Sequence dependent energy transfer from DNA to an anthryl probe: discrimination between GC and IC sequences

Challa V. Kumar* and Emma H. Asuncion

Department of Chemistry, 55 N. Eagleville Road, U-60, University of Connecticut, Storrs, CT 06269-3060, USA. E-mail: cvkumar@nucleus.chem.uconn.edu

Received (in Columbia, MO, USA) 19th January 1999, Accepted 5th May 1999

Singlet–singlet energy transfer from ionosine-cytosine sequences of DNA to an anthryl probe has been observed, but no energy transfer occurs from guanine-cytosine sequences.

Understanding the interaction of small molecules with the DNA double helix and establishing how these interactions vary with the DNA sequence is crucial for deciphering how small molecules may influence DNA structure and function.[1] Metal ions, heterocyclic cations, and natural antibiotics bind to DNA and the DNA binding studies with these ligands have been useful in cancer research.[2] Sequence dependent energy transfer from the DNA bases to *N*-ethyl-9-anthrylmethylamine hydrochloride (*N*-Et-AMAC, Fig. 1) is reported here. Upon excitation of the DNA bases, energy transfer to the anthryl chromophore (Fig. 2) has been observed from ionosine-cytosine sequences but not from guanine-cytosine sequences.

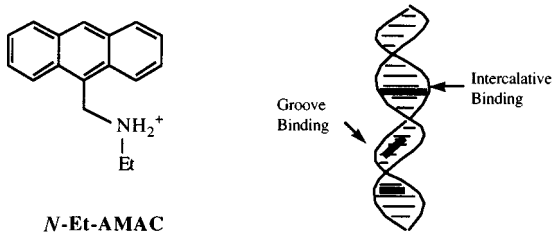

Fig. 1 Structure of the anthryl probe used for energy transfer studies with DNA, and graphical presentation of the intercalative and groove binding of organic ligands to the DNA helix.

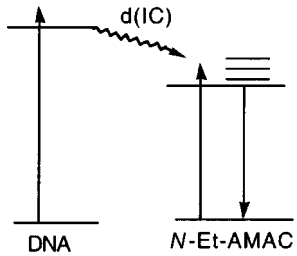

Fig. 2 Energy transfer scheme illustrating the sensitized emission from the anthryl chromophore bound to the DNA double helix.

Addition of calf thymus DNA (CT DNA) to a solution of *N*-Et-AMAC results in dramatic decreases in probe absorbance (Fig. 3). The concentration of the probe was kept constant (5 μM) while varying the DNA concentration from 0 to 400 μM. The large hypochromism (62%) accompanying the binding implies strong electronic interactions between the probe and the DNA bases.[3] No such hypochromism was observed when anthryl probes were allowed to bind to polyelectrolytes, sodium dodecylsulfate micelles, or proteins.[4] The above absorption data were used to construct Scatchard plots and a binding constant of 1.2×10^4 mol^{-1} has been estimated for *N*-Et-AMAC with CT DNA. Marginally higher binding constants have been observed with poly(dI-dC) and poly(dG-dC) (2.4×10^4 and 2.7×10^4 mol^{-1}, respectively) when compared to that with CT DNA. Additional evidence for the binding of *N*-Et-AMAC to the DNA helix was obtained from circular dichroism and from viscometric studies. Strong induced circular dichroism spectra were observed in the anthryl chromophore absorption region, when DNA was added to *N*-Et-AMAC.

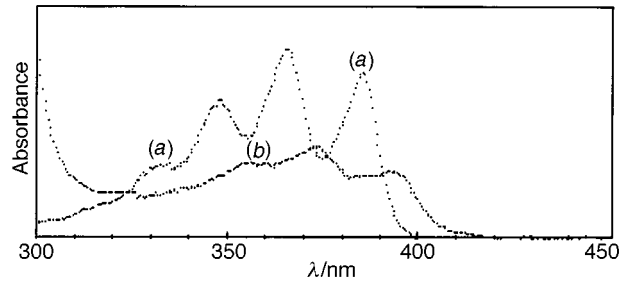

Fig. 3 Absorption spectra of *N*-Et-AMAC (9.7 μM) (*a*) in the absence and (*b*) in the presence of calf thymus DNA (400 μM). Absorbance full scale is 0.1.

The high affinity binding of *N*-Et-AMAC to the DNA helix was exploited in energy transfer studies. Singlet–singlet energy transfer from the DNA bases to 9-anthrylmethylamine hydrochloride was reported from this laboratory, previously.[3] The energy transfer from AT sequences was facile while no energy transfer was observed with GC sequences. In the case of *N*-Et-AMAC, however, energy transfer was observed from ionosine-cytosine (IC) sequences but not from guanine-cytosine (GC) sequences, providing a simple fluorescence method for discrimination between different DNA bases.

Evidence for sequence dependent singlet–singlet energy transfer was obtained from the fluorescence excitation spectra. Emission from *N*-Et-AMAC is monitored at 425 nm while varying the excitation wavelength from 260 to 410 nm (Fig. 4). Excitation in the DNA absorption region (260–300 nm) resulted in strong emission from the anthryl chromophore. Different DNA sequences were used to evaluate the dependence of

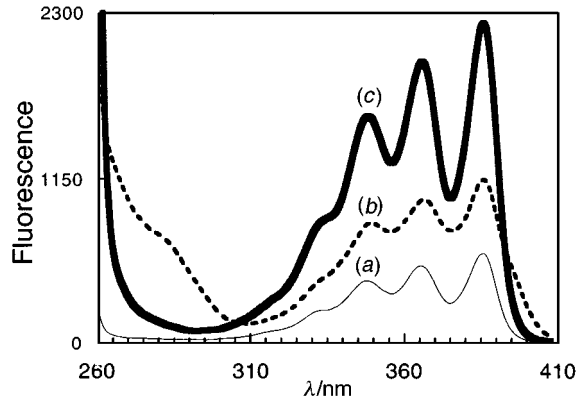

Fig. 4 Fluorescence excitation spectra of *N*-Et-AMAC (2.5 μM) in the presence of (*a*) poly(dI-dC) (99 μM) and (*b*) poly(dG-dC) (97 μM), and (*c*) in the absence of DNA.

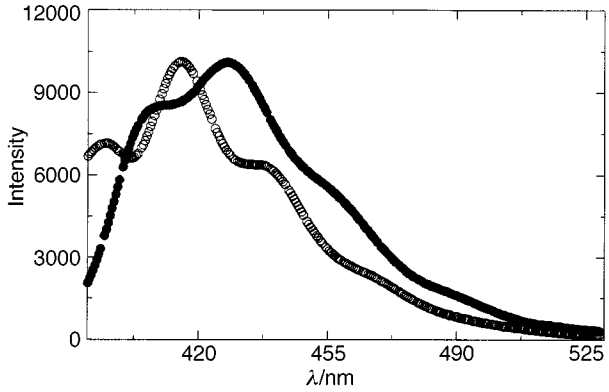

Fig. 5 Fluorescence spectra of *N*-Et-AMAC (5 μM) bound to CT DNA (54 μM) while exciting at (○) 350 and (●) 270 nm. The spectra are normalized to the same height, and no such red shifted emission was observed from *N*-Et-AMAC bound to poly(dG-dC).

energy transfer efficiencies on the DNA sequence and the curves obtained with GC and IC sequences are shown. Light absorption by the probe in the 260–320 nm region is weak, with a broad valley appearing in this window (thick line, Fig. 4). Light absorption by the DNA and subsequent energy transfer to the anthryl excited state is expected to result in sensitized anthryl emission. Accordingly, strong excitation bands in the 260–300 nm region were observed for *N*-Et-AMAC in the presence of IC sequences (dashed line). No such excitation bands appear in the spectrum of *N*-Et-AMAC when bound to poly(dG-dC) (thin line). The fluorescence spectral maxima of *N*-Et-AMAC, under these conditions, correspond to that of the probe bound to DNA (shown below), and the sensitized emission observed with poly(dI-dC) is indeed from the anthryl chromophore.

The *N*-Et-AMAC emission spectra when bound to DNA has distinct peaks at 395, 415 and 440 nm (Fig. 5, direct excitation at 360 nm), similar to those of the free chromophore, and these spectra are independent of the DNA sequence. However, when the excitation was shifted to 270 nm (into the DNA absorption region), new red-shifted emission at 405, 428, and 455 nm was observed (Fig. 5). This new emission was assigned to the chromophores bound to the DNA that are sensitized by the DNA excited states. Such red shifted emission was not observed when *N*-Et-AMAC was bound to poly(dG-dC) (270 nm excitation). These data, clearly suggest sequence dependent sensitization of anthryl fluorescence by the DNA base pairs. If the energy transfer does occur from DNA to the anthryl chromophore, then the excitation bands corresponding to the sensitizer absorption bands should appear in the excitation spectra. These are shown in Fig. 4. *N*-Et-AMAC does not have any absorption bands in the 260–320 nm region and thus, lends itself to the testing of the energy transfer hypothesis. The excitation spectra clearly indicate strong absorption in the 260–300 nm region and this absorption band is absent in the anthryl absorption spectra (bound to DNA or free). This interpretation is strengthened by the fact that the corresponding new excitation bands are absent in the presence of poly(dG-dC) and therefore, the results indicate the sequence dependence of the energy transfer process.

Exothermic energy transfer from all the DNA bases to the anthryl probe may be expected, considering the large singlet excited state energies of DNA bases (34000–35000 cm^{-1}).[5] The strong overlap of the DNA fluorescence spectra (300–400 nm) with the absorption of spectrum of *N*-Et-AMAC, and the fairly large excitation coefficients for the *N*-Et-AMAC absorption transitions suggest facile energy transfer from the DNA singlet excited states to the anthryl chromophore. Hence, the sequence dependence for energy transfer observed here is unexpected. Back energy transfer from the anthryl excited state to the DNA excited states is expected to be highly endothermic and slow. The clear dependence of the energy transfer on the DNA base sequence is suggestive of the ability of the anthryl excited state to differentiate between different DNA base pairs.

Light energy is nearly equally absorbed by all DNA base pairs in the 265–280 nm window. The excitation is known to migrate over a few base pairs along the DNA chain.[5] AT base pairs are four times less likely to trap excitation than GC pairs, and excitation localized at the GC sites is quenched efficiently at the singlet level *via* non-radiative paths, resulting in low fluorescence quantum yields.[5,6] The DNA fluorescence intensity, therefore, decreases with increased GC content of the DNA.[5] Rapid singlet–singlet energy transfer, therefore, is more likely to occur from the AT sites than from the GC sites. Energy transfer from DNA to *N*-Et-AMAC is expected to be sequence dependent and to be least efficient at GC sites. Such sequence dependence, however, was not observed with ethidium bromide (a known intercalator) and one could argue that the energy migration along the helix is slower than energy transfer to ethidium bromide, resulting in no sequence dependence. An alternative explanation for the sequence dependent energy transfer from DNA to *N*-Et-AMAC may have to do with the redox potentials of the bases. The oxidation potentials of different nucleotides are known to vary as GGG < GG < G < A < C < T.[7] One possibility is that the singlet excited states of GC base pairs are quenched by *N*-Et-AMAC by electron transfer rather than by energy trasnfer, due to the low oxidation potential of G compared to other bases.

The sequence dependence of energy transfer may arise from a combination of the excited state properties of the anthryl chromophore as well as those of the DNA bases. All DNA sequences quench the fluorescence from *N*-Et-AMAC with similar efficiencies [quenching constants for CT DNA, poly d(GC) and poly d(IC) sequences are 1.23×10^4, 1.43×10^4 and 1.24×10^4 M^{-1}, respectively). The quenching constants are nearly the same within experimental error and these data cannot account for the new excitation and emission bands presented here. Therefore, the overall sequence dependence may arise from a combination of facile energy transfer from the excited states of IC sequences and rapid electron transfer from the excited GC base pairs. While the exact mechanism of the sequence dependence for energy transfer is beyond the scope of this report, the current observations are being exploited in sequence dependent DNA cleavage studies.[8]

We thank the University of Connecticut Research Foundation for financial support of this work, and Willy B. Tan for experimental assistance.

Notes and references

1 Special issue on RNA/DNA Cleavage, *Chem. Rev.*, 1998, **98**; H. Suenaga and K. Nakashima, *J. Chem. Soc., Perkin Trans. 1*, 1998, 1263; H. H. Thorp, C. C. Cheng and J. G. Goll, *J. Am. Chem. Soc.*, 1995, **117**, 2970; T. D. Tullius, in *Bioorganic Chemistry: Nucleic Acids*, ed. S. M. Hecht, OUP, New York, 1996.

2 H. Tamura and H. Fujita, *Chem. Lett.*, 1997, **8**, 711; M. J. Absalon, W. Wu and J. Stubbe, *Biochemistry*, 1995, **34**, 2076; E. Farinas, J. D. Tan, N. Baidya and P. K. Mascharak, *J. Am. Chem. Soc.*, 1993, **115**, 2996; T. Saito, M. Kitamura and T. Shimidzu, *Nucleosides Nucleotides*, 1994, **13**, 1607.

3 C. V. Kumar and E. Asuncion, *J. Am. Chem. Soc.*, 1993, **115**, 8547; C. V. Kumar and E. Asuncion, *J. Chem. Soc., Chem. Commun.*, 1992, 470.

4 E. H. Asuncion, PhD Thesis, University of Connecticut, 1994.

5 J. Eisinger, *J. Photochem. Photobiol.*, 1968, **7**, 597; S. M. Bishop, M. Malone and D. Phillips, *J. Chem. Soc., Chem. Commun.*, 1994, 871.

6 *Photochemistry and Photobiology of Nucleic Acids*, ed. S. Y. Wang, Academic Press, New York, vol. 1, p. 23.

7 I. Saito, M. Takayama, H. Sugiyama and K. Nakatani, *J. Am. Chem. Soc.*, 1995, **117**, 6406; H. Sugiyama and L. Saito, *J. Am. Chem. Soc.*, 1996, **118**, 7063.

8 C. V. Kumar, W. B. Tan and P. W. Betts, *J. Inorg. Biochem.*, 1997, **66**, 177.

Communication 9/00542K