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Aromatic Oligomers that Form Hetero Duplexes in Aqueous Solution

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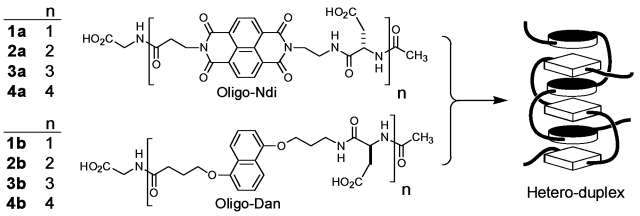
Natural molecular assemblies, for example, double-stranded DNA and protein complexes, possess a range of functions due largely to the organization of linear precursors into defined structures such as hetero duplexes.[1] Recently, the development of nonnatural chains that associate into duplexes has drawn active interest. Duplexes forming synthetic strands have employed hydrogen-bonding,[2] a combination of hydrogen-bonding and aromatic interactions,[3] and metal coordination.[4] Strong associations are generally seen in organic solvents but are often disrupted upon addition of polar protic solvents such as methanol[3a] and water.[3b]

Oligomers of alternating electron-rich 1,5-dialkoxy-naphthalene (Dan) and electron-deficient 1,4,5,8-naphthalene-tetracarboxylic diimide (Ndi) units,[5,6] termed aedamers,[7] were among the first foldamers[8] to demonstrate folding in aqueous solution. The Ndi:Dan intramolecular complexation in aedamers prompted us to explore this aromatic−aromatic interaction[9] in an intermolecular format to create a first-generation hetero-duplex system that self-assembles in water from complementary oligo-Ndi and oligo-Dan chains.



For stacking of aromatic units in aqueous solution, desolvation of stacked structures (i.e., the hydrophobic effect) is important.[5,10a] Electrostatic interactions make significant contributions as well.[10,11] For example, the nature of the aromatic interactions that serve as the basis for aedamer folding was studied with uncharged Ndi and Dan monomers.[5] For this system, desolvation of the aromatic surfaces provides the dominant driving force for complexation. However, the strength of the interaction seems to be modulated by the geometry of the stacked structure which, in turn, is dictated by electrostatic complementarity. Simply put, the complex between the relatively electron-deficient Ndi and relatively electron-rich Dan units exhibits stacking in an electrostatically preferred face-centered geometry allowing for maximum desolvation of the aromatic surfaces in water. On the other hand, electrostatic complementarity would be expected to prefer off-center modes of stacking, or herringbone arrangements, of self-stacked Ndi or Dan units, respectively, thereby limiting a desolvation driving force.[5]

Compounds **1a**−**4a** and **1b**−**4b** were synthesized using Fmoc-based solid-phase peptide synthesis with incorporated aspartic acid residues to provide water solubility. Compounds were dissolved in 50 mM sodium phosphate buffer, pH = 7.0, for all experiments discussed here. A consequence of having solutions buffered at pH = 7.0 is that the desired hetero-duplex formation will involve the assembly of like-charged chains.

Table 1. Binding Data[a]

	K_a (T = 298 K)	$\Delta G°$	$\Delta H°$[b]	$\Delta S°$[b]
1a:**1b**[c]	3.0 (0.1) × 10^2	−3.4	−	−
2a:**2b**[c]	7.5 (0.5) × 10^3	−5.3	−	−
2a:**2b**[d]	7.6 (0.1) × 10^3	−5.3	−10.4 (0.2)	−17.2
	K_a (T = 318 K)	$\Delta G°$	$\Delta H°$	$\Delta S°$
1a:**1b**[c]	1.3 (0.1) × 10^2	−3.1	−	−
2a:**2b**[c]	2.8 (0.1) × 10^3	−5.0	−	−
2a:**2b**[d]	2.7 (0.1) × 10^3	−5.0	−12.3 (0.3)	−23.0
3a:**3b**[d]	4.5 (0.1) × 10^4	−6.8	−17.7 (0.1)	−34.2
4a:**4b**[d]	3.5 (0.2) × 10^5	−8.1	−19.3 (0.2)	−35.3

[a] Units are K_a (M^{-1}), $\Delta G°$ (kcal mol^{-1}), $\Delta H°$ (kcal mol^{-1}), $\Delta S°$ (cal mol^{-1} K^{-1}). $\Delta G°$ calculated from average K_a values and $\Delta S°$ calculated from average $\Delta G°$ and $\Delta H°$ values. [b] For NMR data, $\Delta H°$ and $\Delta S°$ were not calculated.[15] [c] Analyzed by NMR. [d] Analyzed by ITC.

Job plots[12] using the aromatic proton chemical shifts of the Ndi unit provide evidence that the binding stoichiometry follows a 1:1 mode for **1a:1b** and **2a:2b** complexation. Using a 1:1 binding model to fit NMR titrations at T = 298 K results in binding constants of $K_{a(1a:1b)}$ = 300 M^{-1} and $K_{a(2a:2b)}$ = 7500 M^{-1} (Table 1).[13] Binding analysis of the longer oligomers using NMR titration becomes increasingly difficult due to overlapping peaks usually encountered with unit-repeating oligomers. In these cases, isothermal titration calorimetry[14] (ITC) is a more appropriate method to determine association constants. Complexation of **1a** with **1b** is below the sensitivity limit of microcalorimetry, but ITC experiments with **2a** and **2b** at conditions identical to those in the NMR titration experiments afford isotherms that corroborate a 1:1 binding stoichiometry with a $K_{a(2a:2b)}$ = 7600 M^{-1}.

ITC data for duplexes **3a**:**3b** and **4a**:**4b** collected at T = 298 K display binding isotherms that do not satisfactorily exhibit binding saturation and also do not fit any binding models well.[16] When ITC data is collected at T = 318 K, well-behaved, 1:1 binding isotherms for all duplex pairs are acquired (Figure 1A). This suggests that alternate modes of association, such as 1:2 or 2:1 binding and homomeric aggregation, possibly exist at ambient conditions but are disfavored at elevated temperatures.

The data in Table 1 indicate that (a) association improves with increasing chain length, (b) free energy, $\Delta G°$, of duplex formation is roughly additive, with a change of −1.3 to −1.9 kcal mol^{-1} per additional aromatic unit, and (c) association is enthalpically favored. For comparison, Diederich and co-workers[17] studied aromatic ring inclusion by cyclophanes in water and found enthalpy, $\Delta H°$, values ranging from −8 to −13 kcal mol^{-1} and entropy, $\Delta S°$, values ranging from −6 to −22 cal mol^{-1} K^{-1}. The larger entropic cost for the assembly of our duplexes compared to the cyclophane system likely derives from the degrees of freedom lost upon association of our relatively flexible chains.

The hetero duplex **4a**:**4b** displays an association constant of 350 000 M^{-1}, 3 orders of magnitude larger than **1a**:**1b**, despite a

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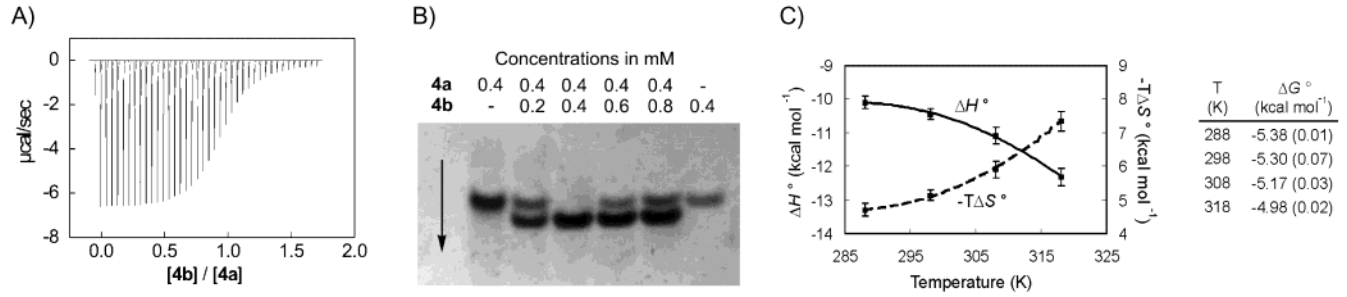

Figure 1. (A) ITC raw data showing heat pulses of 40 injections over 200 min. $T = 318$ K, $[\mathbf{4a}]_{initial} = 0.188$ mM, $[\mathbf{4b}]_{in\ syringe} = 1.59$ mM. (B) Photograph from PAGE experiments. A 20% polyacrylamide gel was used with standard Coomassie staining and visualized with UV shadowing over a fluorescent TLC plate. Arrow indicates direction of band migration. (C) Graph of the thermodynamic parameters of **2a**:**2b** binding and table of the corresponding free energies. Data are from variable-temperature ITC. $[\mathbf{2a}]_{initial} = 0.375$ mM, $[\mathbf{2b}]_{in\ syringe} = 3.21$ mM.

larger charge repulsion between the longer chains. This result emphasizes the relatively strong driving force for duplex formation present in oligoaromatic systems. In fact, negative charge along the backbone of both strands may have advantages. As is thought for DNA, intramolecular charge repulsion might keep oligomer chains "spread out" and more available to interact with a complementary strand.[18] Second, having like-charged strands keeps the final duplex water soluble as is required for NMR and ITC binding analysis.

Polyacrylamide gel electrophoresis (PAGE) experiments were used to visualize the **4a** and **4b** association (Figure 1B). A 1:1 mixture migrates as one band with increased mobility compared to either **4a** or **4b**, consistent with the increased charge density of the **4a**:**4b** complex. It appears that an excess of either **4a** or **4b** remains unbound, underscoring the high degree of chain discrimination seen with this system.

To elucidate the underlying thermodynamic parameters of binding, **2a:2b** association was reexamined from $T = 288-318$ K (Figure 1C). We attempted to calculate the heat capacity, ΔC_p, ($\Delta C_p = \delta\Delta H°/\delta T$) of binding which is often reported for biological systems to determine the extent of hydrophobic driven recognition.[19] ITC experiments reveal a significant temperature dependence of enthalpy that is not linear, thus precluding determination of a single ΔC_p value over the whole temperature range investigated.[20] Linear-fitting over narrower temperature ranges gives ΔC_p values between -50 and -94 cal mol^{-1} K^{-1}, similar to those observed in the Diederich studies of aromatic inclusion.[17] Last, an enthalpy−entropy compensation effect[21] is apparent with $\Delta G°_{(\mathbf{2a:2b})}$ values that decrease only slightly with increasing temperature.

The observed discrimination ability and high affinity between like-charged chains of complementary aromatic donors and acceptors illustrate the potential of this approach for modulating molecular recognition in aqueous solution. Currently, we are working on a structural analysis to allow us to better optimize linkers as well as exploring applications of this new recognition mode in solution and various solid phases.

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Supporting Information Available: Synthesis, details of analyses, and supplemental data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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