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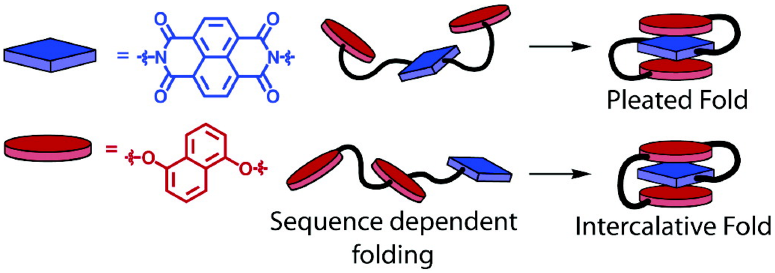

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Altering the Folding Patterns of Naphthyl Trimers

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Abstract: Proteins can adopt helical and sheet-type secondary structures that depend on their primary sequence of amino acids. Nonnatural foldamers have been developed to emulate these protein structures as well as investigate various types of noncovalent interactions. Here we report a strategy to access two distinct folding topologies in aqueous solutions using the inherent recognition properties of aromatic donor/acceptor interactions. These oligomers are constructed of electron-rich 1,5-dialkoxynaphthalene (Dan) and electron-deficient 1,4,5,8-naphthalenetetracarboxylic diimide (Ndi) units. A trimer of the sequence Dan-Ndi-Dan was shown to adopt a pleated fold in solution, while its constitutional isomer, Dan-Dan-Ndi, adopted an intercalative or turn-type fold. UV−vis and NOESY spectroscopy analyses were consistent with the two different conformations. This study illustrates the designability of folding naphthyl oligomers and encourages the use of directed aromatic interactions to construct larger and more complex assemblies in water.

Introduction

Previously, we have studied the aromatic donor/acceptor complexation of electron-rich 1,5-dialkoxynaphthalene (Dan) and electron-deficient 1,4,5,8-naphthalenetetracarboxylic diimide (Ndi) monomers.[1] Dan:Ndi complexation was found to be favored over Dan:Dan and Ndi:Ndi self-associations in aqueous solutions due to complementary electronic differences of the naphthyl rings that modulate the extent of the desolvation driving force for aromatic stacking.[1,2] This association discrimination of Dan and Ndi aromatics suggests that oligomers composed of these two naphthyl moieties could have designable folding and binding behaviors, analogous to the wide array of supramolecular structures created by Stoddart and co-workers.[3]

For instance, the Dan:Ndi interaction has been used in an *intra*molecular fashion by oligomers of the sequence (Dan-Ndi)$_3$ to create single-strand foldamers[4] termed aedamers.[5−8] Additionally, the Dan:Ndi association was found to be effective when exploited in an *inter*molecular format, controlling heteroduplex formation in water between donor Dan$_n$ and acceptor Ndi$_n$ strands.[9] In continuing investigations on the capability of aromatic interactions to direct folding, we report here that a Dan-Ndi-Dan trimer folds into a pleated structure in solution, but an intercalative or turn-type fold is adopted by a trimer with a different sequence of Dan-Dan-Ndi (Figure 1).

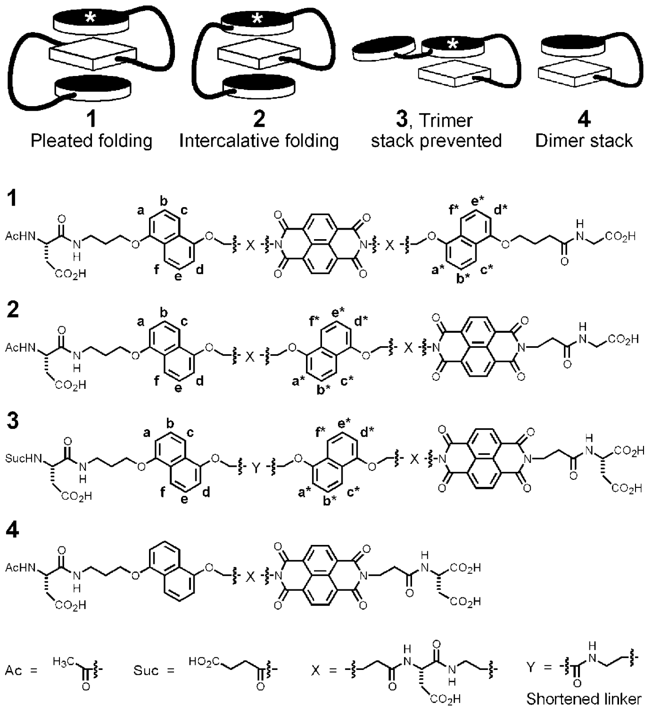

Figure 1. Cartoon representations of the folding patterns targeted by compounds **1-4**. Black disks represent a Dan or Dan* unit. Also shown are the letter designations, a−f and a*−f*, used for NOESY analyses.

The use of aromatic interactions is emerging as a versatile tool for creating folding systems, including turn structures.[10−12] Li and co-workers have reported a series of donor/acceptor based folding systems, including a “zipper-featured” foldamer that adopts a U-turn fold in organic solvents.[11] In addition, Waters and co-workers have extensively studied hairpin peptide

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Table 1. UV−Vis Data[a]

compd	ϵ_{CT} (λ_{max})[b]	$\epsilon_{382\ nm}$[c]
1	700 (532 nm)	12200
2	670 (532 nm)	11200
3	490 (510 nm)	13200
4	380 (526 nm)	14600

[a] Extinction coefficients (ϵ) in $M^{-1}\ cm^{-1}$. [b] Solutions at 1 mM concentrations. [c] Solutions at 40 μM concentrations.

mimics to probe interactions involving aromatic rings.[12] The work reported here extends the use of aromatic interactions, illustrating a strategy to access distinctly different folding patterns just by shuffling the sequence of two residue types.

Natural α-peptides can form both helical and β-sheet structures, along with a variety of different turns. The nonnatural β-peptide foldamers can also form a variety of helical and hairpin structures.[13] The foldamers reported here join the β-peptides as a nonnatural folding system capable of accessing at least two distinct folding topologies by design.

Results

Design and Synthesis. Earlier work indicated that Dan and Ndi moieties prefer to stack in an alternating arrangement in aqueous solution, maximizing the complementary Dan-Ndi facial interactions.[1,7−9] Trimer **1,** with an alternating naphthyl sequence was therefore expected to form a Dan/Ndi/Dan hydrophobic column through a pleated fold (Figure 1) similar to a previously reported naphthyl hexamer.[5] However, a constitutional isomer, trimer **2**, served as the focus of this study because it can maximize Dan:Ndi contacts presumably through a unique intercalative type of turn. Trimer **3** was intended as a nonfolding control because models indicated that its shortened linker between the Dan residues precludes a tri-ring parallel stack. The dimer, compound **4,** was used to calibrate our quantitative UV−vis studies since the folding of Dan/Ndi dimers has been well-established.[7] These four compounds, shown in Figure 1, were synthesized by solid phase synthesis methods. Purification was accomplished by reverse-phase chromatography to give analytically pure fractions and freeze-drying afforded soft pale purple solids.

UV−Vis Spectroscopy. UV−vis data were acquired for aqueous solutions of **1-4** in 50 mM Na phosphate buffer, pH = 7.0 (Table 1). Hypochromism between 45 and 60% of the Ndi absorbance at 382 nm (using the unstacked, monomeric Ndi value $\epsilon_{382} = 26600$[7]) was observed for each compound signifying the interaction of π-systems in a face-to-face-orientation. As reference, face-to-face stacking of DNA bases upon duplex formation typically exhibits 50% hypochromism.[14] A charge-transfer (CT) band also appeared for solutions of each compound with **1** and **2** having an identical λ_{max} of 532 nm and similar extinction coefficients for their CT bands of 700 and 670 $M^{-1}\ cm^{-1}$, respectively. These values are consistent with timers **1** and **2** folding to a similar extent and with the aromatic units in a similar face-centered stacking geometry. However, trimer **3**, which has the same Dan-Dan-Ndi sequence as **2**, but with a restrictive linker, exhibited markedly less hypochromism at 382 nm, a lower ϵ_{CT} compared to **1** and **2**, and a shift of the charge transfer λ_{max} down to 510 nm. As expected based on the shortened linker design of **3**, these values are consistent with significantly less folding and an altered stacking geometry. For comparison, dimer **4** exhibited an ϵ_{CT} of 380 $M^{-1}\ cm^{-1}$ at its λ_{max} of 526 nm and displayed an extinction coefficient of 14600 at 382 nm.

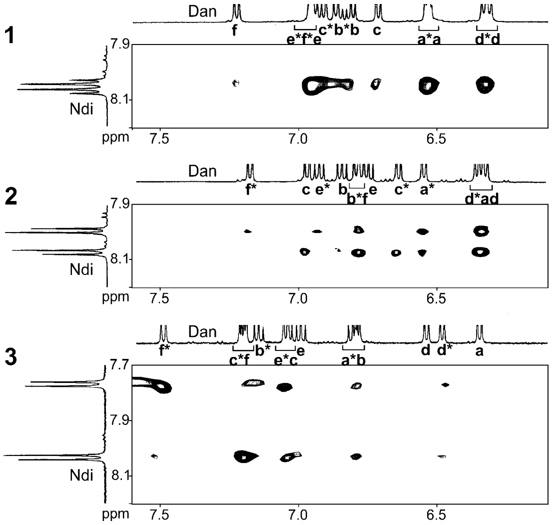

Figure 2. Aromatic region of NOESY spectrum of **1**−**3** exhibiting through-space H/H contacts between the Ndi (vertical axis) and the Dan and Dan* moieties (horizontal axis).

NOESY Spectroscopy. 1D NMR spectra displayed upfield shifts of the aromatic protons relative to Ndi and Dan monomers[7] consistent with the expected ring current effects for face-to-face stacking. Solutions of 1 mM trimer in 90/10% buffered H_2O/D_2O were used for hydrogen assignment purposes and by employing NOESY and TOCSY−NMR[15] methods, the identification of the aromatic resonances of the two Dan units (Dan and Dan*) for each trimer was accomplished.[16] Further, 2D NMR experiments were performed to investigate in detail the specific H/H contacts that occur between aromatic units and the resulting NOESY spectra (Figure 2) for compounds **1**-**3** afforded distinct cross-peak patterns.

The NOESY spectrum of **1** exhibited NOE signals along the entire range of Dan resonances indicating contacts between the Ndi unit and both Dan and Dan* rings. In particular, Ndi proton contacts with Dan protons f and c were unambiguous (since

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(16) NOESY techniques identified through-space correlations across amide carbonyls and alkoxy oxygens while long-range total correlated spectroscopy (TOCSY) methods have been used for aromatic systems before[16] and in the case reported here connects protons a and d for each Dan ring.[8]

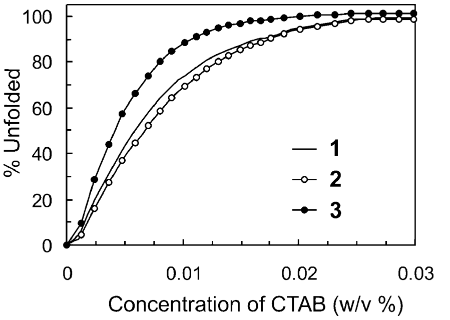

Figure 3. Unfolding curves of trimers **1**−**3**. Complete unfolding occurred at CTAB concentrations above 0.03 w/v % at which point no hypochromism at 382 nm was observed for the solution.

there was no chemical shift overlap for these two protons). As for the Dan* protons, the relative intensities of the cross-peaks at 6.5 and 6.3 ppm compared with the other cross-peaks suggested that a* and d* also made Ndi contacts. Unfortunately this could not be verified because of chemical shift overlap. The primary sequence of compound **2** places the Dan unit far enough from the Ndi moiety so that if the molecule were in an extended or partially unfolded state (a non Dan/Ndi/Dan stack), NOE signals would not be expected between the Ndi protons and the Dan protons. Examining the spectrum of **2** revealed that Ndi/Dan* contacts can be unequivocally identified (f*, e*, c*, and a*).

Importantly, for the Dan unit, contact between proton c and the Ndi unit was clear as well. There may be other Dan protons that make contact with the Ndi ring (in particular protons a and d) but signal overlap hinders this assignment in an unambiguous fashion. Last, the NOESY spectrum of **3**, with unambiguous Ndi hydrogen correlations with f* and d*, indicated an interaction between the Ndi unit and the immediately adjacent Dan* residue. However, as expected for a restricted linker control, no Ndi interactions could be attributed to the more distant Dan residue of **3**.

Unfolding studies were carried out by monitoring the increase in absorbance (i.e. the decrease in hypochromism) at 382 nm with the addition of cetyltrimethylammonium bromide (CTAB), a detergent that was found to disrupt aromatic stacking of related folding molecules.[5] In Figure 3, the percent unfolded was scaled to each molecule with 0% unfolded representing the starting state before CTAB was introduced to the solution and 100% unfolded indicating a final state in which absorbance did not change with higher concentrations of CTAB. As expected, since each trimer has one Ndi unit, 100% unfolded gave the same absorbance for each solution, and corresponded to a value equal to that found for an Ndi monomer solution of the same concentration containing the same amount of CTAB.

Once again, the behavior of compounds **1** and **2** were almost indistinguishable, while that of **3** was different. In particular, **3** unfolded at lower CTAB concentrations, presumably because there are fewer intramolecular stacking interactions for this less folded system.

Computer Modeling. Molecular dynamics simulations of twenty diverse initial structures of models of **1** and **2** each were performed using restraints based on unambiguous NOE data. Various conformations were thus obtained, of which the more stable ones (within 1 kcal of the lowest-energy conformers shown in Figure 4) all displayed face-centered Dan/Ndi/Dan stacking. Differences between structures were largely confined to the torsion angles of the flexible linker regions. This current modeling is in agreement with the idea that a naphthyl oligomer will adopt a conformation to maximize, if geometrically reasonable, the number of Dan/Ndi interactions. The few conformers that did not adopt a face-centered Dan/Ndi/Dan arrangement of rings after energy minimization (severely off-set and edge-to-face oriented structures for instance) resulted in significantly higher energies (>5 kcal/mol) than the representative low energy structures shown in Figure 4. Note that the modeling is not assumed to be quantitative, as no explicit water molecules were included. Rather, it is intended to evaluate relative linker compatibilities with the various folded structures.

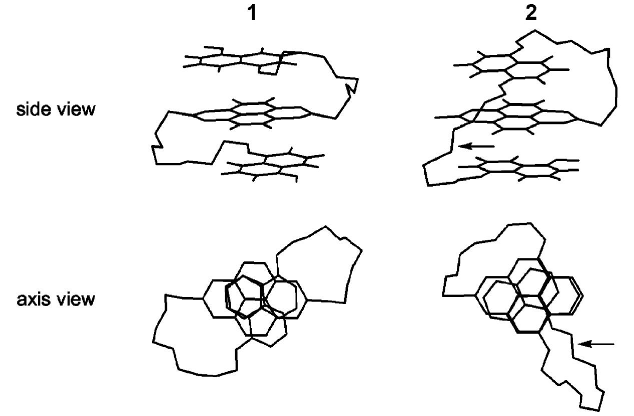

Figure 4. Representative lowest energy conformers of **1** (left structures) and **2**. Only the aromatic rings and carbon atoms in the backbone are shown for clarity. The Dan/Dan linker for **2**, designated by the arrows, appears flexible and long enough to allow for the unstrained insertion of an NDI unit.

Discussion

Due to the similarities of the UV−vis extinction coefficients of **1** and **2**, plus their comparable unfolding curves based on UV absorbance, it is reasonable to believe that these two trimers share a common arrangement of their aromatic rings. Data from supporting 2D NMR and computer modeling experiments indicate that this arrangement of rings consisted of a face-to-face Dan/Ndi/Dan stack. A control trimer **3**, designed to geometrically prevent this type of trimeric stack, gave significantly different UV−vis and NOESY spectra as anticipated.

Indeed, while the NOESY spectra are consistent with a pleated fold structure for **1** and an intercalative fold for **2**, both resulting in a Dan/Ndi/Dan hydrophobic column, it is important to consider alternative interpretations. For example it is possible to imagine a conformation for **2** in which edges of both Dan rings make contacts with the same face of the Ndi unit, in a roughly triangular geometry. Such a scenario is unlikely, however, in light of the observed hypochromism that strongly supports a face-to-face stacking geometry of the aromatic units.[5,14] Moreover such a triangular geometry would have significantly different ring current effects and therefore dramatically different chemical shifts of the aromatic protons for **2** compared with **1**. Such differences were not observed, and in fact, the chemical shifts for the aromatic regions of the spectra for **1** and **2** are comparable.

There are, however, subtle differences in the splitting patterns of the aromatic protons for **1** and **2**, indicating slightly different relative orientations of the stacked rings. These differences are likely due to the unique geometrical preferences imposed by presumed distinct linker topologies (Figure 4). Analogous splitting pattern differences were observed in a previous study of Dan-Ndi dimers tethered with a wide range of linkers.[7]

The aim of computer modeling was to investigate the ability of **2** to adopt a tri-aromatic stack conformation resulting in a new type of intercalative fold. An exhaustive survey of conformational space was considered unnecessary since there was already strong spectroscopic evidence that naphthyl oligomers fold by arranging their rings in close proximity to each other. As important was the decision to generate a set of optimized structures rather than pursue a single, presumed lowest-energy structure with prohibitively long molecular dynamic simulations. This modeling strategy had been previously shown successful for naphthyl oligomers.[7,8]

Overall, the modeling does corroborate the proposed Dan/Ndi/Dan stacking for **1** and **2** (Figure 4). In earlier investigations, we rejected a rigid two-state unfolded/folded model for aedamers and provided evidence that there exists families of stably folded structures, but importantly, all of these conformers were characterized by face-centered stacking.[7,8] The same was indicated by our modeling studies for trimers **1** and **2** where all structures within 1 kcal mol^{-1} of the most stable conformation exhibited a tri-aromatic parallel stack that maximizes Dan:Ndi interactions, consistent with the H−H correlations from the NOESY experiments.

This naphthyl oligomer folding system that is directed by aromatic interactions joins the β-peptides as nonnatural foldamers capable of accessing distinct folding patterns in a predictable fashion.[13]

Conclusions

Combined, UV−vis data, 2D NMR data, and computer modeling are fully consistent with the interpretation that both naphthyl trimers **1** and **2** adopt folded conformations in which a face-to-face Dan/Ndi/Dan stack is attained. A consequence of this stacked arrangement is that the secondary structure of **1** is a pleated fold, while that of **2** can be considered an intercalative turn type of fold. It thus appears that the folding patterns were predicted by the linear sequence of two residue types owing to the strong directionality of the Dan:Ndi association and the designability of naphthyl oligomers. Stability studies of longer naphthyl oligomers that display other types of folding are underway.

Experimental Section

UV−Vis Spectroscopy. UV−vis spectra were taken on a temperature-regulated Hewlett-Packard 8452A diode array spectrophotometer. For the unfolding studies, solutions of trimers were initially at 40 μM before the titration of a 2.7 μM (0.1 w/v %) solution of CTAB. Absorbances were adjusted for volume changes. The hypochromism after each injection of CTAB was measured and divided by the hypochromism of the original solution to give a percent unfolded value. Beer's law studies generated linear plots for all the molecules throughout the concentration ranges investigated by UV−vis spectroscopy, indicating that the reported values are for monomeric species. Similarly, chemical shifts in the NMR spectra displayed no conentration dependence over the concentration range used in these studies.

NOESY Spectroscopy. Samples for 2D NMR experiments were readily soluble in 50 mM sodium phosphate, pD = 7.0 D_2O. Spectra were recorded on a Varian INOVA 500 MHz spectrometer at 1 mM concentrations of compound and TSP-d_4 (3-trimethylsilyl-propionic-*2,2,3,3*-d_4 acid, sodium salt) was used as a standard. Informative NOEs were observed between the Dan and Ndi units when NOESY spectra were acquired with a mixing time of 800 ms.

Computer Modeling. All simulations were performed with the HyperChem software package (Hypercube Inc., Gainesville, FL 32601) using the MM+ force field. To expedite calculations, representative models of **1** and **2** were used in which the side chains were removed. Unrestrained molecular dynamics was used to produce a set of 20 random structures for both models of **1** and **2**, each of which were then annealed over 10 ps with a distance restraint based only on unambiguous NOE signals (additional details can be found in the Supporting Information). Each structure was then subjected to geometry optimization without any restraints using a Fletcher−Reeves conjugate gradient (0.01 kcal mol^{-1}).

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Supporting Information Available: Details of UV−vis, NMR, and molecular modeling analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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