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Rational Design of a Reversible pH-Responsive Switch for Peptide Self-Assembly

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The coiled-coil motif, defined by two or more interwound α-helices, has inspired the design of synthetic peptide systems that self-assemble into structurally defined, supramolecular fibrils on the basis of features programmed into the peptide sequence.[1−5] However, native biomacromolecular assemblies are characterized not only by well-defined structures but also by unique functions that arise as a consequence of those structures. One important function is the ability to sense and respond to incremental changes in environmental conditions. A critical challenge in the creation of synthetic biomolecular assemblies is the rational design of responsive mechanisms that can be coupled to supramolecular structure to re-create the self-assembly behavior that is characteristic of native biological systems. In this Communication, we report the design and characterization of a peptide, **TZ1H** (Figure 1), based on a trimeric coiled-coil motif that reversibly assembles into long-aspect-ratio helical fibrils as a consequence of a pH-induced conformational transition.

Comprehensive structural studies of in-register, coiled coils have established that residues in the core a- and d-positions and the proximal e- and g-positions of the heptad repeats act as determinants of the oligomerization state, the inter-strand orientation, and the thermodynamic stability of the assemblies.[6] These structural criteria can be redefined to accommodate the formation of helical fibril assemblies in which a staggered orientation is enforced between adjacent peptides, corresponding to an integer number of heptad repeats.[3,4] The design of **TZ1H**, a 41-residue peptide comprising six heptad repeats of a coiled-coil structural motif, was based on the amino acid sequence of the isoleucine zipper peptide GCN4-pII.[7] The latter peptide had been demonstrated, on the basis of crystallographic data, to form a three-stranded helical bundle. Consequently, the incorporation of isoleucine residues at the majority of the core positions of **TZ1H** should favor the formation of a trimeric assembly. Lateral registration between adjacent helical protomers in the structure of **TZ1H** was specified through manipulation of the Coulombic interactions between charged residues at the e- and g-positions of the heptad repeats.[8−10] The sequence of the **TZ1H** peptide was designed such that the electrostatic interactions between the e- and g-residues on structurally adjacent protomers would be completely charge-complementary only in a staggered alignment in which the peptides self-assemble into a helical fibril corresponding to a three-stranded rope, as depicted in Figure 1. The pH-responsive element was engineered into **TZ1H** through introduction of histidine residues into the core d-positions of alternate heptads.[11] In the suppositious fibril structure of Figure 1, the core histidine residues should reside at adjacent positions across the helical interface. Protonation of the histidine side chains should trigger an electrostatic destabilization of the helical fibril and provide a mechanism to couple self-assembly to incremental changes in pH near the pK_a of the imidazole group.

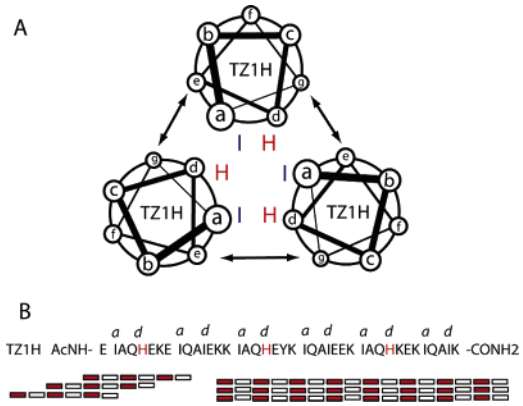

Figure 1. (A) Helical wheel diagram corresponding to a cross section of the trimeric bundle resulting from self-assembly of **TZ1H** into a helical fibril. (B) Amino acid sequence of **TZ1H** depicting core isoleucine (blue) and histidine (red) residues. Below, proposed packing arrangement of peptides in a helical fibril of **TZ1H**. The staggered alignment arises from an axial displacement of two heptad units between adjacent protomers (white, His-containing heptads; red, non-His-containing heptads).

To assess the pH-dependent self-assembly behavior of **TZ1H**, aliquots of peptide were dissolved in 10 mM sodium phosphate buffer across a pH gradient that ranged from pH 4.0 to 8.0 in ca. 0.5 pH-unit increments (cf. Supporting Information). Circular dichroism (CD) spectroscopy of these solutions of **TZ1H** indicated that the peptide conformation depended strongly on the pH of the buffer solution (Figure 2). Peptide samples prepared at pH values between 6.5 and 8.0 displayed a strongly α-helical CD signature with minima at 208 and 222 nm and a limiting value for the fractional helicity of 87% at pH 8.0. In addition, the observed value of the $\theta_{222}/\theta_{208}$ ratio of 1.07 at pH 8.0 was within the expected range for a coiled-coil structure. In contrast, at pH values within the range from 5.6 to 4.0, a loss of the α-helical CD signal was observed with the emergence of a new minimum at approximately 201 nm that was suggestive of a high content of random-coil conformation. A near-isodichroic point was observed in the pH-dependent CD manifold at a wavelength of 203 nm, which is consistent with a helix−coil transition (Figure 2).[12] Moreover, the helix−coil interconversion was found to be completely reversible as a function of the solution pH within the range from 4.0 to 8.0 (cf. Supporting Information).

The helix−coil conformational transition for **TZ1H** occurred within a narrow pH range with an inflection point at pH 5.8, which is significantly less than the pK_a value of 6.88 determined for the imidazole group of histidine in the random-coil peptide Ac-Ala-Ala-His-Ala-NH_2.[13] However, the pK_a values of histidine residues can vary significantly, depending on the identity of spatially

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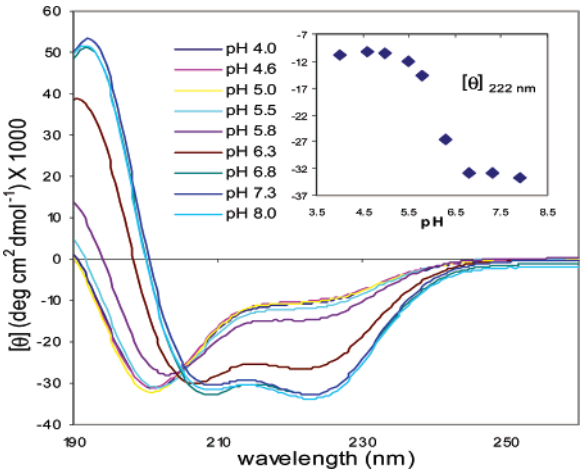

Figure 2. pH dependence of the circular dichroism spectra of **TZ1H** (9.85 μM, 10 mM NaH_2PO_4 buffer) at 4 °C. (Inset) Mean residue ellipticity at 222 nm for **TZ1H** as a function of pH.

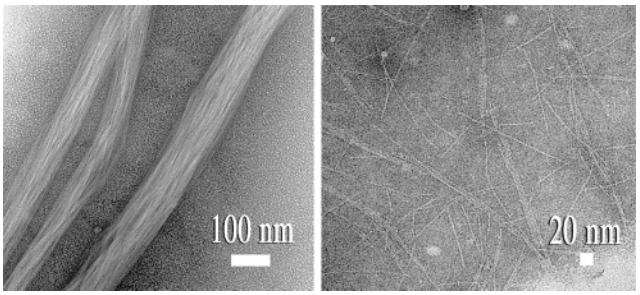

Figure 3. Negative-stain TEM images of annealed specimens of **TZ1H** assembled at pH 8.2. (Left) Large-diameter fiber bundles. (Right) High-magnification image of fibrils of ca. 3 nm diameter.

proximal residues. Histidine residues in hydrophobic environments, such as buried sites within folded proteins, typically display lower pK_a values. For a series of synthetic four-helix bundle proteins, the pK_a values for histidine residues at core d-positions were observed within a range from 5.2 to 6.0, which encompassed the conformational transition of **TZ1H**.[14] In addition, inclusion of polar histidine residues at core positions in the sequence of **TZ1H** influences the thermodynamic stability of the helical assembly, which undergoes a melting transition under basic conditions with a T_m of 63 °C at 15 μM peptide (cf. Supporting Information). The helical form of **TZ1H** is destabilized thermodynamically with respect to the corresponding peptide in which isoleucines populate all of the core positions, which remains fully helical under identical conditions.[12]

Conventional TEM was employed for visualization of **TZ1H** specimens that were prepared at pH values of 4.0 and 8.2, which defined the limits of coil and helix conformations, respectively (Figure 3). At pH 8.2, long-aspect-ratio fibers were observed in which the diameters ranged between 40 and 100 nm — at least an order of magnitude larger than that estimated for fibril assemblies based on a three-helix bundle. Higher magnification TEM images revealed the presence of much thinner fibrils, in which the observed diameter of 3.3 ± 0.7 nm more closely approximates the lateral dimension of trimeric helical bundles.[7] These fibrils were observed either in isolation or in association with each other or the larger fibers. The larger fibers also displayed longitudinal striations of diameter, 2.5 ± 0.3 nm, similar to that of the isolated fibrils, which suggested that the large-diameter fibers corresponded to associated bundles of the smaller-diameter fibrils. Multiple attempts at TEM imaging of **TZ1H** specimens prepared at pH 4.0 provided negligible evidence for the formation of assembled structures, which was in agreement with the hypothesis that self-assembly coincided with the development of the α-helical conformation within **TZ1H**.

Sharp differences in pH-responsive behavior were also observed for **TZ1H** by microrheology.[15] At pH 8.0, the solutions of the **TZ1H** peptide display an elastic storage modulus, $G'(\omega)$, higher than the viscous loss modulus, $G''(\omega)$, while the viscous modulus of the **TZ1H** solution at pH 4.0 closely parallels that of water (cf. Supporting Information). These observations provide further evidence that the **TZ1H** system remained unassociated at low pH, which is in agreement with the proposed correlation between the helical conformation and supramolecular assembly.

In conclusion, these results demonstrate that the self-assembly of a de novo-designed peptide into a supramolecular material can be controlled reversibly through a mechanism that couples histidine side-chain proton-transfer equlibria to peptide conformation. This peptide engineering strategy represents a promising approach to the design of intelligent materials that combine well-defined structures with environmentally responsive self-assembly.[16] A range of synthetic peptide materials can be envisioned in which artificial allosteric sites[17] are introduced at *structurally critical core positions* in the peptide sequence such that self-assembly can be potentiated through incremental changes in environmental stimuli (pH, metal ion, or small-molecule) *within a sharply defined concentration range under physiologically relevant conditions*.

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Supporting Information Available: Material and methods and additional analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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