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Fabrication and Characterization of Hydrogels formed from Designer Coiled-Coil Fibril-Forming Peptides

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Supporting information

Thermodynamics of coiled-coil assembly

Guanidinium chloride (G.HCl) denaturation studies on AFD36 and AFD19 were used to quantify the stability of these two peptide systems. Figure S1 shows the effects of added G.HCl on mean residue ellipticity for 250 μM AFD19 at pH 5.0 and 250 μM AFD36 at pH 6.0, in each case representing concentrations and pH values slightly below gelling conditions. AFD19 and AFD36 are both highly resistant to denaturation, with a G.HCl concentration of approximately 5.3 M required for 50% denaturation of AFD36, while AFD19 required an even higher concentration of 6.0 M. By fitting ECD data to monomer-oligomer models, it was possible to determine thermodynamic parameters for peptide self-assembly, including the free energy of oligomer self-assembly and the coefficient of G.HCl denaturation; results are given in Table S1.

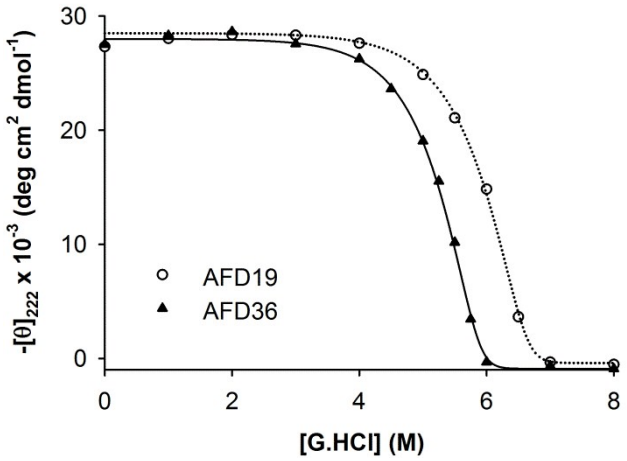

Figure **S1**. Guanidinium chloride denaturation of AFD19 and AFD36 at 20 °C. Filled triangles, AFD36; open circles, AFD19. Lines represent fits to the data obtained as described in the Methods section.

We have previously characterized the thermodynamics of AFD19 self-assembly by a dilution method, obtaining a degree of cooperativity of ~6 and a binding energy of 5.9 kcal mol-1 per monomer for AFD19 self-assembly at pH 5.0 while noting that the monomeric peptide retained a high helix content.1 In the present work, disassembly of oligomeric AFD19 to an unstructured monomer by G.HCl denaturation is associated with an even larger free energy change, amounting to 9.5 kcal mol-1 per monomer if the data are analyzed using a hexamer model. The difference of 3.6 kcal mol-1 represents at least in part the free energy of unfolding of a monomeric α-helix to a random coil, as also studied in model polyalanine systems.2 At the same time, given that the value of ΔG was obtained by a linear extrapolation method, it is possible that non-linear effects relating to ionic strength give a slight overestimate of α-helix stability.3 A small increase in helicity, up to 2 M G.HCl, was observed for both AFD19 and AFD36, suggesting that there is an initial stabilizing effect of added salt prior to the commencement of denaturation. Indeed, AFD36 samples at pH 6.0 show induced gelling in the presence of 2-6 M G.HCl (not shown). However, even given some error in the value of ΔG, the stability of the coiled coil is high compared to other published systems,4 likely reflecting both a high average helix propensity and a high hydrophobic driving force for coiled coil assembly.

Table S1. Thermodynamic stability of AFD19 and AFD36 as determined by G.HCl denaturation

Peptide	pH	Ta	nb	ΔG(H2O)c	md	[θ]mone	[θ]n-mere
AFD19	5.0	20	6	9.5	-0.88	0.4	-28.5
AFD36	6.0	20	5	9.1	-0.95	0.9	-28.0
AFD36	6.0	20	6	9.3	-0.95	0.9	-28.0
AFD36	6.0	20	7	9.2	-0.92	0.9	-28.0
AFD36	6.0	20	12	9.5	-0.90	0.9	-28.0
AFD36	6.0	50	6	10.6	-1.04	0.0	-27.0

a °C
b oligomer state assumed in fitting
c kcal mol-1 per monomer
d kcal mol-1 (M G.HCl)-1 per monomer
e 103 deg cm2 dmol-1 at 222 nm

To assess the effect of assumed oligomerization state on stability values, the AFD36 denaturation data were modeled using pentamer, hexamer, heptamer and dodecamer (based on end-to-end association of two hexamers) models. The stability on a per monomer basis was found to vary only slightly, from 9.1 to 9.5 kcal mol-1 per monomer, indicating that the stability values are relatively insensitive to assumed oligomerization state. Since AFD19 was previously modeled as a hexamer, the hexamer model state was chosen to compare self-assembly across different conditions. AFD36 at pH 6.0 has a stability of approximately 9.3 kcal mol-1 per monomer when analyzed using a hexamer model, slightly lower than AFD19 in a similar charge state at pH 5.0.

The lower stability of AFD36 is surprising given that the serine-to-lysine mutation should favor helix formation. The difference is small, however, and may relate to stabilization of the unfolded state by interactions involving lysine, or slight differences in the pKa values of amino acid sidechains between the two peptides, leading to differences in the actual molecular charge. On increasing the temperature from 20 to 50 °C, the stability of AFD36 is increased to 10.6 kcal mol-1 per monomer, consistent with the strengthening of hydrophobic interactions with temperature,5 and underlining the key role of hydrophobic association in stabilizing the coil.

The value of m, the coefficient of denaturation, can be used to analyze the change in accessible surface area (ASA) on unfolding of peptide structures based on correlations between crystallographic data and measured m-values for a range of proteins.6 Similarly to the stability values, the per-monomer m-value varies little with assumed oligomerization state, from 0.90-0.95 kcal mol-1 (M G.HCl)-1. The m-values obtained here are larger than for a designed coiled-coil tetramer with a comparable number of residues,4 as well as for native proteins of similar molecular weight,6 and for datasets collected at 20 °C correspond to a change in accessible surface area of approximately 20,000 Å2 per AFD36 foldamer and 19,000 Å2 per AFD19 foldamer, or 3,200-3,300 Å2 per helix if a hexamer structure is assumed. Outlier values for m are also known for mutants of staphylococcal nuclease7 (149 residues) and T4 lysozyme8 (163 residues), indicating the relationship between m-values and change in ASA may be more complex than accounted for by these correlations. The large values of m observed for AFD36 and AFD19 may also relate to a higher degree of unfolding than is found in the reference set of proteins, giving a larger exposed surface area on denaturation.[9]

References

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