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Tetrameric β^3-Peptide Bundles

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There is considerable current interest in the design of non-proteinaceous quaternary structures with defined oligomeric states because these materials have potential as nanomaterial scaffolds, drug delivery tools and enzymatic platforms.[1,2] We recently described a series of β^3-peptides, which are exemplified by the sequences of Zwit-1F and Acid-1Y (Figure 1 A), that assemble into octameric β-peptide bundles of known structure and high stability.[3–6] The 3_{14} helices that comprise these octamers form three distinct faces: a leucine face whose side chains form the bundle core, a salt bridge face of alternating β^3-ornithine and β^3-aspartate resides, and an aromatic face that contains two β^3-tyrosine or β^3-phenylalanine residues.[7–9]

It is well established that natural coiled-coil–bundle stoichiometries are controlled by the identity and conformation of the side chains that are buried at the bundle interface.[10] For example, substitution of valine or isoleucine for leucine at the GCN4 dimer interface leads to trimeric and tetrameric bundles.[10] Here we show that the β^3-peptide bundle stoichiometry is also controlled by the side-chain identity within the bundle core. Specifically, by replacing the leucine residues at the octamer interfaces of Zwit-1F and Acid-1Y with valine generates valine derivatives, Zwit-VY and Acid-VY. These second-generation β-peptides assemble into discrete and stable tetrameric bundles that were characterized by analytical ultracentrifugation (AU), circular dichorism spectroscopy (CD), 1-anilino-8-naphthalenesulfonate (ANS) binding, and deuterium exchange NMR spectroscopy.[4,6,11]

First we used analytical ultracentrifugation (AU) to determine whether Zwit-VY and Acid-VY formed bundles that possess a discrete stoichiometry in solution (Figure 1). The Zwit-VY AU data fit best to a monomer–*n*-mer equilibrium in which $n = 4.07$ with an RMSD of 0.00670 (Figure 1 B). A comparable fit (RMSD = 0.00672) resulted when *n* was set to equal 4 (Figure S2 A in the Supporting Information). The $\ln K_a$ value that was

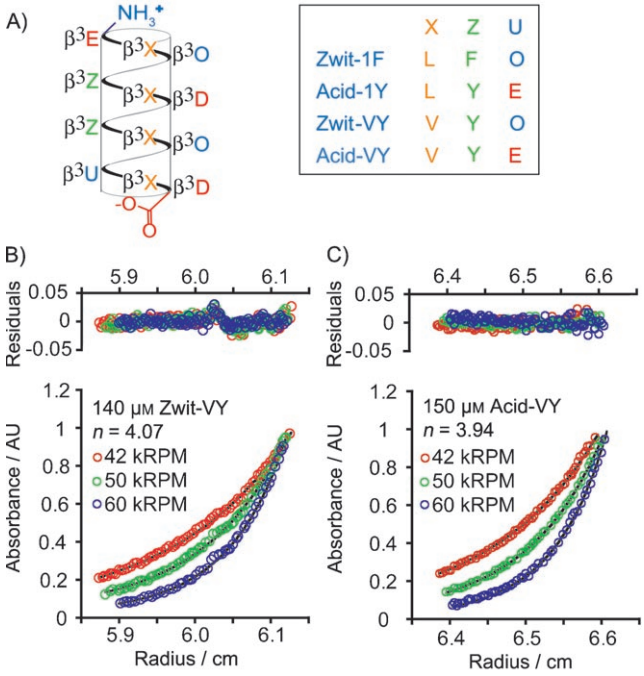

Figure 1. A) Helical net representation of Zwit-1F, Acid-1Y, Zwit-VY and Acid-VY. B, C) Zwit-VY and Acid-VY self-association monitored by analytical ultracentrifugation and fit to monomer-*n*-mer equilibria, where *n* was allowed to vary during fitting. Samples were prepared in 10 mM NaH_2PO_4, 200 mM NaCl (pH 7.1) and centrifuged to equilibrium at 25 °C at the indicated speed. The experimental data points are shown as open circles; lines indicate fits to the indicated monomer-*n* models.

calculated from these two fits are 37.70 ± 0.07 and 38.2 ± 0.8, respectively. Significantly poorer fits (larger RMSD values and residuals with systematic errors) resulted when *n* was set to equal 5, 6 or 8 (Figure S2). The AU data that were collected for Acid-VY fit optimally to a tetramer where $n = 3.94$ (*n* was allowed to vary) with an RMSD value of 0.00861 (Figure 1 C). The Acid-VY data were fit to a variety of other oligomeric assemblies, all of which afforded poorer fits to the AU experimental data, and showed increased RMSD values and systematic errors in the residuals (Figure S2).

We next used wavelength-dependent circular dichroism (CD) spectroscopy to characterize the concentration and temperature-dependent changes in the secondary structures of Zwit-VY and Acid-VY (Figures 2 and S3). Zwit-VY underwent a concentration-dependent increase in 3_{14}-helical structure (as judged by the molar residue ellipticity at 209 nm, MRE_{209}[12]) between 12 and 100 μM (Figure 2 A), which is consistent with a concentration-dependent equilibrium between a partially structured monomer and a folded oligomer. This behavior mimics the behavior of previously characterized β-peptide bundles Zwit-1F, Zwit-1F*, and Acid-1Y.[5,6] A plot of MRE_{209} as a

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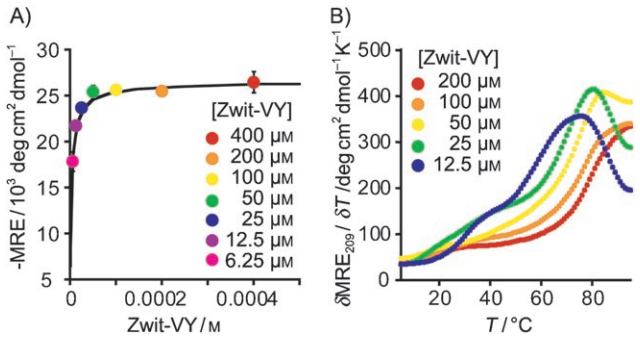

Figure 2. Zwit-VY self-association monitored by circular dichroism spectroscopy (CD). A) Plot of MRE_{209} as a function of $[Zwit\text{-}VY]_{monomer}$ fit to a monomer–tetramer equilibrium. Error bars were generated from the standard deviation that was calculated from three independent trials. B) Plot of $\delta MRE_{209}{\cdot}\delta T^{-1}$ for the concentrations of Zwit-VY shown as a function of temperature (T).

function of the Zwit-VY concentration fit well to a monomer–tetramer equilibrium with $\ln K_a = 38.3 \pm 0.5$ (Figure 2A); this is in agreement with the value calculated from the AU data (38.2 ± 0.8). Acid-VY also displayed a concentration-dependent increase in 3_{14}-helical structure (as judged by the MRE minimum at 209), and fit best to a monomer–tetramer equilibrium with a $\ln K_a$ of 39.3 ± 0.5 (Figure S3). In addition, the temperature-dependent CD spectra of both Zwit-VY and Acid-VY showed concentration-dependent increases in T_m that implied self-association (Figures 2B and S4C).[13] The T_m (defined as the maximum in a plot of $\delta MRE_{209}{\cdot}\delta T^{-1}$) of a 50 μM Zwit-VY solution (88% folded) is 85 °C, and it is also 85 °C for a 80 μM (88% folded) solution of Acid-VY. Unexpectedly, these values are higher than the T_m values of 70 and 78 °C that were previously observed for Zwit-1F (100 μM, 65% folded) and Acid-1Y (100 μM, 92% folded), respectively.[5] In summary, analytical ultracentrifugation measurements, as well as wavelength and temperature-dependent CD spectroscopy experiments indicate that β-peptides Zwit-VY and Acid-VY assemble into a 3_{14}-helical tetramer.

We used 1-anilino-8-naphthalenesulfonate (ANS) to further probe the features of the tetrameric Zwit-VY and Acid-VY hydrophobic cores. The fluorescence of ANS increases upon binding to hydrophobic surfaces.[14] A significant increase in ANS fluorescence (between 60 and 100-fold) upon addition of protein indicates a loosely folded, exposed hydrophobic core as shown for the α-lactalbumin molten globule.[15] Most well-folded or unfolded proteins do not provide favorable ANS-binding sites, and minimal fluorescence changes are observed in these cases.[15] The relative fluorescence of 10 μM ANS increased from a value of 1.1 at 12.5 μM Zwit-VY (79% tetramer) to a value of 1.6 at 350 μM Zwit-VY (98% tetramer, Figure S5). The increase in relative ANS fluorescence upon the addition of Acid-VY ranged from 1.1 at 25 μM (91% tetramer) to 1.2 at 300 μM (98% tetramer). Taken with the CD spectroscopy data, the minimal concentration-dependent increase in ANS fluorescence upon addition of Zwit-VY and Acid-VY suggests that both tetramers possess minimally exposed hydrophobic cores.

Finally, we used hydrogen–deuterium exchange NMR spectroscopy to characterize the kinetic stability of the Zwit-VY and Acid-VY tetramers.[16] The ^{1}H NMR spectra of both samples show dispersion of the amide N–H resonances (0.8 ppm) that is higher than that observed for the β-peptide monomer Acid-$1^{L2A,L11A}$ (Figures 3 and S6)[5] although smaller than that observed for Zwit-1F (1.4 ppm) and Acid-1Y (1.5 ppm). As was observed for the isosteric octameric bundles Zwit-1F and Acid-1Y,[4,6] the pattern of the amide N–H resonances of Zwit-VY and Acid-VY are nearly identical; this suggests that the quaternary structures of the two bundles are similar.[5,6]

The amide (N–H) exchange rate constants (k_{ex}) that were derived from the deuterium exchange NMR spectroscopy experiment correlated with the availability of amide protons to exchange with bulk solvent. Slow exchange rate constants are found for amide protons that are protected from exchange due to participation in stabilizing hydrogen bond interactions. The rate of disappearance of peaks a–d in a 600 μM sample of Zwit-VY (Figure 3A) fit a first-order kinetic model with rate constants (k_{ex}) between 1.6×10^{-3} and 5.5×10^{-4} s^{-1} (Figure 3B). A comparison of these values to the rate constant for exchange of an amide N–H in poly-β-homoglycine (k_{rc}) allows the calculation of a protection factor ($P = k_{rc}/k_{ex}$)[17] that facilitates comparisons between protein and β-peptide quaternary systems.[5,17,18] The protection factors that were calculated for a 600 μM (98% folded) sample of Zwit-VY fall between 4×10^3 and 1.8×10^4.[12] These values are comparable to the range that was calculated for a 1.5 mM (97% folded) solution of Zwit-1F of 3.4×10^3 and 2×10^4.[5,6] A similar trend in protection factors was also observed for Acid-VY.[12] The minimal difference in protection factors of Zwit-VY and Zwit-1F provides additional evidence that the valine β-peptide derivatives assemble into a discrete tetrameric structure that possess kinetically stable hydrophobic cores.[5,16,19,20]

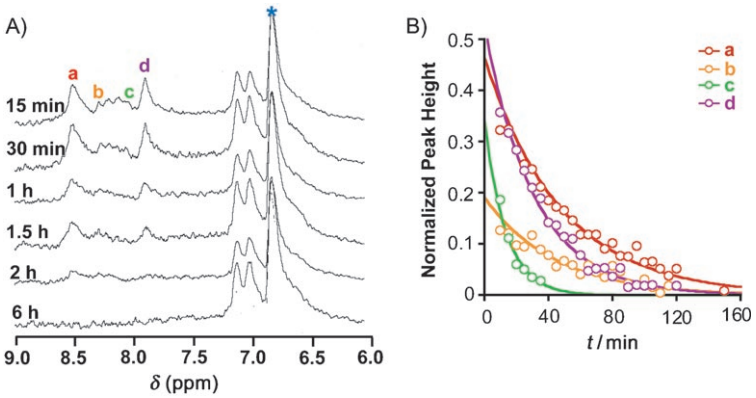

Figure 3. Kinetic stability of the Zwit-VY tetramer as determined by hydrogen–deuterium exchange analysis. A) 500 MHz ^{1}H NMR spectra of 0.6 mM Zwit-VY acquired at the indicated times (t) after a lyophilized Zwit-VY sample was reconstituted in phosphate-buffered D_2O. B) Peak heights of the indicated resonances (normalized to an amide peak at 6.8 ppm, *) fit to exponential decays as described in the Supporting Information.

In summary, here we show that β-peptide bundle stoichiometry can be controlled by the identity of the side chains that are buried at the subunit interface in a manner analogous to that observed in natural coiled-coil proteins.[10] These results provide a second, critical step in the "bottom-up" assembly of β-peptide assemblies that possess defined sizes, reproducible structures, and sophisticated function.[21]

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