J|A|C|S
COMMUNICATIONS

Published on Web 05/22/2002

Approaching Exponential Growth with a Self-Replicating Peptide

Roy Issac and Jean Chmielewski*

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

Received February 25, 2002

Self-replicating peptide systems hold great promise for a wide range of technological applications, as well as to address fundamental questions pertaining to the molecular origins of life.[1] The development of peptide self-replicators, however, requires high catalytic efficiency that is highly dependent on the stability of the product−template complex.[2] Under optimum conditions and in the absence of product inhibition, self-replicating systems should exhibit exponential growth while product inhibition causes growth to be parabolic for dimeric systems. The design of self-replicating compounds capable of high efficiency has remained elusive, although recently Kiedrowski and co-workers reported the development of a self-replicating DNA analogue in which solid-phase cycling circumvented product inhibition.[3] Here we describe the development of a highly efficient peptide self-replicator with a catalytic enhancement that is close to that of known enzymes.

In an effort to improve the catalytic efficiency of the self-replicating peptide E1E2,[4] we sought to destabilize its coiled coil structure. Fairman and co-workers achieved dramatic decreases in stability for tetrameric coiled-coil peptides by shortening the chain lengths,[5] and Hodges and co-workers found similar effects with dimeric coiled coil peptides.[6] With these precedents as a basis, a peptide was designed for self-replication, RI-26, that contains 3 full heptad repeats within the coiled coil, one shorter than the original E1E2 sequence. RI-26 maintained the design principle of E1E2 in that glutamate residues were positioned at the **e** and **g** positions of the helical heptad repeats to achieve pH-based control over helicity (Figure 1). RI-26a and RI-26b, therefore, correspond to the two fragments of RI-26 that may undergo thioester mediated chemical ligation to produce RI-26.[7]

The full length template, RI-26, was found to adopt a helical conformation in a pH-dependent fashion; at pH 7.0 the helical content was only 28% as determined by circular dichroism, and increased to 87% when the pH was lowered to 4.0 as was observed with E1E2.[5] The helicity of RI-26a and RI-26b increased by 45% and 16%, respectively, in the presence of RI-26 at pH 4.0, indicating the ability of RI-26 to act as a template for its fragments. Analytical ultracentrifugation was used to determine the aggregation state of RI-26 at pH 4.0, and interestingly this peptide was found to exist as a tetramer. E1E2 by contrast exists as a dimer under similar conditions.

To determine if RI-26 had self-replicating properties, the fragments RI-26a and RI-26b were incubated at pH 4.0 with and without added RI-26. As is indicative of an autocatalytic system,[2] adding increasing amounts of the template led to a dramatic acceleration in product formation (Figure 2). By contrast, the reaction at pH 7 was insensitive to added template and, therefore, not autocatalytic. The experimental data were analyzed with the program SimFit based on the empirical equations developed by Kiedrowski (Figure 2).[2] The SimFit analysis provided an apparent

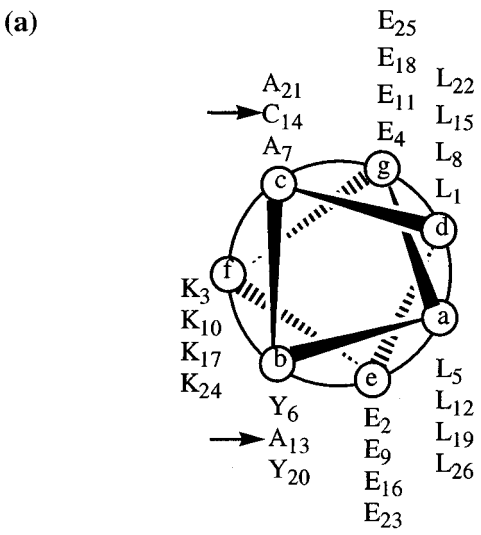

Figure 1. Helical wheel diagram (a) and sequence (b) of RI-26 and its fragments. An arrow indicates the residues where chemical ligation occurs.

catalytic rate constant, k_a, of 50.6 ± 0.5 $M^{-1.91}$ s^{-1} and a noncatalytic rate constant, k_b, of 5.04 ± 0.03 × 10^{-4} M^{-1} s^{-1} with a catalytic efficiency ($\epsilon = k_a/k_b$) of 1.0 × 10^5. This is a remarkably efficient system when compared to other self-replicating molecules; self-replicating peptides and oligonucleotides have displayed catalytic efficiencies in the range of 24 to 3700.[4,8] The efficiency observed with RI-26 is comparable to that observed for some enzymatic systems, such as glutathione transferases.[9] The unin-structed noncatalytic or background reaction, presumably a result of the association between the two fragments, is also much slower in this peptide system than any of the other reported peptide self-replication systems.[4,8e−f] This is most likely due to the presence of fewer leucine residues in the shorter fragments, thereby reducing the hydrophobic interactions between them.

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 ± 0.04. A linear relationship was also found between the initial rate for each reaction as a function of the concentration of the template to the power of 0.91 (Figure 3). Since RI-26 forms a tetramer in solution, one possible ligation complex would be composed of RI26a·RI26b·$(RI\text{-}26)_3$. For a self-replicating tetramer the reaction order (p) would be expected to be 0.75 if the system exhibited product inhibition.[2,8a] For RI-26 a significantly higher reaction order was observed, thereby classifying this replicating system as weakly exponential ($0.75 < p < 1$). This is the first self-replicating system to date that

* Corresponding author. E-mail: chml@purdue.edu.

10.1021/ja026024i CCC: $22.00

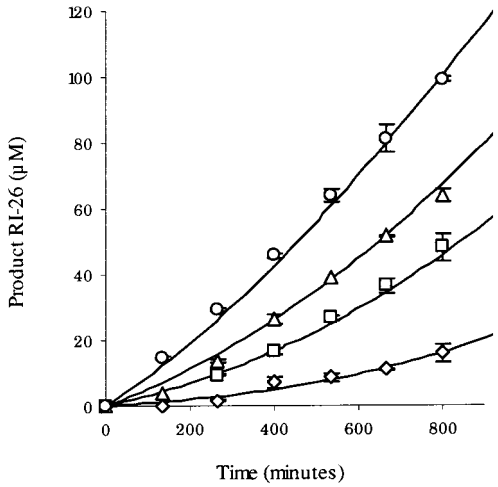

Figure 2. RI-26 production from two fragments, RI-26a and RI-26b (500 μM each), at 23 °C in 100 mM MOPS buffer (with 1% 3-mercaptopropionic acid) at pH 4.0 as a function of time with varying initial concentrations of template: (◇) no template, (□) 10 μM RI-26, (△) 20 μM RI-26, and (○) 40 μM RI-26. Error bars reflect standard deviations of two independent experiments. Curves were generated with SimFit[2] by simulations based on the reaction model: RI-26a + RI-26b → RI-26 (k_b); RI-26a + RI-26b + 0.91 RI-26 → 1.91 RI-26 (k_a).

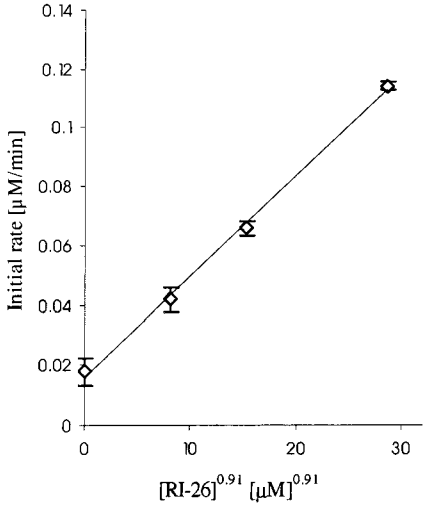

Figure 3. Initial rate of RI-26 formation as a function of the concentration of added template to the power of 0.91.

has attained a reaction order higher than the product inhibited order. One potential cause of the reduction in product inhibition would be a reduction in the stability of the tetrameric product of RI-26 as compared to the dimeric product of E1E2 self-replication. To address this hypothesis the thermal denaturation of both peptides was examined by circular dichroism (Figure 4).[5,6] The melting temperature of RI-26 displayed an approximately 20 °C shift to lower temperature as compared to E1E2, confirming the significant reduction in stability of the coiled-coil of RI-26.

In conclusion, we disclose a successful strategy whereby modulation of coiled-coil stability results in remarkable catalytic

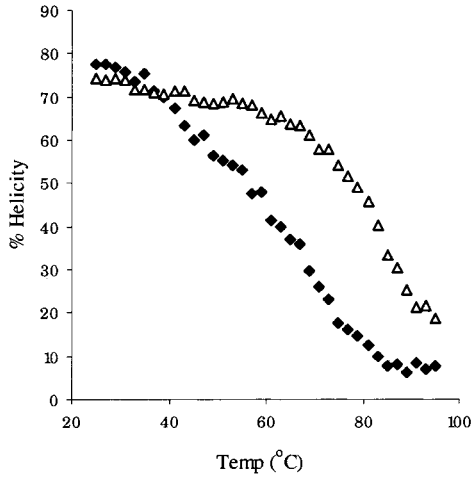

Figure 4. Thermal denaturation of 20 μM E1E2 (△) and RI-26 (◆) in the presence of 6 M GdnHCl in a pH 4.0, 100 mM MOPS buffer.

efficiency for self-replication. By shortening the peptide to the minimum length necessary for coiled-coil formation a highly efficient self-replicating system was obtained due to very low background reaction rates, bringing the efficiency close to naturally occurring enzymes. An added benefit of the reduction in peptide size was a decrease in the stability of the coiled-coil leading to effective suppression of product inhibition. The design of auto-catalytic peptides with high efficiencies and low product inhibition enhances their potential for future technological applications and their consideration as pre-biotic precursor molecules.[10]

Acknowledgment. The authors are grateful to NSF (78923) and NASA for support of this work. We are also grateful to Prof. von Kiedrowski for the generous gift of the software program SimFit.

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

References

(1) Issac, R.; Ham, Y.-W.; Chmielewski, J. A. *Curr. Opin. Struct. Biol.* **2001**, *11*, 458.
(2) von Kiedrowski, G. *Bioorg. Chem. Front* **1993**, *3*, 113.
(3) Luther, A.; Brandsch R.; von Kiedrowski, G. *Nature* **1998**, *396*, 245.
(4) Yao, S.; Ghosh, I. G.; Zutshi, R.; Chmielewski, J. *J. Am. Chem. Soc.* **1997**, *119*, 10599.
(5) Fairman, R.; Chao, H.-G.; Mueller, L.; Lavoie T. B.; Shen, L.; Novotny J.; Matsueda G. R. *Protein Sci.* **1995**, *4*, 1457.
(6) Su, J. Y.; Hodges, R. S.; Kay, C. M. *Biochemistry* **1994**, *33*, 15501.
(7) Dawson, P. E.; Muir, T. W.; Clark-Lewis, L.; Kent, S. B. H. *Science* **1994**, *266*, 776.
(8) (a) von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1986**, *28*, 1235. (b) von Kiedrowski, G.; Wlotzka, B.; Helbing J.; Matzen, M.; Jordan, S. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 423. (c) Huc, I.; Pieters, R. J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 10296. (d) Lee, D. H.; Granja, J. R.; Martinez, J. A.; Severin, K.; Ghadiri, M. R. *Nature* **1996**, *382*, 525. (e) Severin, K.; Lee, D. H.; Martinez, J. A.; Ghadiri, M. R. *Chem. Eur. J.* **1997**, *3*, 1017. (f) Yao, S.; Ghosh, I.; Zutshi, R.; Chmielewski, J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 478.
(9) Hanson, L. O.; Windersten, M.; Mannervik, B. *Biochemistry* **1997**, *36*, 11252−11260.
(10) Rode, B. M. *Peptides* **1999**, *20*, 773−786.

JA026024I