Analytica Chimica Acta 940 (2016) 128–135

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Small molecule probes finely differentiate between various ds- and ss-DNA and RNA by fluorescence, CD and NMR response

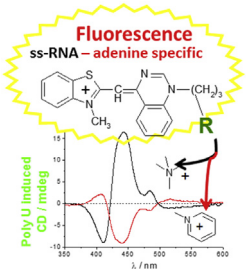
Ivo Crnolatac [a], Iva Rogan [a], Boris Majić [a], Sanja Tomić [a], Todor Deligeorgiev [b], Gordan Horvat [c], Damjan Makuc [d, e], Janez Plavec [d, e], Gennaro Pescitelli [f], Ivo Piantanida [a, *]

[a] Division of Organic Chemistry and Biochemistry, Division of Physical Chemistry, Ruđer Bošković Institute, P.O. Box 180, 10002 Zagreb, Croatia
[b] Faculty of Chemistry and Pharmacy, University of Sofia, Bulgaria
[c] Department of Physical Chemistry, Faculty of Science/Chemistry, Horvatovac 102A, HR-10000 Zagreb, Croatia
[d] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, Ljubljana, Slovenia
[e] EN-FIST Centre of Excellence, Trg Osvobodilne Fronte 13, Ljubljana, Slovenia
[f] Department of Chemistry, University of Pisa, Via Moruzzi 13, Pisa, Italy

HIGHLIGHTS

- Novel dyes emit fluorescence only for poly rA even at high excess of all other ss-RNA.
- Fluorescence response for AT-DNA is 8 times stronger than for GC-DNA.
- Florescence induced by ds-RNA is 20% stronger that emission induced by ds-DNA.
- Intrinsically non-chiral, dyes show strong and characteristic ICD response for poly rU.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form
10 August 2016
Accepted 11 August 2016
Available online 15 August 2016

Keywords:
DNA/RNA recognition
Fluorescence
Induced circular dichroism
Chiral recognition
Molecular modelling

ABSTRACT

Two small molecules showed intriguing properties of analytical multipurpose probes, whereby one chromophore gives different signal for many different DNA/RNA by application of several highly sensitive spectroscopic methods. Dyes revealed pronounced fluorescence ratiomeric differentiation between ds-AU-RNA, AT-DNA and GC-DNA in approximate order 10:8:1. Particularly interesting, dyes showed specific fluorimetric response for poly rA even at 10-fold excess of any other ss-RNA, and moreover such emission selectivity is preserved in multicomponent ss-RNA mixtures. The dyes also showed specific chiral recognition of poly rU in respect to the other ss-RNA by induced CD (ICD) pattern in visible range (400–500 nm), which was attributed to the dye-side-chain contribution to binding (confirmed by absence of any ICD band for reference compound lacking side-chain). Most intriguingly, minor difference in the side-chain attached to dye chromophore resulted in opposite sign of dye-ICD pattern, whereby differences in NMR NOESY contacts and proton chemical shifts between two dye/oligo rU complexes combined with MD simulations and CD calculations attributed observed bisignate ICD to the dimeric dye aggregate within oligo rU.

* Corresponding author.
E-mail address: pianta@irb.hr (I. Piantanida).

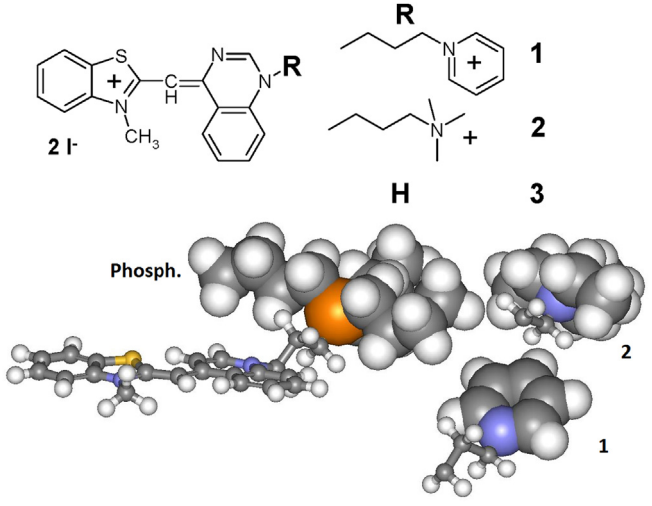
http://dx.doi.org/10.1016/j.aca.2016.08.021
0003-2670/

1. Introduction

Small molecules targeting DNA/RNA by the combination of several different non-covalent binding modes, drew lots of attention in the last decade [1,2]. For instance small molecule dyes relied mostly on thermodynamic equilibrium to achieve selective or even specific sensing of small structural differences of DNA/RNA secondary structure [1–3] or were applied in new supramolecular chromophoric assemblies [3,4]. Both DNA and RNA exhibit a wide range of structural topologies, among which different single stranded (ss–) sequences are quite numerous. For instance, ss–sequences are essential for the RNA folding landscape, and there are stable ss–DNAs, such as hairpins [5] or abasic sites [6]. These latter are often very convenient targets since ds–DNA is protected from reaction with a number of synthetic and biological nucleases [7]. A number of small molecules were designed for specific interaction with abasic lesions with an idea to inhibit the DNA repair system and in that way pronounce the action of antitumor drugs [8]. Several antiviral drugs and fluorescent probes also target viral ss-sequences. Applications of ss-DNA/RNA targeting small molecules can also be extended to new materials, using ss-DNA as a template for arraying multichromophoric systems [3].

Our recent studies revealed that differences in the secondary structure of the several most common long double stranded DNA/RNA can be probed by the thermodynamically controlled complexation of the single small molecule, able to give for each of several types of DNA/RNA different spectroscopic response [9–12]. We also observed intriguing selectivity of phosphonium-cyanine dyes [13] showing very rare, kinetically controlled differentiation between homo-AT-DNA and alternating AT-AT- DNA. Interestingly, our subsequent studies on very close analogues revealed that even minor changes in cyanine chromophore completely abolished aforesaid selectivity and in some cases induced cytotoxicity [14].

Based on the aforesaid results, here elaborated idea was to see for the same cyanine dye-linker combination as in [13], if the volume of positive substituent is decreased and the shape is modified (pyridyl- or ternary ammonium-), how this would influence on the DNA/RNA recognition. The differences in mentioned substituents were graphically compared on Scheme 1, DOWN. Thus novel small molecules (Scheme 1) were characterised by a) voluminous positively charged substituents of varying aromaticity/flexibility for fine control of electrostatic and steric interactions within DNA/RNA binding site; b) chromophore of adjustable geometry prone to aggregation within the DNA/RNA binding site; c) convenient spectroscopic properties to report recognition. Compounds **1** and **2** comprise ideal combination of these properties, including also easy accessibility and strong fluorimetric response upon binding to DNA [15].



Scheme 1. UP: Structures of studied compounds [15] **1** and **2** and reference compound **3** [22]. The model compound **4** used in calculations has R = CH_3. **DOWN**: Comparison of the voluminous positively charged tributyl-phosphonium substituent of previously studied analogue [13] (**Phosph.**) with ternary ammonium- (**2**) and piridyl- (**1**) in CPK style. Cyanine dye as well as propyl chain are in ball-and-stick style.

2. Material and methods

2.1. Material

Compounds **1** and **2** were prepared according to previously reported procedures [15] synthetic details given in Scheme S2 (Supp. Info.).

2.2. Spectroscopy

The UV/Vis spectrophotometric experiments were recorded on Perkin-Elmer Lambda 950 or Varian Cary 100 Bio instruments, CD spectra on a JASCO 815 and fluorescence spectra on a Varian Eclipse instrument. All experiments were performed in 1 cm path quartz cuvettes in thermostated conditions. Synthetic polynucleotides were purchased from Sigma. Polynucleotide concentration was determined spectroscopically as the concentration of phosphates. Titration data were processed by Scatchard equation [16] to give binding constants *Ks* and bound dye/polynucleotide ratio *n*. Thermal denaturation data for DNA, RNA and their complexes with L- and D-5 were determined at 260 nm, absorbance of the ligands was subtracted from every curve, Tm values are the midpoints of the transition curves and *ΔTm* values were calculated subtracting Tm of the free nucleic acid from Tm of the complex.

2.3. ITC

Calorimetric measurements were performed on MicroCal VP-ITC (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) with the polynucleotide solution in the cell and the dye solution in the syringe. Both solutions were prepared in 50 mM Na-cacodylate buffer, pH 7. The obtained results were fitted using built in "Two sets of sites" fitting model in Origin 7.0.

2.4. NMR

Compounds **1** and **2** were dissolved in ^2H_2O. NMR titrations of rU_{12} oligonucleotide were performed in 50 mM sodium cacodylate buffer (pH 7.0). The rU_{12} concentrations were 0.9 mM and 1.1 mM per strand in the presence of **1** and **2**, respectively. 1D and 2D $^1H/^{13}C$ NMR spectra were acquired on 800 and 600 MHz Agilent Technologies NMR spectrometers equipped with cold probes. Sample temperature was set to 298 K. ^1H-^1H NOESY spectra were acquired using mixing time of 200 ms at 273 K. ^{31}P NMR experiments were acquired on 300 MHz Unity Inova NMR spectrometer with broadband ATB probe.

2.5. CD calculations

DFT and TD-DFT calculations on model **4** were run with Gaussian'09 package [17]. The monomer structure was obtained by DFT geometry optimizations with M06–2X/6-31G(d) upon systematic variation of the C–CH and CH=C bonds to produce a 2D energy scan. All the energy minima thus obtained were used for TD-TDT calculations at M06/SVP and M06–2X/SVP level. The starting geometry of the dimer was built from the structure of thiazole orange dimer [18] and optimized at M06–2X/6-31G(d)