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Molecular Recognition of Watson–Crick-Like Purine–Purine Base Pairs

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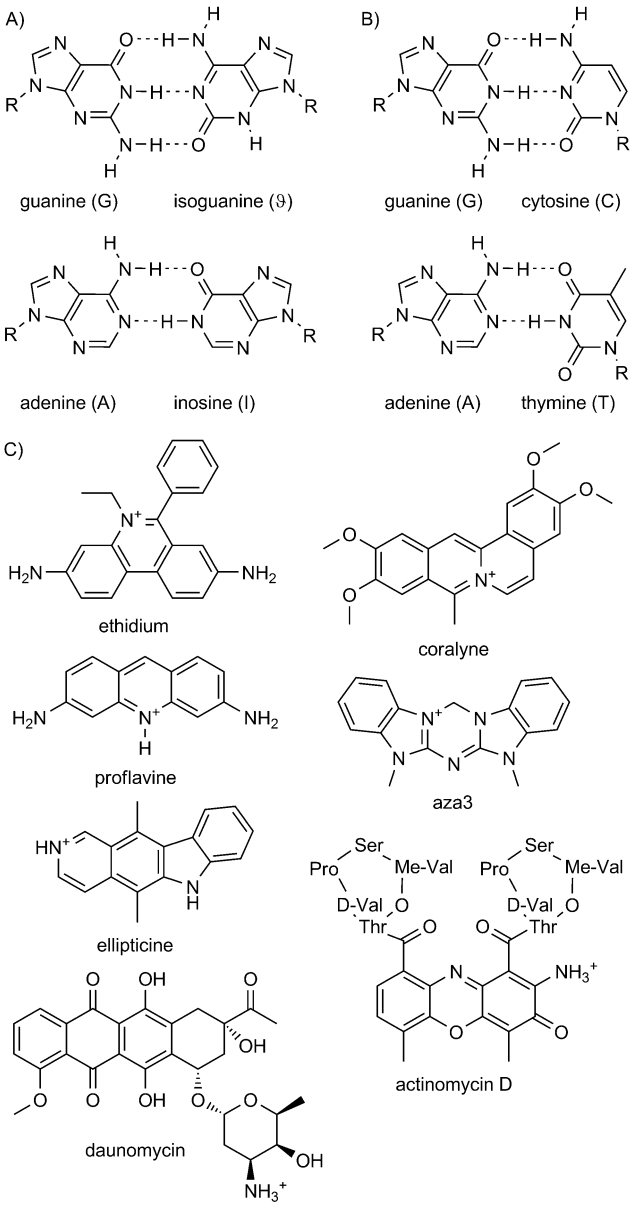
Nucleic acid duplexes containing non-Watson–Crick base pairs are of interest to several fields including structural biology, supramolecular chemistry, origin of life, and synthetic biology.[1] An early observation of a natural non-Watson–Crick base pair was the tRNA–mRNA adenine–inosine wobble pair, and it is now known that purine–purine pairs occur frequently in natural RNAs, including ribosomes and tRNAs.[1a,b] Crick even proposed that purine–purine base pairs might have been the original mode of information transfer in early life,[2] a proposal that recently received support from the observation that duplexes with only purine–purine pairs can be as stable as Watson–Crick duplexes.[3]

Given that small-molecule recognition of nucleic acids is an important element of drug development, biochemical assays, and rationally designed molecular assemblies,[4] and because intercalation might have played a role in the origin of nucleic acids,[5] we have explored the interaction of several nucleic acid intercalators with DNA purine–purine duplexes. Here, we demonstrate the ability of some small molecules to bind duplexes containing guanine–isoguanine and adenine–inosine base pairs (Scheme 1 A) with selectivity over duplexes with Watson–Crick base pairs (Scheme 1 B), and vice versa. This selectivity is consistent with the structural differences expected between these two classes of duplexes, which arise from differences in base-pair size and shape and helix groove geometry. This study represents, to the best of our knowledge, the first demonstration of selective binding of small molecules to an informational, but non-Watson–Crick, duplex.

Ethidium, proflavine, and ellipticine are three classical intercalators of Watson–Crick DNA.[6] These molecules are each characterized by a single positive charge and a planar, multicyclic surface that is approximately the size of a Watson–Crick base pair (Scheme 1 C).[7] However, the structures of these molecules differ in other respects, thereby providing a means to explore the potential for shape-selective binding to purine–purine base pairs. The representative purine–purine duplex used in this study, Pu·Pu, was formed from self-complementary dodecamers with the nucleotide sequence d(ϑAGIAGϑIAϑIG) (I, inosine; ϑ, isoguanine). For all the small-molecule ligands investigated, binding to Pu·Pu was compared with binding to the analogous Watson–Crick duplex, WC, with C in place of ϑ, and T in place of I, that is, d(CAGTAGCTACTG).



Scheme 1. A) Purine–purine base pairs. B) Watson–Crick base pairs. C) Small-molecule ligands used in this study.

Pu·Pu causes a 41 nm red shift of the longest-wavelength absorbance bands of ethidium, which is the same as the shift observed when ethidium binds to WC. The shapes of these two red-shifted absorbance bands are also virtually identical (Figure 1 A). This result suggests a common binding mode of ethidium by Pu·Pu and WC. The same phenomenon is observed for proflavine, which gives a red shift of 22 nm upon binding to either Pu·Pu or WC (Figure 1 B). Although such spectral changes are not definitive indicators of binding mode, red shifts in the longest-wavelength absorption bands are consistent with an intercalative mode of binding.[8] Similarly, ellipticine binding to Pu·Pu is accompanied by changes in its lon-

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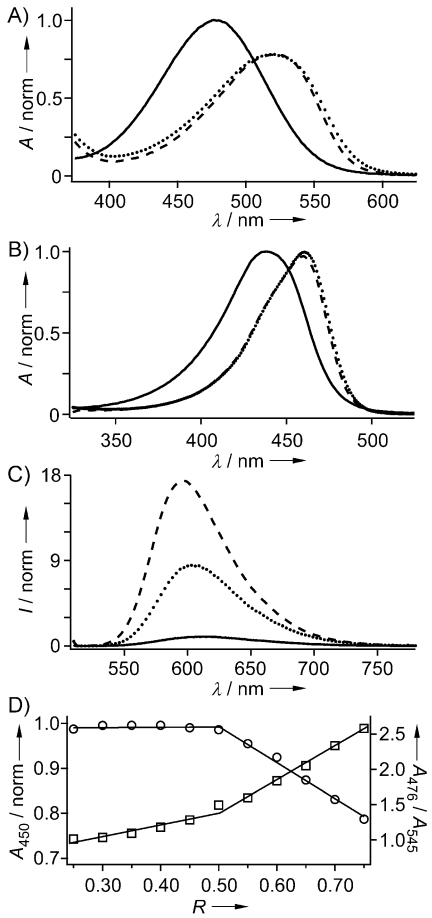

Figure 1. A) UV/Vis spectra of ethidium alone (——), in the presence of Pu•Pu (····), or in the presence of WC (-----). B) UV/Vis spectra of proflavine alone (——), in the presence of Pu•Pu (····), or in the presence of WC (-----). C) Fluorescence emission spectra of ethidium alone (——), in the presence of Pu•Pu (····), or in the presence of WC (-----), all with $\lambda_{ex} = 507$ nm. D) Job plots used to determine the binding stoichiometry of Pu•Pu with proflavine (○, left axis) or with ethidium (□, right axis). *R* is defined as [ligand]/ ([ligand] + [bp]/2). For (A)–(C), spectra within each graph are normalized to the local spectral maximum of each free ligand. Ligands were present at a stoichiometry of two molecules per duplex. Additional experimental details are provided in the Supporting Information.

gest-wavelength absorbance bands, but the spectral shift could not be quantitatively determined due to the low extinction coefficients and partial overlap with a 300 nm absorbance band of isoguanine (the N3-H tautomer, Figure S1 A in the Supporting Information).

Comparing changes in duplex melting temperature (ΔT_m) is a common means to assess the relative binding affinities of small molecules to various nucleic acids. In the absence of a small-molecule ligand, the Pu•Pu duplex melts at 20 °C, while the WC duplex melts at 46 °C. UV–visible-monitored thermal melting reveals that ethidium, proflavine, and ellipticine all increase the T_m of both Pu•Pu and WC (Table 1). The ΔT_m values are distinct for the two duplexes, with ellipticine providing the second overall largest increase for Pu•Pu ($\Delta T_m = 24$ °C), but the second smallest increase for WC ($\Delta T_m = 10$ °C). In contrast, ethidium increased the T_m of WC the most ($\Delta T_m = 25$ °C), but that of Pu•Pu much less ($\Delta T_m = 11$ °C).

Table 1. Melting temperatures, association constants, and association constant ratios for small molecules with Pu•Pu and WC.[a]

Ligand	ΔT_m [°C]		K_A [$\times 10^5$ M^{-1}]		K_A(Pu•Pu)/ K_A(WC)
	Pu•Pu	WC	Pu•Pu	WC	
ethidium	11	25	0.45	3.6	$(7.7)^{-1}$
proflavine	9	20	1.4	3.3	$(2.4)^{-1}$
ellipticine	24	10	15	5.4	2.8
aza3	20	10	0.28	0.22	1.3
coralyne	25	4	6.7	0.9	7.4
daunomycin	n.d.[b]	24	0.13	17	$(132)^{-1}$
actinomycin D	n.d.[b]	n.d.[b]	0.17	2.0	$(12)^{-1}$

[a] See the Supporting Information for melting curves, dilution binding curves, and method of analysis. [b] Not determined due to irreversible melting profile or large hysteresis.

Ethidium, proflavine, and ellipticine association constants were determined by monitoring UV–visible spectra during serial dilution at 4 °C (Table 1).[9] These studies reveal that proflavine binds WC with a K_A that is two times more favorable than that for Pu•Pu (see the Supporting Information for dilution binding curves), whereas ethidium binds WC almost eight times more favorably than Pu•Pu (Table 1). In contrast, ellipticine preferentially binds Pu•Pu almost three times more favorably than it does WC.

Ethidium intercalation of duplex DNA is associated with an increase in fluorescence intensity due to the protection of ethidium from solvent interactions that quench the excited state.[6c] To determine whether ethidium binds to Pu•Pu in the same intercalative mode as to WC, we compared the fluorescence properties of ethidium bound to both. When excited at 507 nm, a wavelength at which free ethidium and the complexes of ethidium with Pu•Pu and WC have the same absorbance, the fluorescence emission of ethidium with WC is 14 times greater than that of free ethidium. The enhancement upon binding to Pu•Pu is 7.4 times greater than that of free ethidium (Figure 1 C). These results are consistent with substantial protection from solvent interactions, as expected for an intercalative mode of binding to both Pu•Pu and WC.[6c]

We also investigated the binding stoichiometry of proflavine and ethidium to Pu•Pu by using Job's method of continuous variation.[10] This analysis revealed a stoichiometry of ethidium and proflavine binding to Pu•Pu of one ligand for every two base pairs (Figure 1 D). This binding stoichiometry is also confirmative of intercalation, as a maximum loading of one ligand per two base pairs is a hallmark of the nearest-neighbor exclusion principle that governs nucleic acid intercalation.[11]

The greater binding affinity of ellipticine to Pu•Pu, as compared to ethidium and proflavine, suggests a more favorable match of the larger ellipticine ring system to the purine–purine base pairs. To further explore this possible source of enhanced purine–purine binding, we analyzed Pu•Pu binding by two additional molecules, coralyne and aza3,[12] that have curved shapes similar to ellipticine and even larger ring systems (Scheme 1 C). We note that the shapes of coralyne and aza3 are close matches to that of a purine–purine base pair (Figure 2). As predicted, both ligands exhibit enhanced binding

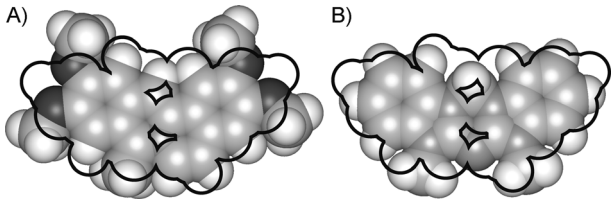

Figure 2. The outline of the guanine·isoguanine base pair superimposed on space-filling representations of A) coralyne and B) aza3. This figure is for size and shape comparison and does not represent an experimentally determined structure.

(K_A) to Pu·Pu over WC at 4 °C, and ΔT_m values for Pu·Pu that are comparable to that of ellipticine (Table 1).

A number of intercalating molecules have functional groups that reside in the DNA minor groove, as does the pendant ring of ethidium.[6d] Given that the width of a purine–purine base pair is approximately 2 Å wider than a Watson–Crick base pair (based on idealized C1′–C1′ distances), the grooves of a purine–purine duplex are expected to be wider than those of a Watson–Crick duplex. Molecular dynamics (MD) simulations with the AMBER8 force field[13] and structure analysis by the Curves + program[14] support this prediction. The energy-minimized Pu·Pu model duplex has a minor groove that is, on average, 2 Å wider than the minor groove of a WC model duplex (Figures S4 and S5). The greater selectivity of ethidium for WC over Pu·Pu over (in terms of K_A and ΔT_m), as compared to proflavine and ellipticine, is consistent with there being interactions between the ethidium pendant ring and the Pu·Pu minor groove, whereas proflavine and ellipticine, lacking pendant rings, do not interact with the minor groove. To further explore this hypothesis, association constants were determined for two known DNA intercalators with extensive minor-groove interactions, daunomycin and actinomycin D. For both cases, binding to Pu·Pu was much less favorable than binding to WC, with actinomycin D binding reduced more than tenfold and daunomycin binding reduced more than 100-fold (Table 1).

Overall, our investigations have demonstrated ligand selectivity between purine–purine and Watson–Crick base pairs of at least two orders of magnitude in K_A, for example, from 7.4-fold in favor of Pu·Pu by coralyne, to 132-fold in favor of WC by daunomycin. Furthermore, these studies have revealed a remarkable 25 °C increase in the T_m of Pu·Pu upon binding of the alkaloid coralyne which, in contrast, only stabilizes WC by 4 °C. Differential stabilization is even seen among classic intercalators, with ellipticine increasing the T_m of Pu·Pu by 24 °C, whereas proflavine only stabilizes Pu·Pu by 9 °C (with the opposite trend observed for WC). These results emphasize the importance of size and shape matching of intercalators with base-pair surfaces and of pendant groups within the minor groove as important contributors to the molecular recognition of duplexes containing Watson–Crick versus non-Watson–Crick base pairs. However, the relative contributions of enthalpy and entropy to the free energy of purine–purine base-pair selectivity by intercalating ligands is not obvious and will require further investigation. For example, previous studies have revealed that changing the RNA backbone from the natural 3′–5′ linkage to the closely related 2′–5′ linkage results in the free energy of proflavine intercalation changing from being primarily entropically driven to being enthalpically driven and entropically disfavored.[15]

In the wider view, our results illustrate how DNA-binding drugs with mixed modes of binding have different reliance on the free energy of intercalation versus groove recognition, as the backbones of the two types of duplexes in this study are chemically identical, but separated by different distances owing to their respective base pairs. The information provided by these studies can be used to aid the targeting of non-Watson–Crick base pairs in natural systems, and in nanotechnology applications where otherwise unstable non-Watson–Crick assemblies could be stabilized by small molecules in the presence of Watson–Crick structures.

Experimental Section

Sample preparation: Pu·Pu was synthesized in trityl-on mode on an automated Expedite DNA synthesizer with standard protocols except that dichloroacetic acid (2.5%) was used for deblocking steps. Deprotected (treatment in aqueous ammonia at 55 °C overnight) Pu·Pu was purified by HPLC, desalted, and cation-exchanged prior to use. WC was purchased from Integrated DNA Technologies (Coralville, IA, USA) and used as received. All experiments were performed with Li-cacodylate buffer (10 mM, pH 6.0) and LiCl (50 mM). Li^+ was used as the DNA counter ion to eliminate any possibility of tetraplex formation, which can occur with both guanine and isoguanine bases.[16] The starting DNA strand concentration was 100 μM.

Data collection: UV/Vis thermal-denaturation experiments and Job plots were performed on an Agilent 8453 spectrophotometer equipped with an Agilent 8909A Peltier temperature-control device. UV/Vis dilution experiments were performed on an Ocean Optics USB 2000 spectrophotometer (Dunedin, Fl, USA) with a custom mount that allows the use of both rectangular and cylindrical cuvettes. Fluorescence experiments were performed on a Jobin–Yvon Horiba Fluorolog-3 spectrofluorometer. Additional details are provided in the Supporting Information.

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