Pergamon

Tetrahedron Letters 42 (2001) 6779–6783

TETRAHEDRON LETTERS

Phenanthridinium cyclobisintercalands. Fluorescence sensing of AMP and selective binding to single-stranded nucleic acids

Ivo Piantanida,[a] Barbara Susanne Palm,[b] Predrag Cudic,[a] Mladen Zinic[a,*] and Hans-Jörg Schneider[b,*]

[a]*Laboratory for Supramolecular and Nucleoside Chemistry, Division of Chemistry and Biochemistry, Rudjer Boskovic Institute, HR 10000 Zagreb, PO Box 1016, Croatia*

[b]*FR Organische Chemie der Universität des Saarlandes, D-66041 Saarbrücken, Germany*

Received 20 June 2001; revised 17 July 2001; accepted 27 July 2001

Abstract—Macrocyclic ligands **1** and **2** containing two positively charged phenanthridinium units and aminobisacetylenic bridges, exhibit significantly higher affinity toward single-stranded rather than double-stranded polynucleotides. The ligands bis-intercalate into the former and show non-intercalative interactions with the latter type of nucleic acids. Both ligands differentiate AMP from the GMP or UMP by significant fluorescence emission increase upon complexation of the first nucleotide in water (log K_s of 1:1 and 1:2 complexes 5.8 and 1.4, respectively), while only a slight emission change is observed in titrations with GMP and UMP.

1. Introduction

Among the number of small synthetic compounds that can bind to nucleic acids, those with selective binding to single-stranded regions (hair pins, bulges)[1,2] or to abasic sites of DNA[3] or those that can selectively interact with single-stranded (ss-) RNA sequences[4–6] are rather rare. Such compounds are of current interest for recognition and marking of single-stranded regions of DNA or RNA, or as selective RNA ligands and hence potential antiviral agents.[7]

Cyclobisintercaland receptors constructed by bridging of two acridinium[8,9] or phenanthridinium[10] intercalator units (Fig. 1, **I**) with relatively short (6–9 atom sequence) aminomethylene or bisacetylene bridges were shown to bind nucleotides strongly in water by π-π stacking interactions between receptor units and the inserted nucleobase. The acridinium cyclobisintercalands with short bridges also exhibited recognition of single stranded domains of nucleic acids.[1,3] However, the bis-acridinium macrocycles with longer (14 atom) bridges were found to bis-intercalate by threading into ds-polynucleotides[11] or to bind in more complex modes.[12] The latter observations reveal that the length and flexibility of connecting bridges may have a decisive influence on the single strand/double strand binding selectivity. With this in mind, we prepared the phenanthridinium cyclobisintercalands **1** and **2** (Fig. 1) possessing two short and rigid aminobisacetylenic bridges located at the long axes ends of the intercalating units.[13] The macrocycles are diastereoisomeric due

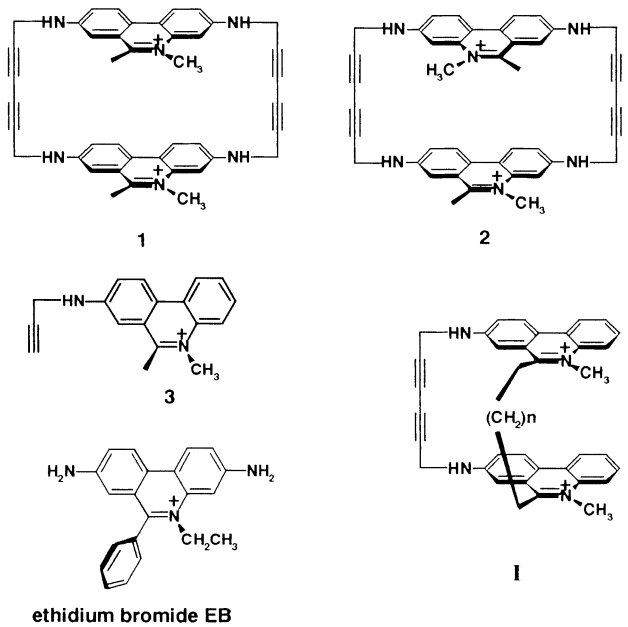

Figure 1.

* Corresponding authors. Tel.: +385 1 46 80 195; fax: +385 1 46 80 195 (M.Z.); tel.: +49 (0)681 302 3209/2269; fax: +49 (0)681 302 4105 (H.-J.S.); e-mail: zinic@rudjer.irb.hr; ch12hs@rz.uni-sb.de

0040-4039/01/$ - see front matter
PII: S0040-4039(01)01386-7

to the different orientation of phenanthridinium units. Structural characteristics of **1** and **2**, such as the relatively short distance between phenanthridinium units (ca. 4.5 Å) that only allows insertion of a single nucleic base as well as the location of bridges, are unfavorable for intercalation into ds-nucleic acids. Consequently, preferred binding to single-stranded polynucleotides or single-stranded regions of nucleic acids may be expected. To prove this assumption, the affinities of **1** and **2** for ss- and ds-polynucleotides of RNA and DNA type were studied by fluorescence, thermal denaturation experiments and viscometry. The results of affinity studies are compared to those determined for monomeric phenanthridinium derivative **3** (Fig. 1) which, as well as ethidium bromide,[14] behaves as a typical ds-nucleic acid mono-intercalator. In addition, binding of nucleotides in water by **1** and **2** was studied to evaluate the strength of interaction with a single nucleobase and to possibly observe the effect of the different mutual orientation of phenanthridinium units in diastereomeric ligands on binding properties and selectivity.

2. Materials and methods

Nucleotides and polynucleotides were purchased from Sigma and Aldrich, and used without further purification. Polynucleotides were dissolved in the respective buffer and their concentration determined spectroscopically[15] as the concentration of phosphates. Electronic absorption spectra were recorded on a Varian Cary 1 spectrometer using quartz cuvettes (1 cm). Fluorescence spectra were recorded on a Perkin–Elmer LS 50 fluorimeter. In fluorimetric titrations excitation wavelengths of 320 and 500 nm were used and changes of emission were monitored at 580 nm. The stability constants (K_s) were calculated by processing titration data using the SPECFIT program.[16] The rather large errors of log K_s (±0.5) are due to the small total change of fluorescence, allowing collection of only 10 data points for $c_{AMP}=10^{-6}$ to 10^{-2} mol dm^{-3}. The stability constants (K_s) and [bound **1**, **2**]/[polynucleotide phosphate] ratio (n) were calculated according to the Scatchard equation[17] by the non-linear least-square fitting method.[15] Values for K_s and n are given in Table 1 and all have satisfactory correlation coefficients (>0.999). Due to the previously observed slow kinetics of macrocyclic bis-acridinium analogues,[11] all titrations were performed in a way that the solution of **1** or **2** was mixed with increasing concentrations of polynucleotide in separate vessels, left to equilibrate for 4 hours (in the dark; room temperature) and then emissions recorded for each vessel starting from lower to higher c (polynucleotide). The use of NMR techniques was hampered by the low solubility of **1** and **2** and their complexes.

3. Spectroscopic properties

In contrast to previously studied phenanthridinium cyclobisintercalands of type **I**,[18] **1** and **2** exhibit very low fluorescence in aqueous solution. Addition of 20% D_2O to the solution of the ligands induced a significant increase in emission intensity. The same effect was described for ethidium bromide and can be explained by a decrease in quenching rate due to partial NH–ND exchange; quenching of ethidium emission was found to occur by NH proton transfer to water molecules in the excited state.[19] Hence, very low emissions for **1** and **2** may be explained by the same quenching mechanism due to the presence of four secondary amino groups. The observed large difference in emission intensity between **1** and **2** and the type **I** ligands[10] indicates a much lower quenching rate by NH proton transfer for the latter due to the presence of only two secondary NH groups. The fluorescence intensity of **1** and **2**, although low, is linearly concentration dependent up to 5×10^{-6} mol dm^{-3}.

4. Interactions with nucleotides

In the fluorimetric titrations, the addition of AMP induced a ca. 90% emission increase of **1** and **2**; in contrast with GMP and UMP where only a very slight emission change could be observed. Processing of the titration data gave the best fit for formation of two different **1**:AMP complexes, that of 1:1 stoichiometry ($c_{AMP}=10^{-6}$ to 10^{-4} mol dm^{-3}, emission increase ca.

Table 1. Binding affinities (log K_s)[a] and ratios n ($c_{\text{bound 1–3}}/c_{\text{phosphate}}$) for **1**, **2** and **3** toward single-stranded and double-stranded polynucleotides[b]

	1		**2**		**3**	
	n	log K_s	n	log K_s	n	log K_s
Poly A	0.05	6.3	0.03	6.8	0.2	4.2
Poly G	0.03	7.1	0.05	6.2	0.09	5.5
Poly U	0.1–1[c]	≈5[c]	[c]	[c]	[c]	[c]
Poly G-C	0.02	5.7	0.05	5.4	0.2	5.1
Poly A-U	[d]	[d]	[d]	[d]	0.2	5.5
Poly dA-dT	0.1	6.0	0.1	5.7	0.2	4.7

[a] The correlation coefficients >0.999 correspond to given ranges of n and log K_s.
[b] Fluorimetric titrations were performed at pH 6.2 (0.01 mol dm^{-3} Na cacodilate or MES buffer).
[c] Estimated value due to small spectroscopic changes.
[d] Systematic discrepancy between experimental and calculated values was observed.

15%) and that of 1:2 stoichiometry ($c_{AMP}=10^{-3}$ to 10^{-2} mol dm^{-3}, emission increase ca. 70%), with log K_s values of 5.8 and 1.4, respectively. The K_s for the 1:1 complex is practically the same as those determined previously for type **I** ligands and nucleotides; the latter complexes were found to form exclusively by π-π stacking interactions between phenanthridinium units and the inserted nucleotide base.[10] Formation of the much less stable **1**:AMP 1:2 complex at large excess of AMP indicates stacking of the second nucleotide base on the 'outside' aromatic surface of the receptor. This conclusion is supported by a similar constant determined for the **EB**:AMP 1:1 stacked complex (log K_s 1.92).[20] This striking difference presents the opposite fluorescence response observed for nucleotide complexes of the two types of phenanthridinium cyclobisintercalands: the emission increase for **1**,**2** and AMP and strong quenching for all four major nucleotide complexes with **I**. The insertion of the electron rich purine base of AMP between positively charged units of **1** and **2** may decrease the acidity of amino protons and hence the quenching rate which results by emission increase upon complexation. The observation that stacking of the second base in the **1**:AMP 1:2 complex contributes 70% to the total emission increase supports this explanation. However, with the ligands of type **I** it seems that stacking interactions between phenanthridinium units and the inserted nucleic base in the complex leads to decreased emission. Interestingly, different mutual orientations of the phenanthridinium units of **1** and **2** gives no significant difference in the binding of AMP. The observed selective fluorescence response of **1** and **2** for AMP and not for GMP and UMP is not fully understood at present; however, it can be of interest for development of fluorescent AMP sensing devices.

5. Interactions with polynucleotides

Under the conditions necessary for UV–vis titrations ($c_{\mathbf{1,2}}=10^{-5}$ mol dm^{-3}) the addition of ds-polynucleotides induced instant precipitation. However, the addition of poly A caused a bathochromic (5 nm) shift in the absorption maxima at 474 nm and 10% of hypochromicity.

A pronounced increase in fluorescence emission at $c_{\mathbf{1,2}}=2\times10^{-6}$ mol dm^{-3} upon addition of polynucleotides allowed titrations to be carried out. No precipitation effects on fluorescence spectra were observed.[21] The K_s values for **1** and **2** with single-stranded poly A, poly G (Table 1) are two orders of magnitude higher than those of monomeric **3**. This result strongly suggests participation of both phenanthridinium units in complexation with a single nucleobase inserted between them. A similar difference in affinity was found between bis-intercalative and mono-intercalative agents.[22]

Fluorimetric changes of **1** and **2** upon addition of double-stranded polynucleotides were found to depend significantly on a base pair composition (Fig. 2). Poly A-poly U induces a much larger emission change than the G-C analogue; also there is an obvious difference in fluorescence response between RNA (A-U) and DNA (dA-dT) ds-polynucleotides. The stability constants (K_s, Table 1) derived from fluorimetric titration data for **1**, **2** and poly G-poly C are almost identical to the K_s of mono-intercalating compound **3**. However, structural characteristics of **1** and **2** exclude the possibility of intercalation of only one phenanthridinium unit into ds-polynucleotide. A somewhat lower affinity of **3** toward poly dA-poly dT, common for the mono-intercalators,[23] is not observed for **1** and **2**. It should be noted, however, that the similar affinities of **1**, **2** and **3** do not necessarily exclude bis-intercalative binding of the former.[24]

Processing of the titration data for poly A-poly U according to the Scatchard equation shows a systematic discrepancy between experimental and calculated values indicating the presence of at least two different binding modes.

In thermal denaturation experiments (Table 2) ΔT_m values obtained for mono-intercalator **3** point to stronger stabilization of RNA than DNA as also found for **EB**.[7] Macrocycle **2** stabilizes RNA polymers almost identically to monomer **3**. In contrast, 2–7 times stronger stabilization is observed for all known bis-intercalators[22] compared to mono-intercalators. This comparison speaks against bis-intercalative binding of

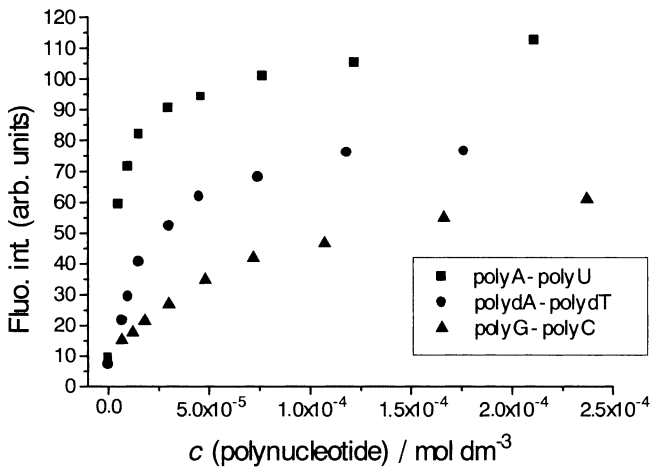

Figure 2. Fluorimetric titration of **1** ($\lambda_{exc}=500$ nm; $\lambda_{em}=580$ nm; $c=2\times10^{-6}$ mol dm^{-3}; pH 6.2, 0.01 mol dm^{-3} Na cacodylate buffer) with double-stranded polynucleotides.

Table 2. Melting temperatures (ΔT_m/°C)[a] of DNA and RNA polymers determined in the presence of **2** and **3**

	r[b]	0.05	0.1	0.2	0.3
2	poly dA dT	0.9	1.8	4.9	6.5
	poly A U	1.7	2.8	6.3	8.7
3	poly dA dT	0.8	1.5	2.6	3.5
	poly A U	1.9	3.6	6.1	8.4

[a] Values have been corrected for absorbance of the tested compound at various r ratios; pH 6.25 (buffer MES, 0.01 M, 0.001 M EDTA).
[b] $r=c_{compound}/c_{polynucleotide\ phosphates}$.

2. The RNA/DNA preference of **2** is much less pronounced than that of **3**.

Viscometric measurements were performed according to a previously described procedure[15] with the modification that aliquots of DMSO stock solutions of the ligands to be studied were added and the viscometry data obtained corrected for DMSO content (final content not exceeding 5% of the total volume). Results have shown that **2** induces much less elongation ($\alpha = 0.3$) of ds-polymer than **3** ($\alpha = 0.9$); this observation also contrasts the effect characteristic for a bis-intercalator.[22]

The results of comparative fluorimetric, thermal denaturation and viscometry studies taken together strongly suggest non-intercalative binding of **1** and **2** with ds-polynucleotides[22,25] and indicate groove binding driven by hydrophobic and electrostatic (double positive charge of **1**, **2**) interactions.

6. Conclusions

Macrocycles **1** and **2** strongly bind AMP in water ($\log K_s$ 5.8) with significant emission increase upon complexation. In contrast with GMP and UMP, only a slight change in fluorescence was observed with **1** and **2**. The observed selective fluorescence response for AMP can be of interest for the development of fluorescent sensing devices for this nucleotide. It should be emphasized that so far no other synthetic receptor molecule is known that gives specific fluorescence responses on complexation to AMP in aqueous media. Low fluorescence emissions for free **1** and **2** and significant emission increases upon binding to polynucleotides makes these derivatives of interest as potential fluorescent markers for nucleic acids. Both **1** and **2** exhibit 25 times higher affinity toward ss-polymer (poly G) compared to ds-polymer (poly G-poly C); the selectivity toward poly A compared to poly dA-poly dT although significant is somewhat lower (2–13 times). This makes **1** and **2** with short lateral bridges connecting two intercalative units strikingly different from classical mono-intercalators and also macrocyclic bis-intercalators possessing long connecting bridges;[11,12] both of the latter ligands were found to bind more strongly to ds-polynucleotides. The observed ss-polynucleotide selectivity with these types of macrocycles may allow the design of new ligands for recognition and blocking of nucleic acid single-stranded domains. On the other hand, strong binding to ss-RNA polynucleotides makes **1** and **2** candidates for testing on antiviral activity.

Acknowledgements

The financial support from the Croatian Ministry of Science and Technology and Deutsche Forschungsgemeinschaft, Bonn (Grants 436KRO113/3 and Schn115/17-1) is gratefully acknowledged.

References

1. Slama-Schwok, A.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Taillandier, E.; Lehn, J.-M. *J. Am. Chem. Soc.* **1995**, *117*, 6822–6831.
2. (a) Wilson, W. D.; Ratmeyer, L.; Cegla, M. T.; Spychala, J.; Boykin, D.; Demennyck, M.; Lhomme, J.; Krishnan, G.; Kennedy, D.; Vinayak, R.; Zon, G. *New J. Chem.* **1994**, *18*, 419–423; (b) Slama-Schwok, A.; Peronnet, F.; Hantz-Brachet, E.; Taillandier, E.; Teulade-Fischou, M.-P.; Vigneron, J.-P.; Best-Belpomme, M.; Lehn, J.-M. *Nucleic Acids Res.* **1997**, *25*, 2574–2581.
3. Berthet, N.; Michon, J.; Lhomme, J.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. *Chem. Eur. J.* **1999**, *5*, 3625–3630.
4. Slama-Schwok, A.; Lehn, J.-M. *Biochemistry* **1990**, *29*, 7895–7903.
5. Jain, R. K.; Sarracino, D. A.; Richert, C. *J. Chem. Soc., Chem. Commun.* **1998**, 423–424.
6. Takenaka, Y.; Manabe, M.; Yokoyama, M.; Nishi, M.; Tanaka, J.; Kondo, H. *J. Chem. Soc., Chem. Commun.* **1996**, 379–380.
7. Wilson, W. D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. *Biochemistry* **1993**, *32*, 4098–4104.
8. Claude, S.; Lehn, J.-M.; Schmidt, F.; Vigneron, J.-P. *J. Chem. Soc., Chem. Commun.* **1991**, 1182–1183.
9. Dhaenens, M.; Lehn, J.-M.; Vigeron, J.-P. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1379–1381.
10. Cudic, P.; Zinic, M.; Tomisic, V.; Simeon, V.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1995**, 1073–1075.
11. Veal, J. M.; Li, Y.; Zimmerman, S. C.; Lamberson, C. R.; Cory, M.; Zon, G.; Wilson, W. D. *Biochemistry* **1990**, *9*, 10918–10927.
12. Yang, X.-lei; Howard, R.; Gao, Y.-G.; Wang, A. H.-J. *Biochemistry* **2000**, *39*, 10950–10957.
13. Cudic, P.; Zinic, M.; Skaric, V.; Kiralj, R.; Kojic-Prodic, B.; Vigneron, J.-P.; Lehn, J. M. *Croat. Chem. Acta* **1996**, *69*, 569–611.
14. (a) LePecq, J. B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87–106; (b) Kapuscinski, J.; Darzynkiewicz, Z. *J. Biomol. Struct. Dyn.* **1987**, *5*, 127–143.
15. Palm, B. S.; Piantanida, I.; Zinic, M.; Schneider, H.-J. *J. Chem. Soc., Perkin Trans. 2* **2000**, 385–392.
16. © SPECFIT GLOBAL ANALYSIS, a Program for Fitting, Equilibrium and Kinetic Systems, using Factor Analysis & Marquardt Minimization: Maeder, M.; Zuberbuehler, A. D. *Anal. Chem.* **1990**, *62*, 2220; Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbuehler, A. D. *Talanta* **1985**, *32*, 257.
17. (a) Scatchard, G. *Ann. NY Acad. Sci.* **1949**, *51*, 660–664; (b) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469–489 and **1976**, *103*, 679–684.
18. Zinic, M.; Cudic, P.; Skaric, V.; Vigneron, J.-P.; Lehn, J.-M. *Tetrahedron Lett.* **1992**, *33*, 7417–7420.
19. Olmsted, III, J.; Kearns, D. R. *Biochemistry* **1977**, *16*, 3647.
20. Odani, A.; Masuda, H.; Yamauchi, O. *Inorg. Chem.* **1991**, *30*, 4484–4486.

21. Precipitation, even to a small degree, causes stray light effects, which generate irreproducibility of spectra, very often decrease of intensity with time and pronounced differences between spectra of old and fresh samples; see Ref. 4 and references cited therein.
22. Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *6*, 275–340.
23. Wilson, W. D.; Wang, Y.-H.; Krishnamoorthy, C. R.; Smith, J. C. *Biochemistry* **1985**, *24*, 3991–3999.
24. Wirth, M.; Buchardt, O.; Koch, T.; Nielsen, P. E.; Norden, B. *J. Am. Chem. Soc.* **1988**, *110*, 932–939.
25. (a) Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 273–279; (b) Dougherty, G.; Pilbrow, J. R. *Int. J. Biochem.* **1984**, *16*, 1179–1192.