Peptide Self-Replication Enhanced by a Proline Kink

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The influence of proline on the conformation of helical regions of proteins and peptides has been well documented.[1] Helices containing proline after the fourth position have a pronounced kink, with bending of the helical axis of approximately 30° away from the side with the proline residue.[1,2] Incorporating proline within a peptide derived from the leucine zipper motif of gp41,[3a] or a model coiled-coil,[3b] demonstrated kinking of the helix in the region of the proline residue and reduced coiled-coil stability. Herein we disclose the effect of a proline kink on coiled-coil, self-replicating peptides.

One of the major challenges in the design of self-replicating molecules is product inhibition.[4] The self-assembly that is exploited to promote self-replication may be its downfall if dissociation of the product/template complex is limited. A self-replicating oligo-nucleotide has been reported that overcomes product inhibition through solid-supported cycling,[5] whereas a highly efficient self-replicating peptide has been described based on reducing the number of heptad repeats within a coiled-coil.[6] The difficulty in limiting product inhibition by weakening the product/template complex is that essential interactions between the peptide fragments and template may also be weakened. Incorporating a proline kink at the center of the template (Figure 1), however, seemed an ideal design as the fragments should maintain all interactions on either side of the kink, but the product of the self-replicating reaction would have reduced affinity for the template.

Two proline-containing, coiled-coil peptides were designed (XL-1 and XL-2) based on the self-replicating peptide E1E2.[7] Within XL-1 a proline replaced Leu 19 of E1E2, whereas in XL-2 the Glu20 residue was replaced with proline. In this way, the effect of the placement of proline within the coiled-coil sequence (hydrophobic d position versus hydrophilic e position) could be evaluated. Two peptide fragments were synthesized for each peptide, one containing a C-terminal thioester (XL-3) and one containing an N-terminal cysteine (XL-4 and XL-5), for the native chemical ligation reaction.[8]

Circular dichroism was used to evaluate the helical content of the proline-containing peptides. XL-1 displayed a somewhat lower helical content as compared to XL-2 (45% and 55%, respectively, at 200 μM). The helical contents of the peptide fragments of XL-1 and XL-2 both increased upon addition of the corresponding template; the fragment XL-3 increased by 39% and 38%, respectively, whereas the fragments XL-4 and XL-5 increased by 42% and 19%, respectively. These data indicate that both peptides adopt a helical conformation that is conducive for templating the fragments, a condition that is necessary for self-replication. For comparison, the helical content of E1E2 under identical conditions was 85%;[7] the replacement of Leu19 or Glu20 with proline did affect the helical content, presumably due to distortions in the helix.

Analytical ultracentrifugation was used to analyze the self-assembly of XL-1 and XL-2. Whereas XL-1 was found to aggregate as an octamer, XL-2 was found to exist as a tetramer. Interestingly, by comparison, E1E2 was found to form a dimeric coiled-coil.[7]
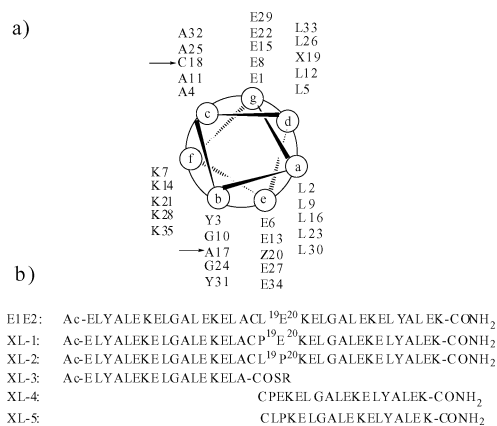

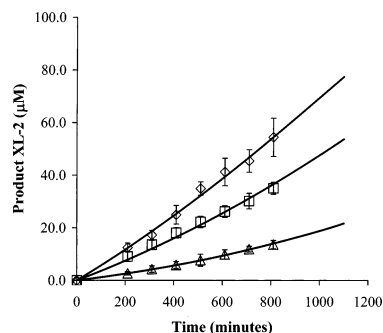**Figure 1.** Helical wheel (a) and sequences (b) of XL-1 and XL-2.

Figure 2. XL-2 production from two fragments (500 μM each) as a function of reaction time with varying initial concentrations of template at pH 4.0 and 23 °C: ($\triangle$) no template, ($\square$) 25 μM template, and ($\diamond$) 50 μM template. Error bars reflect three independent experiments. Curves were generated using the program SimFit.[9] Buffer: 100 mM MOPS with 1% 3-mercapto-propionic acid.

The stability of XL-1 and XL-2 was compared to that of E1E2 using thermal denaturation (all peptides at 20 μM). Melting temperature of 45 and 75 °C were obtained for XL-1 and XL-2, respectively, whereas E1E2 remained greater than 75% folded at 75 °C. These data confirm that the addition of a proline residue to the coiled-coil at the hydrophobic d position has a much greater effect on decreasing the melting temperature than replacement at the hydrophilic e position. A similar decrease has been reported when proline is placed in the f position of a coiled-coil peptide.[3b]

With these data in hand, the self-replicating properties of XL-1 and XL-2 were investigated (Figure 2). Autocatalysis in the formation of XL-2 from XL-3 and XL-5 was unambiguously established by performing the fragment ligation in the presence of increasing amounts of the template XL-2. The reaction was accelerated by the presence of template; increasing the amount of XL-2 in the reaction mixture increased the initial rate of XL-2 formation. Alternatively, the reaction between the XL-3 and XL-4 was very slow, with less than 5% of product formed within 24 h,
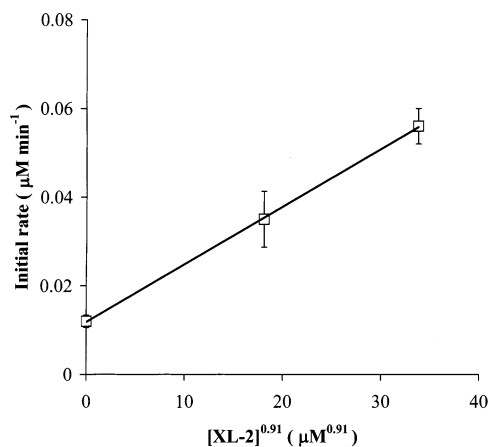
Figure 3. Initial rate of XL-2 formation as a function of added template to the power of 0.91.

Figure 4. Schematic of the effect of proline substitution (black circle) on the direction of the kink within XL-1 (a) and XL-2 (b) with respect to the hydrophobic surface of leucine residues (gray circles).

and added template had no effect on product formation (data not shown).

The experimental data for XL-2 were analyzed with the program SimFit based on the empirical equations developed by Kiedrowski.[9] This analysis provided an apparent autocatalytic rate constant, k_a, of 24.0 ± 1.2 $M^{-1.91}$ s^{-1}, and a noncatalytic rate constant, k_b, of $7.5 \pm 0.4 \times 10^{-4}$ M^{-1} s^{-1}. This corresponds to a catalytic efficiency ($\epsilon = k_a/k_b$) of 3.2×10^4, a value that is 260-fold greater than that obtained for E1E2,[7] and is comparable to the catalytic efficiency obtained when the coiled-coil was shortened to destabilize interactions.[6] The order of the self-replicating reaction was determined by finding the best fit for the catalytic and noncatalytic reaction rates using SimFit, and was found to be 0.91. A linear relationship was also observed between the initial rate for each reaction as a function of the concentration of the template to the power of 0.91 (Figure 3). A self-replicating tetramer such as XL-2 should exhibit a reaction order (p) of 0.75 if the system was subject to product inhibition.[9,10] XL-2, however, displayed a much higher reaction order, thereby classifying the replication as weakly exponential ($0.75 < p < 1$).[9,10]

A very interesting contrast is observed between XL-1 and XL-2 with respect to self-replication. On one hand, both peptides are able to template an increased helical content in their corresponding peptide fragments. On the other hand, the fragments of XL-1 show little propensity for ligation, even in the presence of template, whereas XL-2 demonstrates a high catalytic efficiency for self-replication. These results are quite remarkable when one considers that the only difference between XL-1 and XL-2 is the positioning of the proline at adjacent amino acids (Leu19 and Glu20) within the E1E2 core structure. According to literature precedent, these differences should result in a bending of the helical axis of approximately 30° away from the side with the proline residue.[2] For XL-1 this would create a break in the hydrophobic surface of the coiled-coil, with the two segments being directed away from one another (Figure 4). Presumably this conformation would allow
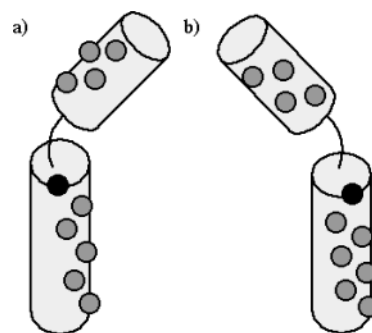
the peptide fragments to bind to each segment of the template, but may place the termini in a poor orientation for ligation. The proline replacement in the hydrophilic face within XL-2, however, would create a bent, but continuous, hydrophobic surface which may be more conducive to ligation between bound fragments.

In conclusion, the use of a proline kink has been shown to promote highly efficient peptide self-replication. However, the placement of the proline kink within the coiled-coil is a critical factor in the success of this strategy. The ability to generate a highly efficient self-replicating system with a single amino acid modification demonstrates the promise for developing replicators with exponential growth in the future.

Supporting Information Available: Characterization data (mass spectral, circular dichroism, SimFit file, and analytical ultracentrifugation) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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