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Fluorescent d(CGCGAATTCGCG): characterization of major groove polarity and study of minor groove interactions through a major groove semantophore conjugate

Dinesh A. Barawkar and Krishna N. Ganesh*

Division of Organic Chemistry, National Chemical Laboratory, Pune-411 008, India

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ABSTRACT

The major and minor groove in duplex DNA are sites of specific molecular recognition by DNA-binding agents such as proteins, drugs and metal complexes and have functional significance. In view of this, understanding of the inherent differences in their environment and the allosteric information transfer between them induced by DNA-binding agents assumes importance. Site-specific incorporation of 5-aminodansyl-dU, (U*) in oligonucleotides d(CGCGAAU*TCGCG) and d(CGCGAATU*CGCG) leads to fluorogenic nucleic acids, in which the reporter group resides in the major groove. The fluorescent observables from such a probe are used to estimate the dielectric constant of the major groove to be ~55D, in comparison to the reported non polar environment of the minor groove (~20D) in poly d[AT]–poly d[AT]. An exclusive minor groove event such as DNA–netropsin association can be quantitatively monitored by fluorescence of the dansyl moiety located in the major groove. This suggests existence of an information network among the two grooves. The fluorescent DNA probes as reported here may have potential applications in the study of structural polymorphisms in DNA, DNA–ligand interactions and triple helix structure.

INTRODUCTION

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 centers in bases are pointed into these grooves (1). Large molecules such as proteins binding to nucleic acids, recognize DNA via specific interactions in the major groove (2); smaller DNA binders such as antibiotics interact with DNA either by intercalating the base pairs or by association in the 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). The expression of molecular forces that dictate and control affinities/specificities of DNA binding agents (proteins/drugs) are modulated by local micro-environments. Hence characterization of the environment in the grooves of DNA complexes, assumes importance in delineating the relative contributions of various molecular interactions in stabilizing DNA complexes. In view of the functional importance of major and minor grooves in DNA recognition, it would be appropriate to study the inherent differences in their environments and the information exchange/transfer that is possible among them upon DNA binding with other molecules. In this paper it is demonstrated that an exclusive minor groove event such as DNA–netropsin association can be quantitatively monitored by changes in dansyl fluorescence as observed from the major groove. The major groove polarity of DNA in oligonucleotides **2–5** has been characterized using an environment sensitive fluoroprobe, dansyl group rigidly linked to C-5 of dU and directed in the major groove (Figure 1) as a 'semantophore'.

MATERIALS AND METHODS

All chemicals used were of the highest purity available. Netropsin was procured from Boehringer Mannheim. The oligonucleotide sequences **2–5** were synthesized by phosphoramidite chemistry on a Pharmacia GA Plus DNA synthesizer using 5-aminodansyl-5′-O-dimethoxytrityl-2′-dU-3′-O-phosphoramidite in place of standard T amidite for **2–5**, as reported earlier (6) and were purified by FPLC and rechecked by RP HPLC. FPLC: (PepRPC HR 5/5, Pharmacia) Buffer A: 5% CH_3CN in 0.1 M TEAA; Buffer B: 30% CH_3CN in 0.1 M TEAA. Gradient 0%B, 3 min; 0–15%B, 5 min; 15–75%B, 35 min; 75–100%B, 1 min. HPLC: Buffers A and B, same as FPLC. Gradient: A to B 20 min.

5-aminodansyl-2′-dU **6** was prepared by detritylation of 5′-O-dimethoxytrityl-5-aminodansyl-2′-dU using p-toluenesulfonic acid in dichloromethane at 0°C for 15 min. The reaction mixture was washed with aqueous $NaHCO_3$, concentrated to

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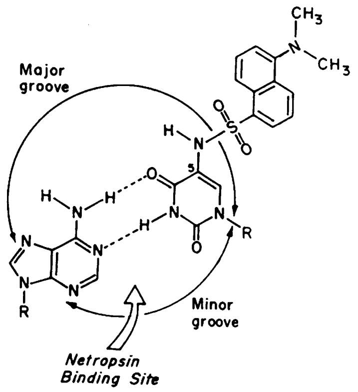
* To whom correspondence should be addressed



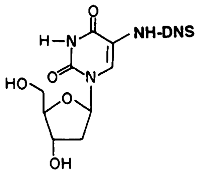


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Figure 1. 5-aminodansyl-dU:dA base pairing with the fluoroprobe in the major groove and netropsin binding site in the minor groove.

dryness followed by column purification over silica gel (100–200 mesh) using dichloromethane:methanol as eluant. ^{1}H NMR (DMSO-d_6): δ 11.4 (s, 1H, N$\underline{\text{H}}$SO$_2$–), 9.7 (s, 1H, N3H), 8.45, 8.3 and 8.1 (3×d, 3H, dansyl Ar-H), 7.75 (s, 1H, H6), 7.6 (m, 2H, dansyl Ar-H), 7.25 (d, 1H, dansyl Ar-H), 6.1 (t, 1H, H1′), 5.3 (d, 1H, 3′OH), 4.9 (t, 1H, 5′-OH), 4.2 (m, 1H, H4′), 3.75 (m, 1H, H3′), 3.4 (m, 2H, H5′and H5″), 2.8 (s, 6H, N(CH$_3$)$_2$), 2.1 and 1.9 (m, 2H, H2′ and H2″).

UV melting experiments on DNA duplexes **2–3** were performed with or without netropsin in 10 mM Tris buffer, pH 7.0, containing 100 mM NaCl and 20 mM MgCl$_2$ using Perkin Elmer Lambda 15 UV/VIS spectrophotometer, fitted with a temperature programmer and heating at a rate of 0.5°C/min. Fluorescence measurements were done on a Perkin Elmer model LS-50 B spectrometer attached to a Julabo programmable water circulator for variable temperature experiments (T_m). The fluorescent DNA samples dissolved in the above buffer were excited at 323 nm and the emission monitored at 500 nm using a spectral bandwidth of 2.5 nm. 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 netropsin and L is the effective netropsin concentration.

For characterization of the major groove polarity, fluorescence spectra of **6** were recorded in mixed organic/aqueous solvent systems prepared by stirring distilled water with appropriate volume percent of 1,4-dioxane (spectroscopic grade). Emission and excitation wavelength maxima were obtained after smoothening each spectrum by using a quadratic polynomial and are accurate to ± 0.5 nm.

RESULTS AND DISCUSSION

We chose dansyl as fluoroprobe because it has large Stoke's shift and further, it responds to perturbations in local environments such as changes in solvation, ligand binding, etc. by undergoing spectral shifts (8). Such alterations in the spectral properties of dansyl fluorophore have been previously used to detect substrate binding to protein (9) and to study interaction of DNA with the Klenow fragment of DNA polymerase I (10).

Synthesis, characterization and stability of 5-aminodansyl-dU-oligonucleotide

We have previously reported successful synthesis of DNA containing 5-amino-dU using trifluoroacetyl as a NH$_2$ protector (6). Fluorescent analogues of these oligonucleotides containing 5-amino-dansyl residue (**2–5**) were synthesized by an identical procedure except that trifluoroacetyl was replaced by dansyl group which serves as both a protector and a fluorescent label. The coupling efficiency of dansyl amidite was similar to the commercial amidites of normal nucleosides. The sulphonamide group is stable to oligonucleotide synthesis and deprotection conditions and hence the final products are fluorescent. The oligonucleotides after purification by FPLC and rechecking by HPLC showed high purity desirable for biophysical studies. The successful incorporation and retention of 5-aminodansyl-2′-dU (**U***) in the final oligonucleotides was confirmed by (i) stability to *Eco*R1 restriction digest since the modification is in recognition site, (ii) base composition analysis by snake venom phosphodiesterase followed by alkaline phosphatase and (iii) UV absorbance at 335 nm (broad) and fluorescence with emission at 500 nm upon excitation at 335 nm due to presence of dansyl group.

Dickerson's dodecamer d(CGCGAATTCGCG) (11) is one of the most well studied oligonucleotides, both in free form and as a complex with a variety of ligands. The two T residues in the above sequence were replaced one at a time by 5-aminodansyl-dU (U*), to yield the oligonucleotides **2** and **3**. Substitution at a C-5 of pyrimidine residue does not significantly affect the standard Watson–Crick mode of hydrogen bonding in dA–dT base pair (12,13) since the substituent projects into the major groove of DNA. The CD spectra of modified oligonucleotides **2** and **3** (not shown) were similar to that of Dickerson's dodecamer d(CGCGAATTCGCG) in the region 220–320 nm (negative Cotton effect at 253 nm, positive Cotton effect at 280 nm with cross over at 270 nm) indicating no major alterations of base stacking in **2** and **3**. The CD profile overall corresponds to the B-form. As seen by temperature dependent UV changes (Figure 2), the dansylated oligonucleotides **2** and **3** gave a lower T_m (48°C) compared to the unmodified dodecamer (60°C) suggesting a slight destabilising effect on duplex, locally induced by aminodansyl group in dU.

The oligonucleotides **2–5** are self complementary and hence their duplexes contain two dansyl groups, one in each strand. Although these differ in the position of dansyl groups, the fluorophores are symmetrically located in the major groove. The observed fluorescence properties are therefore a sum of contribution from both dansyl groups of duplex. In duplexes **2** and **3**, the two dansyls are within the binding site of netropsin (AATT) while in duplex **4** and **5** they are outside the binding site, separated by 10 base pairs in case of **4** and 6 base pairs in **5**. The λ_{em} of **4** and

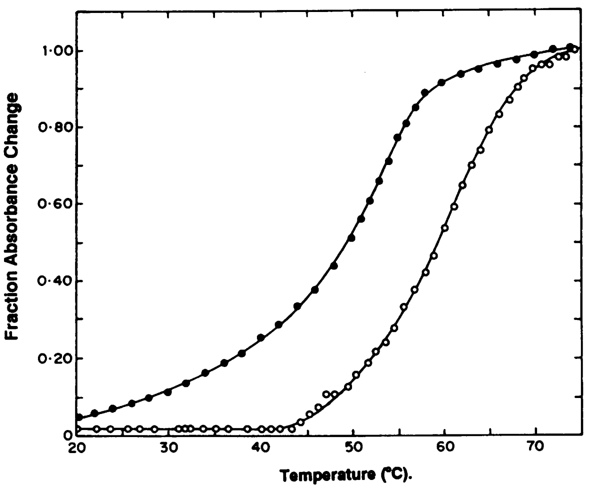

Figure 2. UV melting curves for Dickerson's dodecamer (○) and DNS–DNA 2 (●).

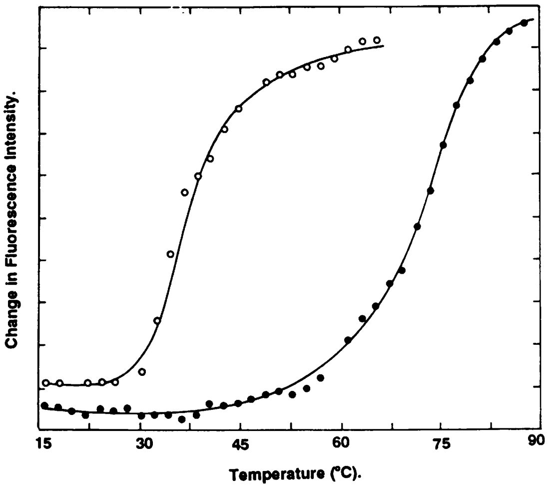

Figure 3. Fluorescence melting curve for DNS–DNA 2 (1 µM) in absence (○) and in presence of equimolar netropsin (●).

5 were blue shifted by 8–10 nm compared to that of 2 and 3. The T_m of 2 and 3 were determined from temperature dependent fluorescence studies. The melting studies showed an enhancement in fluorescence intensity with increasing temperature, the intensity attaining a maximum (Figure 3) just around the melting temperature of oligonucleotide. This rise in fluorescence intensity as a function of temperature is invariant with the oligonucleotide concentration in the range 0.1 µM to 10 µM. From this fact and that of an earlier report (14), the observed temperature of maximum fluorescence intensity was taken as an approximate measure of melting of duplex. This T_m value was in close agreement with that obtained from UV melting studies. The fact that DNS–DNA exists in B-form at room temperature (as seen by CD profile and UV/fluorescence T_m) substantially supports the use of DNS–DNA 2 and 3 as suitable models for studying DNA interactions.

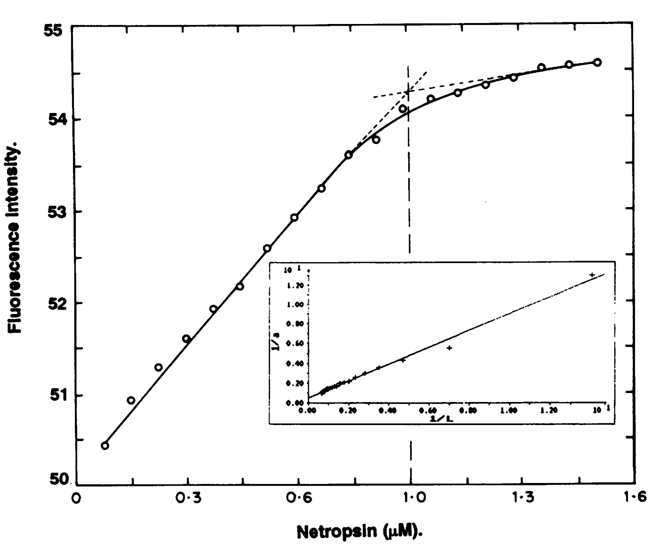

Figure 4. Plot of fluorescence enhancement of DNS–DNA 2 as a function of added netropsin indicating 1:1 stoichiometry, inset: double reciprocal plot of DNS–DNA used for calculating binding constant.

Interaction of netropsin with DNS–DNA

Netropsin is an antiviral minor groove binding drug (3a, 15) and its complexation with B-DNA has been studied by using a combination of spectroscopic and calorimetric techniques. The molecular details of its interaction in minor groove has been established by NMR spectroscopy (5) and X-ray crystallographic studies (11). We sought to examine the effect of netropsin binding to DNA in the minor groove using the fluorescent dansyl probe located in the major groove. Upon stoichiometric addition of netropsin into DNS–DNA 2, an increase in intensity was obtained in the dansyl fluorescence emission at 500 nm as a function of ligand concentration. The fluorescence enhancement reached near saturation at 1:1 stoichiometry (Figure 4). The stoichiometry of binding as computed from a plot of 1/a vs 1/[L] was also close to 1. The modified oligonucleotide 3 behaved in a similar way and in both cases, no appreciable shifts in λ_{ex} and λ_{em} were seen upon netropsin addition. The association constants (Table 1) calculated from the binding isotherm (25°C) were in the range 107–108 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 (15) for netropsin binding to decamer d(GCGAATTCGC) 1. Thus dansyl fluorescent probe present in major groove efficiently monitors netropsin binding in the minor groove. The binding of netropsin with the oligonucleotides 2 and 3 was also established by UV and fluorescence (Figure 3) melting experiments. Compared to DNS–DNA alone, in 1:1 complex of DNS–DNA:netropsin, the T_m as measured by both UV and fluorescence, was enhanced by similar extents ($\Delta T_m \sim 31$ °C) due to thermal stabilization of DNS–DNA by netropsin binding.

The association of netropsin to DNA involves displacement of the spine of hydration in the minor groove corresponding to AATT stretch (11,15). Further, the binding widens the minor groove by 0.5–2.5 Å, accompanied by a bending of helix axis by 8°, but without unwinding or elongation of the double helix (13). The observed increase in fluorescence intensity of DNS located in the major groove of 2 is a resultant of local structural changes induced in DNA upon minor groove binding by netropsin. The oligonucleotide **3** has the fluorophore located on a adjacent base 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 the major groove side to a base pair that is directly involved in hydrogen bonding with netropsin in the minor groove. It is possible that the observed changes in fluorescence properties is a direct consequence of the electronic changes induced in base pairs by netropsin binding. To examine this, the oligonucleotides **4** and **5** which have the fluorophore located outside the region of netropsin binding (AATT) were synthesized. Upon netropsin titration, both **4** and **5** gave a higher percent enhancement (~30%) in intensity compared to **2** and **3** (~10%) and there was no shift in λ_{em} due to complexation (Figure 5). The computed binding constants for oligonucleotides **2–5** are shown in Table 1.

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

Compound No	Oligonucleotide	Ka (M^{-1})	ΔG (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 were performed at 25°C.
#Taken from ref 15.

The above results can only be accounted by the fact that the DNS fluorophore, though located in the major groove, away from the binding site (as in **4** and **5**), still senses the binding event in the minor groove. This may occur through a pathway involving correlated structural changes in DNA, which mediates an information transfer among the two grooves leading to major groove/minor groove 'cross talk'. Such an intergroove conversation has recently been observed in netropsin complexation to DNA triple helix (16). This lead to a decrease in the co-operativity of triplex to duplex transition, in contrast to the stabilization of duplex to single strand transition. The occupancy of the minor groove of DNA by a ligand like netropsin can therefore exert a profound impact on the properties of a resident guest in major groove, for example, an oligonucleotide in a triplex or a fluorophore as in the present case. A study of the conformational perturbations associated with the cross talk among the two main receptor sites of duplex DNA (major and minor groove) may provide an approach for modulating the affinity and specificity of DNA binding agents.

By employing the fluorescent base analogue 2-amino purine, the interaction of netropsin with the fluorescent oligomer has been studied (14) and the drug binding found to be weak due to partial blockage of tight fit of netropsin into the preferred minor groove by the 2-NH_2 group of the host. Such was not the case with the present fluorescent oligonucleotides since the fluoroprobe is located in the major groove through a short and rigid spacer arm.

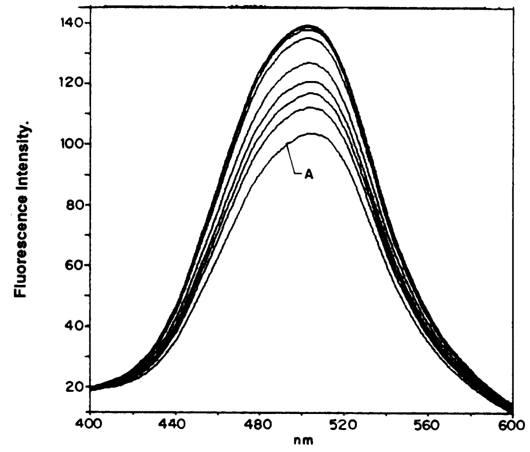

Figure 5. Overlay of fluorescence spectra of DNS–DNA **4**, without netropsin (A) and increasing netropsin concentration.

Characterization of major groove polarity

The fluorescence emission spectra of many fluorophores are sensitive to polarity of their surrounding environment (17). This polarity dependence arises from (i) interaction of dipole moment of the fluorophore in the excited state with the electrical field induced by the surrounding solvent dipoles and (ii) specific chemical interactions such as hydrogen bonding, charge-transfer interactions etc. between the fluorophore and the solvent molecules. The former is a general solvent effect governed by the orientation polarity (f) which is derived from the refractive index (n) and dielectric constant (ε)

$$f = [\varepsilon - 1)/(2\varepsilon + 1)] - [(n^2 - 1)/(2n^2 + 1)]$$

of the medium. The orientation polarity of the solvent reflects the local polarity changes due to reorientation of solvent molecules around the excited fluorophore. In the absence of solvent–fluorophore specific interactions, orientation polarity effects provide a major contribution to the emission spectral shifts. The physico-chemical interactions between fluorophore and the solvent molecules causing the general solvent effects is described by Lippert equation (17).

$$\Delta\nu = (2\Delta\mu^2/hca^3) \times f$$

where Δν is the Stoke's shift (the difference between excitation and emission wavelength, expressed as energy difference in cm^{-1}), Δν is the difference in dipole moment between the excited and the ground states of the fluorophore, h is Planck's constant, c is the speed of light and a is the radius of the cavity in which the fluorophore resides. Since for a given fluorophore, a and Δν remain constant, the above equation implies that the Stoke's shift is directly related to solvent polarity (f) changes. A calibration curve (Lippert plot) may be generated for variation of Stoke's shift (Δν) with respect to orientational polarity (f) and this exhibits a linear behavior if general solvent effects predominate over specific solvent effects. When the fluorophore is in an unknown environment, from the measured Stoke's shift the orientation polarity (f) can be obtained which is interpolated to yield the dielectric constant (ε) of the medium.

It has been reported that HOECHST 33258 which is a highly fluorescent environment-sensitive drug selectively binds to AT

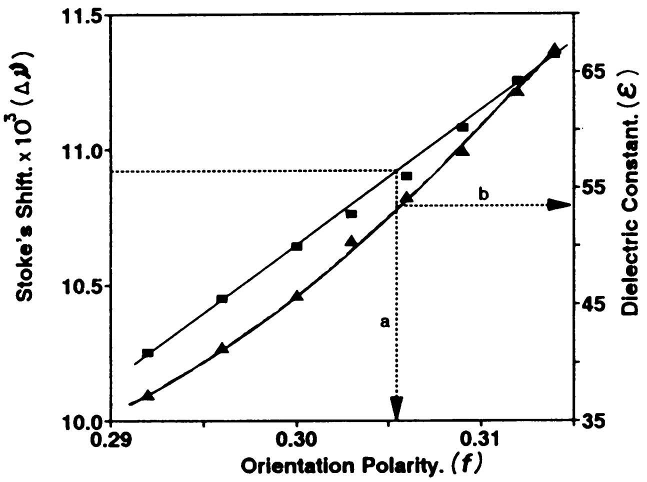

Figure 6. A combined plot of Stoke's shift (ΔV) versus orientation polarity (*f*) (■) and dielectric constant (ε) versus orientation polarity (▲). Arrow a correlates ΔV with *f* for **6** and arrow b interpolates this *f* with ε for DNS–DNA **2–5**.

regions in the minor groove and induces sequence specific structural changes in the resulting DNA complex (18). A comparison of the fluorescent observables of this complex with the corresponding properties of free ligand in different solvent systems of variable polarity (neat organic and mixed organic/ aqueous solvents) has enabled the determination of the polarity of the minor groove which is around 20D.

In order to estimate the dielectric constant of the major groove in DNS–DNA, the fluorescence parameters (λ_{ex} and λ_{em}) of the monomer 5-aminodansyl-dU **6** were first measured in media of different dielectric constants generated by varying ratios of dioxane-water (Table 2). The Stoke's shift Δν calculated from the above parameters, exhibited a linear correlation with the orientation polarity (Figure 6) suggesting the dominant influence of general solvent effect in the observed fluorescence properties of **6**. Assuming that such a correlation of orientation polarity and dielectric constant for 5-aminodansyl-dU **6** is also valid on its incorporation into the major groove of Dickerson's dodecamer, the observed Stoke's shift of DNS–DNA **2–5** can be used to estimate the polarity exhibited by conjugated dansyl in the major groove. The Stoke's shift among **2–5** is spread over a range of 100 cm^{-1}, with an average at 10900 cm^{-1}. Upon interpolation this gave an orientation polarity of ~0.307 (Figure 6, arrow a), which corresponds to a dielectric constant of ~55D (Figure 6, arrow b). This implies that the major groove in **2–5** is non-polar compared to the bulk water (80D), but considerably more polar than the minor groove (~20D). Although slight variation was seen in Stoke's shift among **2–5**, the magnitude of difference (δΔν~ 100 cm^{-1}) is much less to effect significant changes in *f* and hence the value of the interpolated ε. It may be added that, upon netropsin complexation, no changes were seen in λ_{ex} and λ_{em} of DNS–DNA **2–5** and extention of the above principle suggest no appreciable alteration of the polarity of the major groove upon netropsin complexation in the minor groove. This method of polarity estimation using dansyl moiety in **2–5** is valid since the fluoroprobe is regiospecifically and rigidly conjugated to DNA by a sulphonamide bond, without much freedom for flexible averaging over different environments.

Table 2. Stoke's shifts of 5-aminodansyl-2′-dU (6) in media of different dielectric constants generated from varying ratio of dioxane:water*

% 1,4-Dioxane	ε	*n*	*f*	λ_{ex} nm	λ_{em} nm	Δν cm^{-1}
0	78.5	1.333	0.320	330	523	11180
5	72.5	1.338	0.317	328	523	11367
10	67.0	1.343	0.314	330	530	11470
15	63.3	1.349	0.312	332	530	11253
20	58.2	1.354	0.309	334	530	11080
25	**54.2**	**1.359**	**0.306**	**336**	**530**	**10900**
30	50.4	1.364	0.303	338	529	10680
35	45.8	1.369	0.300	338	528	10641
40	41.3	1.374	0.296	340	528	10481
45	37.3	1.379	0.292	342	527	10250
50	32.7	1.383	0.288	342	526	10220
55	28.2	1.389	0.283	343	524	10060
60	24.0	1.392	0.277	343	523	10030
65	20.0	1.397	0.270	343	522	10000
DNS–DNA 2			**0.307**	**323**	**500**	**10959**
DNS–DNA 3			**0.307**	**323**	**499**	**10919**
DNS–DNA 4			**0.307**	**320**	**492**	**10925**
DNS–DNA 5			**0.307**	**320**	**490**	**10842**

*Values for ε, *n*, *f* corresponding to various Dioxane:H_2O compositions are taken from ref 18.

It is well known that fluorescence properties are also influenced by ionic strength and pH of the medium and the exact values of the latter are difficult to obtain for organic/aqueous mixed solvents. In order to examine such effects on dansyl fluorescence, the dependence of Stoke's shift of dansyl in **6** was measured over a range of ionic strength (0.1 M to 5 M) and pH (3 to 7.5). It was observed that the Stoke's shift was invariant under these conditions and hence the induced differences in fluorescence properties in various solvent systems is due to bulk solvent properties. It may be pointed out that the minor groove polarity estimated for DNA complexes with a non-conjugated fluorophore may have significant contribution from the free, uncomplexed ligand and so may lead to substantial errors in polarity estimation. The presently described method employing site-specific, covalent conjugation is devoid of such a shortcoming. The molecular rigidity due to covalent conjugation exhibits negligible effect on the Stoke's shift of a fluorophore since it has been shown that there is little, if any effects on the optical properties of a free fluorescent ligand upon conjugation to a synthetic polymer (18).

CONCLUSION

The molecular recognition of nucleic acids by proteins and drugs mainly occurs from the major and minor grooves, and it is therefore important to know the molecular environment in these regions. We have employed a environment sensitive fluoroprobe, dansyl covalently linked to dU at C5 to quantitate the major groove dielectric constant which turns out to be ~55D in contrast to the more non-polar minor groove (20D). It is also found that significant 'groove cross talk' exists between the major and minor groove as indicated by fluorescence changes in dansyl fluoroprobe in the major groove upon binding of netropsin in the minor groove. Further 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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REFERENCES

1 Saenger, W. (1984). Principles of Nucleic Acid Structure. Springer Verlag. New York.

2 (a) Schleif, R. (1988) *Science*. **241**, 1182–1187; (b) Steitz, T. A. (1990) *Quart. Rev. Biophys*. **23**, 205–280.

3 (a) Zimmer, C. and Wahnert, U. (1986) *Prog. Biophys. Mol. Biol*. **47**, 31–112; (b) Neidle, S., Pearl, L. H. and Skelly, J. V. (1987) *Biochem. J*. **243**, 1–13.

4 (a) Saenger, W. and Heinemann, U. (1989) *Protein–Nucleic Acid Interaction*. Macmillan Press, London.; (b) Travers, A. (1989) *Ann. Rev. Biochem*. **58**, 427–452.

5 Patel, D. J. (1982) *Proc. Natl. Acad. Sci. USA*. **79**, 6424–6428.

6 Barawkar, D. A. and Ganesh, K. N. (1993) *BioMed. Chem. Lett*. **3**, 347–352.

7 Pesce, A. J., Rosen, C. and Pasby, T. L. (1971) Fluorescence Spectroscopy An Introduction for Biology and Medicine. Marcel. Dekker. Inc. New York.

8 (a) Chen, R. F. (1967) *Arch. Biochen. Biophys*. **120**, 609–620. (b) Bramhall, J. (1986). *Biochemistry*. **25**, 3479–3486.

9 (a) Ondera, M., Shiokawa, H. and Takagi, T. (1976) *J. Biochem*. **79**, 195–201. (b) Skorka, G., Shuker, P., Gill, D., Zabicky, J. and Parola, A. H. (1981). *Biochemistry*. **20**, 3103–3109.

10 Guest, C. R., Hochstrasser, R. A., Dupuy, C. G., Allen, D. J., Benkovic, S. J. and Millar, D. P. (1991) *Biochemistry*. **30**, 8759–8770.

11 Kopka, M. L., Yoon, C., Goodsell, D., Pjura, P. and Dickerson, R. E. (1985) *Proc. Natl. Acad. Sci. USA*. **82**, 1376–1380.

12 Barawkar, D. A., Krishna Kumar, R. and Ganesh, K. N. (1992). *Tetrahedron*. **48**, 8505–8514.

13 Kopka, M. L., Yoon, C., Goodsell, D., Pjura, P. and Dickerson, R. E. (1985) *J. Mol. Biol*. **183**, 553–563.

14 a) Patel, N., Berglund, H., Nilsson, L., Rigler, R., McLauglin, L. W. and Graslund, A. (1992) *Eur. J. Biochem*. **203**, 361–366. (b) Lycksell, P. O., Graslund, A., Claesens, F., McLaughlin, L. W., Larsson, U., and Rigler, R. (1987) *Nucleic Acids Res*. **15**, 9011–9025.

15 Breslauer, K. J. and Marky, L. A. (1987) *Proc. Natl. Acad. Sci. USA*. **84**, 4359–4363.

16 a) Park, Y. W. and Breslauer, K. J. (1992) *Proc.Natl. Acad. Sci. USA*. **89**, 6653–6657. (b) Durand, M., Thuong, N. T. and Maurizot, J. C. (1992) *J. Biol. Chem*. **267**, 24394–24399.

17 Lakowicz, J. R. (1983) '*Principles of Fluorescence Spectroscopy*'. Plenum, New York. 187–208.

18 Breslauer, K. J. and Jin, R. (1988) *Proc.Natl. Acad. Sci. USA*. **85**, 8939–8942.