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Random Coils, β-Sheet Ribbons, and α-Helical Fibers: One Peptide Adopting Three Different Secondary Structures at Will

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A common feature of proteins that are involved in neurodegenerative diseases is the ability to adopt at least two different stable secondary structures.[1] Amyloid-forming proteins undergo a conformational transition from the native, mainly α-helical, structure into an isoform with high β-sheet content. Those β-sheet-rich intermediates are supposed to be the immediate precursors for the formation of amyloid fibers and were shown to be the most toxic component in many neurodegenerative diseases.[2] Mutations in the primary structure are a key parameter that makes a protein prone to misfolding. The conformational change can be triggered by protein concentration, but also by environmental conditions, such as pH, metal ions, oxidative stress, chaperons, or an abandoned membrane environment as observed in Alzheimer's disease. Promising approaches for the inhibition of amyloid formation have been reported.[3] However, to unravel the molecular interactions that occur during the transformation from α-helix to β-sheet and the consecutive formation of amyloids on a molecular level is still a challenge. Therefore, the development of small peptide models that can serve as tools for such studies is of paramount importance.

Here we present a de novo designed peptide that contains structural elements qualified for both stable α-helical folding as well as β-sheet formation as competing subunits. We show that the secondary structure formation can be triggered by the variation of the peptide concentration and/or pH.

The design is based on the well studied α-helical coiled coil folding motif.[4] It usually consists of at least two α-helices which are wrapped around each other with a slight superhelical twist. The primary structure is characterized by a periodicity of seven residues, the so-called 4−3 heptad repeat which is commonly denoted (a−b−c−d−e−f−g)*n* (Figure 1). Positions a and d are typically occupied by nonpolar residues that form the first recognition motif by hydrophobic core packing. Charged amino acids in positions e and g form the second recognition motif by interhelical ionic interactions. Positions b, c, and f are solvent exposed and located at the opposite side of the two dimerization motifs in the helical wheel diagram.

Three key features in the design cause the ability of model peptide VW19 to competitively adopt three different secondary structures by adjustment of the environmental conditions (Figure 1). (1) Recognition motifs 1 and 2 have been designed for perfect complementarity to maintain the ability of a stable α-helical coiled coil folding. Positions a and d are exclusively occupied by hydrophobic leucine. Residues in positions e, g and e′, g′, respectively, were arranged to solely form attractive electrostatic interactions in case of a parallel helix alignment. (2) Lysine residues in b and f in combination with position e form a large positively

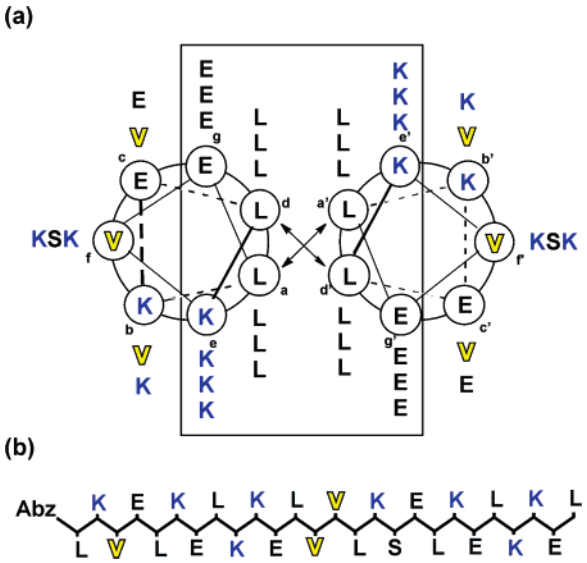

Figure 1. Helical wheel (a) and sequence (b) of model peptide VW19. Frame: positions inducing the α-helical coiled coil structure. Blue: positions to destabilize the helical structure at acidic pH. Yellow: positions favoring a β-sheet conformation.

charged domain once the pH is lowered to 4.0. This excess of positive charges at one side of the helical surface results in a destabilization and unfolding of the α-helix. (3) Amino acids in positions b, c, and f have a minor effect on the stability of the α-helical coiled coil dimer.[5] Therefore, these positions have been used to incorporate three of the β-sheet inducing valine residues.[6] Application of design features 1 and 3 results in a peptide that contains elements for two competing secondary structures, α-helix and β-sheet. However, transition between both secondary structures is only possible due to the peptide's sensitivity to pH changes introduced by design feature 2.

The conformation that VW19 adopts under varying conditions (i.e., for peptide concentrations, c_p, between 150 μM and 1 mM and at pH values 4.0 and 7.4) was studied by CD spectroscopy and by cryo transmission electron microscopy (cryo-TEM). Up to $c_p \sim 250$ μM at pH 4.0 (Figure 2a) and 7.4 (not shown), VW19 remained unfolded over several days. For a single solvated peptide molecule, a theoretical diameter of about 2 nm can be estimated.[7] Cryo-TEM revealed ensembles of very small particles with a typical size in the range of 2.5−3.5 nm, which is in good agreement with the theoretical estimation. The exact particle shape cannot be specified due to the small size. However, the size homogeneity points to a nearly globular state. Increasing c_p above ~300 μM at pH 4.0 induces the transition to a β-sheet conformation. This structure formation is characterized by a slow kinetics, as seen in

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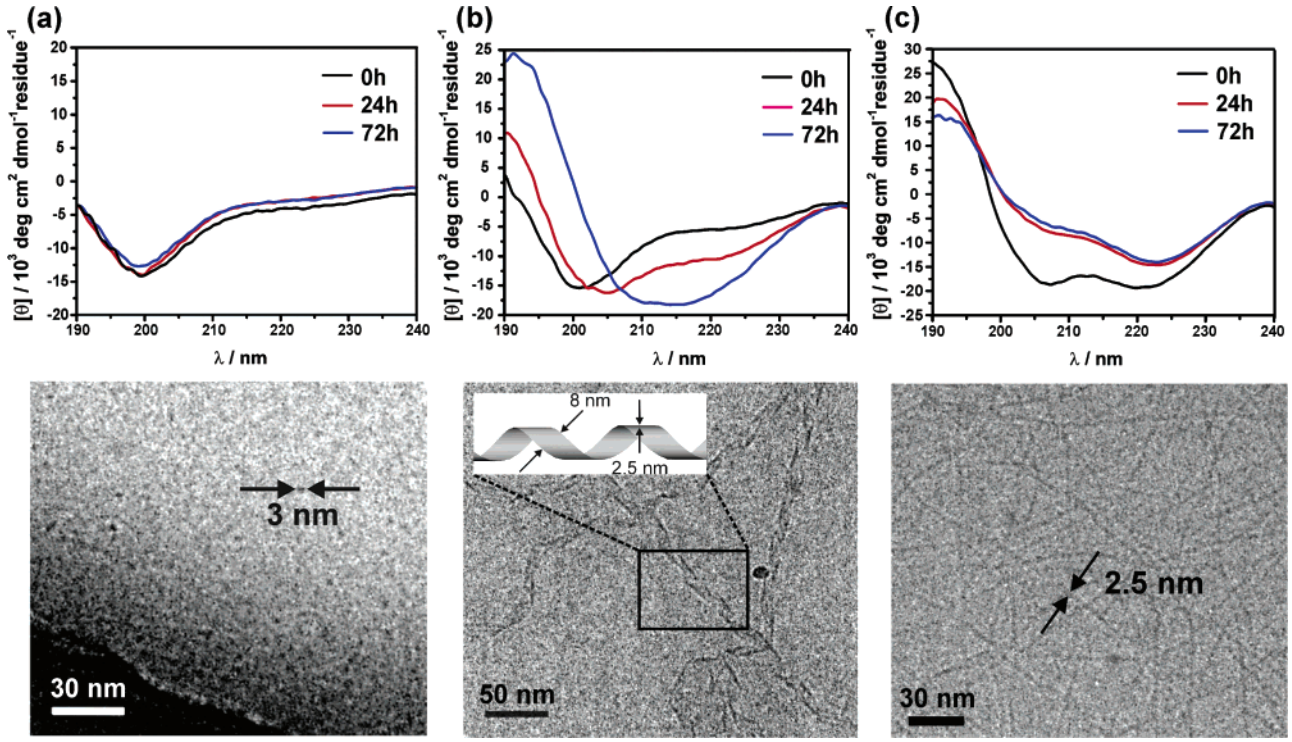

Figure 2. CD spectra and cryo-TEM micrographs of peptide VW19 showing the different secondary structures: (a) 250 μM, pH 4.0 − random coil; (b) 600 μM, pH 4.0 − helically twisted β-sheet ribbons; (c) 600 μM, pH 7.4 − helical fibers. The cryo-TEM micrographs were taken 6 days after sample preparation, when all spectra became invariable.

the CD spectra, indicating the formation of typical minima at 216 nm (Figure 2b). Cryo-TEM images of "matured" solutions reveal regular fibrous aggregates in the order of microns in length. Those aggregates are characterized by helically twisted ribbons with a typical width of 8 ± 2 nm, which is in agreement with a calculated length of 9.1 nm for the 26-residue peptide VW19. A peptide−peptide periodicity of 0.47 nm measured by electron diffraction (Figure S1) supports the evidence of a β-structure organization within the ribbons. The chirality of the peptide monomers induces the ribbon twist. The ribbon thickness of about 2.5 nm estimated from the cryo-TEM micrographs suggests a double-layered packing of peptide molecules that is consistent with current amyloid fibril models (Figure S2).[8]

At pH 4.0 and above $c_p \sim 250$ μM, VW19 adopts an α-helical conformation. Higher concentrated samples (Figure 2c) show decreasing ellipticities at 208 nm over several days, which point to gradual molecular rearrangements.[9] Cryo-TEM images reveal extended fibers with total lengths in the micrometer range and a uniform diameter of 2.5 ± 0.3 nm, independent of c_p. According to the characteristic CD spectrum, an α-helical coiled coil organization of the peptide within the fibers can be assumed. The estimated diameter points to three- or four-stranded assemblies,[10] but to establish a quantitative structure model, more experimental data are needed.

In conclusion, we succeeded in generating a model peptide that, without changes in its primary structure, predictably reacts on changes in concentration and pH by adopting different defined secondary structures. This de novo designed peptide strictly follows the characteristic heptad repeat of the α-helical coiled coil structural motif. Furthermore, it contains domains that favor β-sheet formation and aggregation. As proof of our concept, we showed that the resulting secondary structure of such a peptide will strongly depend on environmental parameters. Thus, this system allows one to systematically study the interplay between peptide and protein primary structure as well as environmental factors for peptide and protein folding on a molecular level.

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Supporting Information Available: Peptide synthesis and purification, CD spectroscopy parameters, cryo-TEM conditions and sample preparation, electron diffraction pattern, and structural models. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Taylor, J. T.; Hardy, J.; Fischbeck, K. H. *Science* **2002**, *296*, 1991−1995.
(2) Stefani, M.; Dobson, C. M. *J. Mol. Med.* **2003**, *81*, 678−699 and literature cited therein.
(3) Pagel, K.; Vagt, T.; Koksch, B. *Org. Biomol. Chem.* **2005**, *3*, 3843−3850.
(4) Mason, J. M.; Arndt, K. M. *ChemBioChem* **2004**, *5*, 170−176.
(5) Tripet, B.; Wagschal, K.; Lavigne, P.; Mant, C. T.; Hodges, R. S. *J. Mol. Biol.* **2000**, *300*, 377−402.
(6) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222−245.
(7) A spherical shape and a partial specific volume of 0.744 cm^3/g have been assumed for the estimate.
(8) Dobson, C. M. *Nature* **2005**, *435*, 747−749.
(9) Pandya, M. J.; Spooner, G. M.; Sunde, M.; Thorpe, J. R.; Rodger, A.; Woolfson, D. N. *Biochemistry* **2000**, *39*, 8728−8734.
(10) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401−1407.

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