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An Antiparallel α-Helical Coiled-Coil Model System for Rapid Assessment of Side-Chain Recognition at the Hydrophobic Interface

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Interactions between α-helical segments constitute a very common theme in protein tertiary and quaternary structure.[1] Thus, elucidation of the factors that control interhelical affinity is essential to understanding protein folding and protein−protein recognition at a fundamental level. The minimum interaction increment, a dimeric coiled-coil, involves just two α-helices.[2] Both parallel and antiparallel α-helical coiled-coil dimers are common; however, biophysical scrutiny has focused almost entirely on parallel dimers.[3] Here we describe the first systematic study of the effect of side-chain variation on the recognition of an α-helical surface by an antiparallel partner. Our findings reveal differences in packing preferences between parallel and antiparallel coiled-coils, and our experimental strategy should be of general utility.

Coiled-coil dimerization is driven largely by burial of hydrophobic surfaces.[4] Sequences that engage in coiled-coil interactions display a characteristic seven-residue repeat pattern in which the first and fourth positions bear hydrophobic side chains. The positions in this "heptad repeat" are conventionally designated *a*−*g*. Hydrophobic side chains, at *a* and *d*, form a continuous stripe along one side of the α-helix; these stripes lie at the core of the dimer interface. We sought an experimental approach that would support rapid evaluation of the effects on antiparallel coiled-coil stability exerted by mutations at *a* and/or *d* positions. Toward this end, we designed a pair of short α-helix-prone segments that could associate to form an antiparallel coiled-coil. These segments were connected with a flexible linker[5] containing a central thioester bond to generate α-helical hairpin molecule **N$_T$-C** (Figure 1a). Thiol−thioester exchange occurs rapidly in aqueous solution at neutral pH; therefore, the equilibrium constant for thiol−thioester exchange (K_{TE}) involving a molecule such as **N$_T$-C** can provide insight on noncovalent attraction between the two α-helical peptide segments (Figure 1b).[6] Mutations at *a* or *d* positions that lead to more or less favorable intramolecular association between the **N**- and **C**-helix segments in the full-length thioester (i.e., larger or smaller K_{CC}) should be manifested as a larger or smaller K_{TE}, which can be measured directly by HPLC. Related studies have been carried out via thiol−disulfide exchange, using peptides bearing Cys residues;[7] however, thiol−*thioester* exchange offers technical advantages relative to thiol−disulfide exchange because careful redox buffering is not required and because the local asymmetry of the thioester group allows one to specify partners (e.g., for heterodimeric pairings).

Several design/analysis cycles were necessary to arrive at the sequence shown for **N$_T$-C**; key issues included optimizing the linker size[8] and avoiding self-association of the full-length molecule. Each segment intended to form an α-helix contains 14 residues (two heptads), with leucine at seven of the eight interface positions. It was necessary to place Arg at one of the *a* positions in **N** to avoid self-association of the full-length molecule.[9] An analogue of **N$_T$-C** in which the thioester is replaced with a carboxamide (i.e., the thioglycolic acid residue is replaced with a glycine residue; **N$_A$-C)** was shown by analytical ultracentrifugation to sediment as a monomer under conditions used for thiol-thioester exchange measurements.[10] All *b*, *c*, *e*, *f*, and *g* positions in **N$_T$-C** are either Arg or Glu, and these residues are arranged so that the maximum number of intra- and interhelical ion pairs is formed in the antiparallel coiled-coil conformation.[11] As expected, the CD spectra of **N$_T$-C**, **N$_A$-C**, **N$_T$-Y**, and **$_{HS}$C** show strong α-helical signatures.[10]

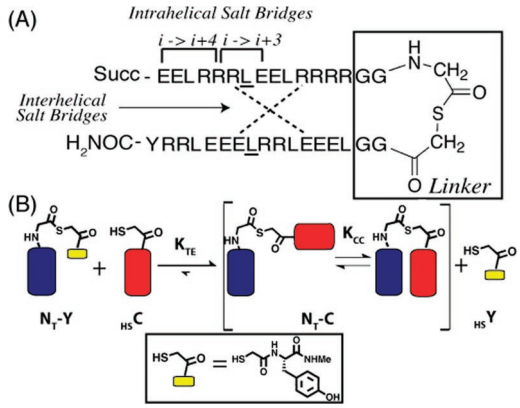

Figure 1. (A) Design and sequence of **N$_T$-C**; Succ = N-terminal succinyl group. Underlined residues are mutated to X/ψ as indicated in Figure 2. (B) Thioester exchange process for **N$_T$-C**. The thioester-thiol pair on the left is comprised of the N− (blue) and C-terminal (red) segments, while the pair on the right contains the full length coiled-coil and a small thiol.

When thiol−thioester exchange is initiated by mixing **N$_T$-C** and **$_{HS}$Y** or by mixing **N$_T$-Y** and **$_{HS}$C** in pH 7 buffer, equilibrium is reached within 40 min, with $K_{TE} = 12.1$. We have previously shown that the folding equilibrium constant (designated K_{CC} here) for the full-length thioester is equal to $K_{TE} - 1$, if there is no energetically significant noncovalent interaction between the Tyr residue and the remaining portion of **N$_T$-Y**.[6] Control studies indicate that this condition is met.[10] The K_{TE} value we measure by HPLC does not vary when thiol/thioester concentrations are varied between 10 and 350 μM, which suggests that intermolecular interactions do not influence this term. The K_{CC} value derived from K_{TE} can be used to estimate the free energy for antiparallel intramolecular association of the two helical segments (ΔG_{CC}) in **N$_T$-C**. Our data indicate that antiparallel coiled-coil formation is favored by 1.4 kcal/mol relative to a more extended state of **N$_T$-C** in which the **N** and **C** α-helices do not interact. This measurement should reflect largely tertiary contact contributions, given that the **N** and **C** segments are highly α-helical in the absence of a partner.[10]

To evaluate the effect of hydrophobic core mutations on antiparallel coiled-coil stability, we selected a central residue for substitution within each helical segment (Figures 1a and 2). Figure 2b shows the noncovalent neighbors of each substitution site (X on the N-terminal segment and ψ on the C-terminal segment), as determined by the knobs-into-holes interdigitation of side chains at the coiled-coil interface.[2] This view reveals that coiled-coil formation should bring substitution sites X and ψ into direct contact.

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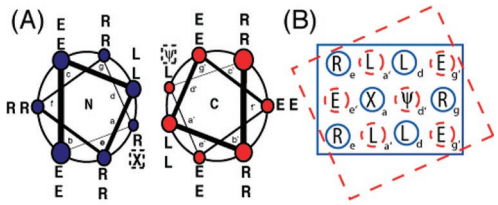

Figure 2. (A) Helical wheel diagram showing the helical regions of $\mathbf{N_T}$-**C**. (B) Partial helical net for $\mathbf{N_T}$-**C**. In each diagram, N-terminal segment is shown in blue and the C-terminal segment is shown in red.

Table 1. Thermodynamic Data (ΔG_{cc})[a] Obtained from Thioester Exchange of $\mathbf{N_T}$-**C** Mutants.

	Ψ = Leu	Ψ = Ile	Ψ = Val	Ψ = Asn	Ψ = Ala
X = Leu	−1.4	−1.3	−0.9	0.2	−0.4
X = Ile	−1.7	−1.0	−0.8	−0.1	−0.9
X = Val	−1.4	−0.8	−0.6	0.0	−0.9
X = Asn	0.3	0.2	0.4	0.5	0.8
X = Ala	−0.7	−0.7	−0.5	0.4	0.0

[a] Values are reported in kcal/mol. Uncertainty ≈ ±0.1 kcal/mol.

Table 1 shows ΔG_{CC} values derived for $\mathbf{N_T}$-**C** and 24 mutants. Five residues were examined at each site, Leu, Ile, Val, Asn, and Ala. Our modular strategy required the synthesis of only 10 short peptides to determine the 25 ΔG_{CC} values. The broad trends among these data are consistent with expectations based on extrapolation from the large literature on parallel coiled-coils and from statistical analysis of residue occurrence in natural antiparallel coiled-coils.[1,12] Thus, the large aliphatic side chains of Leu and Ile participate in the most stable intramolecular pairings, and introduction of a polar Asn side chain causes a substantial loss of folded state stability. These trends support our design hypothesis that the two 14-residue segments engage in antiparallel coiled-coil formation in the full-length thioesters (e.g., $\mathbf{N_T}$-**C**).

Antiparallel coiled-coil formation leads to noncovalent pairing of an *a* position on the N-terminal segment (X) with a *d* position on the C-terminal segment (ψ) (Figure 2b). In contrast, the comparable noncovalent pairings in the hydrophobic core of a parallel coiled-coil are *a*−*a* or *d*−*d*. Given this difference, one expects the impact of core mutations to vary between parallel and antiparallel dimers.[1b,13] Such variations are revealed by comparison of the data in Table 1 with an analogous data set from Vinson et al.[12a] for *a*−*a* pairings in a parallel coiled-coil; some particularly significant distinctions are noted here. (1) In the parallel orientation, the *a*−*a* Ile−Ile pairing is substantially more favorable than any of the other 24 possibilities.[12a] In contrast, we find that Ile-Ile in the antiparallel coiled-coil is a little less stable than Ile-Leu, Leu-Ile, or Leu−Leu. This observation suggests, as might have been expected, that the *a*-*d* pairing in antiparallel coiled-coils confers a packing configuration that differs from the *a*−*a* packing in the parallel orientation. This feature may allow the identity of hydrophobic core residues to influence selectivity for parallel vs antiparallel orientation. (2) Parallel *a*−*a* pairing of Asn with a hydrophobic residue such as Leu is much more destabilizing than the pairing of two Asn residues.[12a] This trend has been rationalized by noting that an Asn/hydrophobic pairing forces the side-chain primary amide group into an environment devoid of H-bonding partners, while paired Asn side chains at *a* positions in a parallel coiled-coil can H-bond to one another.[7a] In contrast, we find that antiparallel Asn-Asn pairing is slightly *more* destabilizing than antiparallel Asn/hydrophobic pairing. This result is consistent with the low frequency of Asn in natural antiparallel coiled-coils.[3,12c] (3) Parallel Ile-Val pairing is significantly more stabilizing (by ca. 50% in terms of $\Delta\Delta G$) than is Ile-Ala,[12a] but we find that antiparallel Ile-Val and Ile-Ala pairings are isoenergetic (both *a/d* arrangements). The comparable stability of these pairings suggests that the matching of β-branched residues with truncated side chains may be especially beneficial in antiparallel coiled-coils, which is consistent with the work of Oakley et al.[14]

The results reported here suggest that the relationship between the identity of paired core residues from partner α-helices and the favorability of the helix−helix interaction varies significantly between parallel and antiparallel orientations. These findings are important because interfacial side-chain packing preferences are likely to contribute to coiled-coil dimerization specificities in vivo, but this aspect of helix−helix recognition is poorly understood at present. The model system introduced here should be useful for continued exploration of helix−helix recognition rules, including evaluation of non-proteinogenic side chains.[15]

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Supporting Information Available: Experimental details, CD analysis, and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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