Toward β-Peptide Tertiary Structure: Self-Association of an Amphiphilic 14-Helix in Aqueous Solution

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ABSTRACT

A major frontier in foldamer research is creation of unnatural oligomers that adopt discrete tertiary structures; at present, only biopolymers are known to fold into such compact conformations. We report an initial step toward helix-bundle tertiary structure in the β-peptide realm by showing that a 10-residue β-peptide designed to adopt an amphiphilic helical conformation forms small soluble aggregates in water. Sedimentation equilibrium data indicate that the aggregated state falls in the tetramer-hexamer size range.

Recent work from many laboratories has shown that oligomers built from a wide range of monomer types can display regular, well-defined conformations.1 These unnatural secondary structures fall into the three classes known from proteins: helix, sheet, and reverse turn. Gaining higher order conformational control is a major current goal in foldamer research. At present, only proteins and nucleic acids are known to fold to specific, compact tertiary structures. Tertiary folding appears to be necessary for many of the sophisticated operations carried out by these biopolymers (e.g., catalysis). Demonstrating that unnatural foldamers can adopt discrete tertiary structures would show that proteins and nucleic acids are not unique in their conformational behavior, and this accomplishment would raise the possibility that unnatural foldamers can be endowed with complex biopolymer-like activities.

Here we report a first step toward creation of a specific tertiary structure with β-amino acid oligomers. We describe a β-peptide that forms an amphiphilic helix2 (lipophilic on one side, hydrophilic on the other), and we show that this molecule undergoes self-association in aqueous solution. Pioneering work from DeGrado, Mutter and others has shown that identifying conventional peptides (α-amino acid residues) that form amphiphilic α-helices and self-associate in small clusters constitutes the initial phase in a “hierarchic” design strategy for helix bundle tertiary structure.3,4 Self-associating α-helices have also formed the basis for self-


replicating systems. Amphiphilic β-peptide helices have found biological applications, but controlled self-association has not previously been observed.

Deca-β-peptide 1 was expected to adopt an amphiphilic helical conformation in aqueous solution on the basis of prior observations. Seebach et al. have shown that β-amino acids (“β3-residues”) lead to formation of a “14-helix”, which is defined by 14-membered ring hydrogen bonds among backbone amide groups (CeO→H–N⋯). We have shown that the 14-helix is promoted even more strongly by β-amino acid residues with a six-membered ring constraint, e.g., trans-2-aminoacyclohexanecarboxylic acid (trans-2-ACHC). The 14-helix has approximately three residues per turn. β-Peptide 1 contains three trans-2-ACHC/trans-2-ACHC/β3-homolysines triads, which should lead to an amphiphilic 14-helix that has all three ammonium side chains aligned on one side of the helix and the six cyclohexyl rings defining a hydrophilic helical face. The predicted disposition of side chains, as viewed along the 14-helix axis, is presented with the illustration of 1. The N-terminal β3-homotyrosine residue facilitates detection and quantification via UV absorbance. Isomeric β-peptide 2 was prepared as a negative control. The 14-helical conformation available to 2 is not amphiphilic because the ammonium side chains are distributed evenly around the periphery. Both β-peptides were prepared by automated solid-phase synthesis, purified by HPLC, and isolated as trifluoroacetate salts. Initial studies were performed in aqueous 10 mM TRIS, pH 8.0, at several rotor speeds ranging from 35 to 60 krpm. Nonamphiphilic isomer 2 was present as a single species at three concentrations under these conditions; linear least-squares regression analysis resulted in molecular weight estimates (averaged over four rotor speeds) of 1330, 1330, and 1330 at 0.2, 1.4, and 1.8 mM, respectively. These values are consistent with the theoretical monomer molecular weight of 1370. Minor discrepancies between observed and calculated values may be attributable to nonideality (particularly applicable to highly charged molecules) or uncertainty in the calculated partial specific volume. Comparable sedimentation behavior was observed for nonamphiphilic β-peptide 2 at 1.7 mM in 100 mM CD3CO2H/CD3CO2Na, 9:1 H2O/D2O, pH 3.8 (these conditions were used for subsequent NMR experiments).

Through similar AU analysis, amphiphilic β-peptide 1 was found to be monomeric at 0.3 and 0.6 mM in 10 mM TRIS, pH 8.0 (molecular weight estimates matched those of 2), but radial distance spectra showed that at least one aggregated state coexisted with the monomer at higher concentrations, as evidenced by nonlinearity of ln(absorbance275) vs (radial distance)2 plots (Figure 1). Nonlinear least-squares fitting to various monomer/n-mer models was performed with concentration distribution data obtained at 0.9, 1.3, and 1.7 mM to estimate aggregate stoichiometry. A monomer–hexamer equilibrium model provided the best fits, as judged

Self-association of 1 and 2 in solution was examined by sedimentation equilibrium analytical ultracentrifugation (AU).

(9) None of the amphiphilic helix-forming β-peptides described in ref 2 has been shown to form discrete, soluble aggregates. Hamau et al. noted that their amphiphilic β-peptides form very large aggregates in the presence of phosphate, and our group has made similar observations with a related 14-helical β-peptide and with the β-peptide reported in ref 2c. Aqvist et al. have described a zwitterionic β-peptide that does not self-associate at concentrations up to 2 mM; the authors commented that this observation “raises the question how to distribute side-chains in order to achieve self-aggregation” of helical β-peptides.


(12) (a) At each speed, full radial spectra were acquired every 2–4 h until three successive spectra were identical. In all cases, complete analysis was performed on data with 0.001 cm step size at rotor speeds of 35, 45, 52, and 60 krpm. (b) Cantor, C. R.; Schimmel, P. R. Biophysical Chemistry; WH Freeman and Company: New York. 1980; Vol. II, pp 590–641. (c) Modern Analytical Ultracentrifugation: Acquisition and Interpretation of Data for Biological and Synthetic Polymer Systems; Schuster, T. M., Laue, T. M., Eds.; Birkhäuser: Boston, 1994.

(13) Data may be found in Supporting Information.

(14) Single species data were analyzed in accordance with the expression (modified from ref 12b) d(ln c)/d(r2) = M(1 – ρω2)/2RT, where d(ln c)/d(r2) is the slope of a ln(concentration) vs (radial distance)2 plot, concentration in all cases is in absorbance units, M is molecular weight, ρ is partial specific volume, ρω is rotor speed in units of radians per sec, R is the universal gas constant, and T is the absolute temperature. A partial specific volume (ρ) of 0.832 mL g−1 was used, as calculated by the method in: Durcshlag, H.; Zipper, P. Prog. Collid Polym. Sci. 1994, 94, 20. Similar results were obtained by nonlinear fits of concentration distribution spectra to (ref 12c) c(r) = c0 exp(r2/σ2), where σ = M(1 – ρω2)/2RT, where c0 is concentration at radial position r, r0 is concentration at an arbitrary reference radius (r0) near the meniscus, and base is a correction included to account for nonsedimenting species (in most cases, set to zero). All other terms are as defined above.
by randomness of residuals. According to this model, 30–40% of 1 is estimated to be hexameric at 1.7 mM. Under buffering conditions used for subsequent NMR studies (100 mM CD$_3$CO$_2$H/CD$_3$CO$_2$Na, 9:1 H$_2$O:D$_2$O, pH 3.8), analysis of AU data for 1 suggests pure monomer at 0.3 mM but multiple species at 1.2 mM. In this case, the best fits were achieved with a monomer-tetramer model.

The variation in apparent aggregate stoichiometry between the two buffer systems may indicate that altering the solvent leads to a change in aggregate structure. Alternatively, this variation may reflect the uncertainty inherent in the fitting process. (For example, two or more aggregated states may be populated.) Whatever the explanation for this variation, however, the important conclusion is that the amphiphilic 14-helix formed by 1 self-assembles into relatively small aggregates that remain highly soluble, rather than forming the large, insoluble aggregates observed for many conventional peptides. The association is weak relative to that of most α-peptides that form amphiphilic helices; however, β-peptide 1 is shorter than most α-peptides designed to form helical bundles. The onset of aggregation for 1 occurs in a concentration range comparable to that of ALPHA-1, a 12-residue α-peptide reported by DeGrado et al. in 1986 as an initial step toward hierarchical design of helical bundles. The stoichiometry range deduced for self-associated 1 is consistent with hydrophobically driven formation of helical bundle aggregates in which nonpolar helix surfaces are buried against one another.

NMR studies were conducted to gain further insight on the self-association of 1. Figure 2 shows NH/aryl CH NMR data for 1 in 100 mM CD$_3$CO$_2$H/CD$_3$CO$_2$Na, 9:1 H$_2$O:D$_2$O, pH 3.8, at various concentrations: (bottom to top) 0.2, 0.4, 0.8, 1.4, 1.8, 4.0, and 8.0 mM.

(15) Sedimentation equilibrium distributions for homogeneous interacting macromolecular systems ($nA \rightarrow A_n$) can be described by (ref 12c) $c_i = c_{0,i} \exp[\sigma_i \cdot r_0] + c_{0,n} \exp[\sigma_n \cdot r_0]$ + base, where $c_{0,i}$ may be simplified to $c_{0,i} = c_0, n^{K_{i,n}}$ $K_{i,n}$. The term $c_{0,i}$ is the concentration of species $i$ (monomer) at reference radius $r_0$ (defined above), $\sigma_i$ is the molecular weight term for species $i$ as defined above, likewise for $c_{0,j}$ and $\sigma_j$ for species $j$, $n$ is the stoichiometry of the aggregate, and $K_{i,n}$ is the dissociation constant for the monomer/n-mer equilibrium. Best results for fitting to monomer/n-mer equilibrium expressions were achieved with base $= 0$.


led to a return to the sharp lines observed for the original 0.4 mM sample, which shows that self-association of 1 is reversible. The broad lines observed at higher concentrations may indicate that equilibration between the monomeric and helical bundle forms of 1 is relatively slow (i.e., the system is approaching decoalescence).

In contrast, a β-peptide composed strictly of acyclic residues (containing three β3-homoleucine/β3-homoleucine/β3-homolysine triads) did not appear to aggregate as monitored by 1H NMR over a similar concentration range (data not shown). From these results, we conclude that cyclic residues (trans-2-ACHC) are important for self-association.

Self-association of amphiphilic α-helical peptides is frequently monitored by circular dichroism (CD), because α-helicity in the monomeric state is much lower than α-helicity in the aggregated state. In contrast, CD is of limited utility for monitoring self-association of 1, because even in the monomeric state this β-peptide displays a high 14-helix population. 14-Helix formation in water by β-peptides containing (R,R)-trans-2-ACHC gives rise to a CD maximum at ca. 215 nm. At 0.1 mM in 10 mM TRIS, pH 8.0, a concentration well below the onset of self-association, 1 exhibits an intense maximum at 213 nm (Figure 3). The CD spectrum of 0.1 mM 1 in methanol displays a slightly shifted maximum (215 nm), and the intensity is slightly lower than observed in aqueous solution. Extensive CD analysis of β-peptides composed exclusively of β3-residues has shown that switching from water to methanol leads to a dramatic increase in 14-helix formation for these flexible oligomers; the similarity between CD spectra of 1 in aqueous buffer and in methanol suggests that this β-peptide is highly folded in both solvents. The CD spectrum of nonamphiphilic isomer 2 at 0.1 mM is nearly identical to that of 1 at 0.1 mM, in both aqueous buffer and methanol, showing that high 14-helix population is not a result of amphiphilicity.

The results described here represent an initial step toward creation of an unnatural helix-bundle tertiary structure. Precedent from de novo protein design suggests that our next steps should involve synthesizing longer β-peptides that presumably will associate more tightly. This step is underway.

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Supporting Information Available: AU and NMR data for 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.