ORGANIC LETTERS

2004
Vol. 6, No. 23
4219−4222

Tuning Stability of Coiled-Coil Heterotrimers by Selection of Steric Matching Partners

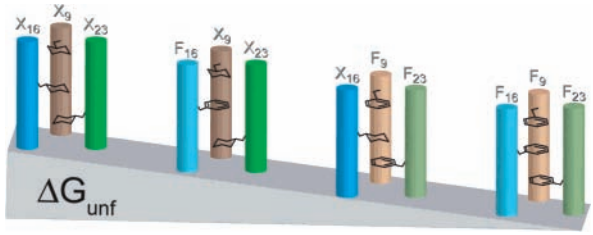
Philip Travisano and Alan J. Kennan*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

kennan@lamar.colostate.edu

Received August 18, 2004

ABSTRACT



Coiled-coil trimers of differing stability have been designed by judicious choice of hydrophobic core side chain identity. Steric matching of two alanines with one large side chain at three core positions affords stable heterotrimers. Use of either cyclohexylalanine (Cha) or phenylalanine (Phe) as the large side chain is effective, and unfolding free energies can be varied by over 3 kcal/mol by mixing Cha- and Phe-containing subunits.

Design of tunable self-assembled systems is of significant current interest. Biopolymers are especially promising components, combining regularity of structure with diversity of possible constructions.[1] This synergism is exemplified by the multitude of designed helical peptide assemblies.[2] Coiled-coils, generated by supercoiling of two or more component α-helical strands, underlie many such efforts.[3] The broad array of natural functions they support attests to their versatility.[4] Here we describe formation of 1:1:1 coiled-coil heterotrimers whose stability varies over a considerable range, governed by selection of specific hydrophobic core residue patterns. Each independent complex exhibits biophysical signatures expected of well-formed coiled-coil trimers.

Extensive study of natural and model coiled-coils has established firm sequence−structure relationships, including the critical influence of hydrophobic core side chains on stability and aggregation number.[5] Recently, we described

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10.1021/ol048352o CCC: $27.50
Published on Web 10/21/2004

the use of core residue steric matching to control specificity of heterotrimer formation.[6]

Favored complexes permit formation of 2:1 alanine−cyclohexylalanine layers at three consecutive *a* positions of the primary sequence heptad repeat (*abcdefg*).[7] To extend the application of this strategy, we have investigated phenylalanine (Phe) as a possible replacement for cyclohexylalanine (Cha) in this arrangement. Besides conferring the potential advantages of purely natural sequences (e.g., easier in vitro expression), such an alternative should allow design of variable-stability trimers by evaluation of mixed-core systems.[8]

The peptides employed contain alanine at two of the central core positions (9, 16, 23) and cyclohexylalanine (X_n) or phenylalanine (F_n) at the other (Figure 1). Within each

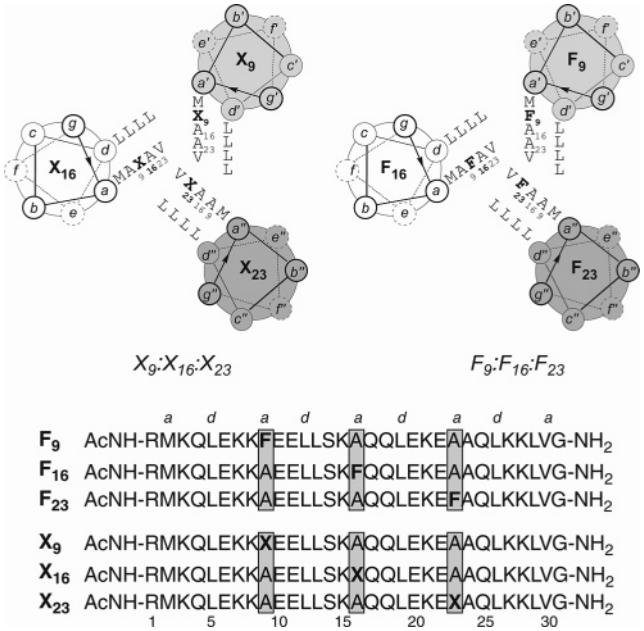

Figure 1. Peptide sequences and helical wheel projections. F_n and X_n peptides have phenylalanine or cyclohexylalanine, respectively, at the indicated positions and alanine at the other two central core *a* layers (modified layers are marked by shaded boxes). Projections of F_9:F_{16}:F_{23} and X_9:X_{16}:X_{23} heterotrimers are also given. Nonhydrophobic core residues are omitted for clarity.

type, peptides differ by location of the large side chain (Cha or Phe), so that a 1:1:1 trimer of X_9:X_{16}:X_{23} or F_9:F_{16}:F_{23} has three sterically matched core layers (two Ala and one Cha/Phe at each). We have previously shown that the former complex is a well-behaved trimeric coiled-coil[6d] and sought to discover if the latter would behave similarly.

Circular dichroism (CD) analysis of a 1:1:1 F_9:F_{16}:F_{23} mixture reveals the high helicity and cooperative thermal denaturation profile expected of coiled-coils (Figure 2). In

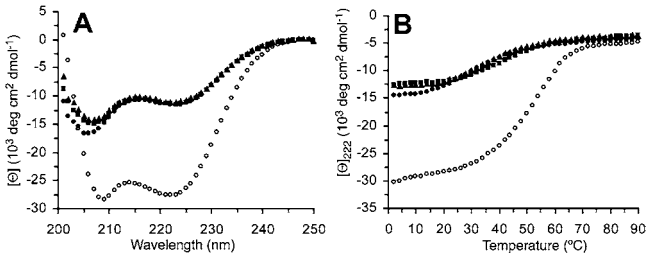

Figure 2. CD analysis of Phe-substituted peptides. Wavelength (A) and thermal unfolding (B) data for F_9 (squares), F_{16} (triangles), F_{23} (circles), and a 1:1:1 F_9:F_{16}:F_{23} mixture (open circles).

contrast, pure solutions of F_9, F_{16}, and F_{23} display dramatically reduced structure. Signals for the pairwise mixtures of any two peptides, though stronger than those of pure components, remain significantly weaker than that of the designed trimer (Figure 3).

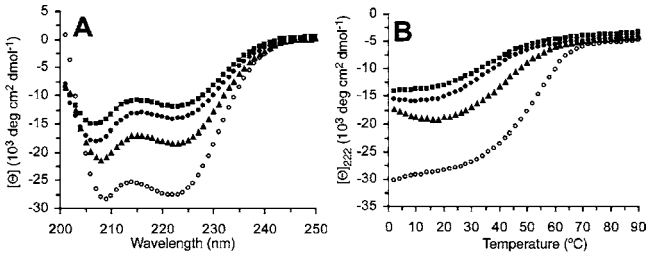

Figure 3. CD analysis of pairwise mixtures. Wavelength (A) and thermal unfolding (B) data for equimolar F_9:F_{16} (squares), F_9:F_{23} (triangles), and F_{16}:F_{23} (circles) and the 1:1:1 F_9:F_{16}:F_{23} mixture (open circles).

The observed inferiority of alternative complexes is in keeping with previous results for the X_9:X_{16}:X_{23} system, particularly the deleterious impact of forming all-alanine core layers. The pure F_n trimers each contain two such layers, which we have demonstrated to be destabilizing by a variety

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of methods.[6] In the pairwise mixtures (F_9:F_{16}, F_9:F_{23}, F_{16}:F_{23}) trimer formation requires separation into two 2:1 complexes. Thus, an equimolar F_9:F_{16} mixture would contain F_9:F_9:F_{16} and F_9:F_{16}:F_{16} assemblies. Each of these has one fully matched layer, one all alanine layer, and one layer with two large side chains and one small one.[9]

Verification of designed trimer stoichiometry was executed using a previously described Ni-affinity method.[6] The F_{16} peptide was derivatized with a Gly-Gly-$(His)_6$ tag, producing a new sequence (F_{16His}) that binds nickel nitrilotriacetic acid agarose beads. Exposure of an initial 1:1:1 F_9:F_{16His}:F_{23} solution to the beads, followed by rinsing to remove nonspecific binders and elution with imidazole buffer, afforded an elution fraction containing 1 equiv of each peptide, supporting the proposed 1:1:1 structure (see Supporting Information for additional details).

Having established the feasibility of phenylalanine/alanine steric matching, we turned to the investigation of mixed-core systems, in which both phenylalanine and cyclohexylalanine are present, in varying ratios. Equimolar mixtures of F_9:F_{16}:F_{23}, F_9:X_{16}:F_{23}, X_9:F_{16}:X_{23}, and X_9:X_{16}:X_{23} should form heterotrimers with only Phe, Phe/Cha/Phe, Cha/Phe/Cha, and only Cha side chains, respectively, at the three core *a* layers (Figure 4). We reasoned that such combinations

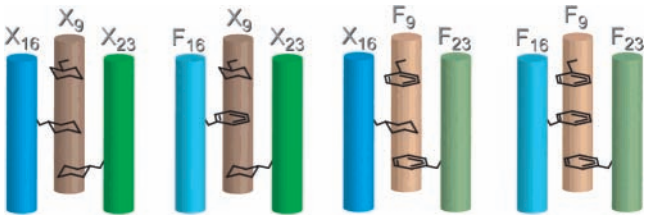

Figure 4. Schematics of mixed-core systems. Large side chains at each of the three modified core layers are depicted. Each layer also contains two small alanine side chains (not shown). Other core layers (including intervening *d* layers between side chains shown) and all noncore positions have been omitted for clarity.

would provide a facile method for tuning heterotrimer stability without sacrificing specificity.

All four mixed-core trimers exhibit similar helicity by CD, but their thermal stabilities vary inversely with the number of Phe residues (Figure 5). Although all four display cooperative unfolding profiles consistent with stable structures, the observed melting temperatures are spread fairly

Table 1. Sedimentation Equilibrium and Thermodynamic Data

sample	T_m (°C)	ΔG_{unf} (kcal/mol)	$M_{r\ obsd}$	$M_{r\ calcd}$
F_9:F_{16}:F_{23}	61	16.27 ± 0.25	10 898	11 535
F_9:X_{16}:F_{23}	67	17.74 ± 0.36	11 353	11 541
X_9:F_{16}:X_{23}	73	18.05 ± 0.28	11 608	11 547
X_9:X_{16}:X_{23}[a]	83	19.60 ± 0.21	10 245	11 556

[a] Data from ref 6d.

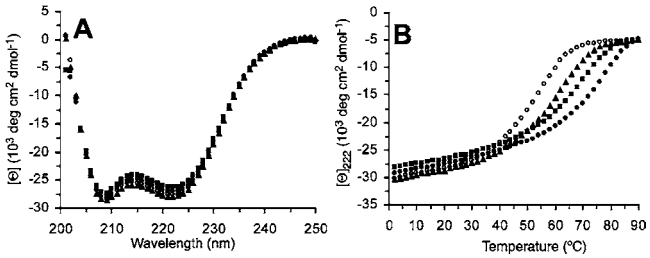

Figure 5. CD analysis of mixed-core systems. Wavelength (A) and thermal unfolding (B) data for 1:1:1 X_9:F_{16}:X_{23} (squares), F_9:X_{16}:F_{23} (triangles), X_9:X_{16}:X_{23} (circles), and F_9:F_{16}:F_{23} mixture (open circles).

evenly over about a 20 °C range (Table 1). Thus, considerable stability variance can indeed be programmed simply by selection of the core makeup.[10]

Further characterization was conducted by chemical denaturation (Figure 6). Each complex displays a cooperative

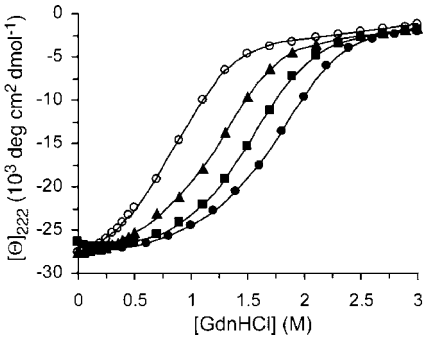

Figure 6. GdnHCl denaturation profiles for 1:1:1 X_9:F_{16}:X_{23} (squares), F_9:X_{16}:F_{23} (triangles), X_9:X_{16}:X_{23} (circles), and F_9:F_{16}:F_{23} mixture (open circles). Lines are fits to the data; for unfolding free energies, see Table 1.

unfolding transition, with significant variation between the samples (Table 1). Unfolding free energies differ by over 3 kcal/mol between the most and least stable assemblies, and the correlation with Phe content holds (i.e., F_9:F_{16}:F_{23} is the least stable at 16.27 kcal/mol, while X_9:X_{16}:X_{23} is the most stable at 19.60 kcal/mol).

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(9) For example, F_9:F_9:F_{16} is 2:1 Phe:Ala at position 9, matched at position 16, and all alanine at position 23.

(10) Wavelength and thermal melt data are very similar for all possible single Cha trimers (X_9:F_{16}:F_{23}, F_9:X_{16}:F_{23}, F_9:F_{16}:X_{23}), as are Ni-affinity results for the X_9:F_{16}:F_{23} and F_9:X_{16}:F_{23} systems (see Supporting Information). Thus, the stability variance appears to be context independent. For dramatic examples of context dependence in hydrophobic core cluster patterns, see ref 5a and: Lu S. M.; Hodges, R. S. *Protein Sci.* **2004**, *13*, 714−726.

Confirmation of trimeric assemblies was obtained by analytical ultracentrifugation. Each of the three Phe-containing complexes (F_9:F_{16}:F_{23}, F_9:X_{16}:F_{23}, X_9:F_{16}:X_{23}) give observed reduced mass values consistent with those calculated for the appropriate trimer molecular weights (Table 1), in analogy with the previously reported data for the X_9:X_{16}:X_{23} complex.[6d]

Overall, these data confirm that phenylalanine side chains can assume the role of large hydrophobic groups in a 2:1 small:large matching scheme. Although complex stability is gradually diminished by increasing Phe content, even the F_9:F_{16}:F_{23} assembly remains a perfectly viable trimeric coiled-coil. Thus, in addition to providing an alternative to cyclohexylalanine for controlling trimer assembly, introduction of phenylalanine side chains establishes a mechanism for smooth variation in complex stability without sacrificing specificity. Such systems could be useful in various applications such as peptidic drug delivery systems where thermal triggering of trimer disassembly at tunable temperatures is desired.

Acknowledgment. This work was supported by an NSF CAREER award to A.J.K. (CHE-0239275). We thank Dr. Nathan A. Schnarr for peptide synthesis and numerous helpful discussions.

Supporting Information Available: Detailed experimental procedures, analytical ultracentrifugation data, and Ni−NTA data. This material is available free of charge via the Internet at http://pubs.acs.org.

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