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Jonathan R. Lai, John D. Fisk, Bernard Weisblum, and Samuel H. Gellman

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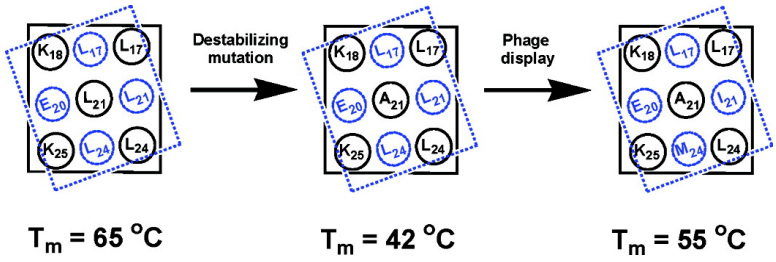

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Hydrophobic Core Repacking in a Coiled-Coil Dimer via Phage Display: Insights into Plasticity and Specificity at a Protein−Protein Interface

Jonathan R. Lai,† John D. Fisk,† Bernard Weisblum,‡ and Samuel H. Gellman*,†,§

Graduate Program in Biophysics, and Departments of Pharmacology and Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Received April 20, 2004; E-mail: gellman@chem.wisc.edu

The coiled-coil,[1] in which two α-helices associate to bury hydrophobic surfaces against one another, is a very common protein dimerization motif that plays a critical role in transcriptional regulation[2a] and other biological processes.[1,2b,c] Elucidation of the factors responsible for dimerization affinity and specificity should improve our understanding of natural coiled-coil function and enhance our ability to design selective self-assembling systems.[3,4] Sequences that form coiled-coil pairs are characterized by a heptad repeat, designated ***abcdefg***, in which the ***a*** and ***d*** residues form the hydrophobic core of the dimer.[1a−c] Most efforts to elucidate pairing preferences in coiled-coils comprising proteinogenic residues have focused on electrostatic interactions among side chains that flank the core, positions ***e*** and ***g***,[5a] and on polar side chains that occur occasionally at core positions.[1c,5b,c] Here we describe the use of phage display[6] to explore the role of packing among nonpolar side chains at core positions in determining heterodimeric coiled-coil partnering preferences.

We began by introducing a destabilizing mutation into one partner of a designed coiled-coil pair, and we then used phage-display to determine whether mutations in the other helix could compensate for this destabilizing alteration. The biologically inspired Base-p1/Acid-p1 parallel heterodimer of Kim et al.,[7] which contains only proteinogenic residues, served as our starting point (Figure 1a). Dimerization is driven by the association of Leu side chains at ***a*** and ***d*** positions on opposing helices. Ionic interactions between Glu residues on Acid-p1 and Lys residues on Base-p1, at ***e*** and ***g*** positions, impart specificity for the heterodimer relative to homodimers. A single pair of buried Asn residues controls both registry and orientation of the two helices,[5b,c] as is commonly observed in natural coiled-coils.[1c] Each of the core side chains (***a*** or ***d***) on one helix is surrounded by four side chains from the opposing helix in dimeric systems.[1a] Figure 1b shows, for example, that Leu_{21} on Base-p1 contacts Leu_{17}, Glu_{20}, Leu_{21}, and Leu_{24} on Acid-p1. We hypothesized that introducing a destabilizing mutation at position 21 on Base-p1 could lead to pairing specificity if a stable partner to this mutant could be selected from an Acid-p1-derived library in which positions 17, 20, 21, and 24 were randomized.

Mutation of Leu_{21} to Ala on Base-p1 (Base-L21A) results in drastic destabilization of the coiled-coil (ΔT_m = 23 °C).[8] We produced a variant of Base-L21A that contains a biotin tag at the N-terminus for immobilization onto streptavidin-coated surfaces. Concurrently, we produced an M13 phage library[6] displaying the Acid-p1 sequence (as a pIII fusion) with the four selected positions randomized. Several rounds of selection led to convergence on a single sequence: pLEIM (Table 1). Of 25 isolated binding clones, 21 proved to be pLEIM.[8] In contrast, panning against biotinylated Base-p1 resulted in a phage population consisting of a variety of

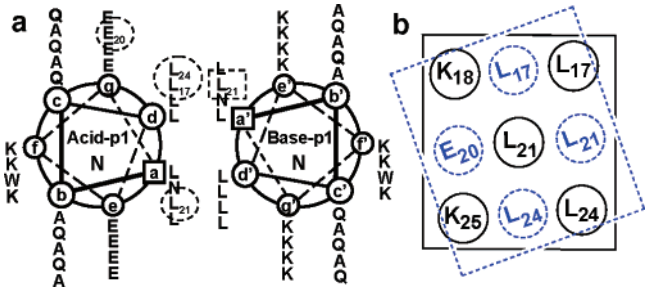

Figure 1. (a) Helical wheel diagram of Base-p1/Acid-p1. In Base-L21A, Leu_{21} (boxed) of Base-p1 is mutated to Ala. The four sites of variation in the Acid-p1-derived phage library are circled. (b) Helical net analysis of Base-p1/Acid-p1. Leu_{21} of Base-p1 (solid black) is surrounded by Leu_{17}, Glu_{20}, Leu_{21}, and Leu_{24} on Acid-p1 (dashed blue).

Table 1. Peptide Sequences

Peptide	Sequence
Base-p1	AQLKKKLQALKKKNAQLKWK**L**QALKKKLAQ
Base-L21A	AQLKKKLQALKKKNAQLKWK**A**QALKKKLAQ
Acid-p1	AQLEKELQALEKENAQ**L**EW**EL**QA**L**EKELAQ
Phage Library	AQLEKELQALEKENAQ**X**EW**XX**QA**X**EKELAQ
pLEIM	AQLEKELQALEKENAQ**L**EW**EI**QA**M**EKELAQ

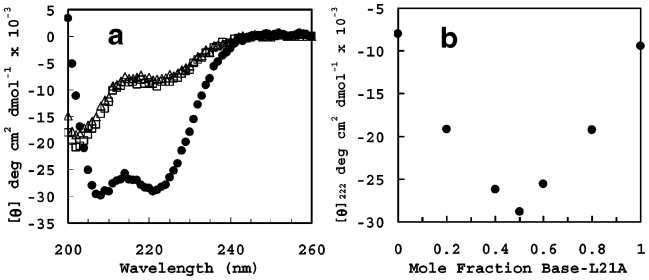

Figure 2. (a) CD spectra of Base-L21A (□), pLEIM (△), and a 1:1 mixture (●) at 10 μM total peptide concentration. (b) Job plot analysis of Base-L21A/pLEIM under similar conditions.

sequences, the majority of which (16 of 25 isolated) contained the motif $L_{17}X_{20}I_{21}Z_{24}$, where X is predominantly polar residues and Z is hydrophobic. The convergence observed upon panning against Base-L21A suggests that Base-L21A should display specificity for pairing with pLEIM over other closely related sequences. In contrast, the panning results imply that Base-p1 is much less selective in partnering preferences.

Characterization of Base-L21A/pLEIM indicates that these peptides form a well-behaved heterodimeric coiled-coil. Circular dichroism (CD) in phosphate-buffered saline (PBS) shows that each component is unstructured in isolation, but a 1:1 mixture results in a strong α-helical CD signature. Job plot analysis (Figure 2b) reveals a discrete aggregate with 1:1 stoichiometry, and ultracentrifugation[8] confirms that Base-L21A/pLEIM is a heterodimer. Thermal and

† Graduate Program in Biophysics.
‡ Department of Pharmacology.
§ Department of Chemistry.

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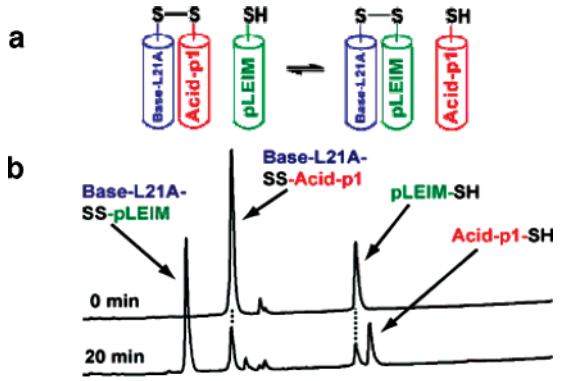

Figure 3. (a) Scheme for the thiol exchange assay. (b) Representative results of the disulfide rearrangement as monitored by RP-HPLC.

Table 2. Stability of Coiled-Coils

coiled-coil	T_m (°C)	ΔG_{unf} (kcal/mol)
Base-L21A/pLEIM	55	10.5
Base-L21A/Acid-p1	42	8.6
Base-p1/Acid-p1	65	11.6
Base-p1/pLEIM	62	11.7

urea denaturations of Base-L21A paired with either pLEIM or Acid-p1 were performed to gain insight into stability and specificity of these coiled-coils (Table 2). Base-L21A/pLEIM is 1.9 kcal/mol more stable than Base-L21A/Acid-p1. This result is consistent with previous host−guest studies involving a different heterodimeric coiled-coil by Vinson et al., who found that ***a-a′*** Ile-Ala pairing is significantly more stabilizing than a comparable Leu-Ala pairing.[9] Base-p1/Acid-p1 and Base-p1/pLEIM are nearly identical in stability; thus, the double-mutation of Leu_{21}→Ile and Leu_{24}→Met has little effect on pairing with Base-p1. Table 2 shows that while a single Leu→Ala mutation in Base-p1/Acid-p1 costs ∼3 kcal/mol in coiled-coil stability, two-thirds of this energy may be regained via appropriate core mutations. The stability of Base-L21A/pLEIM (30 residues/helix) is comparable to that of GCN4-p1, a 33-residue/helix homodimer (reported ΔG_{unf} of 10.6 kcal/mol).[1c,10]

The preference of Base-L21A for pairing with pLEIM vs Acid-p1 was further explored in a thiol-disulfide exchange assay. We prepared variants of these three peptides that a contain Cys-Gly-Gly sequence appended to the N-terminus (Base-L21A-SH, pLEIM-SH, and Acid-p1-SH). Oxidation of Base-L21A-SH with Acid-p1-SH gave the disulfide-bonded heterodimer Base-L21A-SS−Acid-p1. Mixing this dimer with an equal amount of pLEIM-SH in degassed PBS at pH 7.0 resulted in disulfide rearrangement to give Base-L21A-SS-pLEIM as the predominant dimer (Figure 3). The phage-derived coiled-coil pairing (Base-L21A-SS-pLEIM) is preferred over the mismatch dimer by ∼3:1 under these equilibrium conditions. Similar results were obtained by performing the assay in the opposite direction, beginning with Base-L21A-SS-pLEIM and Acid-p1-SH.[8]

Several groups have explored "steric-match" strategies to generate coiled-coils that contain alanine in the hydrophobic core. However, such approaches have produced stable structures only in systems with trimeric or higher oligomerization states[11a,b] or greater peptide length (six heptads).[11c] Furthermore, these studies have focused entirely on interactions between residues at lateral positions (e.g., Leu_{21} on Base-p1 with Leu_{21} on Acid-p1). The apparent ability of a modification in a neighboring side chain layer, Leu_{24}→Met in pLEIM, to participate in accommodating the Leu_{21}→Ala mutation on Base-L21A suggests that analysis of natural sequences and design efforts should not focus exclusively on lateral contacts.

Our results show that heterodimeric coiled-coil pairing selectivity can be profoundly influenced by nonobvious side-chain interactions in the nonpolar core and that phage display is an excellent technique for identifying such interactions. The "compromised" sequence, Base-L21A, appears to be quite discriminating in its pairing preference, but the preferred partner, pLEIM, seems to be promiscuous, as is Base-p1 itself. These observations suggest that insertion of compromising mutations on both partners may be an effective way to generate a coiled-coil in which both partners display high selectivity. Testing of this hypothesis and exploration of other implications of the results described here are underway.

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Supporting Information Available: Phage display procedures, experimental details for peptide synthesis and acquisition of CD data, thermal denaturation and sedimentation ultracentrifugation data. This material is available free of charge via the Internet at http://pubs.acs.org

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