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Toward β-Amino Acid Proteins: A Cooperatively Folded β-Peptide Quaternary Structure

Jade X. Qiu,[†] E. James Petersson,[†] Erin E. Matthews,[†] and Alanna Schepartz*[,†,‡]

Departments of Chemistry and Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8107

Received May 5, 2006; E-mail: alanna.schepartz@yale.edu

Folded polymers in nature are assembled from relatively simple building blocks but adopt complex structures through intricate networks of stabilizing noncovalent interactions. These interactions operate locally and globally to define secondary and tertiary structures, respectively. In certain cases, discrete secondary or tertiary elements associate with one another to form multi-subunit quaternary structures that can possess remarkably sophisticated functions. Nonnatural folded polymers, or foldamers, have potential for similar structural versatility.[1−4] Foldamers composed of β^3-amino acids,[5−8] for example (β^3-peptides), can adopt stable 14-helical secondary structures in methanol[6,8] or water[9−15] solutions. Moreover, β^3-peptide dimers stabilized covalently by a disulfide bond[16a] or non-covalently by Watson-Crick base pairing,[16b] as well as self-assembling β^3-peptide nanotubes[17] have been reported. Here we describe a pair of short β^3-peptides that assemble noncovalently into a well-defined quaternary structure characterized by an integral stoichiometry, highly stabilized secondary structure, and a cooperative melting transition.

Previous work has shown that appropriately designed β^3-peptides can populate a secondary structure known as a 14-helix, characterized by 14-membered ring hydrogen bonds between the amide at position i and the carbonyl of position $i + 2$, a left-handed helical twist, and three discrete faces.[6,8] The β^3-peptide sequences studied here were chosen to increase 14-helix propensity, decrease nonspecific aggregation, and favor hetero-oligomerization (Figure 1). All β^3-peptides contain free N- and C-termini and an alternating pattern of positively and negatively charged side chains on one helical face to improve 14-helical structure within a monomer[9,10,12] and disfavor nonspecific aggregation events reported previously.[18,19] In addition, all contain β^3-homoleucine residues at positions i and $i + 3$ and a pair of β^3-homophenylalanine residues at positions 4 and 7 to favor potential interhelical interactions. Finally, each β^3-peptide contains β^3-homoornithine or β^3-homoglutamic acid at positions 1 and 10 to favor heterodimer formation in a manner reminiscent of Fos/Jun.[20] All molecules were prepared using solid-phase methods, purified to homogeneity by HPLC, and characterized by mass spectrometry, circular dichroism (CD), and analytical ultracentrifugation.[21]

CD spectroscopy indicated that β^3-peptides Acid-1F and Base-1F are minimally 14-helical in buffered aqueous solution at a 25 μM concentration. By contrast, the CD spectrum of an equimolar mixture of Acid-1F and Base-1F at 25 μM concentration suggests a high level of 14-helix structure (Figure 2A). Analysis of the molar residue ellipticity at 210 nm (MRE_{210}) as a function of the mole fraction (X) of Acid-1F defined a Job plot[22] with a minimum at $X = 0.5$, suggesting that the 14-helical structure detected by CD was a hetero-oligomer containing a 1:1 ratio of Acid-1F and Base-1F (Figure 2B). This hetero-oligomer is surprisingly stable: the T_m of

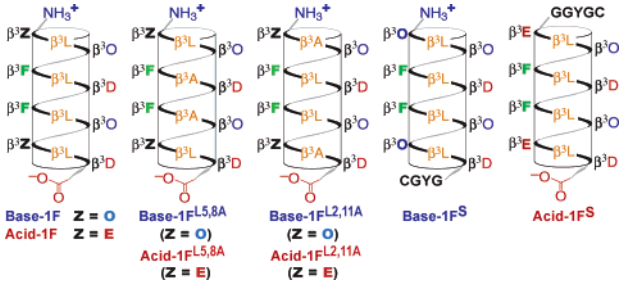

Figure 1. Helical net representation of β^3-peptides described in this work. β^3-homoamino acids are identified by the single letter code used for the corresponding α-amino acids. O signifies β^3-homo-ornithine.

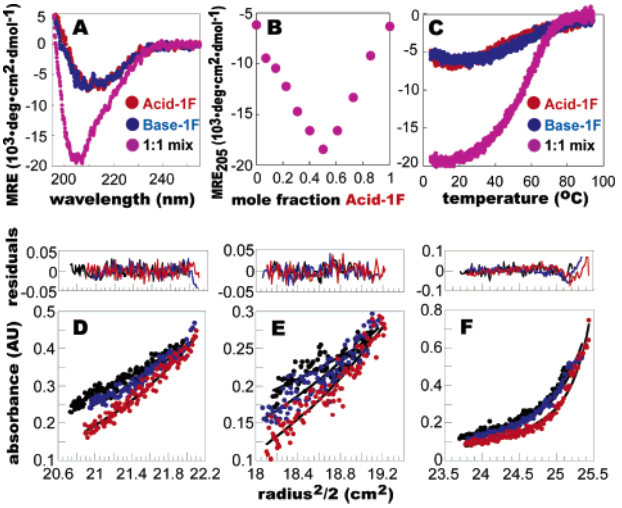

Figure 2. Isothermal (A,B) or temperature-dependent (C) CD analysis of Acid-1F, Base-1F, or a 1:1 mixture thereof. Sedimentation equilibrium analysis of (D) Acid-1F fit to a monomer model, (E) Base-1F fit to a monomer model, or (F) a 1:1 mixture thereof fit to a monomer−octamer model acquired at 42 000 (black), 50 000 (blue), and 60 000 (red) rpm. All experiments were performed at a β^3-peptide concentration of 25 μM in PBC buffer (1 mM phosphate, 1 mM borate, and 1 mM citrate (pH 7.1)).

an equimolar mixture of Acid-1F and Base-1F at 25 μM is 58 °C, close to the values observed, for example, for leucine zipper proteins.[23,24] Sedimentation equilibrium studies indicate that whereas both Acid-1F and Base-1F are monomers at 25 μM, the equimolar mixture of Acid-1F and Base-1F is fit equally well by a monomer−hexamer equilibrium or a monomer−octamer equilibrium (Figure 2F).[21,25] Moreover, the thermally induced unfolding of the hetero-oligomer is sigmoidal (Figure 2C), with a van't Hoff enthalpy of approximately 1.8 kcal·(mol·residue)$^{-1}$, comparable to values measured for natural proteins (1.9−4 kcal·(mol·residue)$^{-1}$).[26]

To probe the contribution of the β^3-homoleucine side chain to bundle formation, we prepared a set of variants of Acid-1F and Base-1F in which the β^3-homoleucine residues at positions 2 and

[†] Department of Chemistry.
[‡] Department of Molecular, Cellular, and Developmental Biology.

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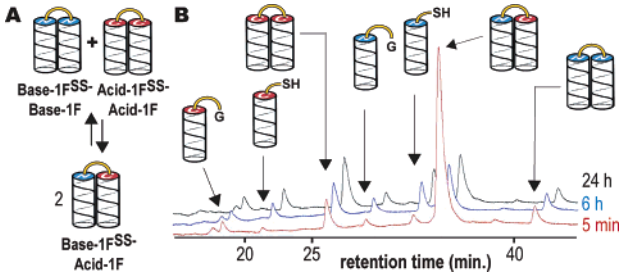

Figure 3. (A) Equilibrium between Base-1F^{SS}Base-1F and Acid-1F^{SS}Acid-1F homodimers and Base-1F^{SS}Acid-1F heterodimer under redox conditions (PBC buffer (pH 7.1) containing 500 μM reduced glutathione and 125 μM oxidized glutathione). (B) HPLC analysis of species observed upon incubation of 25 μM Base-1F^{SS}Acid-1F in redox buffer for 5 min and 6 and 24 h. G indicates glutathione.[21]

11 (Acid-1F^{L2,11A} and Base-1F^{L2,11A}) or 5 and 8 (Acid-1F^{L5,8A} and Base-1F^{L5,8A}) were substituted with β^3-homoalanine (Figure 1). The CD spectrum of each Acid−Base pair—in isolation or mixed in a 1:1 molar ratio—showed modest (−7500 deg·cm^2·dmol^{-1}) values of MRE$_{210}$ that were concentration-independent between 25 μM and 200 μM and resembled closely the CD spectra of the parent β^3-peptides in isolation.[21] Moreover, sedimentation equilibrium analysis indicated that each of the variant β-peptide was a monomer, in isolation or as a pair.[21] These data suggest that formation of the hetero-oligomer demands multiple interactions along the β^3-homoleucine face.

Next we made use of a widely applied disulfide exchange assay[27,28] to determine whether there existed a preferred orientation of the Acid-1F and Base-1F helices within the hetero-oligomer (Figure 3). Variants of Base-1F and Acid-1F containing a single cysteine at the N- or C-terminus, respectively (Base-1F^S and Acid-1F^S, Figure 1), were converted into disulfide-linked homodimers (Base-1F^{SS}Base-1F and Acid-1F^{SS}Acid-1F) and a heterodimer (Acid-1F^{SS}Base-1F) followed by HPLC purification.[21] The Acid-1F^{SS}Base-1F heterodimer (Figure 3B) or a 1:1 mixture of the homodimers[21] was incubated at room temperature in a redox buffer to facilitate disulfide exchange, and the reaction mixture was monitored by HPLC. As expected, at $t = 5$ min, the reaction starting with the Base-1F^{SS}Acid-1F heterodimer contained only a small amount of disulfide-linked homodimers. Over the course of 24 h, however, we observed a systematic decrease in heterodimer Base-1F^{SS}Acid-1F and a concomitant increase in homodimers Base-1F^{SS}-Base-1F and Acid-1F^{SS}Acid-1F. No additional changes were observed up to 72 h. Incubation of a 1:1 ratio of Base-1F^{SS}Base-1F and Acid-1F^{SS}Acid-1F homodimers in redox buffer generates a similar mixture of homodimers and heterodimers.[21] The predominance at equilibrium of both homo- and heterodimers, irrespective of the directional approach to equilibrium, suggests a hetero-oligomer composed of both parallel and antiparallel helices whose precise arrangement must await high-resolution analysis. This work demonstrates that β^3-peptides can assemble into defined, cooperatively folded, quaternary structures and constitutes an important step toward designing protein-like assemblies from nonnatural polymers.

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Supporting Information Available: β-peptide synthesis, CD, analytical ultracentrifugation and disulfide exchange assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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