Conformational Modularity of an Abiotic Secondary-Structure Motif in Aqueous Solution

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Dedicated to *Dieter Seebach* on the occasion of his 65th birthday

We have investigated an abiotic secondary structure based on the stacking of alternating electron-rich (1,5-dialkoxynaphthalene (Dan)) and electron-deficient (1,4,5,8-naphthalene-tetracarboxylic diimide (Ndi) = benzo[*lmn*][3,8]phenanthroline-1,3,6,8(2*H*,7*H*)-tetrone) aromatic units. Previously, the specifics of conformational behavior were uncovered in the minimal folding unit, namely the dimer, consisting of one Dan and one Ndi unit linked through various amino acid residues. Here is reported the investigation of a series of larger oligomers (trimers and tetramers) composed of selected dimer units. We determined that some of the larger oligomers displayed conformational modularity, that is, the persistence of subunit-conformational propensities when those subunits were used as components of larger structures. Conformational modularity can be viewed as a desirable property of folding molecules because it simplifies not only the design of larger, more complex oligomers, but also the structural analyses of such species.

Introduction. – The topological complexity and functionality of most large biomolecules are achieved through the folding of linear oligomers. Emulation of this strategy opens up exciting new realms of chemistry through access to a vastly greater array of large-molecule designs. Multiple research groups are now designing and synthesizing nonbiological oligomers with well-defined conformations (*i.e.* folding) [1–5]. Among these synthetic folding molecules, often referred to as 'foldamers', the β-peptides have been particularly well-studied through the pioneering work of the groups of *Seebach* and *Gellman*. There is even a growing list of β-peptide derivatives that exhibit biological activity [6–10].

We have previously demonstrated an abiotic pleated secondary structure (*Fig. 1*) based on aromatic stacking interactions in aqueous solution [11]. Oligomers consisting of alternating 1,5-dialkoxynaphthalene (Dan) and 1,4,5,8-naphthalene-tetracarboxylic diimide (Ndi; = benzo[*lmn*][3,8]phenanthroline-1,3,6,8(2*H*,7*H*)-tetrone) residues exhibit spectroscopic behavior consistent with the existence of distinct structures [11–14]. Recent studies have addressed the overall driving force for folding in aqueous solution [13] as well as the specifics of conformational behavior in the minimal folding unit, namely the dimer, consisting of one Dan and one Ndi unit linked through various amino acid residues [14][15]. In this latter study, comprehensive NMR and computational analyses were consistent with a dynamic situation in which the folded conformations, in terms of relative Dan/Ndi orientation, appear to be dictated by the linkage composition in ways that can be predicted *via* molecular modeling.

Larger, more elaborate Dan-Ndi oligomers will be necessary to achieve the ultimate goal of functional designs. Unfortunately, the type of conformation analysis

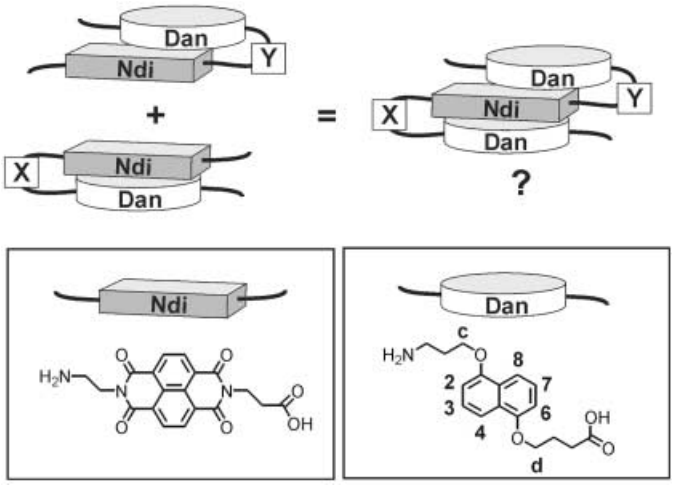

Fig. 1. *Schematic representation of conformational modularity in Dan-Ndi oligomers*

used with the dimers [14] becomes prohibitive with increasing size. Thus, it was of interest to investigate whether the conformational properties of larger oligomers could be predicted from the sum of their components, in other words, as chimeras of individual folding dimer modules. This type of behavior could be designated as *conformational modularity*, *i.e.*, the persistence of subunit conformational propensities when those subunits are incorporated into larger ('foldamer') structures (*Fig. 1*). Conformational modularity has at least two potential advantages. First, it can greatly facilitate the design of much larger folding systems and, second, it simultaneously obviates the need for their *de novo* structural analysis.

Results. – Conformational modularity in Dan-Ndi oligomers was explored by synthesizing several higher-order oligomers (**4**–**7**) that incorporate linkages previously examined in the context of the reference dimers (**1**–**3**) (*Fig. 2*) [14]. Specific linkages were chosen that exhibited easily distinguishable and characteristic ^{1}H-NMR signals in the context of the dimers. Compounds **4**–**7** were synthesized on solid supports with Ndi and Dan monomers described elsewhere [14].

Aqueous solutions of **1**–**7** all exhibit significant UV-hypochromism, charge-transfer absorbance bands in the VIS region, and upfield (negative) ^{1}H-NMR chemical-shift changes ($\Delta\delta$) compared to isolated Ndi or Dan monomers. These spectroscopic changes are consistent with face-to-face aromatic stacking and, thus, folding. Previous investigations demonstrated that $\Delta\delta$ values induced by the ring-current effects of the aromatic Dan and Ndi residues were particularly valuable for elucidation of conformational information [14].

^{1}H-NMR Resonances were assigned by means of a combination of 1-D, NOESY, and TOCSY experiments at 500 MHz. Spectra were obtained in phosphate-buffered D_2O and D_2O/H_2O solutions at 25–27° with analyte concentrations of 0.3–3.0 mM. NOESY and TOCSY mixing times were 200 and 150 ms, respectively. Long-range H,H

Component Dimers

1 RN-Asp-**Ndi**-**Dan**-Gly-OH
2 AcN-Asp-**Dan**-Asp-**Ndi**-Gly-OH
3 RN-Asp-**Dan**-Inp-**Ndi**-Gly-OH

Trimers

4 RN-**Dan**-Inp-**Ndi**-**Dan**-Asp-OH
5 RN-**Dan**-Asp-**Ndi**-**Dan**-Asp-OH
6 RN-Asp-**Ndi**-**Dan**-Asp-**Ndi**-Gly-OH

Tetramer

7 RN-**Dan**-Asp-**Ndi**-**Dan**-Asp-**Ndi**-Asp-OH

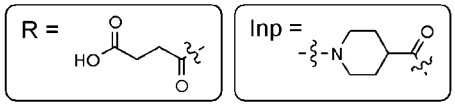

Fig. 2. *Higher-order Dan-Ndi oligomers investigated in this study and their component dimers*

couplings (between adjacent rings in each naphthalene system) were observable *via* TOCSY experiments. This allowed us to unambiguously assign each Dan residue when combined with NOESY connections to the linkage methylenes ($(CH_2(c)$ or $CH_2(d)$, see *Fig. 1*). The spectroscopic behavior of **4**–**7** is essentially concentration-independent below *ca.* 1 mM indicating *intramolecular* interactions.

Key $\Delta\delta$ values for the Dan residues of **4**–**7** are summarized in *Figs. 3* and *4*. The Ndi-Dan 'halves' of trimers **4** and **5** exhibit $\Delta\delta$ values very similar to one another and to those of the Ndi-Dan dimer **1** (*Fig. 3*, right brackets). Likewise, the Dan-Inp-Ndi 'half' of **4** and the Dan-Asp-Ndi 'half' of **5** display values comparable to those of their component dimers **3** and **2**, respectively (*Fig. 3*, left brackets). The strong similarities observed between trimers **4** and **5** and their components are consistent with the existence of conformational modularity. In other words, the behaviors of the Dan-Y-Ndi and Ndi-X-Dan *subunits* in Dan-Y-Ndi-X-Dan are equivalent to those of the Dan-Y-Ndi and Ndi-X-Dan *dimers* (*Fig. 1*).

Trimer **6** also exhibits $\Delta\delta$ values consistent with modular behavior, but not to the same degree as **4** and **5**. Cyclophane studies have demonstrated that ring-current effects are essentially additive in aromatic stacks [16]. Thus, the shielding effects experienced by the Dan residue of trimer **6** should be well approximated by the sum of $\Delta\delta$ values from its component dimers **1** and **2**. The Dan residue of trimer **6** presents several $\Delta\delta$ values that are, in fact, roughly comparable to the sum of $\Delta\delta$ values of the corresponding protons in its constituent dimers **1** and **2** (*Fig. 4*,*a*). However, there are also some $\Delta\delta$ values that are significantly lower (*i.e.* H–C(6), H–C(7), and H–C(8)).

This result could be interpreted as a diminished degree of folding for trimer **6** compared to its component units. However, the $\Delta\delta$ values observed for the aromatic proton resonances of the Ndi residues (-0.47 and -0.56) are significantly larger than those of the corresponding dimers (-0.35 each). Thus, a decrease in the folded equilibrium does not seem to provide a satisfactory explanation. Rather, it appears that a different folded conformation of **6** is responsible for the $\Delta\delta$ differences.

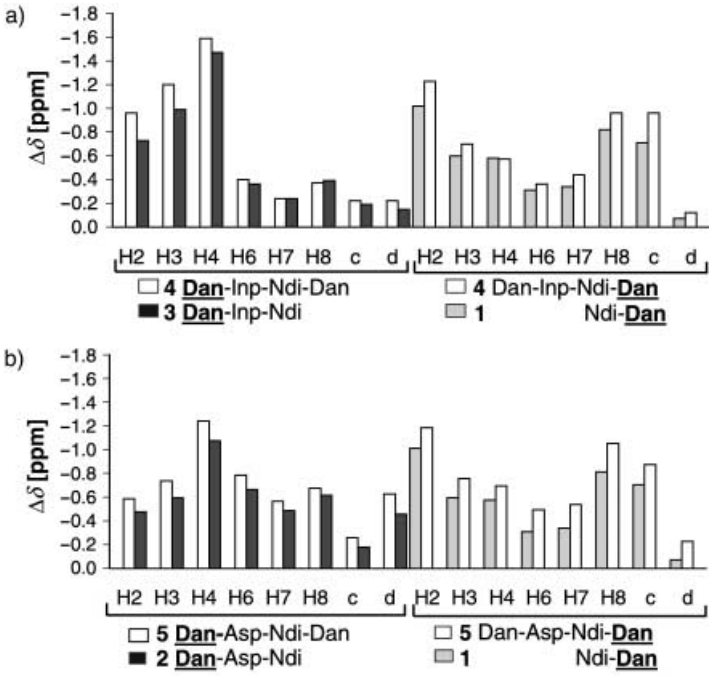

Fig. 3. *Summary of key* ^{1}*H-NMR chemical-shift changes* ($\Delta\delta$) *for the 1,5-dialkoxynaphthalene* (Dan) *residues of trimers* **4** *and* **5** *compared to their component dimers* **1**–**3**. See [14] for reference chemical shifts.

Examination of low-energy molecular-modeling structures obtained for dimers **1**–**3** reveals a possible explanation for a modified folded structure. It was noted that the linkages of dimers **1** and **2** always project *above* the plane of the Dan ring [14]. In trimers of the type Dan-X-Ndi-Y-Dan (**4** and **5**), this protrusion above the plane of Dan apparently has no consequence being on the outside of the aromatic stack (*Fig. 5*,*a*). However, in Ndi-X-Dan-Y-Ndi type trimers (**6**) this projection points inward and could lead to steric clashes and conformational rearrangement (*Fig. 5*,*b*). Such alteration of the relative Dan/Ndi orientation would thus affect the observed $\Delta\delta$ values.

Interestingly, trimer **6** seems to retain much of its conformational behavior when it is incorporated as a subunit of a larger structure. The interior Dan residue of tetramer **7** displays $\Delta\delta$ values very similar to those of the Dan residue in trimer **6** (*Fig. 4*,*a*). In contrast, the end Dan residue of tetramer **7** exhibits only moderate similarity with equivalent Dan residues in dimer **2** and trimer **5**, again indicating a possible steric clash with the interior linker that is apparently relieved by movement of the terminal Dan residue relative to the adjacent Ndi residue (*Fig. 5*,*c*).

Discussion. – The present study has demonstrated how insight obtained from analysis of the minimal folding unit, namely the dimer, can be applied towards the design and understanding of more elaborate Ndi-Dan oligomer systems such as trimers and tetramers. Through examination of the conformational tendencies of 'component'

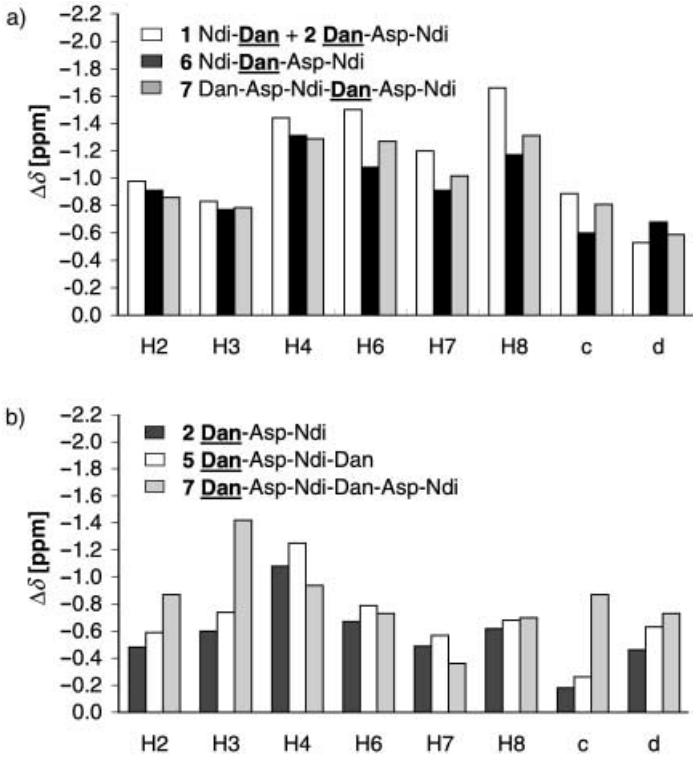

Fig. 4. *Summary of key* ^{1}*H-NMR chemical-shift changes* ($\Delta\delta$) *for the 1,5-dialkoxynaphthalene* (Dan) *residues of trimer* **6** *and tetramer* **7** *compared to their component units.* See [14] for reference chemical shifts.

dimers, much of the apparent behavior of higher-order oligomers could be rationalized. For the parts of systems in which conformational modularity appears to break down to some extent, linkage protrusion above the plane of the Dan moiety has been proposed to cause steric clashes with adjacent folded residues. The essential point is that this insight would have been dramatically more difficult to obtain if a *de novo* conformational analysis of the larger structures were attempted.

Conclusions. – Conformational modularity is a highly desirable feature of folding-molecular systems as their size and complexity increases. Further refinement of the conformational modularity concept could lead to the development of various 'building blocks' with reliable yet varied conformational features. Such building blocks could serve as a foundation for the design *and* conformational characterization of more sophisticated, and, ultimately, functional next-generation folding molecular systems.

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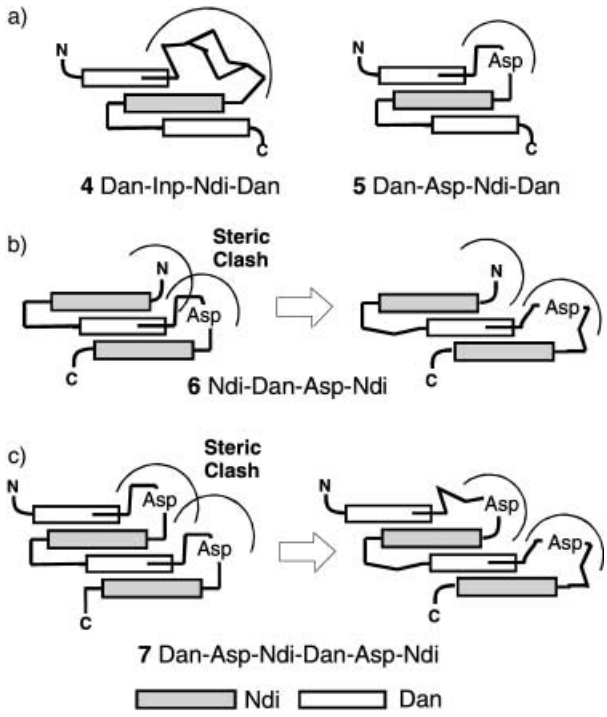

Fig. 5. *Schematic representation of how steric clashes between linkages could disrupt the conformational preferences of a component unit*

Experimental Part

General. Commercially available reagents and solvents were used without further purification unless noted otherwise. $AcNMe_2$ for amide couplings was stored under dry Ar with 4-Å molecular sieves. All aq. solns. of Ndi-Dan oligomers were prepared in 50 mM sodium phosphate buffer (pH or pD *ca.* 7).

UV-VIS Spectra: *Hewlett-Packard 8452A* diode-array spectrophotometer. NMR Spectra: *Varian Inova-500*; chemical shifts in ppm rel. to $Me_3SiCD_2CD_2CO_2Na$. Mixing times for NOE experiments were 200, 500 ms. TOCSY mixing times of 150 ms were used for assignment purposes. Concentrations for NOE and TOCSY experiments were 0.3–3.0 mM.

Solid-Supported Synthesis of Oligomers **4**–**7**. The synthesis of Ndi and Dan amino acid monomers has been detailed elsewhere [14]. All procedures were performed in polypropylene syringes fitted with porous frits (*Torviq*, Tucson, AZ). The syringe outlet was capped and the syringe agitated during couplings, deprotections, and cleavage with an oscillating shaker. First, 100 mg of *Fmoc-Asp-Wang* resin (*ca.* 0.7 mmol/g; 0.18 mmol) was treated with 40% piperidine/DMF for 20 min. It was then rinsed with DMF (3×5 ml), i-PrOH (3×5 ml), and DMF (3×5 ml). Couplings were performed with 2–3 equiv. of amino acid, 3 equiv. of PyBOP (= [(1*H*-benzotriazol-1-yl)oxy]tris(pyrrolidino-1-yl)phosphonium hexafluorophosphate), and 6 equiv. of *N*-methylmorpholine for 1 h in $AcNMe_2$. Coupling was followed by rinsing, deprotection with 40% piperidine/DMF for 0.5 h, and additional rinsing. The terminal N-atoms of all oligomers were capped with 0.200 g of succinic anhydride dissolved in 4 ml of DMF and 1 equiv. of $Et(i\text{-}Pr)_2N$. Cleavage was afforded by treatment with TFA/PhOH 95 : 5 for 1–2 h. The resin was then rinsed with several ml of TFA/CH_2Cl_2 1 : 1 until colorless. The combined rinses were concentrated to give a dark brown oil. The oil was precipitated with 100 ml of cold Et_2O, sonicated for 0.5 h, filtered on a medium sintered glass funnel, and rinsed with 100 ml of additional Et_2O to give **4**–**7** as dark purple solids. The crude oligomers were dissolved in 0.1M sodium phosphate buffer, loaded onto a prep. C18 column (1×8 cm) and eluted with 10 mM NH_4OAc/MeCN (0 to 100% MeCN, 120 min. gradient, 0.75 ml/min).

Chromatographic fractions were analyzed for homogeneity by HPLC (diode-array detector), combined, and lyophilized to give **4**–**7** as purple solids. ^{1}H-NMR signals are summarized in *Figs. 3* and *4*.

Data of **4**: FAB-HR-MS (pos.): 1393.514 ($C_{71}H_{77}N_8O_{22}$, $[M+H]^+$; calc. 1393.515).

Data of **5**: FAB-HR-MS (pos.): 1281.436 ($C_{65}H_{67}N_7O_{21}$, $[M+H]^+$; calc. 1281.439).

Data of **6**: HR-FAB-MS (pos.): 1475.430 ($C_{71}H_{67}N_{10}O_{26}$, $[M+H]^+$; calc. 1475.423).

Data of **7**: HR-FAB-MS (pos.): 1875.580 ($C_{92}H_{91}N_{12}O_{32}$, $[M+H]^+$; calc. 1875.586).

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