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pH-Triggered Strand Exchange in Coiled-Coil Heterotrimers

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The capacity for programmed assembly of specific supramolecular aggregates is a powerful tool for molecular design. When coupled with a mechanism for dynamic control of aggregate stability, these methods hold promise for the design of stimulus-responsive assemblies. The intrinsic order and self-affinity of biopolymers offers myriad prospects for such design efforts. In particular, the α-helical coiled-coil provides a promising proving ground. Herein we describe the use of pH as an environmental variable capable of modulating the preferred stoichiometry of 1:1:1 coiled-coil heterotrimers. By suitable matching of ligand and pH, any component strand can be selectively replaced. The formation and disruption of peptidic cross-links between heterotrimers is controlled by similar methods.

The α-helical coiled-coil, formed by superhelical twisting of two or more component helical peptides, has been a popular target for protein design.[1,2] Extensive studies have established the primary role in complex formation of hydrophobic core residues at the first and fourth (*a*/*d*) positions of a primary sequence heptad repeat (*abcdefg*).[3] Additional influence of electrostatic interactions in positions flanking the core residues (*e*/*g*) has also been documented.[4]

Recently we demonstrated formation of complex peptide assemblies by simultaneous use of unnatural hydrophobic and natural hydrophilic helix interfaces.[5] Stable 1:1:1 heterotrimers contain sterically matched hydrophobic cores (2:1 juxtaposition of alanine/cyclohexylalanine side chains at three *a* layers) and are electrostatically matched at all three *e*/*g* helical interfaces (all Glu/Lys contacts). Analogous complexes with two Glu/Lys and one Lys/Lys (but not Glu/Glu) interactions are also feasible.

Studies of pH sensitivity in related peptide complexes demonstrate that pH variation can alter the relative stabilities of single *e*/*g* interface mismatches.[4] To verify similar effects in our heterotrimer systems, circular dichroism (CD) spectra were recorded between pH 2 and 10 for complexes bearing one Glu/Glu (T_9E, $T_{16}K$, $T_{23}E$), one Lys/Lys (T_9K, $T_{16}E$, $T_{23}K$), or fully matched (T_9K, $T_{16}E/K$, $T_{23}E$) electrostatic interfaces (Figure 1). Above neutral pH the relative helicity (and hence stability) of acidic and basic complexes is as before.[5] In contrast, at pH 5 and below the Glu/Glu contact is preferred. The fully matched complex is relatively unaffected in this window.

These observations provide a mechanism for pH-triggered exchange of heterotrimer components. (Figure 2). A preformed heterotrimer bearing one Lys/Lys contact at high pH can be transformed to one featuring a Glu/Glu interface by reduction of pH and addition of a suitable new peptide. Since each complex must simultaneously maintain proper steric matching in the hydrophobic core, only one specific peptide is replaced. This level of substitution precision is available only through the combined impact of both interfaces. More generally, any complex featuring a pH-sensitive interface is subject to the same specific replacement strategy.

To test these principles we have employed acidic (T_nE), basic (T_nK), and hybrid (T_nE/K) derivatives of our original T_9:T_{16}:T_{23}
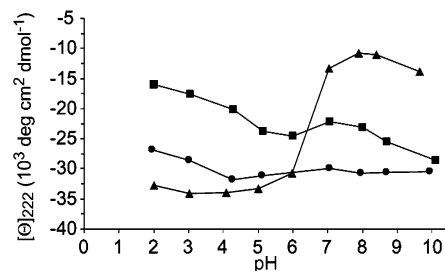

Figure 1. pH titration of heterotrimer helicity. Value of $[\Theta]_{222}$ as function of pH for equimolar solutions of T_9K:$T_{16}E/K$:$T_{23}E$ (all matched, circles), T_9K:$T_{16}E$:$T_{23}K$ (one Lys/Lys, squares), and T_9E:$T_{16}K$:$T_{23}E$ (one Glu/Glu, triangles). All solutions are 10 μM total peptide in PBS buffer (10 mM phosphate, 150 mM NaCl).
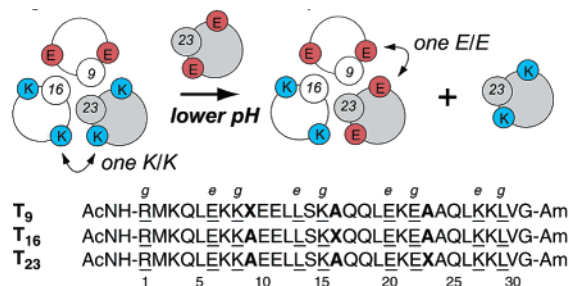

Figure 2. pH-triggered helix exchange. Initial solution favors complex with single Lys/Lys *e*/*g* interface. pH reduction destabilizes Lys/Lys contact compared to Glu/Glu. Simultaneous addition of suitable replacement peptide affords new specific complex. Numbers indicate position of core cyclohexylalanine (X). Parent sequences also given. Acidic (T_nE), basic (T_nK), and hybrid (T_nE/K) derivatives contain Glu, Lys, or both in all *e*/*g* positions (underlined). Key core positions are in bold type.

heterotrimer, whose stability profiles have been recorded at neutral pH (Figure 2). In keeping with the method outlined above, we reasoned that $T_{23}K$ could be displaced from a preformed 1:1:1 T_9E:$T_{16}K$:$T_{23}K$ heterotrimer (one Lys/Lys contact) by addition of $T_{23}E$ at reduced pH (Figure 2). The new complex features one Glu/Glu interface, which can be achieved in principle by replacement of either $T_{16}K$ or $T_{23}K$. The need to simultaneously maintain a sterically matched hydrophobic core engenders the additional specificity for $T_{23}K$.

Verification of the proposed process was conducted as described previously.[5] One trimer component is functionalized with a $(His)_6$-Gly-Gly sequence that binds nickel-nitrilotriaceticacid (Ni-NTA) groups. When shaken with Ni-NTA agarose beads, tagged peptides and their specific binding partners are retained. Washing of the resin and subsequent elution with imidazole buffer permits HPLC determination of bound peptides. Analysis of the original supernatant identifies unbound material. When a derivatized $T_{16}K$ ($T_{16}K_{His}$) is employed in the above experiment, observed fraction compositions are consistent with specific component displacement (Figure 3). The supernatant contains principally ejected $T_{23}K$, while eluted material is composed of peptides from the new complex.[6]
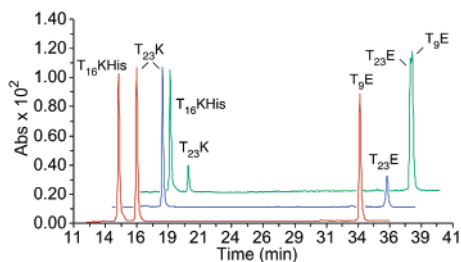
Figure 3. Affinity tag analysis of exchange experiment (see Figure 2). Traces reflect composition of initial 20 μM solution of $T_9E:T_{16}K_{His}:T_{23}K$ heterotrimer before mixing with beads (front, pH 7.7), supernatant after treatment with $T_{23}E$ (middle, pH 5.8), and material eluted from resin after $T_{23}E$ addition (back, pH 5.8). All traces normalized to same height.
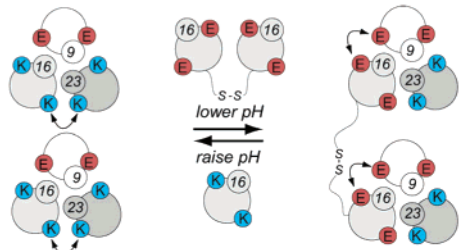
Figure 4. Peptide cross-links. Reduction of pH destabilizes initial complex containing one Lys/Lys contact (doubleheaded arrows), and addition of bifunctional disulfide-linked peptide $T_{16}E$-SS-$T_{16}E$ produces a cross-linked pair of heterotrimers bearing the now favored Glu/Glu contact. The cross-link can be disrupted by selective displacement of the bifunctional peptide at high pH in the presence of $T_{16}K$.

***Table 1.*** Characterization of Cross-link System[a]

sample	T_m (°C)	MW_{obs}	MW_{calc}
$T_9E:T_{16}E:T_{23}K$	89	11 881	11 559
$T_9E:T_{16}E$-SS-$T_{16}E:T_{23}K$	75	22 835	23 568

[a] All samples 10 μM in PBS, pH 5.

This demonstrated capability for selective replacement of a given helix can be further exploited to create more complicated cross-linked assemblies. A suitably complementary bifunctional sequence, comprising two helical segments linked by disulfide or other means, can in principle displace a peptide from each of two distinct heterotrimers, affording a chemically cross-linked pair of complexes (Figure 4).

These principles have now been verified. A derivative of $T_{16}E$, functionalized with an N-terminal Gly-Gly-Cys sequence, was employed as the bifunctional component. The corresponding disulfide ($T_{16}E$-SS-$T_{16}E$) was expected to displace $T_{16}K$ from a preformed $T_9E:T_{16}K:T_{23}K$ heterotrimer at low pH (Figure 4). The reverse process was also investigated.

Before assaying the prospects for dynamic cross-linking, we sought to verify that linked complexes retain appropriate stability and aggregation profiles. Although the isolated $T_9E:T_{16}E:T_{23}K$ system was expected to be precisely analogous to previously studied ones, no such guarantee existed for the pentameric complex formed by cross-linking the two trimers. Gratifyingly, comparison of CD (wavelength and thermal denaturation) and analytical ultracentrifugation data for trimer and pentamer strongly support the proposed model (Table 1). In particular, despite an apparent reduction in thermal stability, the cross-linked system exhibits the expected mass increase by centrifugation.
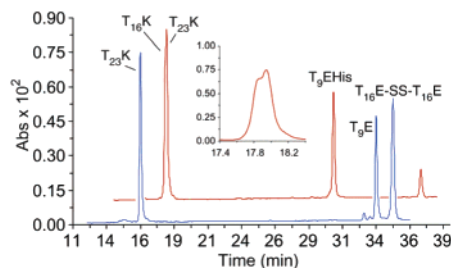
Figure 5. Affinity tag analysis of peptide cross-linking. Front trace reflects supernatant composition after treatment of 20 μM $T_9E:T_{16}K_{His}:T_{23}K$ heterotrimer with $T_{16}E$-SS-$T_{16}E$ (pH 5.4). Back trace obtained by elution of bound material after treatment of $T_9E_{His}:T_{16}E$-SS-$T_{16}E:T_{23}K$ complex with $T_{16}K$ at pH 9.1. Inset reveals presence of two peaks in 17.4−18.2 min region (offset time scale). Traces are normalized to the same height.[7]

Having characterized viable structures, we investigated their dynamic switching. Treatment of a $T_9E:T_{16}K_{His}:T_{23}K$ heterotrimer with disulfide-linked $T_{16}E$-SS-$T_{16}E$, followed by analysis of unbound material, reveals the expected composition (Figure 5). Conversely, beginning with the linked system (differing only in that T_9E_{His} was used in place of $T_{16}K_{His}$), addition of $T_{16}K$ at high pH results in disruption of the cross-link. Only monofunctional components are significantly retained in the elution fraction.

This capacity for reversible, noncovalent cross-linking of peptide subunits should be of particular significance in the design of peptide hydrogel networks that obviate the need for chemically imprecise linking reactions whose toxic byproducts are difficult to remove. The level of precision in helix replacement required for this purpose is not achievable with simple natural sequences. Only the combined specificity of sterically matched hydrophobic cores and electrostatically matched hydrophilic interfaces makes these assemblies possible.

Supporting Information Available: Detailed sequence, CD, sedimentation equilibrium, and Ni-NTA affinity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Due to uncertain absolute resin loading, residual excess $T_{23}E$ and $T_{23}K$ are observed in the supernatant and elution traces, respectively.

(7) Cross-link disruption was also demonstrated using related peptides that give better separation. See Supporting Information.

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