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EXPLORING DNA MINOR GROOVE INTERACTIONS THROUGH A PROBE CONJUGATE IN MAJOR GROOVE : FLUORESCENCE STUDIES ON NETROSIN COMPLEXATION WITH dU-5-AMINODANSYL-DNA †

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Abstract The binding of netropsin to 5-Aminodansyl-dU-DNA (**2-5**) in minor groove is shown to correlate with fluorescence changes of dansyl fluorophore located in major groove. This enables a study of a minor groove binding event through crosstalk with conjugated probe in major groove. The dielectric constant of major groove estimated from fluorescence properties of (**2-6**) is about 55D in contrast to the literature reported minor groove dielectric constant of 20D in the poly[d(AT)]-poly[d(AT)] duplex.

An understanding of the molecular basis of DNA recognition by proteins, drugs and various ligands is crucial to discern the chemistry underlying the basic cellular processes, their regulation and rational design of drugs. The major and minor grooves in duplex DNA act as conduits of molecular information required for DNA association with other molecules since hydrogen bonding recognition centers in bases are pointed into these grooves (1). Many macromolecular proteins binding to nucleic acids, recognise DNA via specific interactions in major groove (2); the smaller DNA binders such as antibiotics interact with DNA either by intercalating the base pairs or by association in minor groove or both (3). Extensive X-ray crystallographic studies have indicated specific structural changes induced in DNA upon complexation with other molecules (4) and are well supported in many cases by spectroscopic studies in solution (3-5). Considering the functional importance of major/minor grooves in DNA recognition, it would be appropriate to study the inherent differences in their environments and information exchange/transfer that is possible among them upon DNA binding with other molecules. This communication addresses at this problem by employing dansyl moiety which is rigidly linked to the C-5 of dU and positioned in the major groove as a "semantophore". It is demonstrated that in 5-aminodansyl con-

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taining DNA **2-5**, an exclusive minor groove event such as netropsin binding can be monitored by changes in dansyl fluorescence seen from the major groove.

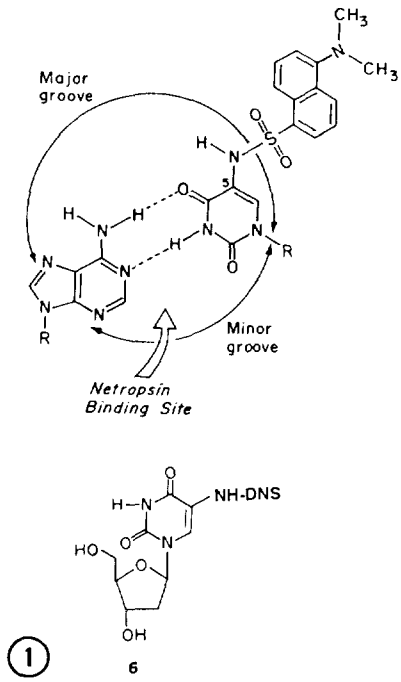
MATERIALS AND METHODS

All chemicals used were of the highest purity available. Netropsin was purchased from Boehringer Mannheim. The oligonucleotide sequences **1-5** were synthesized by β-cyanoethyl phosphoramidite chemistry on a Pharmacia GA Plus DNA synthesizer and using modified dansyl amidite in place of standard T amidite for **2-5**, as reported earlier (6) and were purified by FPLC and rechecked by RP HPLC.

UV melting experiments on DNA duplexes **1-3** were performed with or without netropsin in 10mM Tris buffer, pH 7.0, containing 100mM NaCl and 20mM $MgCl_2$ using Perkin Elmer Lambda 15 UV/VIS spectrophotometer, fitted with a temperature programmer and using a heating rate of 0.5°/min. Fluorescence measurements were done on a Perkin Elmer model LS-50 B spectrometer attatched to a Julabo programmable water circulator for temperature variable experiments (Tm). The DNA samples dissolved in the above buffer were excited at 323nm and the emission monitored at 500nm at a spectral bandwidth of 2.5nm. CD spectra were recorded on a Jobin Yvon instrument at pH 7.0. Association constants were calculated (7) from the 1/a Vs 1/L plots, where a is the fraction of oligonucleotide bound to the netropsin and L is the effective netropsin concentration.

RESULTS AND DISCUSSION

The synthetic oligonucleotides used in this study are analogues of the Dickerson's dodecamer, d(CGCGAATTCGCG), **1**, well studied in both free form and as a complex with netropsin, an established antitumor antibiotic, which binds to B-DNA specifically in the minor groove (8). Both Thymines in this sequence were replaced, one at a time, by modified dU which is 5-aminodansyl-dU (U*), to obtain the oligonucleotides **2** and **3**. This substitution does not significantly alter the standard Watson-Crick hydrogen bonding pattern of dA-dT base pair (9) with C-5 linked dansyl fluorophore projecting into the major groove of DNA (Figure 1). The CD spectra of modified oligonucleotides **2** and **3** (not shown) were identical with that of unmodified DNA **1**, indicating no major alterations in base stacking in **2** and **3** which still exist in B-form. The Tm of modified DNA **2** (48°C) and **3** (48°C) was about 12° lower than that of **1** (60°C) as measured by temperature dependent UV absorbance. The Tm of **2** and **3** were also determined from temperature dependent fluorescence spectra which showed an enhancement in fluorescence intensity upon melting (Figure 2) and the Tm, equivalent to the temperature of maximum fluorescence intensity, was identical to the UV Tm. The fluorescence spectra and the derived Tm were invariant with oligonucleotide concentration in the range 0.1 μM to 1.0 μM. Upon 1:1 binding of **2** and **3** with netropsin, Tm, as measured by both UV and fluorescence increased by same extent (85°C), indicating stabilisation of duplex. These experiments indicate that fluorescence change is a good monitor of structural perturbations in DNA and support the usage of DNS-DNA **2** and **3** as suitable models for studying DNA interactions. Since the oligonucleotide sequences are self complementary, each duplex has two dansyl groups symmetrically located in the major groove and the fluorescence changes seen is the total contribution from both dansyl groups.



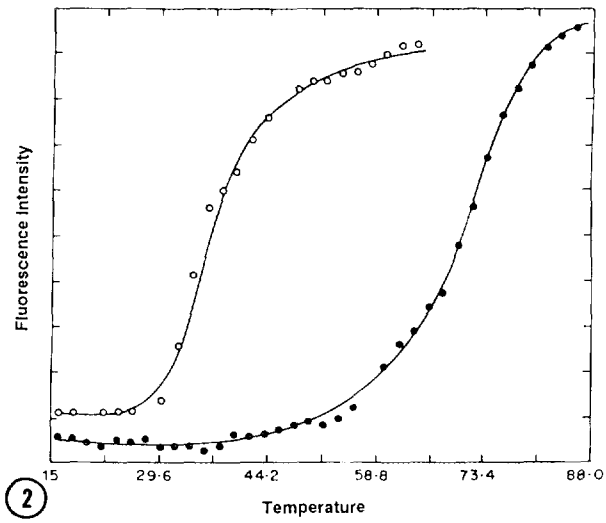


Figure 1. dA : 5-aminodansyl-dU base pairing pattern indicating fluoroprobe in the major groove and netropsin binding site in the minor groove.

Figure 2. Fluorescence melting curve for DNS-DNA **2** (1μM) in absence (o) and in presence of equimolar netropsin (●).

Interaction of Netropsin with DNS-DNA

Upon stoichiometric addition of netropsin into DNS-DNA **2**, an increase in intensity of dansyl fluorescence emmision at 500 nm was observed as a function of netropsin concentration. The fluorescence enhancement reached a saturation at 1:1 stoichiometry and beyond (Figure 3). The modified oligonucleotide **3** also behaved in a similar way and in both cases, no appreciable shifts in λ_{ex} and λ_{em} were observed upon netropsin addition. The association constants (Table 1) calculated from the binding isotherm (25°C) were in the range 10^7-10^8 M^{-1}, corresponding to a binding free energy, ΔG of -10.8 to -12.1 kcal M^{-1} which is in close agreement with the literature reported value (10) for netropsin binding to d(GCGAATTCGC). The stoichiometry of binding computed from a plot of 1/a vs 1/[L] was close to 1. Thus dansyl fluorescent probe present in major groove efficiently monitors netropsin binding in minor groove.

The association of netropsin to DNA involves displacement of spine of hydration in minor groove corresponding to AATT stretch (8,10). Further, the binding widens the minor groove by 0.5 - 2.5 Å, accompanied by a bending of helix axis by 8°, without unwinding or elongation of the double helix (11). The observed increase in fluorescence intensity of DNS located in major groove in **2** is a resultant of local structural changes induced in DNA upon

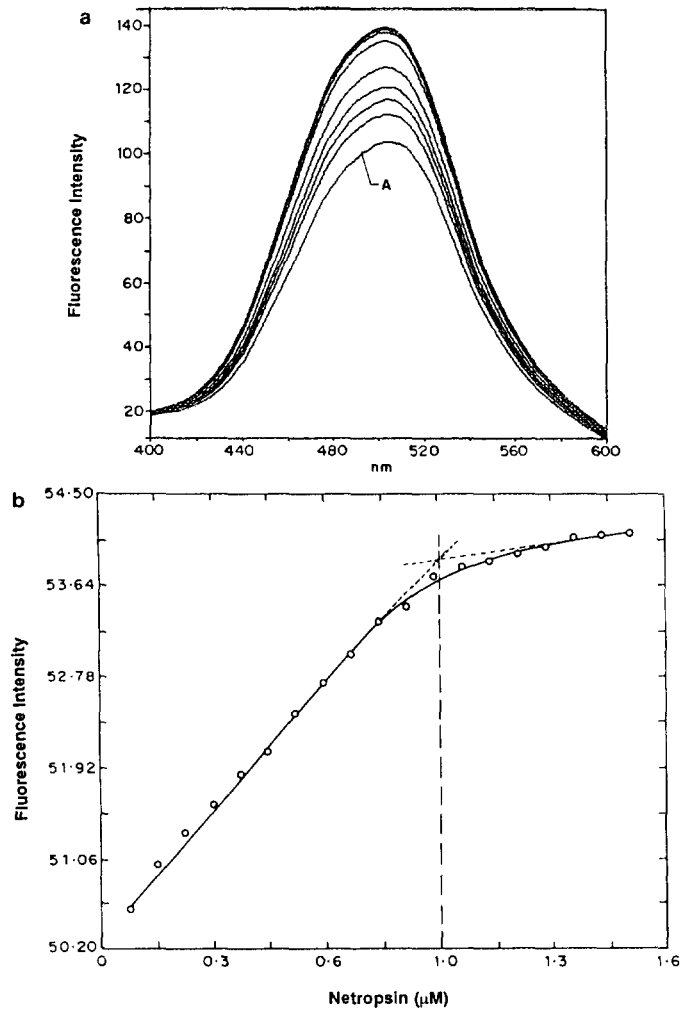


Figure 3. (a) *Fluorescence spectra of DNS-DNA* **2** (A) *in absence of netropsin and increasing netropsin concentration.* (b) Plot of fluorescence enhancement of DNS-DNA **2** as a function of added netropsin indicating 1:1 stoichiometry.

netropsin binding in minor groove. The oligonucleotide **3** has the fluorophore located on position adjacent to that in **2** and the fluorescence titration of **3** with netropsin also gave similar results. In both **2** and **3**, dansyl is linked from major groove side to a base pair that is directly involved in hydrogen bonding with netropsin in minor groove and it is possible that the observed changes in fluorescence properties is a direct consequence of electronic changes induced in base pairs by netropsin binding. To check this, the oligonucleotides **4** and **5** which have the fluorophore located outside the region of netropsin binding (AATT) were synthesised, **4** and **5** gave a higher percent enhancement in intensity compared to **2** and **3**. The calculated binding constants for oligonucleotides **4** and **5** are shown in Table-1. The results can only be accounted by the fact that the DNS fluorophore, though located in major groove, distant from the binding site (in **4** and **5**), still senses the minor groove

Table 1. Association constants and free energy changes for netropsin binding with fluorescent DNA ‡

Compound No.	Oligonucleotide	Ka (M^{-1})	Δ G (kcal M^{-1})
1	GCGAATTCGC#	2.8×10^8	- 11.5
2	CGCGAAU*TCGCG	5.9×10^7	- 10.8
3	CGCGAATU*CGCG	7.5×10^7	- 10.9
4	GCU*GTGAATTCACAGC	1.5×10^8	- 11.1
5	GCTGU*GAATTCACAGC	7.8×10^8	- 12.1

‡ All binding experiments performed at 25°C.
# Taken from ref 10.

event. This may occur through a pathway involving correlated structural changes in DNA, which mediates the information transfer among the two grooves.

By employing the fluorescent base analogue 2-amino purine, (12) interaction of netropsin with the fluorescent oligomer has been studied and found that the drug binding is weak due to partial blockage of tight fit of netropsin into the prefered minor groove, by the NH_2 group of the host. Such was not the case with the present fluorescent oligonucleotides since the fluoroprobe is located in major groove.

Polarity of Major Groove

It has been earlier reported (13) that a fluorescent, environment-sensitive drug, HOECHST 33258, interacts with poly[d(AT)]-poly[d(AT)] in minor groove, which in the complex, is quite non-polar with a local dielectric constant of about 20D. The presently used fluorophore DNS also possesses polarity-dependent fluorescence properties (14) and is therefore a good environment-probe (15). The orientation polarity *f*, is a useful means for characterising the bulk properties of each solvent system and can be obtained from the dielectric constant ε and refractive index *n* (16). The fluorescence parameters (λ_{ex} and λ_{em}) of monomer 5-aminodansyl-dU **6** were measured in media of different dielectric constants generated by varying ratios of dioxane-water. The stokes shifts $\Delta\mu$ derived from the above parameters exhibited a linear correlation with the orientation polarity for **6** (not shown). Comparison of the stokes shift for DNS in **2** and **3** with this correlation, indicated the dielectric constant of the major groove environment as sensed by DNS to be 55D (Table 2), which is thus considerably more polar than the minor groove (~20D). Such polarity measurments are valid since dansyl moiety is regiospecifically conjugated to DNA by a rigid sulphonamide bond, without much scope for flexible averaging over different environments.

In summary, this communication demonstrates a novel application of site specifically labelled fluorescent DNA probes in studying DNA-drug interactions. In particular, it is shown that long range exchange of structural information is possible across the two grooves of B-DNA, which enables monitoring of minor groove association using a major groove probe.

Table 2. Stoke's shift ($\Delta\mu$) and dielectric constant (ε) of **6** in dioxane-water mixed solvent *

Dioxane %	0	10	20	**25**	30	40	50	60	**DNA 2,3**
ε	78.5	67.0	58.2	**54.2**	50.4	41.3	32.7	24	**~55**
$\Delta\mu$	11180	11470	11080	**10900**	10680	10481	10220	10030	**10959#**

* All fluorescence spectra recorded at 25°C.

\# Calculated from fluorescence spectra of **2** and **3**.

The possible potential applications of such fluorescent DNA probes include study of structural polymorphism in DNA, DNA-peptide interactions and investigation of triple helix formation by using fluorescent DNA as the Hoogsteen strand and studies in these directions are in progress.

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