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Fluorescent probes based on side-chain chlorinated benzo[*a*]phenoxazinium chlorides: Studies of interaction with DNA

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ABSTRACT

The interaction of DNA with six water soluble benzo[*a*]phenoxazinium chlorides mono- or di-substituted with 3-chloropropyl groups at the *O* and *N* of 2- and 9-positions, along with methyl, hydroxyl and amine terminal groups at 5-positions, was investigated by photophysical techniques. The results indicated that almost all compounds intercalated in DNA base pairs at phosphate to dye ratio higher than 5. At lower values of this ratio, electrostatic binding mode with DNA was observed. Groove binding was detected mainly for the benzo[*a*]phenoxazinium dye with $NH_2 \cdot HBr$ terminal. The set of six benzo[*a*]phenoxazinium chlorides proved successful to label the migrating DNA in agarose gel electrophoresis assays. These finding proves the ability of these benzo[*a*]phenoxazinium dyes to strongly interact with DNA.

1. Introduction

Small fluorescent molecules serve as important tools in quantification and identification of living cells and organisms [1]. They also find numerous applications in drug delivery [2], sensing [3], monitoring of chemical interactions of biomolecules [4], diagnostic imaging [5], and therapeutics [6]. Some of the fluorescent dyes selectively localize and stain a cellular target organelle such as mitochondria, lysosomes, endoplasmic reticulum, or Golgi apparatus. Besides, some ligands possess dual behaviour when interacting with DNA, such as a fluorescence enhancement or quenching based on the flanking base sequence [7,8]. In specific, the most widely used dyes for nuclear staining are 4′,6-diamidino-2-phenylindole (DAPI) [9], ethidium bromide [10] and Hoechst 33258 [11], which emit strong fluorescence when bound to DNA. Among the fluorescent probes [12,13], phenoxazine and its derivatives interact with DNA mainly *via* noncovalent interactions or by π-π stacking which render stability to these planar polycycles. [14,15]. Analytical techniques such as X-ray crystallography [16], mass spectrometry [17], UV–visible [18] and fluorescence spectroscopies [19] are employed to study the binding properties of fluorophores with DNA.

In this perspective, Nile Blue (NB) derivatives possess the required structural framework for intercalation with biomolecules, mainly due to low toxicity and good sensitivity for DNA quantification purposes [20]. Moreover, NB was used as a marker for the detection of DNA in gel electrophoresis experiments [21,22]. Continuing our research towards the synthesis and photophysical studies of fluorescent molecules [23–31], the present work is focused to study the DNA interaction with a set of benzo[*a*]phenoxazinium dyes bearing chlorinated terminals at the 2- and 5-positions of the heterocycle. Additionally, we evaluated the influence of methyl, hydroxyl or the amino group terminals at 5-position of the polycyclic systems [31] and photophysical studies of the interaction with DNA and in agarose gel electrophoresis assays were studied.

2. Experimental

2.1. Materials

Tris(hydroxymethyl)aminomethane (Tris) and HCl (37%) were purchased from Sigma-Aldrich. Natural double-stranded salmon sperm DNA was obtained from Invitrogen as a 10 mg/mL aqueous solution with an average size of 2000 bp. Tris/Acetic acid/EDTA (TAE) buffer solution was obtained from BioRad Laboratories GmbH. The strength of the 1× solution: 40 mM Tris, 20 mM acetic acid, 1 mM EDTA was used. Stock solutions of salmon sperm DNA were prepared in 10 mM Tris-HCl buffer (pH = 7.4), with 1 mM EDTA. The purity of DNA was checked by the absorption spectrum and the ratio of absorbance $A_{260}/A_{280} = 1.95$ (good-quality DNA has A_{260}/A_{280} ratio higher than 1.8) [32]. The DNA concentration in number of bases (or phosphate groups, [P]) was determined from the molar absorption coefficient $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ at 260 nm [33]. Appropriate amounts of 10^{-4} M ethanolic solutions of compounds **1–6** were added to DNA solutions at the desired concentrations.

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The solutions were stored for 24 h to stabilize. All other chemical reagents and solvents (spectroscopic grade) were commercially available and used without further purification.

2.2. Synthesis of benzo[a]phenoxazinium chlorides **1**–**6**

Benzo[*a*]phenoxazinium chlorides **1**–**6** (Fig. 1) were synthesized by condensation of the corresponding nitrosophenol hydrochloride, 5-(bis(3-chloropropyl)amino)-2-nitrosophenol hydrochloride (compounds **1** and **2**) or 5-((3-chloropropyl)amino)-2-nitrosophenol hydrochloride (compounds **3** to **6**), with *N*-alkylated naphthalen-1-amine or *N*- and *O*-alkylated 5-aminonaphthalen-2-ol, namely *N*-propylnaphthalen-1-amine, 6-(3-chloropropoxy)-*N*-propyl naphthalen-1-amine, 3-(naphthalen-1-ylamino)propan-1-ol, N^1-(naphthalen-1-yl)propane-1,3-diamine hydrobromide in acidic (HCl) ethanol, as previously reported [31]. The required nitrosophenol was obtained by nitrosation of the corresponding 3-aminophenol precursor with sodium nitrite and hydrochloric acid in aqueous ethanol [34]. All the intermediates and benzo[*a*]phenoxazines obtained were characterised by ^{1}H NMR, ^{13}C NMR and HRMS [31].

2.3. Instrumentation and methods

Absorption spectra (200–800 nm) were recorded on a Shimadzu UV-3101PC UV/vis/NIR spectrophotometer. Fluorescence measurements were performed using a Spex Fluorolog 2 spectrofluorometer, equipped with double monochromators in both excitation and emission, Glan-Thompson polarizers and temperature-controlled cuvette holder. Spectra were corrected for the instrumental response of the system.

Fluorescence quantum yields (Φ_F) were determined using the standard method (Eq. (1)) [35,36] with Oxazine 1 in ethanol as reference, $\Phi_r = 0.11$ [37]:

$$\Phi_s = \frac{A_r F_s n_s^2}{A_s F_r n_r^2} \Phi_r \quad (1)$$

where *A* is the absorbance at the excitation wavelength, *F* the integrated emission area and *n* the refraction index of the solvents used. Subscripts (r) and (s) denotes the reference and sample compounds.

The steady-state fluorescence anisotropy, *r*, is calculated by Eq. (2),

$$r = \frac{I_{VV} - G I_{VH}}{I_{VV} + 2G I_{VH}} \quad (2)$$

where I_{VV} and I_{VH} are the intensities of the emission spectra obtained with vertical and horizontal polarization, respectively (for vertically polarized excitation light), and $G = I_{HV}/I_{HH}$ is the instrument correction factor, where I_{HV} and I_{HH} are the emission intensities obtained with vertical and horizontal polarization (for horizontally polarized excitation light).

Fluorescence quenching studies with iodide ion were modelled using Eq. (3),

$$\frac{I_0}{I} = \frac{f_{int}\varepsilon_{int}\Phi_{int} + (1-f_{int})\varepsilon_w\Phi_w^0}{f_{int}\varepsilon_{int}\Phi_{int} + (1-f_{int})\varepsilon_w\Phi_w} \quad (3)$$

where I_0 and I are the fluorescence intensities in the absence and presence of quencher, respectively; f_{int} is the fraction of intercalated molecules in DNA and Φ_{int} their fluorescence quantum yield; Φ_w^0 and Φ_w represent the fluorescence quantum yields (in water) in the absence or presence of quencher, respectively; ε_w and ε_{int} are the molar absorption coefficients of the dye in water or when intercalated in DNA, respectively.

Using $\alpha = \frac{\varepsilon_w\Phi_w^0}{\varepsilon_{int}\Phi_{int}}$, Eq. (3) simplifies to Eq. (4),

$$\frac{I_0}{I} = \frac{f_{int} + (1-f_{int})\alpha}{f_{int} + (1-f_{int})\alpha\Phi_w/\Phi_w^0} \quad (4)$$

and, by application of the Stern-Volmer relation, we obtain

$$\frac{I_0}{I} = \frac{f_{int} + (1-f_{int})\alpha}{f_{int} + (1-f_{int})\alpha/(1 + K_{SV}[KI])} \quad , \quad (5)$$

K_{SV} being the Stern-Volmer constant and [KI] the concentration of potassium iodide.

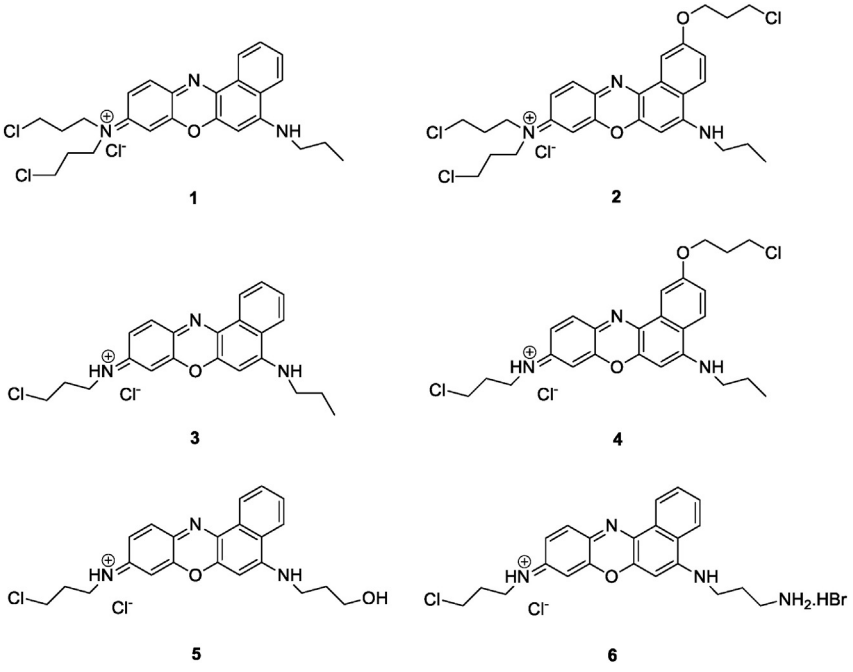

Fig. 1. Structures of benzo[*a*]phenoxazinium chlorides **1**–**6**.