Specific binding to polyA of a naphthalene diimide carrying thymine groups

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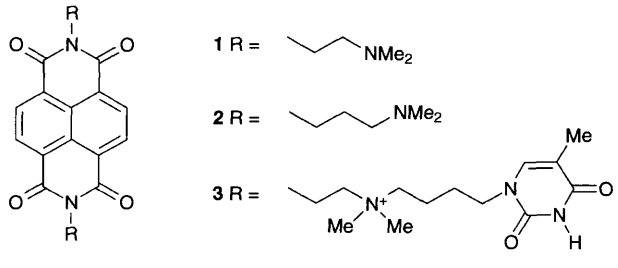
A naphthalene diimide carrying two thymine moieties at the termini of its substituents exhibited enhanced polyA binding affinity.

RNA adopts a variety of structures such as base bulges and hairpins incorporated into the helical forms and these structures are believed to be associated with the several activities of ribozymes and RNA viruses.[1] Thus, it is important to develop compounds which interact with specific RNA structures. Despite considerable effort, few small molecules have been known to show specificity for RNA. Wilson and co-workers[2] reported an ethidium derivative which can bind a bulge in the TAR region of the HIV-1 RNA genome with high specificity. Here, we report specific binding of naphthalene diimide carrying thymine substituents to polyA. Naphthalene diimides have been known as threading-type intercalators, one of whose substituents needs to thread into adjacent base pairs of DNA or RNA. Although naphthalene diimides carrying bulky substituents are hard to thread into double-stranded nucleic base pairs,[3–5] they may instead bind to single-stranded DNA or RNA specifically. If these diimides are furnished with a substituent which can interact with nucleic bases through some additional forces, their binding to single-stranded nucleic acids may be altered. On the basis of this consideration, a naphthalene diimide carrying two thymine moieties at the termini of substituents (**3**) was designed.

Compound **3** was prepared by the reaction of 1-(4-iodobutyl)thymine with naphthalene diimide **1**.† Compound **2** was also synthesized as a reference. The molar extinction coefficients of **2** and **3** were 27 300 and 29 300 $dm^3\ mol^{-1}\ cm^{-1}$ at 382 nm for the diimide chromophore, respectively, suggesting the absence of intra-molecular stacking of the thymine with the naphthalene diimide ring.

The interaction of compounds **2** and **3** with polyA:polyU (double-stranded RNA), polyU, polyA (single-stranded RNA), and polydA (single-stranded DNA) was studied in 10 mmol dm^{-3} MES [(2-*N*-morpholino)ethanesulfonic acid] and 1.0 mmol dm^{-3} EDTA (ethylenediaminetetraacetic acid) buffer at pH 6.24 and 25 °C. Compounds **2** and **3** exhibited nearly 60% of hypochromic shift together with a few nm of bathochromic shifts for the naphthalene diimide chromophore at 382 nm in absorption spectra upon binding to polyA:polyU, indicative of the intercalation into RNA duplex. Isosbestic points were observed for those titrations with polyA:polyU. Comparison of the experimentally obtained Scatchard plots with the theoretical plots generated by the binding equation of McGhee and von Hippel[6] allowed estimation of the binding constant (K), binding site size (n) and cooperativity parameter (ω) (Table 1). Data for **2** and **3** with polyA were analysed analogously. Data with polyU and polydA were not amenable to such an analysis because of the low affinity of ligands for these polymers and were analysed by the Benesi–Hildebrand equation[7] with a good linear correlation ($R > 0.9998$).



The binding constant (K) of **3** for polyA:polyU was 2.5 times smaller than that of **2** due presumably to the steric bulk of the thymine moieties. On the other hand, the binding constants of **2** and **3** for polyU were identical and were 10–20 times smaller than those with polyA:polyU. These results prove that the adenine of polyA is mainly responsible for the binding with the naphthalene diimides and that the thymine of **3** is not involved in this binding. However, **3** shows a significantly increased affinity for polyA. Thus, the binding constant of **3** with polyA is 15 times larger than that of **2** and 16 times larger than that with polyA:polyU. This result demonstrates that the thymine moieties of **3** contribute to the interaction with polyA through the adenine–thymine hydrogen bonding. Compound **3** binds to polydA 53 times weaker than to polyA and compound **2** cannot bind to polydA at all. The difference in the binding ability of **3** with polyA and polydA may derive from a difference in the stacking ability of adenine bases, because the adenine bases of polydA stack more tightly than those of polyA.[8]

To ascertain the specificity of **3** for polyA, circular dichroism (CD) spectra of 120 μmol dm^{-3} of polyA were determined in the absence and presence of 20 μmol dm^{-3} of **2** or **3** (Fig. 1). Given the binding constants for **2** and **3**, all of the ligand is bound by polyA under these conditions. The CD band at 280 nm of polyA decreased by complexation with these ligands, but the magnitude of decrease was larger for **3** than for **2**. The difference in the magnitude of the CD band of polyA in the presence of **2** and **3** shows the interaction of thymine moieties with polyA, because the difference of **3** and **2** is the existence of thymine moieties.

The binding affinity of intercalators is larger for double-stranded DNA than for single-stranded DNA and RNAs,[9]

Table 1 Binding affinity of naphthalene diimides **2** and **3** for RNA or DNA polymer[a]

Compound	DNA or RNA	$K/10^{-5}$ mol^{-1} dm^3	n	ω
2	polyA:polyU	2.82[b]	3	0.4
	polyU	0.13[c]		
	polyA	1.13[b]	1	0.5
	polydA	—[d]		
3	polyA:polyU	1.12[b]	2	0.4
	polyU	0.13[c]		
	polyA	16.0[b]	1	0.2
	polydA	0.31[c]		

[a] Experiments were performed in 10 mmol dm^{-3} MES and 1.0 mmol dm^{-3} EDTA buffer at pH 6.24 and 25 °C. [b] K, n and ω values were determined by a non-linear least-squares fit using the binding equation of McGhee and von Hippel.[6] [c] K was obtained by Benesi–Hildebrand plots.[7] All plots showed a good linear correlation with $R > 0.9998$. [d] The hypochromicity of **3** in the presence of 100 molar excess of polydA was smaller than 0.1%.

though some of the intercalators such as proflavine have the same binding affinity for both single and double-stranded DNA.[10] Nevertheless, no intercalators are known to bind to single-stranded RNA preferentially. The preference of **3** for single-stranded polyA originates from specific adenine–thymine pairing which results in the stabilization of the complex.

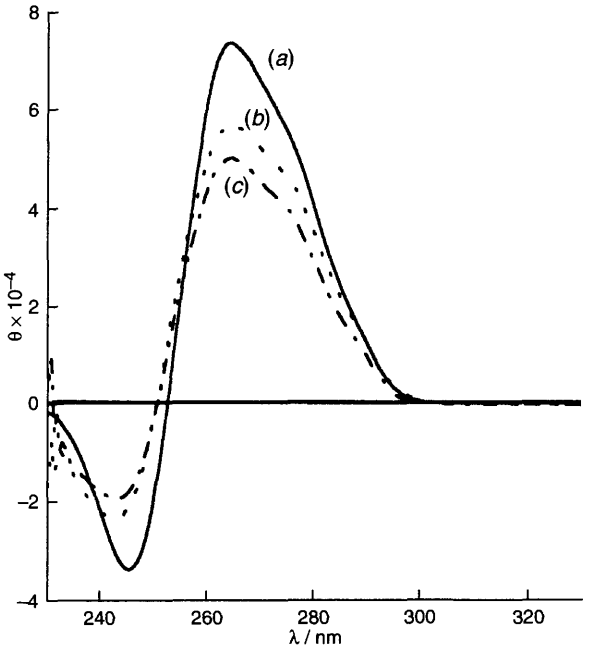


Fig. 1 CD spectra of 120 mmol dm^{-3} polyA in the absence (*a*) and presence of **2** (*b*) and **3** (*c*). The concentrations of **2** and **3** were 20 mmol dm^{-3}. All experiments were performed in 10 mmol dm^{-3} MES, 1.00 mmol dm^{-3} EDTA buffer and 2% Me_2SO at pH 6.24 and 25 °C.

Moreover, compound **3** was able to discriminate polyA from polydA by a large margin.

In conclusion, our results may be useful to design compounds which show specificity for single-stranded RNA with a sequence different from polyA.

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Footnote

† The iodide of compound **3** was exchanged with hexafluorophosphate and the resulting salt was purified by recrystallization from water. Satisfactory spectral data and elemental analyses of compounds **1**–**3** were obtained.

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