ORGANIC LETTERS

2008
Vol. 10, No. 17
3797−3800

Simultaneous Directed Assembly of Three Distinct Heterodimeric Coiled Coils

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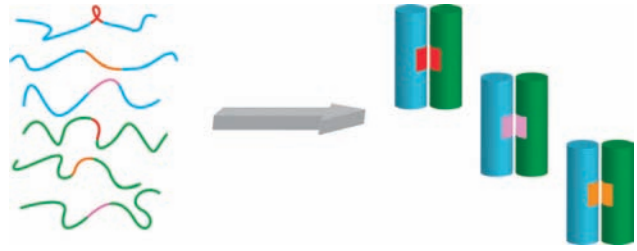
Received June 30, 2008

ABSTRACT



We describe simultaneous formation of three distinct heterodimeric coiled coils from a mixture of six different peptides. The choice among electrostatically viable complexes is governed by alignment of buried core residues, including a fundamentally new interaction that exploits urea-terminated side chains. Buried urea/urea contacts lead to extremely stable dimeric coiled coils, with T_m values between 63 and 79 °C. Core ureas can also form stable complexes with a variety of other polar groups, including guanidines, acids, and amides.

Programmed molecular self-assembly is a powerful route to otherwise prohibitively complicated structures, consistent with its extensive application in biological systems. Such natural scaffolds provide building blocks for advanced design.[1] As components are simplified, strategies for selective recognition become correspondingly more complicated. As such, introduction of new specificity elements, particularly those that complement rather than replace existing ones, significantly enhances downstream design capabilities.

Among nature-inspired recognition architectures, design and application of alpha helical coiled coils has drawn considerable attention. Such systems bundle supercoiled helical peptides, driven by hydrophobic packing of side chains alternately spaced by 3 or 4 residues. This pattern divides the sequence into seven-residue heptads ($a-g$) in which hydrophobic a/d side chains are often flanked by polar e/g residues that establish interhelical polar contacts.

Fundamental studies have produced well-characterized coiled coils of varying peptide number and orientation, formed by homo- or heteromeric strand binding and exhibiting a broad range of stabilities up to and exceeding those found in natural systems.[2] In turn, these basic efforts have facilitated application in protein folding models, materials chemistry, and biotechnology.[3]

This widespread utility underscores the value of new methods for specific coiled coil construction. We have recently deveolped new motifs derived from a well-known

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10.1021/ol801461a CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/12/2008

basic strategy: alignment of buried polar groups. A single polar side chain in a core *a* or *d* position of each component peptide reliably favors complexes juxtaposing those groups.[4] In this vein, we have recently demonstrated that variable-length guanidinylated core residues support formation of well-defined dimeric coiled coils with a variety of binding partners.[5] In particular, guanidine (Gdn)/aspartate pairs proved compatible with dimer formation in the presence of alternate partners bearing core asparagines, adding a significant dimension to possible assembly systems. Given this success, we sought to discover additional viable recognition partners and in particular focused on neutral pairs that might emulate the strong thermal stability of Asn/Asn contacts.

Urea/urea recognition has a vast portfolio of applications in synthetic self-assembly systems. Here we report application of buried urea recognition in heterodimeric coiled coil formation, with thermal stabilities up to and even slightly exceeding that of the corresponding Asn/Asn system ($T_m = 51-79$ °C vs 77 °C for the Asn pair). Most strikingly, use of a citrulline pair allows simultaneous formation of three distinct heterodimers from a six-peptide mixture, a problem of exponentially higher complexity than even the already-difficult four-component system previously reported.

Peptide sequences for probing core urea interactions were derived from the Acid-p1/Base-p1 heterodimer reported by Kim and co-workers, itself inspired by the coiled-coil domains of natural proteins (Fos, Jun, GCN4).[7] Each peptide contains a core urea side chain at position 14, spaced from the backbone by one (pUr), two (pUr*), or three (pCit) methylenes (Figure 1). Acidic and basic derivatives of each

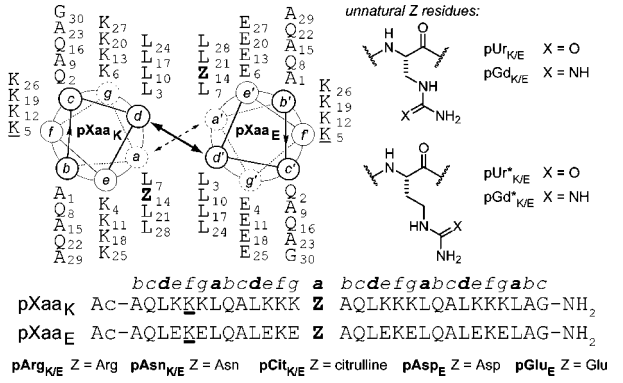

Figure 1. Peptides used. Helical wheel projection demonstrates interactions in a heterodimer containing one acidic (pXaa$_E$) and one basic (pXaa$_K$) peptide (general sequences below). Structures of unnatural buried polar residues for Ur/Ur*/Gd/Gd* peptides are as shown (position 14 in the sequence, denoted with a Z). Peptides with natural core polar residues are also listed. Underlined lysine side chains are capped with acetamidobenzoyl groups as spectroscopic labels.

sequence were prepared (denoted with E or K subscripts, respectively), in which all *e*/*g* positions contain Glu or Lys, to discourage homodimer formation. Interactions with natural polar residues and chain-shortened Arg derivatives were measured using some of our previously reported sequences.[5] Peptides were prepared via standard solid-phase methods, augmented by our methods for on-resin urea formation during solid-phase peptide synthesis.[8]

Our determination of urea-core feasibility began with evaluation of urea/urea contacts. By design, each acidic peptide (Glu at *e*/*g*) can pair only with one of the three basic ones (Lys at *e*/*g*), affording nine possible mixtures. Equimolar solutions of each combination examined by circular dichroism (CD) spectroscopy exhibited wavelength and thermal denaturation profiles consistent with coiled coil formation (Figure 2).[9] Significantly, spectra for 1:1 mixtures exhibited

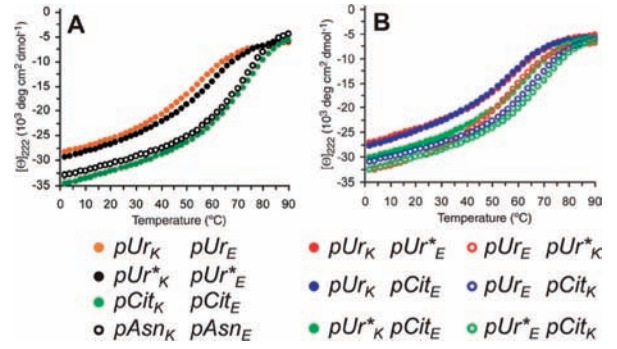

Figure 2. Coiled coils with buried urea side chains. Thermal denaturation CD curves for the indicated equimolar peptide mixtures with identical (A) or distinct (B) urea side chains at a core *a* position. In (B), closed/open circles of the same color denote complexes that differ by exchange of polar residue between acidic and basic sequences. The Asn/Asn dimer is given in (A) for comparison.[6]

room-temperature helicities and thermal stabilities well in excess of calculated component weighted average signals, consistent with complex formation.[10] Analytical ultracentrifugation supports dimer formation in all three like-group pairs, although the pUr$_K$/pUr$_E$ complex shows some evidence of higher-order behavior.[10]

(4) (a) Straussman, R.; Ben-Ya'acov, A.; Woolfson, D. N.; Ravid, S. *J. Mol. Biol.* **2007**, *366*, 1232–1242. (b) Lear, J. D.; Gratkowski, H.; Adamian, L.; Liang, J.; DeGrado, W. F. *Biochemistry* **2003**, *42*, 6400–6407. (c) Schneider, J. P.; Kretsinger, J. *J. Am. Chem. Soc.* **2003**, *125*, 7907. (d) McClain, D. L.; Gurnon, D. G.; Oakley, M. G. *J. Mol. Biol.* **2002**, *324*, 257–270. (e) Akey, D. L.; Malashkevich, V. N.; Kim, P. S. *Biochemistry* **2001**, *40*, 6352–6360. (f) Oakley, M. G.; Kim, P. S. *Biochemistry* **1998**, *37*, 12603–12610. (g) Zeng, X.; Herndon, A. M.; Hu, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3673–3678. (h) Schneider, J. P.; Lear, J. D.; DeGrado, W. F. *J. Am. Chem. Soc.* **1997**, *119*, 5742. (i) Gonzalez, L., Jr.; Woolfson, D. N.; Alber, T. *Nat. Struct. Biol.* **1996**, *3*, 1011–1018. (j) Lumb, K. J.; Kim, P. S. *Biochemistry* **1995**, *34*, 8642–8.

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(6) Solutions for all CD experiments contained 10 μM total peptide in PBS buffer (150 mM NaCl, 10 mM phosphate, pH 7.0). See Supporting Information for corresponding wavelength traces.

(7) O'Shea, E. K.; Lumb, K.; Kim, P. S. *Curr. Biol.* **1993**, *3*, 658–667.

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(9) See Supporting Information for wavelength traces and plots of 1:1 mixtures vs individual components and weighted averages.

(10) See Supporting Information for further details.

In general, use of a neutral polar group had the desired effect on heterodimer stability (T_m = 63−79 °C, Table 1).

Table 1. Observed T_m Values for Urea Core Pairs

sample	T_m (°C)	sample	T_m (°C)
pUr_K/pUr_E	63	$pUr_K/pCit_E$	63
pUr^*_K/pUr^*_E	63	$pUr^*_K/pCit_E$	67
$pCit_K/pCit_E$	79	$pUr_E/pCit_K$	71
pUr_K/pUr^*_E	63	$pUr^*_E/pCit_K$	75
pUr_E/pUr^*_K	65		

In particular, the $pCit_K/pCit_E$ sample displayed a T_m nominally higher than the parent $pAsn_K/pAsn_E$ system (79 vs 77 °C), a significant improvement over our previously most stable non-Asn core system (featuring a guanidine/carboxylate pair with T_m = 67 °C).[5] Any influence of polar residue side chain length on overall stability is subtle, with a general trend favoring longer chains that is less pronounced than it was in our earlier guanidine systems. In some contexts, however, chain length can be significant. Both the Ur/Cit and Ur*/Cit complexes are much more stable (ΔT_m = 8 °C in each case) when the shorter chain is attached to the acidic parent sequence (i.e., complexes formed using pUr_E or pUr^*_E rather than pUr_K or pUr^*_K). The analogous experiments with buried guanidine pairs revealed a much lower preference for one configuration over the other.[5]

After establishing the fundamental viability of urea-substituted core residues, we began to explore interactions between each of the three urea side chains and other known polar groups. To begin, we paired each urea with one of the three guanidinylated residues from our earlier work (positioning the guanidine 1−3 CH_2 groups from the backbone).[5] Thermal unfolding CD curves for the 18 possible combinations (nine each with the urea residues on the acidic or basic peptides, respectively) exhibit cooperative transitions and a broad range of melting temperatures (T_m = 51−75 °C).[10]

These guanidine/urea pairs exhibit an interesting preference for parent sequence identity, with universally higher T_m values observed when the guanidine residue is on the basic rather than acidic peptide. The strength of this effect is quite variable, with ΔT_m ranging from 2 to 13 °C.[10] A similar preference is exhibited by Gdn/Asn pairs but with less variability (ΔT_m = 6−10 °C).[5] The source of this selectivity is not obvious but may be useful as an empirical guide to future designs.

Given the selectivity in Gdn/Asn pairs, we next investigated heterodimers positioning each urea derivative opposite an asparagine. In contrast to the interaction of either group with guanidines, amide/urea pairs were insensitive to the parent strand, producing stable complexes in each case (T_m = 67−71 °C).[10]

Finally, we evaluated mixtures of each basic urea peptide and either $pAsp_E$ or $pGlu_E$, containing the indicated carboxylic acid side chain at position 14. Again, stable structures were observed (T_m = 55−67 °C), albeit somewhat less stable than the amide/urea ones. As with the guanidine core residues, little to no preference was exhibited for binding to either Asp or Glu partners.

Comparing the melting curves for each urea derivative with its nonurea partners reveals some general trends (Figure 3). The short-chain guanidines were generally the weakest

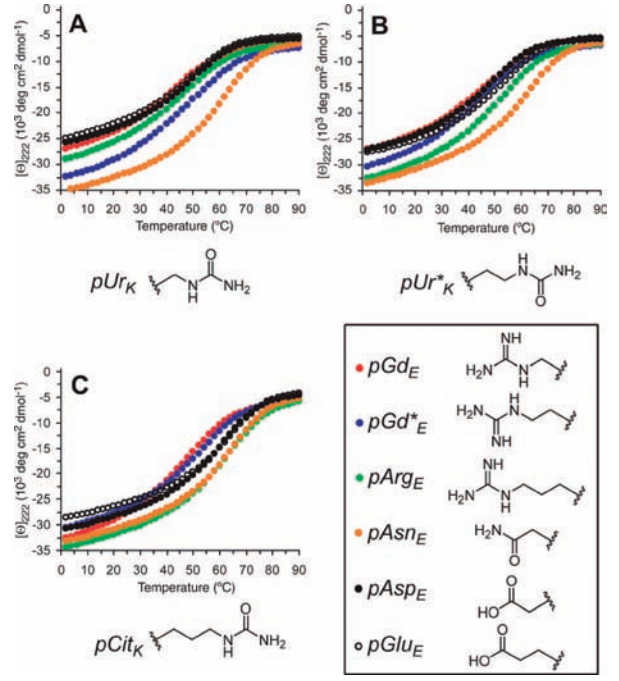

Figure 3. Urea/polar group interactions. Thermal stability of heterodimers formed between the indicated basic urea peptides and acidic peptides bearing a variety of polar groups.[6]

affinity partners, but similar length Asp and Glu residues were only a bit better. The neutral Asn contact consistently gives rise to the most stable complexes, but overall discrimination between groups tends to compress as the urea side chain lengthens, as evidenced by the similar affinity of $pCit_K$ for either $pArg_E$ or $pAsn_E$. Even pGd_E and pGd^*_E are reasonable partners for $pCit_K$, emphasizing that subtle structural changes can materially alter recognition profiles.

With all of these new buried polar group interactions catalogued, we turned our attention to their implementation as directing elements in multicomponent mixtures. The preference for self-recognition in the urea-functionalized peptides suggested that a urea/urea pair might be added to already successful four-component systems. The resulting six-peptide mixture can form nine different complexes that differ only by buried core residue juxtaposition (pairing each acidic and basic peptide). We hoped that orthogonal recognition would winnow observed dimers down to the designed three.

To determine binding preferences, we employed an affinity assay in which one peptide of the complex to be tested is derivatized with an N-terminal $GlyGly(His)_6$ sequence. An equimolar mixture of the tagged peptide and its intended

binding partner(s) is then exposed to His-binding Ni-NTA agarose beads. Following supernatant removal and washing, bound material is recovered by treatment with imidazole buffer, and each fraction is analyzed by HPLC.[10] Untagged peptide(s) stick to the beads (and appear in the elution fraction) only by virtue of a specific interaction with the tagged one. Those with no such affinity remain in the original supernatant.

Impressively, appropriate six-component systems do indeed form three independent heterodimeric coiled-coils, with little or no contamination from competing complexes (Figure 4). Mixtures of $pAsn_K/pAsn_E$, $pCit_K/pCit_E$, and $pGdn_K/pAsp_E$

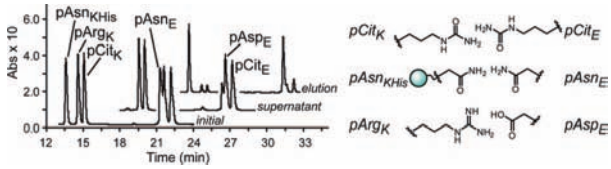

Figure 4. Ni-NTA analysis of six-component self-assembly. HPLC analysis of an equimolar $pAsn_{KHis}/pAsn_E/pCit_K/pCit_E/pArg_K/pAsp_E$ mixture demonstrates specific recognition: the elution fraction is dominated by Asn peptides. The initial mixture was 20 μM total peptide, pH 7.0, 150 mM NaCl, 10 mM phosphate. See Supporting Information for the other successful cases.

were investigated ($pGdn_K$ = any of the three guanidine peptides). The four noncitrulline components were shown previously to be specific, and in this case again tagging $pAsn_K$ peptides affords an elution fraction containing essentially only $pAsn_E$. All four of the other peptides remain in the supernatant. Separate analysis demonstrates that each undesired dimer is intrinsically viable absent the other peptides and that the four supernatant components exhibit high fidelity as well.[10] The overall system thus displays impressive selectivity. The specific guanidine peptide seems relatively unimportant.[11]

The difficulty in constructing a viable six-component recognition system is underscored by replacing the $pAsp_E$ peptide in the successful mixture with $pGlu_E$ (Figure 5).

(11) The results seem a bit cleaner with pArgK, though the four-component analysis of the non-asparagine peptides is slightly less selective in that case. See Supporting Information for details.

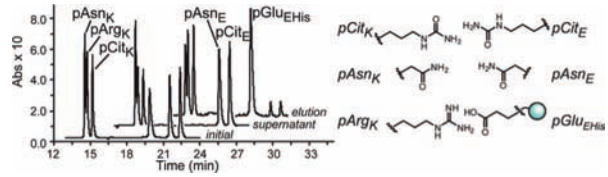

Figure 5. Unsuccessful six-component recognition experiment. An equimolar mixture of $pAsn_K/pAsn_E/pCit_K/pCit_E/pArg_K/pGlu_{EHis}$ fails to exhibit specific recognition. The elution fraction contains substantial amounts of all three basic peptides, demonstrating that Glu is not an appropriate binding partner for Arg in this context, despite the success of Asp. Initial solutions were prepared as in Figure 4.

Despite the subtle structural change, essentially all specificity is removed. Each of the two other possible binding partners for each component of the tagged dimer are observed in both supernatant and elution fraction.

The results above dramatically expand the capacity of simultaneous recognition designs in dimeric coiled coil formation. Buried ureas are compatible with exceptionally stable heterodimers, and the peptides are trivially synthesized. Ureas are also good partners for guanidines, amides, and acids, enabling many new designs. Although dimer stability generally increases with polar side chain length, the correlation is loose, and considerable T_m variance is observed with subtle modifications. This permits need-specific tuning of complex stability. As a demonstration that new polar pairs enable otherwise challenging recognition events, we have demonstrated simultaneous and specific recognition of up to three distinct heterodimers driven solely by core matchups. Given the ubiquitous application of coiled coil formation in fundamental and applied contexts, this capacity should find considerable use.

Acknowledgment. This work was supported by an NSF CAREER award to A.J.K. (CHE-0239275).

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

OL801461A