Pergamon

Tetrahedron Letters 39 (1998) 3911–3914

TETRAHEDRON LETTERS

A Novel Method to Synthesize Cyclic Peptides

Yang Shao, Wuyuan Lu and Stephen B. H. Kent

Gryphon Sciences, 250 E. Grand Ave. Suite 90, South San Francisco, CA 94080

Received 29 October 1997; revised 26 February 1998; accepted 1 April 1998

Summary: *Successful synthesis of cyclic peptides resembling the binding loop of Eglin c, a serine protease inhibitor, provides extended native chemical ligation as a novel method to synthesize cyclic peptides.* © 1998 Elsevier Science Ltd. All rights reserved.

Cyclic peptides, being constrained conformationally and more resistant to protease digestions than their linear counterparts, thus having increased biological specificity, activity and metabolic stability, have been applied extensively in biological studies.[1] The conventional ways to cyclize peptides were limited to either formation of cyclic lactam on protected peptides in organic solution[2] or formation of disulfide bonds on unprotected peptides in aqueous solution.[3] Recently, Tam and coworkers[4] synthesized cyclic peptides through thiazolidine formation of unprotected peptides in aqueous solution. Interestingly, no by-products from polymerization was observed, presumably due to the more favored intramolecular reaction. Native chemical ligation[5] has been used[6] to achieve the same goal even without introducing a pseudo-proline residue at the cyclization site.[4] However, as was in the case of the thiozalidine chemistry, a cysteine residue at the site of cyclization is an absolute necessity. Herein, we describe a novel method to synthesize cyclic peptides by chemoselective ligation in aqueous solution without the necessity for cysteines in the amino acid sequence.

Extended native chemical ligation[7] has been proven useful in ligating short unprotected peptide segments at the site of Xxx-Gly (Xxx = amino acid), thus extending the native (amide-forming) chemical ligation technique beyond the Xxx-Cys ligation site. A removable auxillary group, namely oxyethanethiol, on the α-amino group of the N-terminal glycine is employed to mimic the side chain of N-terminal cysteine in native chemical ligation.[5] We have applied the same auxillary functional group chemistry to cyclizing peptides. The sequence of the cyclic peptides reported here was adopted from the Leu37 to Arg53, the binding loop region of Eglin c, a serine protease inhibitor.[8] N^{α}(oxyethanethiol)-glycine was introduced at the amino terminus and glycyl-, alanyl- or phenylalanyl-α-carboxythioester was introduced at the carboxy terminus of the peptides, respectively (Scheme 1). During the process of cyclization, a cyclic thioester intermediate was produced via the thioester transesterification between the amino terminal thiol and carboxy terminal thioester, which then underwent S-N acyl rearrangement to give the amide-linked cyclic peptide, as also reported by Canne and coworkers[7] in their

PII: S0040-4039(98)00725-4

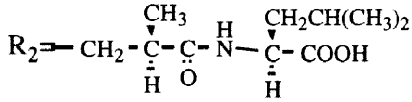
study of ligation of two linear peptides. The N-α-oxyethanethiol group was then removed by zinc dust reduction in dilute acidic solution to give the native backbone structure of the cyclic peptide (Scheme 2).

Scheme 1. Sequence of the Peptides*

X-LeuProGluGly•SerProValThrLeu•AspLeuArgTyrAsn•ArgValArg-NHCHR$_1$CO-SR$_2$

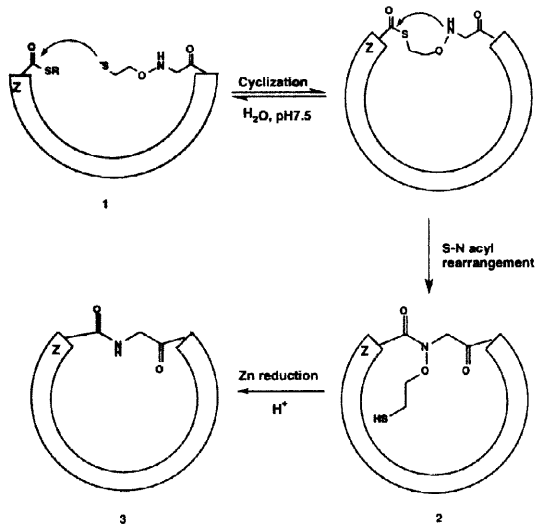
1

X = N$^{\alpha}$(oxyethanethiol)-glycine; 1a: R$_1$ = H, b: R$_1$ = CH$_3$, c: R$_1$ = CH$_2$Ph;



*All amino acids are in L configuration unless specified otherwise.

Scheme 2. Synthesis Of Cyclic Peptides Using Extended Native Chemical Ligation

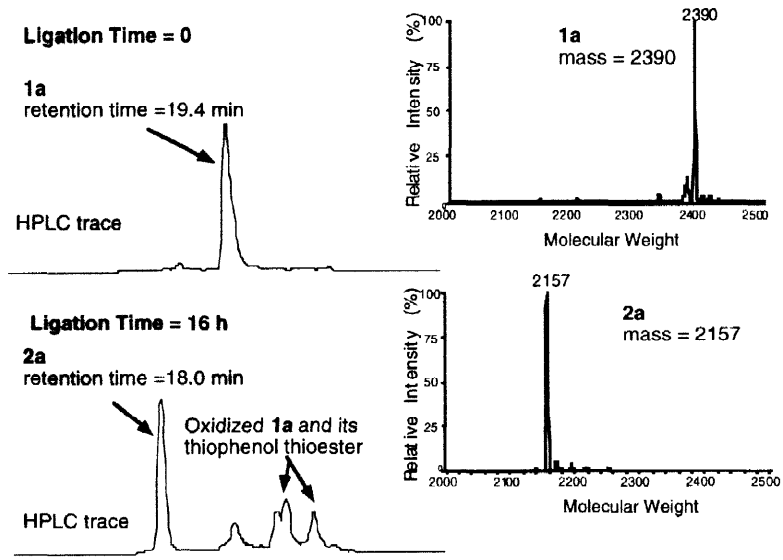


3a: Z = Gly; b: Z = Ala; c: Z = Phe

Interestingly, differences in the rates of S-N acyl rearrangement during the cyclization of these peptides were observed. In Gly-Gly ligation, the rearrangement was complete in 16 h (Figure 1). However, in the cases of Ala-Gly and Phe-Gly ligations, overnight reaction gave two products of the same mass, presumably one with the amide and one with the thioester backbone (data not shown). Only lowering the pH to 4.5 and incubating for 48 hours could drive the rearrangement to completion.[7] No by-product from polymerization was observed in either case, presumably for the same reason as outlined in Tam's method.[4,6]

In order to address the possibility of racemization of the C-terminal residue of the thioester peptides under ligation conditions, peptide 1c containing D-Phe$^{\alpha}$COSR at the ligation site was also synthesized and cyclized in the same way as peptide 1c containing L-Phe$^{\alpha}$COSR. An analysis using the C18 reverse-phase HPLC column found the two cyclic diastereomers comigrated on the column. However, after trypsin digestion of the cyclic petides, the resulting two linear diastereomeric 14 residue peptides could be separated under a gradient of 5-65% B over 30 min (data not shown), thus enabling us to conclude that no detectable (<5%) amount of recemization at Phe had occured under the ligation/cyclization conditions used. This is consistent with a previous study of ligation of linear peptides in which no detectable (<1%) racemization was found.[9]

Figure 1. Cyclization and S-N Acyl Rearrangement of Peptide **1a**.



Binding affinities to chymotrypsin of the eglin c related cyclic peptides produced in this study were measured and found to be five orders of magnitude weaker than that of wild-type Eglin c (Table 1),[10] presumably due to the lack of protein molecular scaffold to stablize the structure of the binding loop.

Table 1. Summary of Synthesis of Cyclic Peptides

Cyclic peptide	Cyclization	Rearrangement	HPLC Tr (min)	Mass	Yield (%)*	Ka
3a	4 hrs	O/N	17.0	2081.1	41.2	10^6
3b	O/N	48 hrs at pH 4.5	16.7	2095.1	18.1	10^6
3c	O/N	48 hrs at pH 4.5	18.6	2171.2	35.8	10^6

*Based on the recovered weight of purified **3**.

Synthesis of the N^{α}-oxyethanethiol C-α-carboxythioester peptides: C-α-carboxythioester linker was coupled to the resin by the method of Canne.[7] Peptides were synthesized in stepwise fashion by manual solid phase methods using in situ neutralization/HBTU activation protocols for Boc chemistry[11] until the penultimate amino acid was coupled to the resin. Then N^{α}-oxyethanethiol glycyl residue was coupled to the resin by the method of Canne.[7] Peptides were deprotected and simultaneously cleaved from the resin by treatment with HF containing 5% *p*-cresol for 1 h at O°C. After HF was removed under reduced pressure, the crude peptide was precipitated by anhydrous Et_2O, dissolved in HPLC buffer (50%B), lyophilized and purified by HPLC.

Cyclization of the peptides by chemical ligation and removal of N^{α}-oxyethanethiol from cyclic peptides: purified N^{α}-oxyethanethiol C-α-carboxythioester peptides were dissolved in 6 M guanidine•HCl, 0.3 M sodium phosphate, pH 7.5 buffer solution to a 1-2 mmol concentration. 0.5% v/v thiophenol was added and the mixture was stirred at room temperature for 8-16 hours, Then in the case of 1b and 1c, 4 volumes of 6 M guanidine•HCl, 0.1 M sodium acetate, pH 4.0 buffer solution was added and the mixture was stirred at room temperature for 48 hours. Cyclic peptides were isolated by HPLC and N^{α}-oxyethanethiol group was removed by zinc reduction in acetic condition by the method of Canne.[7]

In summary, we have shown that extended native chemical ligation provides a novel method to synthesize cyclic peptides with amide backbones, without the neccesity of a Cys residue.

References and Notes:

1. Hruby, V. J. *Life Science* **1982**, *31*, 189-199
2. Chorev, M.; Roubini, E.; McKee, R. L.; Gibbons, S. W.; Goldman, M. E.; Caulfield, M. P.; Rosenblatt, M. *Biochemistry* **1991**, *30*, 5968-5974
3. Scarborough, R. M.; Naughton, M. A.; Teng, W.; Rose, J. W.; Phillips, D. R.; Nannizzi, L.; Arfsten, A.; Campbell, A. M.; Charo, I. F. *J. Biol. Chem.* **1993**, *268*, 1066-1073
4. Botti, P.; Pallin, T. D.; Tam, J. P. *J. Am. Chem. Soc.* **1996**, *118*, 10018-10024
5. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776-779
6. Zhang, L.; Tam. J. P. *J. Am. Chem. Soc.* **1997**, *119*, 2363-2370
7. Canne, L. E.; Bark, S. J.; Kent, S. B. H. *J. Am. Chem. Soc.* **1996**, *118*, 5891-5896
8. (a) Bode, W.; Papamokos, E.; Musil, D. *Eur. J. Biochem.* **1987**, *166*, 673-692 (b) McPhalen, C. A.; James, M. N. G. *Biochemistry*, **1988**, *27*, 6582-6598
9. Lu, W.; Qasim, M. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **1996**, *118*, 8518-8523
10. Qasim, M. A.; Ganz, P. J.; Saunders, C. W.; Bateman, K. S; James, M. N. G.; Laskowski, Jr., M. *Biochemistry* **1997**, *36*, 1598-1607
11. Schnölzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Peptide Protein Res.* **1992**, *40*, 180-193