of Allahabad, Allahabad-211002, India
2. Centre for Cellular and Molecular Biology, Hyderabad - 500 007, India

**Abstract:** A novel intercalator, 4-nitro(N-hexylamine)1,8-naphthalimide, was synthesised and its DNA binding and photoinduced DNA cleavage properties were studied. The DNA unwinding results show that it binds through intercalation. Absorption and fluorescence spectroscopy reveal the preference for A/T base pairs as compared to G/C base pairs for the binding. The intercalator produces photoinduced single strand scissions in double helical DNA.



## INTRODUCTION

DNA-ligand interaction has been characterized by intercalation, groove-binding or electrostatic interaction [1-4]. Since the early work of Peacocke and Skerrett [5], interacalation with DNA has been extensively studiedand because of significant pharmacological application of intercalators [6]. However, several aspects, such as DNA sequence and structural specificity, chemical structure and biological activity relationships, the role of side chains and substituents on the ligand, and the nature of site exclusion remain largely unclear. Interaction of DNA with several non-fluorescent naphthalimides and diimides has been reported earlier [7, 8]. Since fluorescence is an excellent handle to follow interaction at molecular and cellular level, we chose 4-substituted naphthalimide, a well known fluorescent system [9] as the core molecule to develop a molecular assembly which can recognize and modify DNA double helix [10]. We report here the synthesis and DNA binding properties of 4-nitro(N-hexylamine)-1,8-naphthalimide, Figure 1. This

*For correspondence: Laboratoire de Biophysique Moleculaire, Université de Bordeaux II, 146 rue Léo-Saignat, 33076 Bordeaux Cédex, France

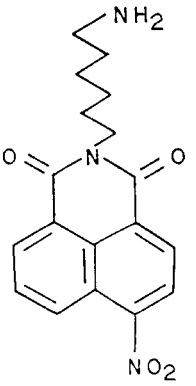


FIG 1. Naphthalimide derived fluorescent intercalator, 4-nitro(N-hexylamine)1,8-naphthalimide.



intercalator with two functional groups across it can be categorized with those having an intercalating site and an additional non-intercalating site, that can bind to the surface of the double-helix [11-14]. It also shows distinct preference for A/T base pairs over G/C base pairs and thus falls in a less common class of A/T specific intercalators [15].

The groove binders and intercalators have been used recently in sequence specific targeting of DNA and RNA [16-18]; in probing DNA conformational microheterogeneity and also in targeting the modification in genomic DNA [17, 19, 20]. Photochemical cleavage of DNA with various intercalators has been reported previously, e.g., methylene blue [21, 22], acridine orange [23], porphyrins [24], nitrobenzamide derivatives of amino acridine [25], neocarzinostatin [26], transition metal complexes of bleomycin and triphenanthrolines [27, 28], etc. We report that the fluorescent intercalator, 4-nitro(N-hexylamine) 1,8-naphthalimide, produces single strand nicks in the DNA double helix upon irradiation with visible light. The possible mode of interaction of this intercalator with DNA and its potential applications are discussed based on the sequence preference for binding, DNA cleaving ability and the functional groups across the intercalator molecule.

## RESULTS

*Interaction with Calf-thymus DNA.* Interaction with ctDNA was monitored spectroscopically by observing changes in its visible absorption and fluorescence emission characteristics. At low DNA/ligand ratio, the 450 nm band of absorption spectrum shows a 30% hypochromism (Figures 2 & 3) and when this ratio is increased, hyperchromism with

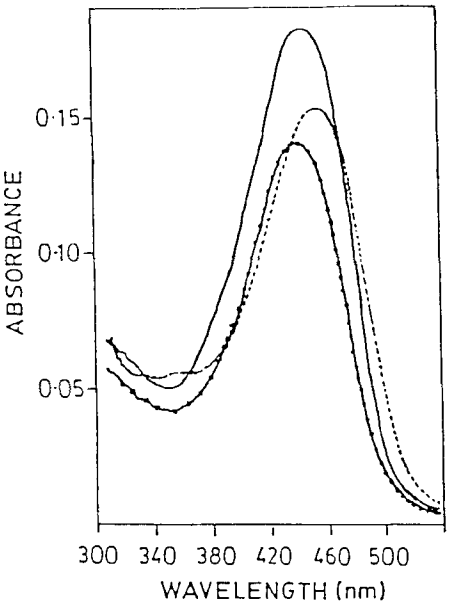


FIG 2. Limiting visible absorption spectra of the ligand (43 μM) with ctDNA. DNA/ligand molar ratio, 0(———); 1(•••••); 12 (·····) in 10 mM Tris HCl (pH 8.0), 1 mM EDTA and 0.1 M NaCl.

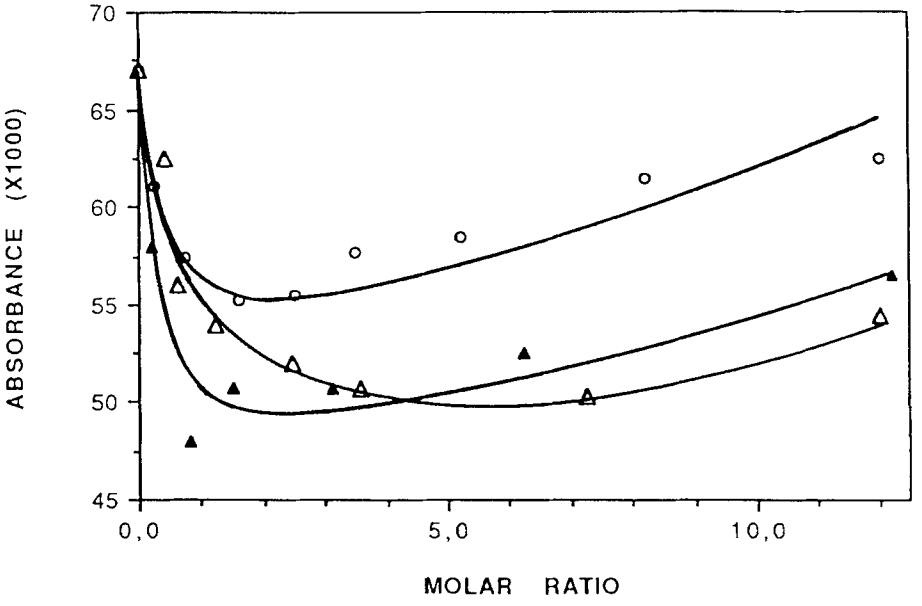


FIG 3. Changes in the visible absorbance at varying DNA/ligand ratio. Changes in 450 nm band of 43 μM solution of the ligand in10 mM Tris HCl (pH 8.0), 1 mM EDTA and 0.1 M NaCl, are recorded with ctDNA, (▲-▲); poly[d(A-T).d(A-T)], (--o--); poly[d(G-C).d(G-C)], (—▲—).

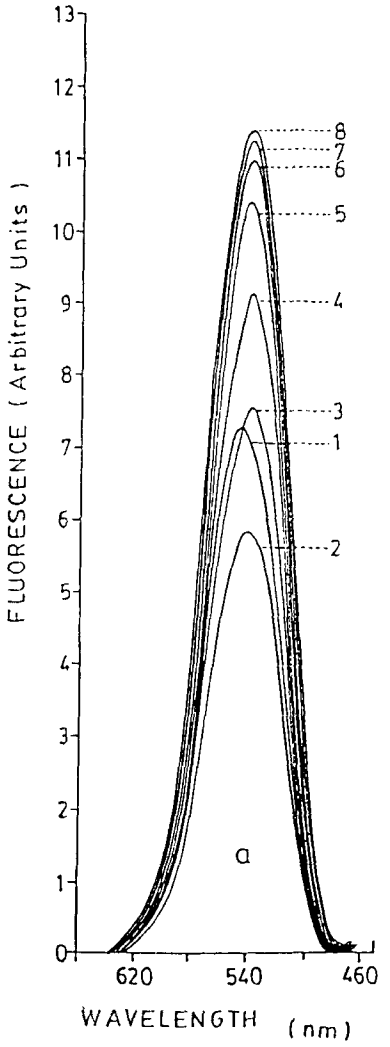


FIG 4. Changes in fluorescence emission spectra of the ligand (25 μM) in10 mM Tris HCl (pH 8.0), 1 mM EDTA as a function of increasing ctDNA/ligand ratio (spectra 2-8); emission spectra of the free intercalator (spectra 1). The 10 nm blue shift in the emission spectra is seen at all DNA:ligand ratios.

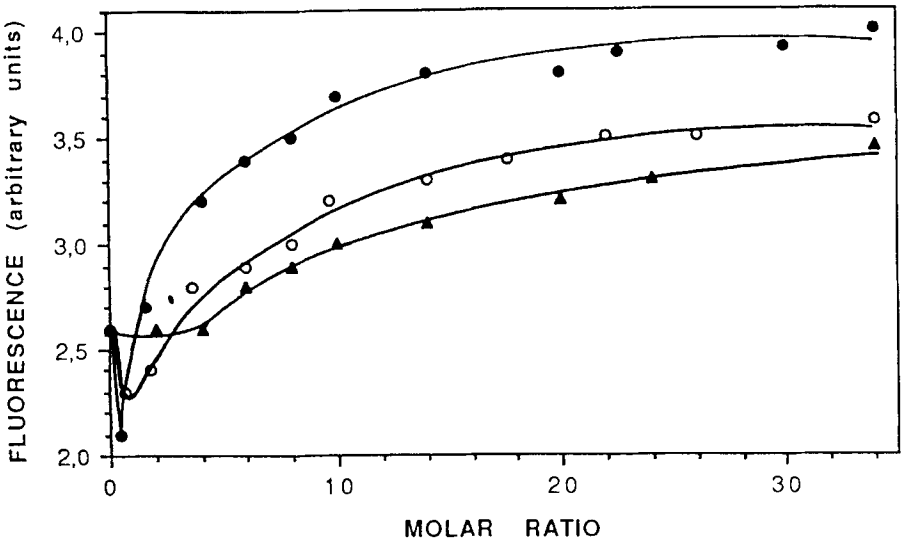


FIG 5. Changes in fluorescence emission with increasing calf-thymus DNA/ligand ratio. 25 μM solution of intercalator in10 mM Tris HCl (pH 8.0), 1 mM EDTA was used. Concentration of NaCl, 0 M (-●-); 0.1 M, (-●-); 0.2 M, (-▲-).



a 10 nm red shift is observed which reaches saturation at DNA/ligand ratio greater than 12. The titration pattern lacks a common isosbestic point which may indicate existence of one or more intermediates in the process or heterogeniety due to hexyl amine side chain flexibility.

Figures 4 and 5 show the changes in the fluorescence emission characteristics upon interaction with ctDNA. At low DNA/ligand ratio there is a sharp quenching of fluorescence and the emission spectra is shifted about 10 nm towards the blue end. As the DNA/ligand ratio is increased an increase in the fluorescence intensity is observed. Results show that the bound intercalator fluoresces 1.5 times more than the free intercalator. The mode of binding leading to the fluorescence quenching is susceptible to salt and is diminished at 0.2 M NaCl, suggesting that it consists, largely, of electrostatic interactions. At high salt concentration fluorescence enhancement is also lesser and the ratio of DNA/ligand required for saturation of this mode of binding is higher.

*Interaction with synthetic polynucleotides.* Synthetic polynucleotides provide a homogeneous lattice to study the interaction of ligands with DNA as a function of base

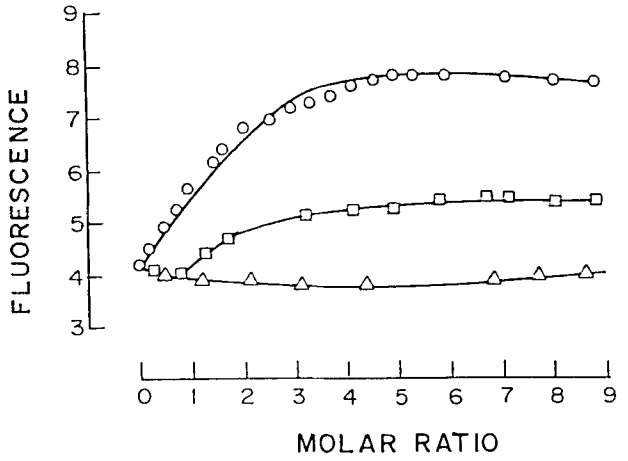


FIG 6. Changes in fluorescence emission in presence of synthetic polynucleotide at increasing DNA/ligand ratio. 10 μM solutions of intercalators in 10 mM Tris HCl (pH 8.0), 1 mM EDTA and 0.1 M NaCl was used. poly[d(A-T).d(A-T)],(-o-o-); poly[d(A-C).d(G-T)], (-□-□-); poly[d(G-C).d(G-C)], (-Δ-Δ-).



pairs. We have studied the interaction with poly[d(A-T).d(A-T)]; poly[d(G-C).d(G-C)] and poly[d(A-C).d(G-T)], Figure 6. Changes in the visible absorption are analogous to those observed in the case of ctDNA, i.e., hypochromism and red shift in the absorption spectrum and a blue shift in the fluorescece emission maxima accompanied by initial quenching and then enhancement in the emission with increasing DNA/ligand ratios. However, the DNA/ligand ratios at which saturation is reached are different.

It is evident that changes observed with poly[d(G-C).d(G-C)] are at higher ratios of DNA/ligand as compared to poly[d(A-T).d(A-T)]. In the presence of 0.1 M NaCl, poly[d(A-T).d(A-T)] and poly[d(A-C).d(G-T)] show only one kind of interaction, leading to 1.8 and 1.2 times fluorescence enhancement, respectively. As with ctDNA, a 10 nm blue shift in the emission spectra is observed here too. On the other hand, poly[d(G-C).d(G-C)] showed very little interaction at this ionic strength. Titrations in absence of salt with both, poly[d(A-T).d(A-T)] and poly[d(A-C).d(G-T)], showed fluorescence quenching at lower DNA/ligand ratios (up to 1), with a 10 nm blue shift, and enhancement at higher ratios. Poly[d(G-C).d(G-C)], however, showed only fluorescence quenching with a 10 nm blue shift (data not shown).

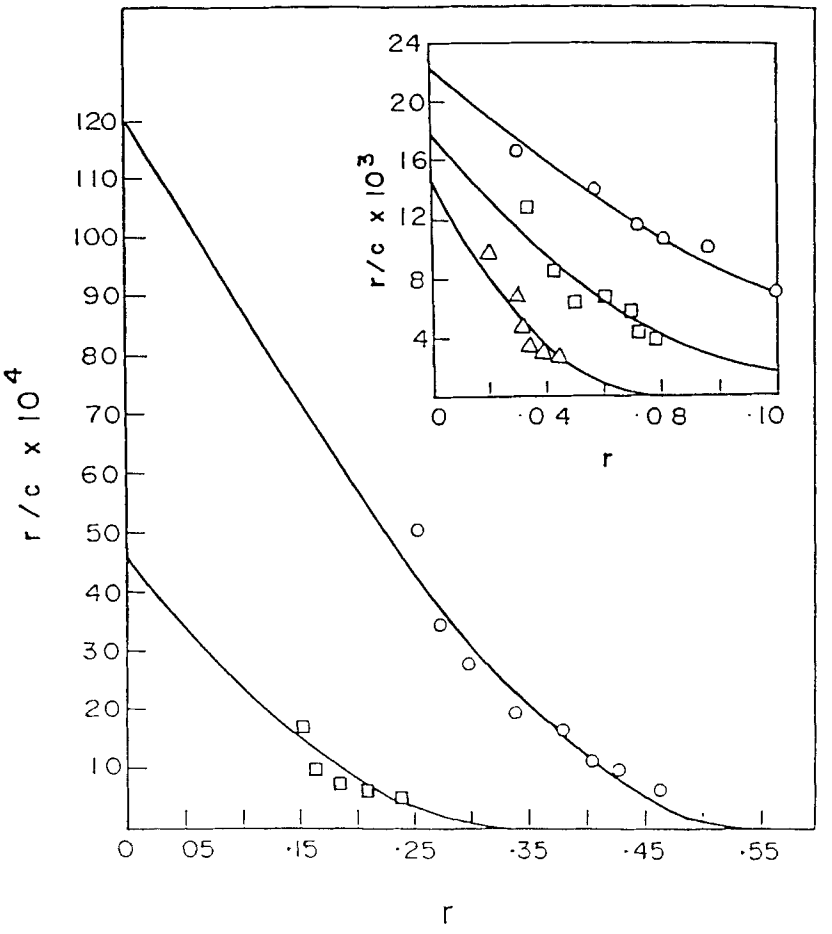


FIG 7. Scatchard plot for the binding to poly[d(A-T).d(A-T)], (-o-); poly[d(A-C).d(G-T)], (-□-). Inset, calf-thymus DNA in NaCl, 0.0 M (-o-); 0.1 M (-□-) and 0.2 M (-△-). Data points were taken from Figures 5 and 6.

Data in the linear portion of Figure 5 & 6 (pertaining to only intercalative mode of binding) have been used to calculate values of r, K and n using the plot r/c vs. r, Figure 7. Table 1 shows the values of K and n with synthetic polynucleotides and ctDNA. Due to very little change in the fluorescence upon interaction with poly[d(G-C).d(G-C)], the data was not used to estimate K and n for this polynucleotide. The K values at 0.1 M NaCl are compared in Table 1. The affinity of the ligand for poly[d(A-T).d(A-T)] is 2.7 and 68 fold greater than that for poly[d(A-C).d(G-T)] and ctDNA, respectively. The excluded binding site (n) for poly[d(A-T).d(A-T)] is close to 2, which is ideal for a homogeneous lattice of

Table 1. Binding constants (K) and excluded site sizes (n).

| S. NO. | DNA | K x $10^4$ (n) 0.0 M NaCl | 0.1 M NaCl | 0.2 M NaCl |
|---|---|---|---|---|
| I. | Poly[d(A-T).d(A-T)] | — | 127 (1.88) | — |
| II. | Poly[d(A-C).d(G-T)] | — | 45 (3.01) | — |
| III. | Calf Thymus DNA | 2.24 (4.06) | 1.76 (7.2) | 1.48 (11.9) |

Comparision of K at 0.1 M NaCl: I/II = 2.7; I/III = 68; II/III = 25

synthetic DNA. The ligand although shows higher affinity for poly[d(A-T).d(A-T)] and significantly lower value of K for poly[d(A-C).d(G-T)], both K and n values do not correspond linearly to the A/T and G/C content of the two polynucleotides. Calculation of n and K using a different (model-independent) method [29] also gave values very much similar to what we report here.



*Life-time measurement of the excited state:* Fluorescence life time measurements in free and bound form reflect the molecular environment of the ligand. 4-Nitro(N-hexylamine)1,8-naphthalimide has mono exponential decay with a life-time of 3.76 ns in the free form. This is increased to 11.32 ns when complexed with poly[d(A-T).d(A-T)] and to 9.67 ns with poly[d(G-C).d(G-C)]. The greater increase in the life-time upon binding with A/T than that with G/C may also indicates that the fluorophore is less accessible when complexed with A/T DNA

*Interaction with supercoiled DNA and Photoinduced Cleavage.* Addition of 4-nitro(N-hexylamine)1,8-naphthalimide in the electrophoresis buffer leads to the unwinding of double helical DNA and thus a decrease in supercoiling of the plasmid molecule. This results into separation of various topoisomers on a 1% agarose gel, Figure 8. The upward shift of the topoprofile is more pronounced as the concentration of the ligand is increased. This unwinding of the DNA double helix demonstrates the intercalation of this compound into the double helical DNA.

The ligand shows DNA nicking property upon irradiation with high pressure or low pressure gas discharge lamp as the light source. However, high pressure gas discharge lamp is almost hundred fold more efficient (data not shown). To see the effect of various parameters on the cleavage, the slow cleaving low pressure gas discharge (fluorescent) light source was used for the cleavage reactions, Figure 9.

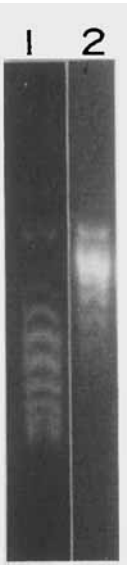


FIG 8. Generation of pBR322 topoprofile on 1% agarose gel in presence of 2 μg/ml (panel 1) and 4 μg/ml (panel 2), in TBE.

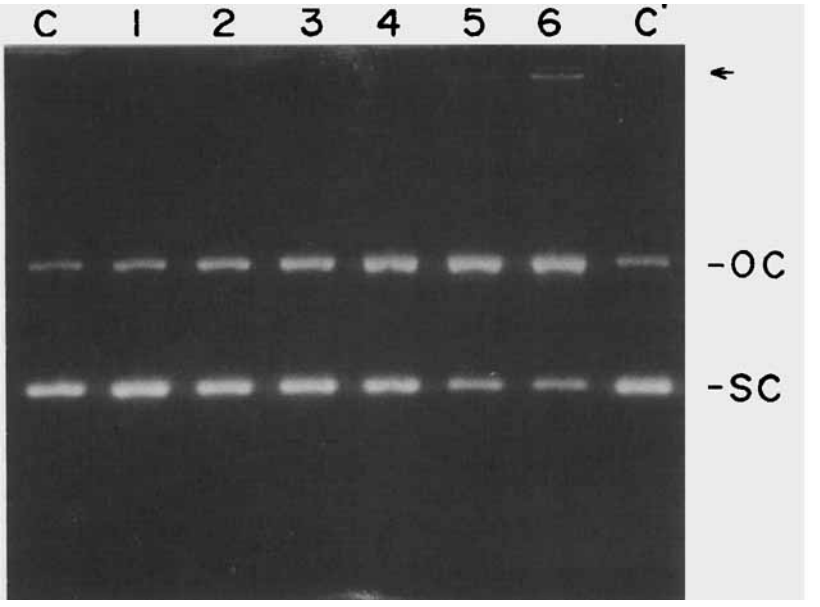


FIG 9. Concentration dependent DNA-cleavage by intercalator. Reactions were carried out using 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mM of intercalator (lanes 1-6); lanes C and C' are only DNA before and after irradiation, respectively. Duration of irradiation was 20 minutes in all the reactions using low pressure gas discharge lamp as the light source. The arrow indicates formation of crosslinked / aggregate product at higher cleaver concentration.

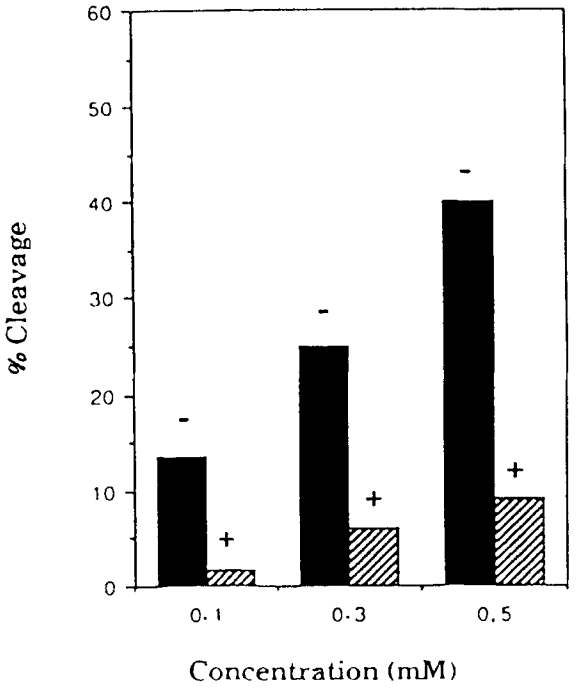


FIG 10. Inhibition of cleavage by thiourea. Cleavage reactions were carried out using 0.1, 0.3 and 0.5 mM of intercalator, bars with (-) and (+) represent reactions in absence or presence of 10 mM thiourea. Irradiation for40 min in all the reactions.



The extent of cleavage is increased by higher ligand concentration or irradiation dosage. Presence of protein (BSA) up to 50 mg/ml, change in pH ranging from 5 to 9 (sodium phosphate buffer for pH 5 to 7 and Tris-HCl for pH 7 and above), ions (sodium, magnesium, copper, sulfate, chloride, acetate) and metal ion chelator (EDTA, upto 1 mM) do not influence the cleavage efficiency (data not shown). To analyze the possible mechanism of cleavage, we carried out reactions in presence of sodium azide or thiourea. As shown in Figure 10, the cleavage efficiency decreases in presence of 10 mM thiourea. At a fixed concentration of cleavage agent and irradiation dosage, increasing concentration of thiourea has greater inhibitory effect on the cleavage efficiency. At concentrations of 20 mM and above, thiourea completely inhibits the cleavage of DNA caused by 30 minutes of irradiation in presence of 1mM ligand. Sodium azide upto 20 mM concentration, however, has no effect on the cleavage efficiency (data not shown).

## DISCUSSION

4-Nitro(N-hexylamine)1,8-naphthalimide shows DNA unwinding property (Figure 8) demonstrating that its interaction with DNA is by mode of intercalation [30]. Results from visible absorption and fluorescence spectroscopic studies with ctDNA and synthetic polynucleotides indicate that the ligand occupies two different, spectroscopically distinguishable, sites on DNA double helix. We interpret our results using a two-step intercalation model based on previous studies [31-33], suggesting two binding sites, one at the surface of the double helix and the other in between the base pairs. The interaction on the surface of the helix is largely electrostatic, while the one between the base pairs is predominantly hydrophobic. At low DNA/ligand ratio, the sugar phosphate backbone may provide stacking sites for the intercalator leading to the observed hypochromic effect and corresponding fluorescence quenching under these conditions. As the DNA/ligand ratio increases, the ligand which is already in the vicinity of the double helix tends to slip in between the base pairs. The ligand acquires a hydrophobic environment in between the base pairs which results in fluorescence enhancement. This assumption is supported by the observation that the fluorescence and absorption characteristics of this ligand show a similar trend with solvents of increasing hydrophobicity [34].



Considering the structure of the intercalator and other reports [6, 35], we propose that the ligand approaches the DNA from the minor groove and while the planar portion of the molecule intercalates in between the base pairs, the alkyl chain lies in the minor groove. At higher DNA/ligand ratio, where all the binding is by intercalation mode and not by aggregation on the surface of the helix, a hyperchromism in the absorption spectra is observed. This could be the result of facile transitions due to the restricted mobility and altered $\pi$-electron environment of the molecule. The red shift in absorption spectrum also indicates a strong association of the ligand with DNA base pairs.

Results with synthetic polynucleotides show that the fluorescence enhancement is a function of A/T content of the DNA, although, there is no linear relationship between the two. Values of K, Table 1, suggest that the intercalator has greater affinity for synthetic polynucleotides than that for ctDNA. We have used very low concentration of ligands in fluorescence titrations at the ionic strength where apparently only one kind of change in intensity is observed, viz., the one due to intercalative mode of binding. Although the heterogeneity of sites can be detected by fluorescence spectroscopy with certainty, the estimates of r, K and n can be less certain when estimated by this method due to a difference in quantum yield of the fluorophore at different binding sites. However, since

G/C base pairs seem to have very little influence on fluorescence property of this intercalator at 0.1 mM NaCl (Figure 5), only A/T base pairs might contribute to the measurements. Therefore, the difference in the binding constant and exclusion site size signify differential affinity for poly[d(A-T).d(A-T)] and poly[d(A-C).d(G-T)]. By comparing the values in Table 1, we propose that 4-nitro(N-hexylamine)1,8-naphthalimide has marked preference for poly[d(A-T).d(A-T)] which is lowered by 2.7 times when A/T content is reduced to 50% as in the case of poly[d(A-C).d(G-T)]. Reduction in binding affinity does not linearly correspond to the A/T content which suggests that the context of A/T base pair also important. For example, A/T when flanked by A/T, as in poly[d(A-T).d(A-T)], offers a better binding site as compared to the A/T flanked by G/C, as in poly[d(A-C).d(G-T)].

The life-time measurements also suggest that under these conditions, the fluorophore is embedded in the molecular lattice provided by the DNA double helix and that the freedom of molecular motion and/or accessibility of quenchers, viz., water molecules, etc., is denied. The differential increase in life time may indicate that intercalation is only partial with G/C base pairs and hence relatively more affinity for A/T base pairs. Alternatively, the complex may be better stabilised in A/T context. Wilson and co-workers [15] proposed a mechanism for A/T specific intercalators involving a water mediated hydrogen bond between C-2 carbonyl of the thymine and CO group on the ligand. The nitro or carbonyl groups on this intercalator may be involved in determining its preference for the A/T base pairs. A two step intercalation model proposed by Wilson and co-workers [15] can be envisaged here also.

The visible light, which does not have any detectable effect on DNA, causes strand scission in the presence of this intercalator. Since thiourea acts as an inhibitor of the cleavage reaction and the cleavage is independent of the effect of salt, pH and sodium azide, we are tempted to suggest that the reaction takes place via a free radical mechanism [36]. Predominant single strand scission in supercoiled DNA also suggests that the free radical generated upon irradiation, having a short life-time, is capable of cleaving only one close by strand. The process of diffusion that could lead to the cleavage of other strand is slower and free radical is quenched in the process by the surrounding molecular environment, eg., water molecules. The intercalator remains chemically unaltered, as its spectral characteristics (UV and florescence) are the same even after prolonged irradiation. This also explains why the increase in cleavage efficiency is observed for the same concentration of intercalator at increased irradiation dosage.

Intercalation of a ligand into the DNA double helix causes elongation of the double helix. This immediately suggests that double helical stretch that can resist deformation (i.e., elongation of the helix) would be less amenable for intercalation. There have been reports of probing such microheterogeneity of DNA double helix by chemical means [37]. The differential affinity of this compound for poly[d(A-T).d(A-T)]; poly[d(G-C).d(G-C)] and poly[d(A-C).d(G-T)] and the quantitative non conformity of the interaction to A/T content suggest that it might discriminate between the stretches of DNA double helix with different helical stability. Since the photochemical cleavage does not get affected by a considerable range of pH or ions, this reagent has good prospects of being used as footprinting agent to study DNA-protein interactions under varying conditions. Also, the intercalator has nucleophilic amino function at the end of hydrocarbon chain which can be attached to DNA recognition elements to render sequence specificity.

## EXPERIMENTAL



4-Nitro-1,8-naphthalic anhydride was synthesized from acenaphthene by published procedures [38]. Calf-thymus (ct) DNA was purchased from Sigma. Poly[d(A-T).d(A-T)]; poly[d(G-C).d(G-C)]; poly[d(A-C).d(G-T)] and BSA were purchased from Pharmacia. Thiourea and sodium azide were from Aldrich. Closed circular supercoiled pBR322 DNA was prepared by alkali lysis method and separated on CsCl density gradient [39].

*Synthesis of 4-nitro(N-hexylamine)1,8-naphthalimide.* Hexane 1,6-diamine (2.32 gm; 0.02 mole) was taken in a round bottom flask containing absolute ethanol (20 ml) and 4-nitro-1,8-naphthalic anhydride (4.86 gm; 0.02 mole) was gradually added to it at room temperature with stirring. The mixture was gently refluxed for 4 hrs. The solution was treated with animal charcoal, filtered, concentrated *in vacuo* and precipitated with ethyl acetate. The crude precipitate was dissolved in methanol and purified on a silica gel column by eluting with ethyl acetate and increasing concentration of methanol. The desired fluorescent product eluted at ethylacetate: methanol ratio of 1:1 and was crystallised in a ethanol-ethylacetate mixture. Yield 3.96 g, 58%; mp 187°C; $\lambda_{max}$ 450 , 285 and 260 nm; $E_{max}$ ($H_2O$) at 450 nm 1577 $\pm$39 $M^{-1}cm^{-1}$; Rf 0.4 (methanol : acetic acid :: 9 : 1) $\lambda_{ex}$ 450 nm and $\lambda_{em}$ 548 nm; IR (KBr) 3400, 3350, 1600, 1470 $cm^{-1}$; $^1$HNMR ($D_2O$) $\delta$ 7.8(d, J = 7.0 Hz,1H), 7.7(d, J = 7.0 Hz,1H), 6.2(t,1H), 7.1(t,1H), 7.6(d, J = 8.0 Hz,1H), 1.2(q, 4H, 2x$CH_2$), 1.4(t, 4H, 2x$CH_2$), 2.7(t, 4H, 2x$CH_2$), 1.7(s, 2H). Elemental analysis, calculated for $C_{18}H_{19}O_4N_3$, C, 63.3; H, 5.57; N, 12.31; found: C, 62.51; H, 5.92; N, 12.20.

*Gel electrophoresis.* Supercoiled pBR322 DNA was electrophoresed on 1% agarose gel in 89 mM Tris Borate (pH 8.0), 2 mM EDTA (TBE). The gel was equilibrated for several hours with the running buffer containing 2 $\mu$g/ml or 4 $\mu$g/ml of the intercalator prior to use. After the run, the gel was washed several times with TBE to remove the intercalator. The gel was then stained with ethidium bromide and photographed over a UV transilluminator.

*Spectroscopic studies of intercalator binding to DNA.* The intercalator shows a tendency to self-associate at concentrations higher than 150 $\mu$M in aqueous medium [34]. Therefore, all interaction studies were done at a lower concentration. DNA solutions were prepared in

10 mM Tris HCl (pH 8.0), 1 mM EDTA and concentrations were determined spectrophotometrically using the following molar extinction coefficients (in $M^{-1}cm^{-1}$), $E_{260nm}$ = 6600 for ctDNA, plasmids, poly[d(A-T).d(A-T)] and poly[d(A-C).d(G-T)]; $E_{260nm}$ = 8100 for poly[d(G-C).d(G-C)] [40]. Absorption measurements were performed using Pye-Unicam S-220 spectrophotometer at 25°C in quartz cells of 1 cm path length. Titrations were done in buffer containing 10 mM Tris HCl (pH 8.0), 1 mM EDTA and 100 mM NaCl. Changes in absorption characteristics of the ligand upon binding to natural DNA or synthetic polynucleotides were determined at varying DNA/ligand ratio.

Fluorescence titration experiments were performed on a Hitachi 650-105 spectrophotometer at room temperature using 1450 nm for excitation and measuring the emission at 548 nm. The concentration of bound ligand was calculated as follows [41];

$$C_b = (I_1 - I_0) / ((V - 1) k)$$

Here, $C_b$ denotes concentration of bound ligand. $I_1$ and $I_0$ are the total fluorescence intensities in the presence and absence of DNA, respectively. V is the ratio of the fluorescence intensity emitted by the bound and free ligand. Constant k is the ratio between fluorescence intensity and concentration. Fluorescence titrations were performed with ctDNA and synthetic polynucleotides at different ionic strengths. Life-time measurements were performed in the presence of poly[d(A-T).d(A-T)] and poly[d(G-C).d(G-C)] to see the effect of A/T and G/C base pairs on the excited state of the ligand, using a Photon Technology International LS-100 nanosecond fluorescence spectrometer using hydrogen lamp as the light source for excitation.



*Analysis of binding data.* Based on fluorescence measurements, scatchard binding isotherms [42] (plots of r/c vs. r) were generated for the ctDNA and synthetic polynucleotides, Figure 7. All the titrations were repeated several times and each point was within a 5% range of the variation. The points in the figure are average of at least three experiments. The solid lines are determined by a nonlinear least-squares computer program using the site exclusion equation [43];

$$r/c = K (1\text{-}nr) [(1\text{-}nr)/(1\text{-}(n\text{-}1)r)]^{n-1}$$

where, r is the moles of ligand bound per nucleotide, c is the free ligand concentration, K is the equilibrium binding constant and n is the number of nucleotides per binding site [44].

*Cleavage reaction.* The cleavage reaction mixture essentially consisted of supercoiled plasmid DNA (200 ng) and the cleavage reagent in a 20 µl buffer of 10 mM Tris HCl (pH 8.0) and 1 mM EDTA. All the additions were carried out in dark and the reaction tube was covered with aluminium foil. After incubating at 25°C for 10 minutes, the reaction mixture was directly irradiated from the top of the open reaction tube using a high pressure gas discharge light source, PULSARC 505 HP (Xenon arc lamp, 5000 candles/$cm^2$, 3000 lumens), NEMS CLARKE INC., USA or low pressure gas discharge lamp (60 watt, 600 lumen) of Philips, Holland. The distance of the light source was 50 cm in case of high pressure gas discharge lamp and 5 cm in case of low pressure gas discharge lamp. All the reactions were carried out at 25°C. After irradiation, the reaction mixture was extracted with equal volume of phenol (distilled and equilibrated over water) followed by equal volume of ether (saturated with water) and analysed on 1% agarose gel in 89 mM Tris borate (pH 8.0), 2 mM EDTA buffer. After the electrophoresis, the gel was stained with (1 µg/ml) ethidium bromide solution for 30 minutes. The gel was exposed to UV light for few minutes and restained in for 30 minutes. This staining procedure was followed to obtain quantitative comparison of DNA in supercoiled and relaxed forms. The gel was photographed over UV transilluminator and scanned on a Densitometer 300A (Molecular Dynamics) using the software Molecular Dynamics Image Quant V3.0.

**Acknowledgements:** Authors are thankful to J.J. Toulmé for critically reading the manuscript. Helps of D. Chatterji in life-time measurement, S.C. Rao in Scatchared analysis and G. Jyothirmai in spectroscopy are gratefully acknowledged.

## REFERENCES

1. Baguley, B.C. (1982) Mol. Cell. Biochem. **43**, 167.
2. Dougherty, G. (1984) Int. J. Biochem. **16**, 1179.
3. Feigon, J.; Denny, W.A.; Leupin, W.; Kearns, D.R. (1984) J. Med. Chem. **27**, 450.
4. Neidle, S.; Abraham, Z. (1984) CRC Crit. Rev. Biochem. **17**, 73.
5. Peacocke, A.R.; Skerrett, J.N.H. (1956) Trans. Faraday Soc. **52**, 261.
6. Wilson, W.D.; Jones, R.L. (1981) Adv. Pharmacol. Chemotherapy **18**, 177.
7. Waring, M.J.; Gonzalez, A.; Jimenez, A.; Vazquez, D. (1979) Nucleic Acids Res. **7**, 217.
8. Yen, S.-F.; Gabbay, E.J.; Wilson, W.D. (1982) Biochemistry **21**, 2070.
9. Middleton, R.W.; Parrick, J. (1985) J. Heterocycl. Chem. **22**, 1567.
10. Mehrotra, J.; Misra, K.; Mishra, R.K. (1991) Nucleosides & Nucleotides **10**, 609.
11. Pigram, W.J.; Fuller, W.; Hamilton, L.D. (1972) Nature (New Biol) **235**, 17.
12. Henry, D.W. (1976) in Cancer Chemotherapy (Sartorelli AC, Ed) p. 15, American Chemical Society, Washington, DC.
13. Quigley, G.J.; Wang, A.H.-J.; Ughetto, G.; van der Marel, G.; van Boom, J.H.; Rich, A. (1980) Proc. Natl. Acad. Sci. USA **77**, 7204.
14. Sobell, H.M.; Reddy, B.S.; Bhandray, K.K.; Jain, S.C.; Sakre, T.D.; Seshadri, T.D. (1977) Cold Spring Harbor Symp. Quant. Biol. **42**, 87.
15. Wilson, W.D.; Wang, Y.H.; Kusuma, S.; Chandrsekaran, S.; Yang, N.C.; Boykin, D.W. (1985) J. Amer. Chem. Soc. **107**, 4989.
16. Asseline, U.; Delarue, M.; Lancelot, G.; Toulme, F.; Thoung, N.T.; Montenay-Garestier, T.; Helene, C. (1984) Proc. Natl. Acad. Sci. USA **81**, 3297.
17. Moser, H.E.; Dervan, P.B. (1987) Science **238**, 645.
18. Strobel, S.A.; Dervan, P.B. (1990) Science **249**, 73.
19. Le Doan, T.; Perrouault, L.; Chassignol, M.; Thuong, N.T.; Helene, C. (1987) Nucliec Acids Res; **15**, 8643.
20. Strobel, S.A.; Dervan, P.B. (1991) Science **350**, 172.
21. Friedman, T.; Brown, D.M. (1978) Nucleic Acids Res. **5**, 615.
22. OhUigin, C.; McConnel, D.J.; Kelly, J.M.; van der Putten, W.J.M. (1987) Nucleic Acids Res. **15**, 7411.

23. Bowler, B.E.; Hollis, L.S.; Lippard, S.J. (1984) J. Amer. Chem. Soc.**106**, 6102.
24. Praseuth, D.; Gaudemer, A.; Verlhac, J.-B.; Kraljic, I.; Sissoeff, I.; Guille, E. (1986) Photochem Photobiol **44**, 717.
25. Nielson, P.E.; Jeppesen, C.; Egholm, M.; Buchardt, O. (1988) Biochemistry **27**, 6338.
26. Kappen, L.S.; Goldberg, I.H. (1984) Proc. Natl. Acad. Sci. USA **81**, 3312.
27. Chang, C.-M.; Meares, C.F. (1984) Biochemistry **23**, 2268.
28. Subramanian, R.; Meares, C.F. (1985) Biochem. Biophys. Res. Comm.**133**, 1145.
29. Schwarz, G.; Watanabe, F. (1983) J. Mol. Biol. **163**, 467.
30. Wang, J.C. (1974) J. Mol. Biol. **89**, 783.
31. Li, H.J.; Crothers. D.M. (1969) J. Mol. Biol. **39**, 461.
32. Bresloff, J.L.; Crothers, D.M. (1975) J. Mol. Biol. **95**, 103.
33. Marcandalli, B.; Stange, G.; Holzwarth, J.F. (1988) J. Chem. Soc. Faraday Trans. **84**, 2807.
34. Mehrotra, J.; Misra, K.; Mishra, R.K. (1993) Ind. J. Chem. **32B**, 540.
35. Wilson, W.D.; Jones, R.L. (1982) in Intercalation Chemistry (Whittingham, S., & Jacobson, A., Ed.) chapter 14, p. 445, Acad Press, New York.
36. Mehrotra, J.; Misra, K.; Mishra, R.K. (1992) Curr. Sci. **62**, 693.
37. Sigman, D.S.; Spassky, A.; Rimsky, S.; Buc, H. (1985) Biopolymers **24**, 183.
38. Morgan, G.T.; Harrison, H.A. (1930) J. Soc. Chem. Trans. **49**, 413.
39. Sambrook, J.; Fritsch, E.F.; Maniatis, T. (1989) in Molecular Cloning, A Laboratory Mannual, (Nolan, C., Ed.) 2nd edition, Cold Spring Harbor Laboratory Press.
40. Allen, F.S.; Gray, D.M.; Roberts, G.P.; Tinoco, I. (1972) Biopolymers **11**, 853.
41. LePecq, J.-B.; Paoletti, C. (1967) J. Mol. Biol. **27**, 87.
42. Scatchard, G. (1949) Ann. N.Y. Acad. Sci. **51**, 660.
43. McGhee, J.D.; von Hippel, P.H. (1974) J. Mol. Biol. **86**, 469.
44. Wilson, W.D.; Lopp, I.G. (1979), Biopolymers **18**, 3025.

Received 7/25/93
Accepted 11/11/93