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6-Aminoacridizinium bromide: a fluorescence probe which lights up in AT-rich regions of DNA†‡

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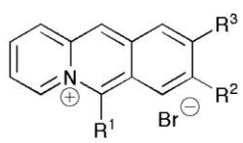
The title compound exhibits a selective enhancement of its fluorescence intensity in the presence of AT-rich DNA.

Fluorescence probes[1] whose emission intensity increases significantly upon complex formation with biologically or medicinally relevant analytes are useful, because the binding event with the host molecule may be readily followed by the appearance of a strong emission intensity ("light-up probes"). Thus, the detection of nucleic acids may be performed with appropriate fluorescent dye molecules which bind to DNA.[2] Such probes are especially useful if they lead to the selective detection of particular base sequences, so that structural motifs, which allow such selectivities, are still needed. During our studies of fluorescence sensors on the basis of amino-substituted acridizinium derivatives,[3] we discovered that the known 6-aminoacridizinium bromide (**1a**)[4] represents one of the few examples of DNA fluorescence sensors, which selectively light up in AT-rich regions.[5]



1a–**d**

1a: $R^1 = NH_2$, $R^2 = R^3 = H$
1b: $R^2 = NH_2$, $R^1 = R^3 = H$
1c: $R^3 = NH_2$, $R^1 = R^2 = H$
1d: $R^1 = R^2 = R^3 = H$

The absorption spectrum of **1a** in aqueous solution exhibits a broad, but partially structured band in the visible spectrum with a long-wavelength maximum at $\lambda = 427$ nm. The absorption properties are slightly solvatochromic, *i.e.* the wavelength of the absorption maxima varies from 426 nm in ethanol, acetonitrile, or 2-propanol to 433 nm in DMSO. Nevertheless, the wavelength of the absorption maxima cannot be correlated with common solvent parameters.[6] **1a** is only weakly fluorescent ($\phi_{fl} \ll 0.01$). Since the fluorescence quantum yield is low in protic and aprotic solvents, the radiationless deactivation is unlikely to be caused by hydrogen bonding. Also, the emission intensity is independent from the counterion, *i.e.* both the bromide or tetrafluoroborate salt are almost non-fluorescent. Thus, we propose that the radiationless deactivation of the excited state may result from a lengthening of the exocyclic C(6)–N bond in the excited state and subsequent rotation around this single bond to avoid steric repulsion between the amino functionality and the 4-H and 7-H protons. In fact, the analysis of the solid-state structure of **1a** reveals a C(6)–N bond length of 132.0 pm,[7] which reflects a high degree of double-bond character.

The interaction of the acridizinium **1a** with DNA was monitored by spectrophotometric and spectrofluorimetric titrations (Fig. 1).[8] Upon addition of ct DNA (calf thymus DNA) to the acridizinium **1a**, a red shift of the absorption maxima was observed ($\Delta\lambda = 8$ nm) along with a decrease of the absorbance (35%). The same effect was observed when (poly[dA-dT]-poly[dA-dT]) or poly[dG-dC]-poly[dG-dC]) were added to salt **1a** (*cf.* ESI). Such behaviour is well known to result from an association of dye molecules to the nucleic acid. Moreover, isosbestic points, which usually reveal one preferential, almost exclusive binding mode, appear during the titration of **1a** with ct DNA or (poly[dA-dT]-poly[dA-dT]). Upon addition of poly[dG-dC]-poly[dG-dC]) to **1a**, however, no isosbestic points were observed, which may reflect that at least two independent binding modes are occupied by **1a** when bound to this synthetic polynucleotide. The respective binding constants (K_b) and the binding-site sizes (n) were estimated from the spectrophotometric titrations with a Scatchard plot (r/c *versus* c) and analysis of these data according to McGhee and von Hippel (*cf.* ESI).[9] **1a** binds with slightly higher selectivity to poly[dG-dC]-poly[dG-dC]) ($K_b = 2 \times 10^5$ M^{-1}, $n = 1.9$) compared to (poly-[dA-dT]-poly[dA-dT]) ($K_b = 3 \times 10^4$ M^{-1}, $n = 3.8$) and ct DNA ($K_b = 3 \times 10^4$ M^{-1}, $n = 4.5$). The fit of the data to the theoretical model is only moderate ($r = 0.93$–0.94), nevertheless, fitting of the experimental data to the "two-site model"[10] was not possible at all.

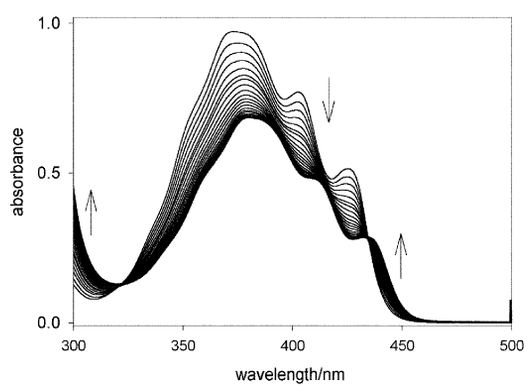


Fig. 1 Spectrophotometric titration of **1a** with ct DNA in phosphate buffer (10 mM, pH = 7.0); c(**1a**) = 10^{-4} M; titration interval: 0.5 molar equivalents of DNA [DNA : dye from 0 (top line at λ_{max}) to 10 (bottom line at λ_{max})]; the arrows indicate the decreasing/increasing absorption during the course of the titration.

To determine the binding mode of **1a** with DNA, linear-dichroism (LD)-spectroscopic investigations were performed (Fig. 2):[11] The addition of salmon testes DNA (st DNA) to **1a** resulted in a negative LD signal in the long-wavelength

† This communication is dedicated to Prof. Wolfgang Malisch, University of Würzburg, on the occasion of his 60th birthday.

‡ Electronic supplementary information (ESI) available: spectrophotometric titrations and Scatchard plots; LD spectra, and an illustration of the visible effect of the light-up probe. See http://www.rsc.org/suppdata/ob/b3/b305439j/

§ X-Ray diffraction analysis.

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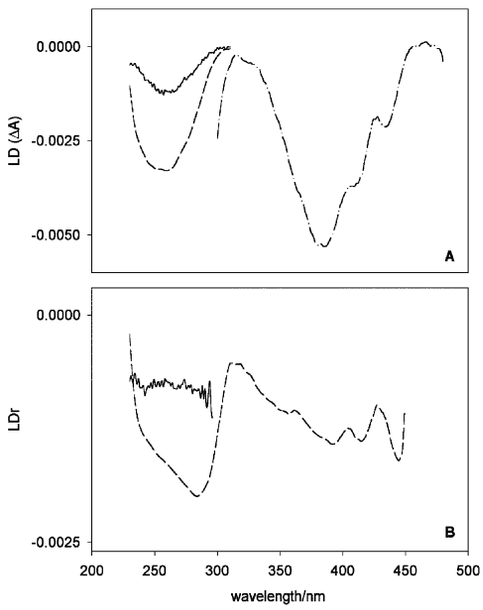

Fig. 2 LD (**A**), and LD_r (**B**) spectra of mixtures of st DNA and 6-aminoacridizinium bromide (**1a**) at [**1a**]/[DNA] = 0 (solid line) and 0.20 (dashed line); in ETN buffer, 10 mM, pH = 7.0).

absorption region of the chromophore. Also, the reduced LD (LD_r) signal intensities of the DNA bases ($\lambda = 260$ nm) resemble those of the long-wavelength absorption of the dye **1a** ($\lambda = 320$–450 nm) and both LD bands are almost wavelength independent at any acridizinium : DNA ratio (0, 0.04, 0.08, 0.20), as is usually observed for a homogeneous binding mode. These observations are in agreement with the appearance of isosbestic points during spectrophotometric titration and give evidence that **1a** intercalates into DNA without any or only marginal contribution of other binding modes. Notably, the LD band of DNA increases significantly in the presence of **1a**. Such an effect usually results from a better orientation of the macromolecule in the flow field because of a stiffening of the nucleic acid upon intercalation.

The weak emission of aminoacridizinium **1a** increases significantly in the presence of DNA (Fig. 3), which is in sharp contrast to the behaviour of acridizinium salts **1b**–**1d**, whose fluorescence is quenched upon DNA addition.[3] Most notably, the titration of (poly[dA-dT]-poly[dA-dT]) resulted in a much higher increase of the emission quantum yield ($I/I_0 = 33$ at [DNA] : [**1a**] = 28) than the addition of poly[dG-dC]-poly[dG-dC]) ($I/I_0 = 2$) or ct DNA ($I/I_o = 3$) — an effect which may be seen by the naked eye (*cf.* ESI). The increase in emission intensity upon DNA addition may be rationalized by a significant suppression of the conformational flexibility of the dye within the complex.[12] It may be assumed that the geometrical constraint within DNA hinders the deactivating rotation around the C_{ar}–NH_2 bond of **1a**. Nevertheless, this does not explain why the emission intensity is about ten times larger in the

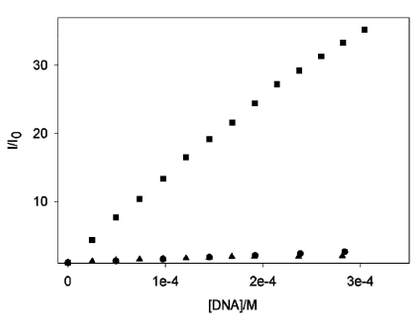

Fig. 3 Relative fluorescence intensities of **1a** upon addition of ct DNA (●), (poly[dA-dT]-poly[dA-dT]) (■) and (poly[dG-dC]-poly[dG-dC]) (▲), in phosphate buffer (10 mM, pH = 7.0); c(**1b**) = 10^{-5} M.

presence of (poly[dA-dT]-poly[dA-dT]) compared to poly-[dG-dC]-poly[dG-dC]) or ct DNA. To understand the different influence of GC and AT base pairs, it may be considered that fluorescence quenching by electron-transfer (ET) reaction between the excited dye and the DNA is exergonic, when the reduction potential of the excited dye (E^*_{Red}) is larger than the oxidation potential of the nucleic bases.[13] The reduction potential of **1a** is −0.8 V (in CH_3CN, *vs.* NHE). With the 0–0-transition energy of 2.8 V, the E^*_{Red} is estimated to be *ca.* 2.0 V.[14] Considering the oxidation potentials of guanine (1.47 V) and adenine (1.94 V) in CH_3CN,[13] these data lead to the proposal that the excited aminoacridizinium **1a** may be able to oxidize guanine, whereas with adenine the ET reaction is energetically disfavored. Thus, in ct DNA and poly[dG-dC]-poly[dG-dC]), two effects with opposite results take place: a) the emission intensity increases due to conformational restriction and b) the emission intensity decreases due to ET with the guanine bases. Since the latter effect is not possible in AT-rich regions, a much higher fluorescence is observed therein. The weak emission enhancement in the presence of ct DNA is likely due to the higher binding affinity of **1a** towards GC base pairs. Moreover, guanine moieties may quench the emission of **1a** even when they are separated by several base pairs. Thus, the acridizinium is always positioned in close proximity to a guanine and an ET reaction takes place. Notably, the above described effect does not take place with the derivatives **1b**–**d**. This difference may be explained by the fact that these acridizinium salts already exhibit a pronounced fluorescence which is not efficiently quenched by C–N-bond rotation as in **1a**. Thus, upon binding to DNA, no emission enhancement due to steric constraints (a) takes place and fluorescence quenching by ET (b) is the only effect.

Most AT-selective light-up probes exhibit decreasing emission because the fluorophore is shielded from solvent[5e] or due to restricted conformational flexibility upon binding to DNA. The latter may inhibit intramolecular protonation as in DAPI.[5d] Also, in one example, a donor moiety of a PET probe is protonated within the acidic microenvironment of the DNA to restore fluorescence.[5a] The emission of **1a** is also enhanced within DNA due to restricted conformational freedom; but in contrast to most other probes, its enhancement in AT-rich DNA results from selective electron-transfer reactivity towards the base pairs and not from selective binding to AT-rich regions. Such a role of the oxidation potential of the nucleic bases for AT-selective probes has been suggested recently for ruthenium complexes,[5a] nevertheless, in contrast to those groove-bound compounds, **1a** is intercalated with significantly closer contact to the nucleic bases. Thus, the electron-transfer reaction should play a more important role in the latter case.

In summary, we have discovered a useful fluorescence probe for DNA detection. Its emission properties are drastically switched by a change of the base composition within the binding pocket because of a delicate balance between emission and redox properties. Thus, this compound constitutes a useful platform for a new generation of selective DNA-sensing probes.

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