JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by University of Birmingham | http://www.library.bham.ac.uk

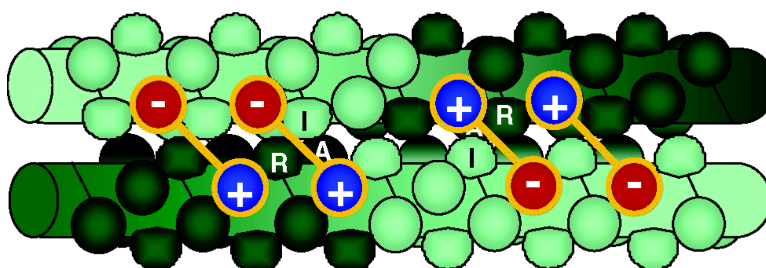
Communication

Design and Characterization of a Homodimeric Antiparallel Coiled Coil

Daniel G. Gurnon, Jennifer A. Whitaker, and Martha G. Oakley

J. Am. Chem. Soc., **2003**, 125 (25), 7518-7519• DOI: 10.1021/ja0357590 • Publication Date (Web): 03 June 2003

Downloaded from http://pubs.acs.org on March 29, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S COMMUNICATIONS

Published on Web 06/03/2003

Design and Characterization of a Homodimeric Antiparallel Coiled Coil

Daniel G. Gurnon, Jennifer A. Whitaker, and Martha G. Oakley*

Department of Chemistry, Indiana University, 800 East Kirkwood Avenue, Bloomington, Indiana 47405-7102

Received April 23, 2003; E-mail: oakley@indiana.edu

Coiled coils consist of two or more α-helices wound around one another and occur in nature as the dominant motif in fibrous proteins and as mediators of oligomerization.[1,2] Applications of coiled coils[3,4] range from affinity purification[4c] to the inhibition of viral membrane fusion.[4d] In addition, coiled coils have been utilized as controllable cross-linking agents in peptide-based hydrogels.[5,6] The wide range of coiled coil applications underscores the need for topological control of designed peptides. Although naturally occurring parallel homodimers have been successfully applied to protein fusion and materials applications,[4a,b,6] no naturally occurring antiparallel coiled coil has yet been shown to be suitable for such uses.[7] Here we report the successful design of a stable, homodimeric, antiparallel coiled coil.

The primary sequence of coiled coils is characterized by a heptad repeat of amino acid residues, labeled **a**−**g**.[3,8] Residues at the **a** and **d** positions are predominantly hydrophobic, whereas residues at the **e** and **g** positions are frequently charged and can participate in interhelical Coulombic interactions.[2,3,8] The alignment, partner specificity, and oligomerization state of a coiled coil are influenced by the interactions that occur among the **a**, **d**, **e**, and **g** positions of opposing helices (Figure 1).[2,3,7,8]

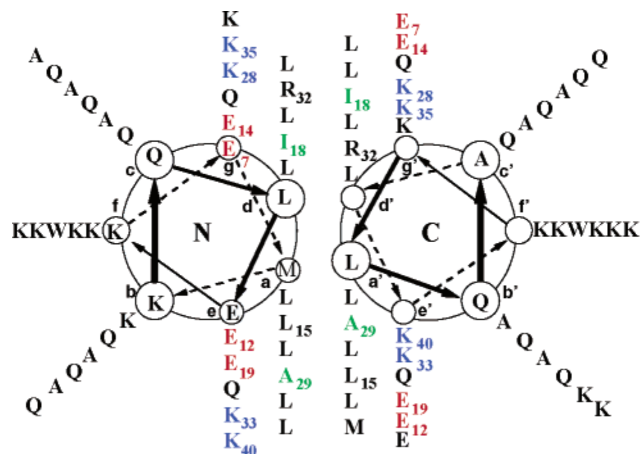


Figure 1. Helical wheel representation of the antiparallel homodimer APH. The view is shown looking down the superhelical axis from the N-terminus of the monomer on the left and the C-terminus of the monomer on the right. Basic (blue), acidic (red), and hydrophobic (green) residues expected to contribute to a preference for the antiparallel alignment are indicated.

A significant challenge in the design of an antiparallel homodimer is specifying a two-stranded structure. A buried polar interaction between a pair of Asn residues on opposite strands of a coiled coil can specify, at a cost in stability,[9,10] a preference for a dimeric state,[10] as well as for a particular helix orientation[11,12] or partner preference.[2,4b] Specificity for a dimer is most likely due to the greater solvent accessibility of the **a** and **d** positions in a two-stranded structure relative to higher order oligomers.[13] However, because the cost of burying polar residues in a nonpolar environment is minimized when Asn residues interact,[4b,9,14] helices align to place Asn residues across from one another in the hydrophobic core.[11,12,15] Because **a**−**a′** and **d**−**d′** contacts occur in parallel but not antiparallel coiled coils (Table 1), an interaction between equivalent Asn residues in a homodimer would favor a parallel alignment.[15] A buried polar interaction is therefore not useful for the design of an antiparallel homodimer.

Table 1. Interhelical Interactions Based on Alignment of APH

Antiparallel Alignment			
e–e′	g–g′	a–d′	d–a′
$E_{12}-K_{40'}$	$E_{7}-K_{35'}$	$A_{29}-I_{18'}$	$I_{18}-A_{29'}$
$E_{19}-K_{33'}$	$E_{14}-K_{28'}$	$L_{15}-R_{32'}$	$R_{32}-L_{15'}$
$K_{33}-E_{19'}$	$K_{28}-E_{14'}$		
$K_{40}-E_{12'}$	$K_{35}-E_{7'}$		
Parallel Alignment			
e–g′	g–e′	a–a′	d–d′
$E_{12}-E_{7'}$	$E_{7}-E_{12'}$	$A_{29}-A_{29'}$	$I_{18}-I_{18'}$
$E_{19}-E_{14'}$	$E_{14}-E_{19'}$	$L_{15}-L_{15'}$	$R_{32}-R_{32'}$
$K_{33}-K_{28'}$	$K_{28}-K_{33'}$		
$K_{40}-K_{35'}$	$K_{35}-K_{40'}$		

We and others have recently shown that incorporation of a single charged residue at an interior position is sufficient to specify a dimeric structure at a lower cost in stability than two buried Asn residues.[16] Our homodimer design therefore incorporates a single Arg residue at a **d** position (Figure 1). Although this design results in the pairing of Arg residues in a parallel alignment (Table 1), recent studies suggest that differential packing of charged and hydrophobic residues is unlikely to contribute to helix orientation preference.[14] Similarly, potential **d**−**g′** interactions that can arise from a buried charged residue provide little to no contribution to helix orientation,[16a] prompting additional strategies for specifying an antiparallel alignment.

We designed the homodimer to favor an antiparallel alignment of α-helices through the simultaneous application of Coulombic and hydrophobic components.[19] Coulombic interactions between **e** and **g** positions have been shown to influence helix orientation in coiled coils.[3,20] Our design features Glu residues at the N-terminal **e** and **g** positions and Lys at C-terminal **e** and **g** positions, resulting in eight potential Coulombic attractions in an antiparallel alignment (Figure 1, Table 1) and eight potential Coulombic repulsions in a parallel alignment (Table 1).

Our design also favors the antiparallel orientation through steric matching of β-branched and truncated side chains in the hydrophobic core.[3,8,18,21] Opposing Ile residues at **d** positions are poorly accommodated in a parallel homodimer.[13] In an antiparallel homodimer, the Ile is accommodated by placement of an Ala residue at the opposing **a′** position (Figure 1, Table 1).[3,8] Indeed, similar **d**−**a′** layers composed of Ile-Gly and Ile-Leu are observed in naturally occurring antiparallel coiled coil dimers.[17,18] The potential formation of a destabilizing Ala-Ala "hole" in the interior of the

10.1021/ja0357590 CCC: $25.00 © 2003 American Chemical Society

parallel homodimer is also expected to contribute to the antiparallel preference (Table 1).[2,3,8,22]

Many de novo designed coiled coils are not amenable to expression in a host cell.[4a] Despite this observation, overexpression of a 45 residue construct incorporating the design described above was achieved in *Escherichia coli*.[23] The resulting peptide, APH, forms a highly helical homodimer as judged by CD (Figure 2a) and equilibrium sedimentation experiments.[23] The stability of APH ($\Delta G°_{unf} = 11.9 \pm 0.3$ kcal (mol dimer)$^{-1}$, Figure 2b) is roughly 2 kcal (mol dimer)$^{-1}$ greater than that of GCN4, a four-heptad naturally occurring parallel coiled coil.[9,24]

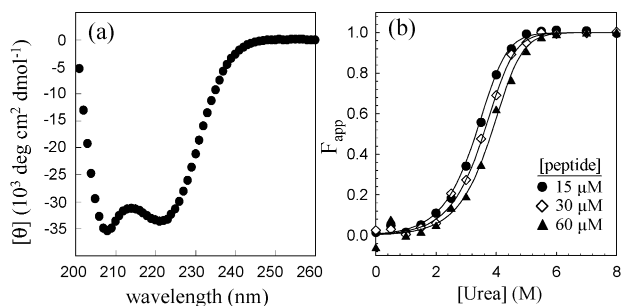


Figure 2. (a) CD spectrum of the antiparallel homodimer APH (15 μM peptide, 37 °C, pH 7.0). APH is ca. 90% helical under these conditions. (b) Dependence of the apparent fraction of unfolded peptide, F_{app}, on the concentration of urea at pH 7.0 and 25 °C for APH. Curves represent fits of the data to a two-state model using eq 3.[23] Global analysis of three peptide concentrations yields an apparent $\Delta G°_{unf}$ of 11.9 ($\pm$ 0.3) kcal (mol dimer)$^{-1}$ at a standard state of 1 M peptide.

Two additional peptides were synthesized to probe the relative helix alignment in the APH homodimer. The sequence Gly-Gly-Cys was appended to the N or C terminus of the monomer to produce APH-N and APH-C. Disulfide-linked peptides APH-NC (antiparallel) and APH-CC (parallel) were obtained by air oxidation.[25] Importantly, oxidation of APH-N and APH-C at neutral pH produced a single major peak observed by HPLC, corresponding to the antiparallel species (Figure 3a). This observation demonstrates a strong preference for an antiparallel orientation under these conditions.

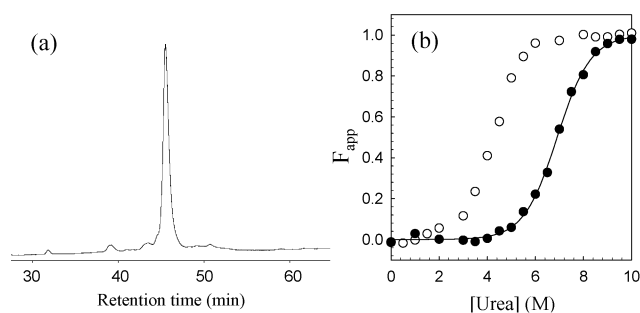


Figure 3. (a) HPLC chromatogram showing results of disulfide bond formation between equimolar amounts of APH-C and APH-N at neutral pH. Elution times are 33 min (APH-C), 39 min (APH-N), 44 min (APH-CC), 45 min (APH-NC), and 50 min (APH-NN). Mass spectral analysis of peaks indicates that of three possible oxidized products, only the antiparallel species is detected. (b) Dependence of the apparent fraction of unfolded peptide, F_{app}, on the concentration of urea at pH 7.0 and 25 °C for the disulfide linked peptides APH-CC (10 μM, ○) and APH-NC (10 μM, ●). The curve represents fitting of the data for APH-NC to a two-state model using eq 4.[23] The data yield an apparent $\Delta G°_{unf}$ of 5.9 ($\pm$ 0.4) kcal (mol)$^{-1}$.

Chemical denaturation of the covalently constrained dimers shows that the antiparallel peptide APH-NC is significantly more stable than the parallel species APH-CC (Figure 3b). Equilibrium sedimentation experiments[23] demonstrate that APH-NC is a disulfide-linked dimer across an order of magnitude in concentration. In contrast, the parallel-constrained APH-CC aggregates under the experimental conditions, precluding a quantitative comparison of the stabilities of the parallel and antiparallel homodimers. The simplest explanation for this observation is that APH-CC forms intermolecular antiparallel coiled coils. Similar higher order association has been observed for naturally occurring parallel coiled coils constrained in an antiparallel orientation[25] and is indicative of an overwhelming preference for a given helical alignment.[20b]

The antiparallel preference of this homodimer is therefore sufficiently strong that APH can be used in fusion proteins to control the topology of interacting protein domains or subdomains.[4a,b] Successful expression in bacterial cells distinguishes APH from other model coiled coils and promotes its use in a biological setting. Indeed, these properties suggest that this self-complementary peptide may also be suitable for the development of novel synthetic biomaterials.[26]

Acknowledgment. We thank A. Hansen for assistance with mass spectrometry. This work was supported by the National Institutes of Health [Grant GM-57571].

Supporting Information Available: Experimental procedures, peptide sequences, equilibrium sedimentation data, and thermal denaturation data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Lupas, A. *Trends Biochem. Sci.* **1996**, *21*, 375−382. (b) Burkhard, P.; Strelkov, S. V.; Stetefeld, J. *Trends Cell Biol.* **2001**, *11*, 82−88.
(2) Vinson, C.; Myakishev, M.; Acharya, A.; Mir, A. A.; Moll, J. R.; Bonovich, M. *Mol. Cell. Biol.* **2002**, *22*, 6321−6335.
(3) Kohn, W. D.; Hodges, R. S. *Trends Biotechnol.* **1998**, *16*, 379−389.
(4) (a) Muller, K. M.; Arndt, K. M.; Alber, T. *Methods Enzymol.* **2000**, *328*, 261−282. (b) Rieker, J. D.; Hu, J. C. *Methods Enzymol.* **2000**, *328*, 282−296. (c) Chao, H. M.; Bautista, D. L.; Litowski, J.; Irvin, R. T.; Hodges, R. S. *J. Chromatogr. B* **1998**, *715*, 307−329. (d) Eckert, D. M.; Kim, P. S. *Annu. Rev. Biochem.* **2001**, *70*, 777−810.
(5) Petka, W. A.; Hardin, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389−392.
(6) Wang, C.; Stewart, R. J.; Kopecek, J. *Nature* **1999**, *397*, 417−420.
(7) Oakley, M. G.; Hollenbeck, J. J. *Curr. Opin. Struct. Biol.* **2001**, *11*, 450−457.
(8) Betz, S. F.; Bryson, J. W.; DeGrado, W. F. *Curr. Opin. Struct. Biol.* **1995**, *5*, 457−463.
(9) Knappenberger, J. A.; Smith, J. E.; Thorpe, S. H.; Zitzewitz, J. A.; Matthews, C. R. *J. Mol. Biol.* **2002**, *321*, 1−6.
(10) Lumb, K. J.; Kim, P. S. *Biochemistry* **1995**, *34*, 8642−8648.
(11) O'Shea, E. K.; Lumb, K. J.; Kim, P. S. *Curr. Biol.* **1993**, *3*, 658−667.
(12) Oakley, M. G.; Kim, P. S. *Biochemistry* **1998**, *37*, 12603−12610.
(13) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401−1407.
(14) Acharya, A.; Ruvinov, S. B.; Gal, J.; Moll, J. R.; Vinson, C. *Biochemistry* **2002**, *41*, 14122−14131.
(15) O'Shea, E. K.; Klemm, J. D.; Kim, P. S.; Alber, T. *Science* **1991**, *254*, 539−544.
(16) (a) McClain, D. L.; Gurnon, D. G.; Oakley, M. G. *J. Mol. Biol.* **2002**, *324*, 257−270. (b) Campbell, K. M.; Lumb, K. J. *Biochemistry* **2002**, *41*, 7169−7175.
(17) Cusack, S.; Berthet-Colominas, C.; Hartlein, M.; Nassar, N.; Leberman, R. *Nature* **1990**, *347*, 249−255.
(18) Zuccola, H. J.; Rozzelle, J. E.; Lemon, S. M.; Erickson, B. W.; Hogle, J. M. *Structure* **1998**, *6*, 821−830.
(19) (a) DeGrado, W. F.; Summa, C. M.; Pavone, V.; Nastri, F.; Lombardi, A. *Annu. Rev. Biochem.* **1999**, *68*, 779−819. (b) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 667−671.
(20) (a) McClain, D. L.; Binfet, J. P.; Oakley, M. G. *J. Mol. Biol.* **2001**, *313*, 371−383. (b) McClain, D. L.; Woods, H. L.; Oakley, M. G. *J. Am. Chem. Soc.* **2001**, *123*, 3151−3152.
(21) (a) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 9779−9783. (b) Gernert, K. M.; Surles, M. C.; Labean, T. H.; Richardson, J. S.; Richardson, D. C. *Protein Sci.* **1995**, *4*, 2252−2260.
(22) Wagschal, K.; Tripet, B.; Lavigne, P.; Mant, C.; Hodges, R. S. *Protein Sci.* **1999**, *8*, 2312−2329.
(23) See Supporting Information.
(24) A four-heptad construct with design features similar to those of APH was not stable at room temperature (see Supporting Information).
(25) O'Shea, E. K.; Rutkowski, R.; Kim, P. S. *Science* **1989**, *243*, 538−542.
(26) Holmes, T. C. *Trends Biotechnol.* **2002**, *20*, 16−21.

JA0357590