Design and Characterization of a Heterodimeric Coiled Coil that Forms Exclusively with an Antiparallel Relative Helix Orientation

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Coiled coils are formed by two or more α-helices that align in a parallel or an antiparallel relative orientation. In spite of the growing biological importance of antiparallel coiled coils, the study of this class of molecules has been hampered by the lack of a suitable model system. We report here the successful design of a well-behaved antiparallel coiled-coil heterodimer. The antiparallel helix orientation preference for this model coiled coil is similar in magnitude to the parallel preference of naturally occurring leucine zipper peptides, providing a useful tool for controlling the relative orientation of heterologous protein subdomains.

Coiled coils are found both as the dominant motif in fibrous proteins and as oligomerization domains in a variety of globular proteins.[1] The α-helices of naturally occurring coiled coils have generally been assumed to be parallel. However, a growing number of proteins have been shown to contain antiparallel coiled-coil domains.[2,3]

The discovery that the basic region−leucine zipper class of transcription factors[4] contains short coiled-coil dimerization domains[5] has provided an invaluable, tractable model system for parallel coiled-coil formation. These parallel coiled coils also have been used to assemble complexes of heterologous domains for structural and functional studies.[6] In contrast, no naturally occurring coiled coil has proven suitable as an antiparallel counterpart to the leucine zipper peptides.

It has been shown recently that a single buried polar interaction can confer an antiparallel preference of approximately 2.3 kcal/mol to the model coiled coil Acid-a1−Base-a1.[7] A similar coiled coil has been used to reassemble the N- and C-terminal domains of the green fluorescent protein into a functional complex.[8] However, the modest antiparallel preference of this coiled coil is likely to preclude its use in applications for which the heterologous domains do not influence helix orientation preference.

Coiled-coil sequences are characterized by a heptad repeat of amino acid residues, denoted **a**−**g**.[9] The residues at positions **a** and **d** are predominantly apolar, with charged residues occurring frequently at the **e** and **g** positions.[9] Residues at these four positions participate in interhelical hydrophobic and Coulombic interactions.[10] The relative positions of residues expected to engage in Coulombic interactions are known from the three-dimensional structures of coiled coils.[2,10] In parallel coiled coils, residues at the **e** position can interact with **g′** residues on the opposite strand. In antiparallel coiled coils, **g** residues interact with **g′** residues, while **e** residues can interact with **e′** residues. Potentially attractive and repulsive Coulombic interactions between residues at these positions have been shown to influence partner strand specificity.[11] Similarly, such interactions have been reported to affect orientation preference in disulfide-linked model coiled coils.[12] However, the extent to which a single or pair of differential interactions can contribute to helix orientation preference is unknown.

Because Acid-a1 contains only Glu residues and Base-a1 only Lys residues at both the **e** and **g** positions,[7] only potentially attractive interhelical Coulombic interactions are expected in either orientation. We substituted a single residue at a **g** position in each peptide such that all potentially attractive interactions are expected in the antiparallel orientation. In contrast, two potentially repulsive Coulombic interactions are expected in the parallel orientation. The buried polar interaction between interior Asn residues can occur only in the antiparallel orientation (Figure 1).

An equimolar mixture of the resulting peptides, Acid-K**g** and Base-E**g**, forms a stable heterodimer, as demonstrated by CD (Figure 2A) and equilibrium sedimentation studies (data not shown). To probe the relative helix orientation in this heterodimer, three additional peptides with flexible Cys-containing tripeptides at the N- or C-terminus were synthesized: Acid-K**g**N, Base-E**g**N, and Base-E**g**C. CD studies show that both the antiparallel, covalently linked heterodimer, Acid-K**g**N−Base-E**g**C, and its parallel counterpart, Acid-K**g**N−Base-E**g**N, are highly helical at 25 °C. Nonetheless, the antiparallel heterodimer is substantially more stable to thermal denaturation than its parallel counterpart (Figure 2B), strongly suggesting that the antiparallel orientation is preferred.

The preference for an antiparallel helix orientation under equilibrium conditions was monitored with use of a thiol−disulfide exchange assay[7,13] (Figure 3). Acid-K**g**N−Base-E**g**N (10 μM) and Base-E**g**C (11 μM) were mixed (PBS buffer, pH 7) and allowed to equilibrate (Figure 3C). The observed equilibrium

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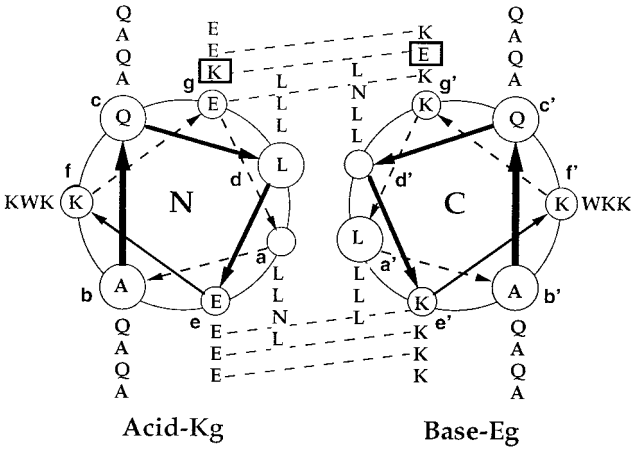

Figure 1. Helical wheel representation of the antiparallel Acid-K**g**-Base-E**g** heterodimer. The sequences of the peptides are the following: Acid-K**g** = Ac-AQLEKELQALEKKLAQLEWENQALEKELAQ-NH_2 and Base-E**g** = Ac-AQLKKKLQANKKELAQLKWKLQALKKKLAQ-NH_2. The view is shown looking down the superhelical axis from the N-terminus of Acid-K**g** and from the C-terminus of Base-E**g**. Residues involved in differential electrostatic interactions are indicated by boxes. Pairs of residues connected by dotted lines can participate in interhelical Coulombic interactions.

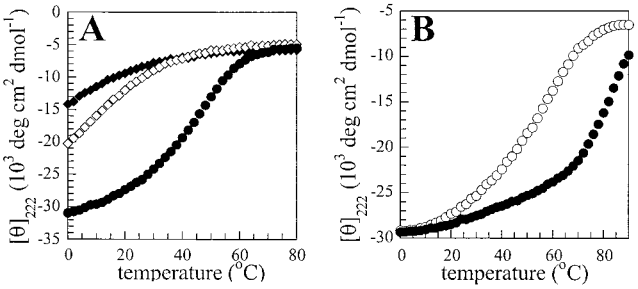

Figure 2. (**A**) Temperature dependence of the cirular dichroism (CD) signal at 222 nm for Base-E**g** (◆), Acid-K**g** (◇), and an equimolar mixture of Acid-K**g** and Base-E**g** (●) [20 μM total peptide concentration, phosphate buffered saline (PBS), pH 7]. Base-E**g** and Acid-K**g** are largely unfolded, but the equimolar mixture undergoes a cooperative thermal transition ($T_m \sim 40$ °C). (**B**) Temperature dependence of the CD signal at 222 nm for Acid-K**g**N−Base-E**g**C (●) and Acid-K**g**N−Base-E**g**N (○) (10 μM peptide, PBS, pH 7, and 1 M urea). The T_m of the thermal unfolding transition is ∼ 84 °C for the antiparallel species and ∼56 °C for the parallel species.

reflects the relative stabilities of the parallel and antiparallel disulfide-linked heterodimers. After the rearrangement is complete, the only detectable heterodimer in solution is the antiparallel heterodimer. Moreover, when the antiparallel heterodimer is allowed to equilibrate with 3 equiv of Base-E**g**N, only a trace amount of parallel heterodimer is observed. Thus, the antiparallel orientation is strongly preferred for Acid-K**g**-Base-E**g**. In contrast, substantial quantities of the parallel heterodimer are observed for the Acid-a1−Base-a1 heterodimer under these conditions.[7]

Because the thiol−disulfide rearrangement assays use an unequal ratio of monomers, aggregates that are not present in an equimolar mixture of peptides can form, complicating the quantitative interpretation of this assay.[7] Nonetheless, a lower limit for the antiparallel preference of Acid-K**g**−Base-E**g** can be estimated from these data. An apparent equilibrium constant for

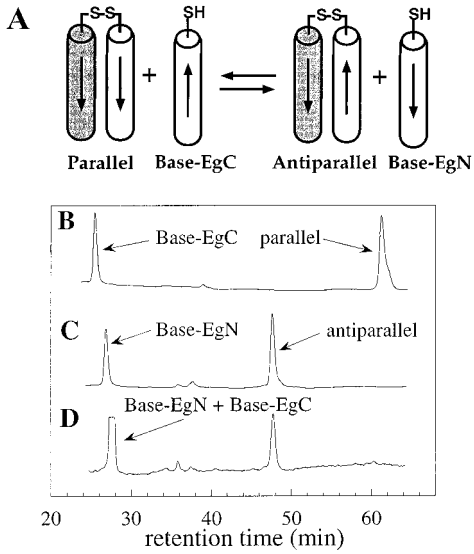

Figure 3. (A) Schematic view of the equilibrium thiol−disulfide exchange assay. Acid-K**g** species are shaded. Base-E**g** species are shown in white. Arrows indicate the direction of the peptide chain from N- to C-terminus. (B−C) HPLC chromatograms showing Base-E**g**C (11 μM) and Acid-K**g**N−Base-E**g**N (10 μM) before (B) and after (C) thiol−disulfide rearrangement. (D) HPLC trace showing rearrangement products of a 3:1 mixture of Base-E**g**N (12 μM) and Acid-K**g**N−Base-E**g**C (4 μM). Base-E**g**N and Base-E**g**C have the same retention time under these conditions. The Base peak has been truncated in trace D for clarity.

the rearrangement reaction (Figure 3A) can be calculated from the known initial concentrations and the ratio of the antiparallel and parallel species after rearrangement (≥96:4 in the presence of 3 equiv of Base-E**g**N). The resulting apparent equilibrium constant of ≥1700 suggests that the antiparallel orientation is preferred to the parallel orientation by at least 4.4 kcal/mol.

Sedimentation equilibrium experiments (data not shown) indicate that the antiparallel heterodimer does not aggregate under the conditions used in these experiments. In contrast, the apparent molecular weight of the parallel Acid-K**g**N−Base-E**g**N heterodimer in solution is concentration dependent, suggesting higher-order aggregation. A likely explanation for this observation is the formation of intermolecular, antiparallel coiled coils. Similar higher-order association has been observed for parallel coiled coils[5] that were constrained in an antiparallel relative orientation. Such behavior is not seen for Acid-a1−Base-a1.[7] Thus, the model coiled coil Acid-K**g**−Base-E**g** appears to have as strong a preference for an antiparallel arrangement of its helices as the leucine zipper peptides have for a parallel relative helix alignment. This model coiled coil should therefore prove useful as a means to constrain domains and subdomains of protein complexes in an antiparallel relative orientation for structural and functional studies.

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

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