Biophysical Characterization of a β-Peptide Bundle: Comparison to Natural Proteins

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We recently described the high-resolution X-ray structure of a helical bundle composed of eight copies of the β-peptide Zwit-1F (Figure 1A,B).1 Like many proteins in nature, the Zwit-1F octamer contains parallel and antiparallel helices, extensive inter-helical electrostatic interactions, and a solvent-excluded hydrophobic core. Here we explore the stability of the Zwit-1F octamer in solution using circular dichroism (CD) spectroscopy, analytical ultracentrifugation (AU), differential scanning calorimetry (DSC), and NMR. These studies demonstrate that the thermodynamic and kinetic properties of Zwit-1F closely resemble those of natural α-helical bundle proteins.

CD spectroscopy indicates that Zwit-1F is minimally 3i,β-helical in dilute solution (as judged by the molar residue ellipticity at 205 nm, MRE205)2 but undergoes a large increase in helical structure between 20 and 200 μM (Figure 1C). The concentration dependence of MRE205 fits a monomer–octamer equilibrium with an association constant of 4.0 × 109 M−1 (ln Ka = 70.5 ± 1.9).3 This value matches the result of AU analysis, which fits a monomer–octamer equilibrium with ln Ka = 71.0 ± 0.9.4 Taken together, the AU and CD data support a model in which unfolded Zwit-1F monomer is in equilibrium with folded octamer.4

Examples of natural octameric proteins include the histones5 (hetero-octamer), TATA binding protein6 (octamer in 1 M KCl), and the thermodynamically and structurally characterized hemerythrin (ln Ka = 84).7 Although Zwit-1F is less stable than hemerythrin, it is smaller in mass (13.1 vs 110 kDa) and interaction surface area (7000 vs 15 (000 Å2).8 To compare the stability of Zwit-1F to that of proteins of diverse size and stoichiometry, we calculated the free energy of association per Å2 of buried surface area (ΔGarea). Issues of molecularity aside, the ΔGarea of Zwit-1F is higher than that of hemerythrin, the tetrameric aldolase, and hemerythrin, it is smaller in mass (13.1 vs 110 kDa) and interaction surface area (7000 vs 15 (000 Å2).8 To compare the stability of Zwit-1F to that of proteins of diverse size and stoichiometry, we calculated the free energy of association per Å2 of buried surface area (ΔGarea). Issues of molecularity aside, the ΔGarea of Zwit-1F is higher than that of hemerythrin, the tetrameric aldolase, and

Figure 1. (A) Helical net representation of the Zwit-1F monomer. β3. Amino acids are designated by the single letter corresponding to the equivalent α-amino acid. O signifies ornithine. (B) Zwit-1F octamer structure determined by X-ray crystallography.1 (C) Plot of MRE205 as a function of [Zwit-1F] (μM). The Zwit-1F octamer equilibrium with an association constant of 4.0 × 109 M−1 (ln Ka = 70.5 ± 1.9).3 This value matches the result of AU analysis, which fits a monomer–octamer equilibrium with ln Ka = 71.0 ± 0.9.4 Taken together, the AU and CD data support a model in which unfolded Zwit-1F monomer is in equilibrium with folded octamer.4

Table 1. Comparison of Protein Association Parameters

<table>
<thead>
<tr>
<th>protein (stoichiometry)</th>
<th>MWmonomer (Da)</th>
<th>ΔGarea (kal mol−1 Å−2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zwit-1F (8)</td>
<td>1.6</td>
<td>5.9</td>
</tr>
<tr>
<td>hemerythrin (8)</td>
<td>13.8</td>
<td>3.37</td>
</tr>
<tr>
<td>aldolase (4)</td>
<td>39.2</td>
<td>3.911</td>
</tr>
<tr>
<td>GCN4 (2)</td>
<td>4.0</td>
<td>4.812</td>
</tr>
<tr>
<td>ROP (2)</td>
<td>7.2</td>
<td>2.5, 3.011</td>
</tr>
</tbody>
</table>

The values of ΔGarea for Zwit-1F are very similar to those of natural helical bundle proteins GCN4 and ROP (Table 1). In fact, ΔGarea for Zwit-1F is close to the average value (7.0 ± 2.8 cal mol−1 Å−2) observed for protein complexes burying at least 1000 Å2 of surface area upon association.9,10 The comparison between Zwit-1F and hemerythrin implies that the lower affinity of Zwit-1F is due to its small size and not an inherent instability of β3-peptide complexes.

Temperature-dependent CD studies (Figure 2A) show Zwit-1F to exhibit a concentration-dependent Tαm, an inherent property of protein quaternary structure.14 The Zwit-1F Tαm, which increases from 57 °C at 50 μM to 95 °C at 300 μM, is comparable to Tαm values of thermostable proteins such as ubiquitin (Tαm = 90 °C) and bovine pancreatic trypsin inhibitor (Tαm = 101 °C).15 The Zwit-1F Tαm is significantly higher than the Tαm of GCN4 (41–78 °C at 1–880 μM)16 and ROP (58–71 °C at 0.5–160 μM).17 We note, however, that the unfolding of Zwit-1F is less cooperative: the width of the temperature derivative of the CD signal at half-maximum is 40 versus 20 °C for GCN4 or 15 °C for ROP.16,17

A high Tαm is not a definitive measurement of thermodynamic stability, so DSC was used to further characterize Zwit-1F unfolding (Figure 2B). At 300 μM concentration (where Zwit-1F is 87% octameric), the temperature-dependent heat capacity (Cp) peaks near the Tαm identified by CD. This peak is embedded in a sloping baseline (ΔCp/ΔT = 5.1 cal mol−1 K−1 Å−2 = 3.1 kcal g−1 K−2) that is similar to the Cp versus temperature plot of monomeric β3-peptides, for which no cooperative unfolding peak has yet been observed.2 For most natural proteins, (ΔCp/ΔT) is about 1 kcal g−1 K−2 in the folded state,15 but GCN4 (ΔCp/ΔT = 3.6 kcal g−1 K−2)16 and some
ROP mutants \(\Delta C_p/\beta T = 4-5 \text{ kcal g}^{-1} \text{K}^{-1}\) have sharply sloped pretransition baselines like Zwit-1F.

The DSC data fit well to a process defined by a two-state transition with dissociation of eight subunits using the program EXAM.^{3,11} The fitted enthalpy and heat capacity change per mole octamer are 107.4 ± 0.3 kcal mol⁻¹ and 1.4 ± 0.1 kcal mol⁻¹ K⁻¹, respectively. Substituting these values into the Gibbs–Helmholz equation\(^2\) yields an equilibrium constant of \(5.3 \times 10^{31}\) (in \(K = 73.3 \pm 1.4\)) at 25 °C, in excellent agreement with values derived from CD and AU data. The integrated calorimetric unfolding enthalpy (\(\Delta H_{cal}\)) for Zwit-1F is 7.2 cal g⁻¹, within the range observed for natural globular proteins (5.2–11.8 cal g⁻¹).^{20} but somewhat lower than GCN4 (7.7 cal g⁻¹) and ROP (9.5 cal g⁻¹).^{17}

The NMR spectra of many well-folded natural and designed proteins are characterized by differentiated amide resonances and slow hydrogen/deuterium exchange. These results indicate that the Zwit-1F fold in solution creates a remarkably protein-like stepping stone in the path toward fully synthetic mimics of biological molecules.

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**Supporting Information Available:** Experimental procedures, Table 1 calculations, and data fits (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References


2. CD extrema between 205 and 215 nm have been attributed previously to the \(3_{14}\)-helix. For details, see: Seebach, D.; Beck, A. K.; Bierbaum, D. J. Chem. Biodiversity 2004, 1, 1111–1299.

3. See Supporting Information.

4. The MRE₆₅₆ concentration dependence of Zwit-1F also fits a monomer–hexamer equilibrium, as did previous AU data. Although the Zwit-1F X-ray structure shows an octamer, we cannot currently exclude the presence of hexameric forms in solution.


14. Sturtevant, J. M. Annu. Rev. Phys. Chem. 1987, 38, 463–483 and The recent high-resolution structure of Zwit-1F, these studies show that \(\beta\)-amino acid heteropolymers can assemble into quaternary complexes that resemble natural proteins in both solid-state structure and solution-phase stability. We note that our characterizations do not preclude some molten globule character of the Zwit-1F core in solution.\(^27\) Nonetheless, these studies establish Zwit-1F as a remarkably protein-like stepping stone in the path toward fully synthetic mimics of biological molecules.

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