COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Interactions of phenanthridinium–nucleobase conjugates with polynucleotides in aqueous media. Recognition of poly U†

Iva Juranović,[b] Zlatko Meić,[a] Ivo Piantanida,*[a] Lidija-Marija Tumir[a] and Mladen Žinić*[a]

[a] *Laboratory of Supramolecular and Nucleoside Chemistry, Department of Chemistry and Biochemistry, Rudjer Boskovic Institute, HR 10000 Zagreb P.O.B. 1016, Croatia*

[b] *Laboratory of Analytical Chemistry, Department of Chemistry, Faculty of Science, Strossmayerov trg 14, HR 10000 Zagreb, Croatia*

Received (in Cambridge, UK) 20th March 2002, Accepted 14th May 2002
First published as an Advance Article on the web 5th June 2002

Adenine containing conjugates 4 and 5 exhibit specific spectroscopic changes and two orders of magnitude higher affinity toward poly U than uracil conjugates 2 and 3 and the reference compound 1 due to the existence of specific interactions between adenine and uracil, possibly Watson-Crick hydrogen bonding between the bases stacked on the phenanthridinium moiety.

Single stranded domains of DNA and RNA play an essential role in a number of processes in living cells including those involving viruses.[1,2] Some recent reports describe preferred binding to single-stranded (ss-) polynucleotides compared to double-stranded (ds-) ones by macrocyclic compounds of the cyclobisintercaland type.[3] The recognition of uracil and thymine polynucleotides has been realised by formation of coordinative cyclen–Zn^{2+}–nucleobase complexes.[4] A more general approach that would allow the recognition of each of the major nucleobases relies on the construction of intercalator–nucleobase conjugates containing a nucleobase attached on the intercalator by a flexible spacer of a suitable length.[5] Such conjugates may bind by intercalation and provide the recognition of the complementary base of the ss-polynucleotide by the Watson-Crick type of hydrogen bonding. The latter approach was studied more intensively, and various intercalators [6–9] were used for construction of conjugates. The influence of a spacer on binding and recognition properties of such conjugates is often neglected. In these rather complicated systems not only base-pair hydrogen bonding is involved but also electrostatic interactions of positively charged spacers with phosphates and hydrophobic interactions may have an important role as well.[10] We have shown recently that the spacer length in phenanthridinium–nucleobase conjugates **2**–**5** (Fig. 1) controls stability and binding stoichiometry of their complexes with nucleotides in aqueous media.[11] However, with these conjugates we were not able to observe the recognition of the complementary nucleotides; the expected hydrogen bonding between the conjugate and the nucleotide complementary nucleobases stacked upon the phenanthridinium surface does not occur due to the strong competition of bulk water. We have anticipated that the base pairing could be possible upon the conjugate binding into the complementary ss-polynucleotide; the latter providing more a lipophilic environment for hydrogen bonding compared to the conjugate–mononucleotide system. Consequently, such conjugates may bind more strongly to the complementary than to the non-complementary ss-polynucleotides.

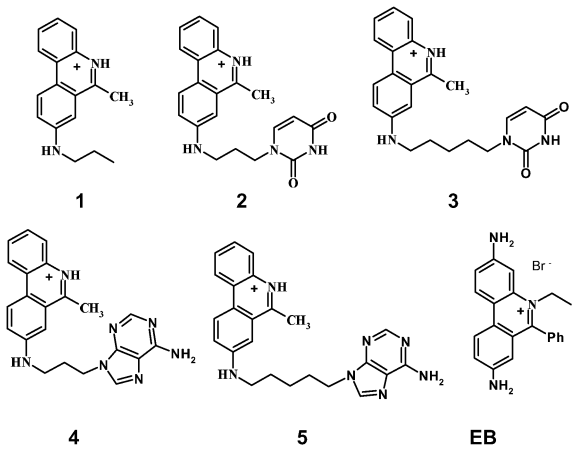

Fig. 1 Phenanthridinium–nucleobase conjugates **2**–**5**, the reference phenanthridinium derivative **1** and ethidium bromide (**EB**).

Interactions of **1**–**5** with DNA as the representative of ds-polynucleotides were studied by fluorimetric titrations. Addition of *calf thymus* (ct-) DNA increased emission of the reference compound **1** 2.8 times and of derivatives **2**–**5** 1.1–1.4 times, while the fluorescence of **EB**[12] was increased 20 times. Binding constants of **1**–**5** (log *Ks* = 5.6–6 dm^3 mol^{-1}) and [bound ligand]/[polynucleotide phosphate] ratios (n = 0.05–0.14) calculated from titration data according to the Scatchard equation[13] are the same as those found for **EB** (log *Ks* = 6.1 dm^3 mol^{-1}, n = 0.2) within the error of the method.[14]

These results show that the conjugates bind to *ct* DNA by intercalation with similar affinity as **EB** and that the presence of a spacer and nucleobase in the former does not considerably reduce their intercalation ability. Somewhat lower values of n found for **2**–**5** indicate a less dense intercalation than predicted by the 'neighbour exclusion' principle.[15] Upon addition of *ct* DNA to **1**–**5** bathochromic and hypochromic effects in UV/Vis spectra were observed, in accord with the intercalative binding mode. The effects were more pronounced for the reference compound **1** than for the nucleobase conjugates **2**–**5**. We have found that **2** and **3** and to a higher extent **4** and **5** form the intramolecularly base-on-phenanthridinium stacked conformations in aqueous media.[11] This intramolecular stacking results in small bathochromic shifts and hypochromicity of the phenanthridinium absorbance of **2**–**5** compared to the reference compound **1** lacking a nucleobase. Since the magnitude of bathochromic and hypochromic effects induced by addition of *ct* DNA depend on the spectroscopic difference between free and intercalated compound, effects must be smaller for **2**–**5** than for the reference **1** having higher absorbance and non-shifted phenanthridinium band in the free state.

In contrast to titrations with *ct* DNA, the effects in UV spectra induced by additions of ss-polynucleotides were strongly dependent on both, the type of nucleobase present in **2**–**5** and the type of polynucleotide added (Table 1). The bathochromic shifts and the hypochromicity effects induced by poly A are stronger for **1**–**3** than for **4** and **5** and can be explained as in the case of *ct* DNA by stronger intramolecular base stacking in the latter conjugates. In contrast, poly U induced significantly stronger effects in the UV spectra of **4** and **5** bearing the complementary adenine than in those of the reference **1** and the non-complementary **2** and **3**. This difference points to important additional interactions between the uracils of poly U and the

† Electronic supplementary information (ESI) available: materials and methods and CD titrations. See http://www.rsc.org/suppdata/cc/b2/b202615e/

DOI: 10.1039/b202615e

Table 1 Spectroscopic properties of **1**–**5** complexes with single-stranded polynucleotides[a]

	poly A		poly U	
	UV/Vis $\Delta\lambda_{max}/\Delta A^b$	Fluorescence ΔI^b	UV/Vis $\Delta\lambda_{max}/\Delta A^b$	Fluorescence ΔI^b
1	30/−20%	+330%	[c]	[c]
2	25/−15%	+260%	[c]	−2%
3	20/−20%	+288%	[c]	[c]
4	10/< −5%	+110%	10/−15%	−10%
5	10/< −5%	+120%	10/−15%	−11%

[a] Spectroscopic titrations were performed at pH = 5 (I = 0.1 mol dm^{-3}, Na cacodylate buffer), λ_{max}(Abs) = 440 nm. [b] Calculated as $\Delta A(\Delta I) = [(A_0(I_0) - A(I)/A_0(I_0)] \times 100$. [c] Spectroscopic changes close to the error of the instrument.

adenines of **4** and **5**. The fluorimetric titrations of **1**–**5** with poly U reveal significantly higher affinity of adenine conjugates **4**, **5** than those of the reference **1**, the non-complementary uracil conjugates **2** and **3** and of **EB** (log *Ks* < 3)[12] (Table 2). Quenching of phenanthridinium emission by poly U is much stronger for **4** and **5** than for **1**–**3** (Table 1) pointing to the stronger stacking interactions of the former conjugate–poly U complexes. Since in the intramolecularly stacked conformations of **4** and **5**[11] the adenines cover only a part of the phenanthridinium surface, the insertion of the uncovered part between the uracils of poly U can be expected (Fig. 2).

We have shown recently that **5** having adenine attached by the longer pentamethylene spacer forms a sandwich-like stacked complex with UMP in water. In the complex, the uracil is inserted between the adenine and phenanthridinium units.[11] However, **4** with the shorter trimethylene spacer does not form such a complex. Based on these results the bis-intercalative binding of **5** to poly U (Fig. 2, upper) cannot be excluded, especially because of the somewhat higher affinity compared to **4**. However, for **4** the binding mode shown in Fig. 2 (lower) remains the most possible explanation.

The addition of poly A resulted in significant changes in UV/Vis spectra of **1**–**5** (Table 1). A well defined tendency depending on the type of covalently attached nucleobase can be seen, bathochromic and hypochromic effects increase in the order **4**,**5** < **2**,**3** < **1**. In fluorimetric titrations, the emission increase is of the same tendency (Table 1). It should be noted that the reversed order for UV/Vis effects and quenching of emission is obtained for poly U. Fluorimetric titrations of **1**–**5** with poly A give similar binding affinities for all studied compounds. Taken altogether, the UV/Vis and fluorescence effects point toward intercalation of phenanthridinium unit into poly A as the dominant binding mode for all conjugates; for **2** and **3** bearing uracil no increased affinity toward poly A could be observed.

The recognition of poly U by **4** and **5** with attached complementary adenines and lack of recognition of poly A by **2** and **3** with appended uracils point to the complexity and

Table 2 Binding affinities (log *Ks*) and ratios n ($c_{\text{bound }\mathbf{1}\text{–}\mathbf{5}}/c_{\text{phosphate}}$)[a] for **1**–**5** toward single-stranded polynucleotides[b]

	poly A		poly U	
	n	log *Ks*	n	log *Ks*
1	0.1 ± 0.07	5.1 ± 0.4	[c]	< 3[c]
2	0.1 ± 0.07	5.4 ± 0.2	[c]	< 3[c]
3	0.1 ± 0.05	5.3 ± 0.4	[c]	< 3[c]
4	0.1 ± 0.03	5.3 ± 0.4	0.1 ± 0.03	4.5 ± 0.4
5	0.8 ± 0.11	5.2 ± 0.1	0.1 ± 0.03	5.5 ± 0.4

[a] The correlation coefficients > 0.999 correspond to given ranges of n and log *Ks* calculated according to the Scatchard equation.[13] [b] Fluorimetric titrations were performed at pH = 5 (I = 0.1 mol dm^{-3}, Na cacodylate buffer). [c] Estimated value due to less than 20% of complex formed.

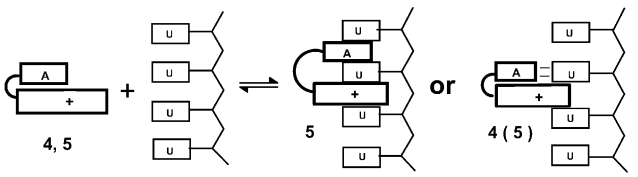

Fig. 2 Schematic presentation of possible bis-intercalative (upper) or mono-intercalative with A–U pairing (lower) binding modes of **4** and **5** to poly U.

sensitivity of these systems on the structural properties of both the conjugates and the polynucleotides. In aqueous solution, poly A forms a well organized single-stranded helix stabilised by stacking interactions between adenines while poly U forms a random coil due to absence of any appreciable stacking between uracils.[1b] On the other hand, the intramolecular stacking of uracil and phenanthridinium in **2** and **3** is less pronounced than the adenine phenanthridinium stacking in **4** and **5**.[11] Consequently, the former conjugates bind to complementary poly A (as well as to double helix of *ct* DNA) only by intercalation of a free phenanthridinium unit. In contrast, **4** and **5** with intramolecularly strong-stacked adenines can intercalate into poly U only by a part of the free phenanthridinium surface. In this case the additional stabilisation of the complex could be provided by stacking interactions and/or hydrogen bonding between A and U enabled by conformational adaptation of a less organised and flexible polynucleotide.

The results clearly demonstrate that conjugates **4** and **5** exhibit two types of binding, depending on structural properties of the polynucleotides: *i*) the mono-intercalation for well organised helical ds- or ss-polynucleotides and *ii*) the intercalation supported by additional A–U interactions in the case of weakly organised and flexible poly U. The observations presented could be of considerable importance for construction of new molecules for recognition of specific structural motifs on nucleic acids or targeting of selected nucleobases.

Notes and references

1 (*a*) W. Saenger, in *Principles of Nucleic Acid Structure*, Springer, New York, 1988; (*b*) C. R. Cantor and P. R. Schimmel, in *Biophysical Chemistry*, Freeman, San Francisco, 1980; vol. 3: pp. 1109–1181.
2 K. M. Weeks and D. M. Crothers, *Cell*, 1991, **66**, 577–588.
3 (*a*) N. Berthet, J. Michon, J. Lhomme, M.-P. Teulade-Fichou, J.-P. Vigneron and J.-M. Lehn, *Chem. Eur. J.*, 1999, **5**, 3625–3630; (*b*) I. Piantanida, B. S. Palm, P Čudić, M. Žinić and H.-J. Schneider, *Tet. Letters*, 2001, **42**, 6779–6782and references cited therein.
4 E. Kimura, M. Kikuchi, H. Kitamura and T. Koike, *Chem. Eur. J.*, 1999, **5**, 3113–3123.
5 J.-F. Constant, J. Fahy and J. Lhomme, *Tetrahedron Lett.*, 1987, **28**, 1777–1780.
6 F. Thomas, J. Michon and J. Lhomme, *Biochemistry*, 1999, **38**, 1930–1937 and references cited therein.
7 W. D. Wilson, L. Ratmeyer, M. T. Cegla, J. Spychala, D. Boykin, M. Demeunynck, J. Lhomme, G. Krishnann, D. Kennedy, R. Vinayak and G. Zon, *New J. Chem.*, 1994, **18**, 419–423.
8 S. Takenaka, M. Manabe, M. Yokoyama, M. Nishi, J. Tanaka and H. Kondo, *Chem Commun.*, 1996, 379–380.
9 P. Kubat, K. Lang, P. Anzenbacher, K. Jursikova, V. Kral and B. Ehrenberg, *J. Chem. Soc., Perkin Trans. 1*, 2000, 933–941.
10 W. D. Wilson, in, *Nucleic Acids in Chemistry and Biology*, eds. M. Blackburn and M. Gait, IRL Press, Oxford, 1989; ch. 8.
11 L. Tumir, I. Piantanida, P. Novak and M. Žinić, *J. Phys. Org. Chem.*, 2002 in press.
12 I. Piantanida, B. S. Palm, M. Žinić and H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2*, 2001, 1808–1816.
13 (*a*) G. Scatchard, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 660–664; (*b*) J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469–489.
14 H. W. Zimmermann, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 115–196.
15 J. L. Butour, E. Delain, D. Couland, J. B. LePecq, J. Barbet and B. P. Roques, *Biopolymers*, 1978, **17**, 872–86.

Typeset and printed by Black Bear Press Limited, Cambridge, England