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Long-Range Interactions Stabilize the Fold of a Non-natural Oligomer

Richard P. Cheng† and William F. DeGrado*

Johnson Research Foundation, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6059

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The accelerating rate of protein structure determination is providing continuing insights into the features stabilizing the three-dimensional structures of proteins. A potential outgrowth of this understanding might be the design of nonbiological polymers (foldamers[1] and tyligomers[2]) that fold into predictable tertiary structures.[1−4] Here we report an important step toward this goal through the design of a "β-oligomer" composed of β-amino acids that adopts a cooperatively folded structure.

Short peptides derived from the sequences of proteins generally show little secondary structure in the absence of long-range interactions that stabilize their folded conformations,[5] and what little secondary structure is present generally unfolds in a broad thermal transition.[6,7] The introduction of covalent links between elements of secondary structure leads to cooperative stabilization of a well-defined tertiary structure and sharp thermal transitions reflecting the large size of the cooperative unit.[8] Thus, a folded tyligomer should show enhanced stability and thermal cooperativity for the full-length synthetic oligomer relative to its constituent elements of secondary structure.

To demonstrate that secondary structure can be stabilized by long-range tertiary contacts within a β-oligomer, we designed a two-helix bundle derived from β-amino acids. Pioneering efforts of Gellman[1] and Seebach[9] have demonstrated that appropriately designed synthetic β-oligomers form a "14-helical" conformation, with a 3.2−3.5 residue repeat.[10−15] We have computationally designed a C_2-symmetrical pair of interacting 14-helical β-oligomers (Figure 1). A model was generated starting with a monomeric 14-helix (torsion angles for the N−C^1, C^1−C^2, C^2−C^3, and C^3−N′ are −139.9°, 60°, −134.3°, and 180°).[13] A dimerized two-helix bundle with its axis parallel to the helical axes and a 10.3 Å helix separation was created by application of a C_2 symmetry operator. Side chains were then added to efficiently fill the interhelical space, and the structure was minimized using the CVFF force field in Insight (Accelrys, CA). As in natural proteins, the interaction interface is stabilized primarily by the packing of hydrophobic side chains in a geometrically complementary manner. Previous studies have shown that amphiphilic β-oligomers associate in aqueous solution,[16] although oligomers with too many hydrophobic side chains nonspecifically associate, giving rise to broad thermal unfolding curves.[17] Thus, here we restricted the hydrophobic interface to four residues (from N-terminus: (*S*)-3-aminovaleric acid (hB), (*S*)-3-amino-5-methylcaproic acid (hL), (*S*)-3-aminobutyric acid (hA), and (*S*)-3-aminovaleric acid (hB)). A β^3-homotyrosine (hY) serves as a spectroscopic probe and may also help to sequester the hL from solvent. The remaining charged side chains are arranged to stabilize electrostatically the folded structure through intra- and interhelical interactions. Previously, appropriately charged residues at i and $i + 3$ were shown to electrostatically stabilize the formation of 14-helices;[18,19] two such interaction pairs were included in the design of each helix. Additionally, two symmetry-related interhelical electrostatic interactions were included in the design. We examined both enantiomers of both Cys and β^3-homoCys residues to support disulfide formation. L-Cys was selected on the basis of its ability to form an unstrained disulfide[20] in the model.

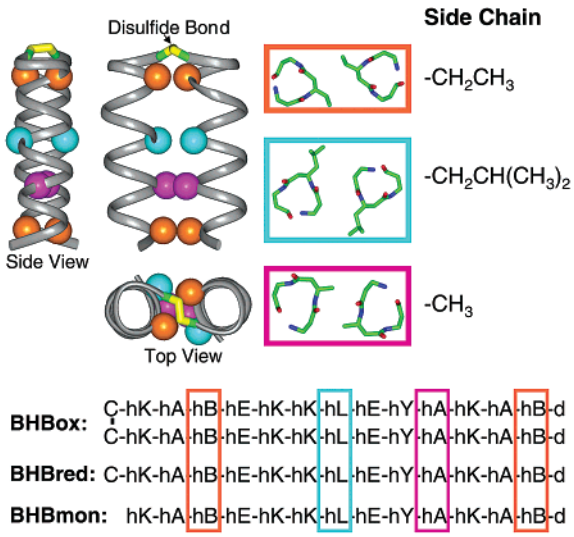

Figure 1. Design of a parallel helical bundle. Interacting residues lie in layers one turn apart; the four interhelical interaction layers are color-coded (orange, blue, and magenta). The corresponding side chains and energy-minimized models are shown on the right. The sequences are depicted on the bottom; the β^3-homo-amino acids are named according to the natural L-residues by adding a preceding "h" to the one letter code ("hB" stands for (*S*)-3-aminovaleric acid, "C" for (*S*)-cysteine, and "d" for (*R*)-aspartic acid). For all designs, both termini were left uncapped, resulting in an amine and carboxylic acid for the N- and C-termini, respectively.

The two helices were stapled together via a disulfide bond; the reduced synthetic β-oligomer, BHBred, represents the individual, isolated helices, while the oxidized form, BHBox, provides a quantitative measure of the extent of stabilization via long-range interhelical interactions. We also prepared a third monomeric synthetic β-oligomer, BHBmon, to avoid inadvertent disulfide formation in the thermal unfolding studies.

All three β-oligomers exhibit circular dichroism (CD) spectra characteristic of a 14-helix (Figure 2).[1,9,21] Furthermore, CD spectroscopy revealed that BHBox showed a dramatic increase in secondary structure relative to the monohelical controls, BHBred and BHBmon (Figure 2). The intensity of the mean residue ellipticity at 210 nm indicated that BHBox was essentially fully 14-helical,[21] while the monohelical controls had a 3-fold lower signal. Further, the CD signal of BHBox was invariant with respect

* To whom correspondence should be addressed. E-mail: wdegrado@mail.med.upenn.edu.
† Current address: Department of Chemistry, University at Buffalo, SUNY, Buffalo, NY 14260-3000.

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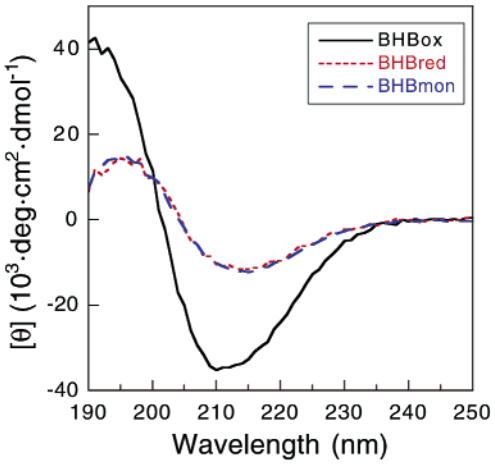

Figure 2. Circular dichroism (CD) spectra of BHBox (16.5 μM), BHBred (16.4 μM in the presence of 51.7 μM TCEP, tris[2-carboxyethylphoshine] hydrochloride), and BHBmon (96.8 μM) in mean residue ellipticity (θ). The spectra were collected on samples in 2 mM PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]) buffer at pH 7.0 with 1 mm path length cells.

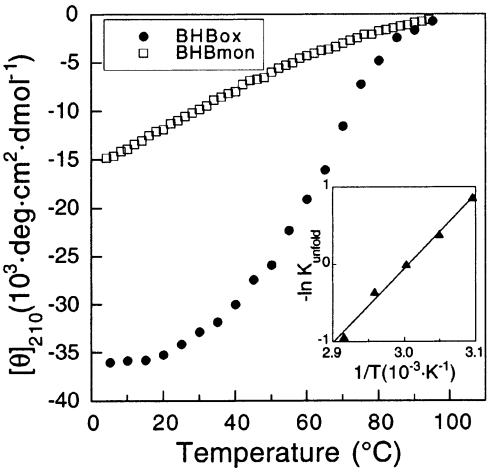

Figure 3. Thermal unfolding curves for BHBox (16.5 μM) and BHBmon (96.8 μM) monitored at 210 nm by CD spectroscopy. The ellipticity is indicated on a per-residue basis. (Inset) Plot of the natural log of the two-state equilibrium constant for unfolding (K_{unfold}) for BHBox versus the inverse temperature. Depending on the method used to treat the baselines, the van't Hoff enthalpy ranged from 19 to 27 kcal/mol. The spectra were collected on samples in 2 mM PIPES buffer at pH 7.0 with 1 mm path length cells.

to the salt concentration between 0 and 120 mM NaCl, and decreased by only 20% at 250 mM NaCl. This behavior contrasts with the results from monomeric 14-helical β-oligomers, whose conformations were stabilized primarily by electrostatic interactions and showed a much more drastic dependence on ionic strength.[18,19] This finding is consistent with the design of BHBox, which also includes packing of apolar side chains for conformational stability.

BHBox showed a sigmoidal thermal unfolding curve (Figure 3), whose steepness is appropriate for a folded macromolecule of this size. Natively folded proteins typically show substantial unfolding enthalpies, while molten globule and other non-native conformations tend to show lower values associated with a non-cooperatively stabilized structure. The per-residue van't Hoff enthalpy is approximately 0.7 kcal/(mol·residue), within the range observed in native proteins (0.6−1.1 kcal/(mol·residue) at 60 °C).[8] By contrast, BHBmon shows a broad thermal transition, typical of multistate unfolding for monomeric helices.[6,7,22] Analytical ultracentrifugation[23] showed that BHBmon and BHBox were monomeric at concentrations ≤800 and 280 μM, respectively. Thus, BHBmon was unable to self-associate to a higher order aggregate at these concentrations. Also, the enhanced helicity of BHBox could be attributed to intramolecular helix−helix interactions rather than formation of a higher order aggregate.

In summary, BHBox is a monomolecularly folded tyligomer whose secondary structure is stabilized by long-range interactions between covalently connected helices. Structural studies will define the extent to which the folded conformation resembles the design.

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(23) Sedimentation equilibrium experiments were performed at 25 °C on samples in 10 mM PIPES buffer at pH 7.0 using a Beckman XL-I analytical ultrcentrifuge (loading concentrations between 0.1 and 0.4 mM). The samples were centrifuged for lengths of time sufficient to achieve equilibrium. Data obtained by UV absorption at 280 nm were analyzed by nonlinear least-squares fitting of radial concentration profiles using the Marquardt−Levenberg algorithm implemented in Igor Pro (Wavemetrics). Using the calculated partial specific volumes (0.787 and 0.777 mL/g for BHBmon and BHBox, respectively, from the method by Durchschlag, H.; Zipper, P. *Prog. Colloid Polym. Sci.* **1994**, *94*, 20−39), the data were fit to a single molecular weight species model. The resulting apparent molecular weights in solution for BHBmon and BHBox were 1984 and 3649 amu, respectively. For both cases, values within a ±10% range of the apparent molecular weights gave reasonable fits and physical parameters. These values are consistent with the theoretical monomeric molecular weights for BHBmon (1745 amu) and BHBox (3695 amu).

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