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Sequence Dependence in Solid-Phase-Synthesis-Cyclization-Cleavage for *Cyclo*(-arginyl-glycyl-aspartyl-phenylglycyl-)

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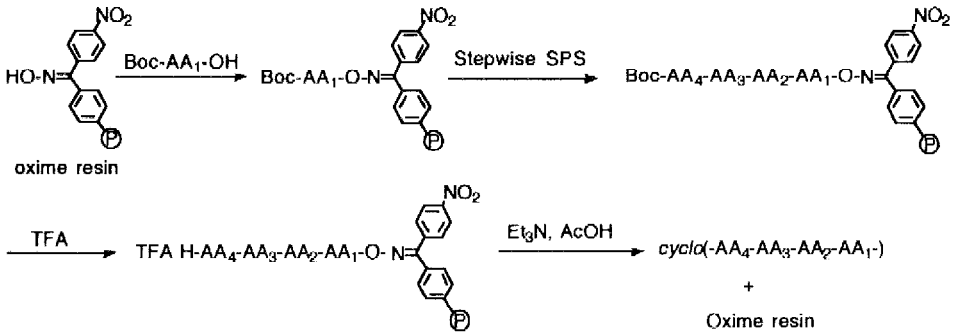
Key Words: peptide synthesis; oxime resin; solid-phase-synthesis and cyclization-cleavage; cyclic tetrapeptide, Arg-Gly-Asp sequence

Abstract: *A cyclic tetrapeptide with inhibitory activity toward cell adhesion , cyclo(-Arg-Gly-Asp-Phg-) (phg, phenylglycine) (IC_{50} = 10 μM), was synthesized. The cyclization-cleavage of the protected precursor from the oxime resin significantly depended on the sequences of the linear tetrapeptides assembled by solid-phase synthesis on the resin.*

A convenient system for the synthesis of cyclic peptides has been recently reported by Ösapay *et al.*[1] It utilizes the lability of the ester linkage on the Kaiser's oxime resin[2] to the nucleophilic attack. We have also independently found out the high utility in the solid-phase-synthesis and cyclization-cleavage (SPS-CC) method by the same principle and applied for the syntheses of some cyclic peptides which include a gramicidin S analog[3], aminosuberic acid-containing cyclic portions of deaminodicarba-oxytocin and elcatonin[4], and iturin A analogs.[5]

After an appropriate peptide sequence is assembled by SPS on the resin, the deprotected amino group can react mildly with its anchoring active ester linkage to close the loop. These continuous procedures allow us to omit the activation of the *N*-protected linear peptide as an active ester (for instance, *N*-hydroxysuccinimide ester) and high dilution condition to avoid the polymerization. Accordingly, for the further demonstration of the great advantages of the SPS-CC method in practical preparation of cyclic peptides, we chose *cyclo*(-Arg-Gly-Asp-Phg-) (Phg, phenylglycine), a possible inhibitor toward cell adhesion, which was synthesized by the conventional method with 10% yield in the cyclization step.[6] The cyclization of such a short peptide usually faces the steric problem and often results in low yield. During the optimization of the condition in SPS-CC for cyclic tetrapeptides containing Arg-Gly-Asp sequence, we experienced significant difference in yield depending on the sequence.



AA_4	AA_3	AA_2	AA_1	
Arg(Tos)	Gly	Asp(OcHex)	Phg	**1**
Phg	Arg(Tos)	Gly	Asp(OcHex)	**2**
Asp(OcHex)	Phg	Arg(Tos)	Gly	**3**
Gly	Asp(OcHex)	Phg	Arg(Tos)	**4**

Fig. 1. Solid-phase-synthesis and cyclization-cleavage for *cyclo*(-Arg(Tos)-Gly-Asp(OcHex)-Phg-). AA, amino acid; cHex, cyclohexyl; Phg, phenylglycine; Tos, *p*-toluenesulfonyl.

Therefore, to investigate systematically the sequence dependence in cyclization which is not described in the literatures,[1] four different tetrapeptides (Fig. 1) were separately assembled by the SPS on *p*-nitrobenzophenone oxime resin with the [(benzotriazol-1-yl)oxy]-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent.[7,8] The Kaiser test[9] for each coupling step indicated that the reaction proceeded quantitatively. After the *N*-terminal Boc groups were removed with 25% trifluoroacetic acid (TFA) in dichloromethane (DCM), Et_3N and AcOH (2.0 equiv. each) were added to the suspension of the peptide resin in dimethylformamide (DMF). The reaction mixtures were shaken at room temperature and periodically analyzed by reversed-phase (RP)-HPLC to monitor the progress of the cleavage reaction.

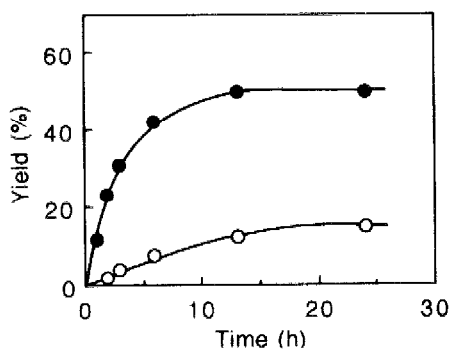


Fig. 2. Time course of the cyclization-cleavage for the protected cyclic tetra- (●) and octa-peptides (○).

The typical progress curves for the CC for *cyclo*(-Arg(Tos)-Gly-Asp(OcHex)-Phg-) and concurrent appearance of the cyclic dimer, *cyclo*(-Arg(Tos)-Gly-Asp(OcHex)-Phg-)$_2$, from the sequence **1** were given in Fig. 2.[10] After 24 h, the products were isolated by the removal of the solvent and precipitation with water, and analyzed for purity. The total yields and contents of the monomer and the dimer were summarized in Table 1. The sequence **1** with Phg at the *C*-terminal gave the best result among the four cases with abundance in monomer (75%) and with little dimer (20%) in the good total yield (65%). The cyclization from the sequence **2** was less successful. The sequence **3** gave increased amount of the dimer with the decrease in the yield of the monomer. The sequence **4** produced only trace amount of cyclic peptides. The majority of the product was eluted much faster than the cyclic peptides in RP-HPLC.

Table 1. Yields and Contents of the Cyclic Tetrapeptide Synthesized from Four Different Sequences by the SPS-CC.

Sequence	Yield (%)	Content (%)[a]	
		monomer	dimer
1	65	75	20
2	65	50	25
3	70	40	40
4	53	10	ND[b]

[a] Determined by RP-HPLC. [b] Not detected.

The varying proportions of the contents of cyclic monomer and dimer in Table 1 suggest that the dimerization on the resin happened when the particular tetrapeptide sequence resists the intramolecular cyclization probably due to the steric problem. However, the result of the last case seemed to be caused by other reason. Accordingly, the behaviour of the Arg(Tos) moiety tethered to the oxime resin was studied further. When Boc-Arg(Tos)-oxime resin was treated in the same condition of the CC, the Boc-Arg(Tos) lactam was liberated as fast as that of the cleavage of cyclic peptides (The half time of the formation was 15 h.). Thus, it should be recommended that Arg is not chosen as the *C*-terminal amino acid on the oxime resin, because the elimination of the peptide segment during the coupling reaction is so much probable.

To elucidate the sequence dependence of the CC, ^{1}H-NMR study of *cyclo*(-Arg(Tos)-Gly-Asp(O*c*Hex)-Phg-) was further carried out. The chemical shifts and the temperature dependence of the amide protons of the cyclic tetrapeptide in $(CD_3)_2SO$ were given in Table 2. There observed two groups of amide protons in $A\delta/\Delta T$ values. The amide protons of Asp(O*c*Hex) and Phg strongly suggest to be engaged in hydrogen bonds. This fact leads us to the illustration in Fig. 3, in which there are two β-turns with hydrogen bonds between Phg CO and Asp(O*c*Hex) NH, and Arg(Tos) CO and Phg NH. Therefore, the latter β-turn similarly formed in the linear sequence **1** would allow the easy access of the amino group of Arg(Tos) to the carbonyl carbon of Phg. Things are same in the sequence **2**, but the reactivity of the amino group may be hindered by the side chain phenyl group. However, when the Gly is tethered to the oxime resin, no hydrogen bond stabilizes the β-turn structure, but the stranded conformation may be preferable for the sequence **3** to dimerize with each other in neighborhood in the significant occurrence. The Phg-Asp(O*c*Hex) sequence in **4** seems to be more rigidly stretched, which is even worse for cyclization. This may be also the reason why the tetra- and octapeptides scarcely cyclized from the sequence **4**.

Table 2. Chemical Shifts and Temperature Dependence of Amide Protons in ^{1}H-NMR Spectrum.[a]

NH	δ (ppm)	$\Delta\delta/\Delta T$ x10^3 (ppm/°C)
Arg(Tos)	8.55	-4.60
Gly	8.20	-4.48
Asp(O*c*Hex)	8.04	-2.25
Phg	8.49	-2.70

[a] Assignment of chemical shifts was carried out by the 2D COSY method on a JEOL JNM GX-400 spectrometer.

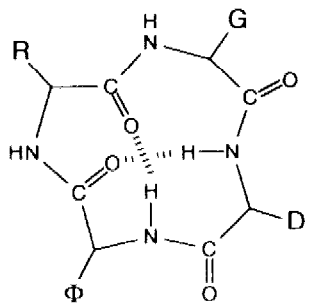


Fig. 3. Illustration of two possible hydrogen bonds. R, Arg(Tos); G, Gly; D, Asp(O*c*Hex); Φ, Phg.

On the other hand, the extent of dimerization can be reduced by employing the resin with low content of the first amino acid[1]. However, the diluted condition on the resin sacrifices the advantages of the SPS-CC method, the efficiency and the applicability to the large scale synthesis. For general application, we recommend

to use the regularly functionalized resin (about 0.5 mmol of the anchoring moiety/g resin) with full incorporation of the first amino acid and to examine the sequences of linear peptide precursors to find out the best condition for desired cyclic peptides.

Finally, the mixture of the cyclic tetra- and octapeptides was deprotected with anhydrous HF,[11] then two peptides were purified by RP-HPLC, and identified by FAB-MS analysis.[12] Both peptides showed the same inhibitory activity toward the cell attachment onto fibronectin-coated wells[13] with IC_{50} value of 10 μM.

REFERENCES AND NOTES

1. a) Ösapay, G.; Taylor, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6046-6051. b) Ösapay, G.; Profit, A.; Taylor, J. W. *Tetrahedron Lett.* **1990**, *31*, 6121-6124. c) Ösapay, G.; Bouvier, M.; Taylor, J. W. *Techniques in Protein Chemistry II*; Academic Press: New York, 1991; pp. 221-231.
2. a) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295-1300. b) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1982**, *47*, 3258-3261. c) Nakagawa, S. H.; Kaiser, E. T. *J. Org. Chem.* **1983**, *48*, 678-685. d) Nakagawa, S. H.; Kaiser, E. T. *J. Am. Chem. Soc.* **1985**, *107*, 7087-7092. e) Kaiser, E. T.; Mihara, H.; Laforet, G. A.; Kelly, J. W.; Walters, L.; Findeis, M. A.; Sasaki, T. *Science* **1989**, *243*, 187-192. f) Sasaki, T.; Findeis, M. A.; Kaiser, E. T. *J. Org. Chem.* **1991**, *56*, 3159-3168.
3. Xu, M.; Nishino, N.; Mihara, H.; Fujimoto, T.; Izumiya, N. *Chem. Lett.* in press.
4. Nishino, N.; Xu, M.; Mihara, H.; Fujimoto, T.; Ohba, M.; Ueno, Y.; Kumagai, H. *J. Chem. Soc. Chem. Commun.* in press.
5. Nishino, N.; Sano, Y.; Mihara, H.; Fujimoto, T. manuscript in preparation.
6. Unpublished data of Asahi Glass, Co., Ltd. group.
7. Rivaille, P.; Gautron, J. P.; Castro, B.; Milhaud, G. *Tetrahedron* **1980**, *36*, 3413-3419.
8. The tetrapeptide resins were prepared manually by the stepwise SPS with the BOP reagent. The Boc group in Boc-Phg-oxime resin (2.0 g, 0.46 mmol/g resin)was deprotected with 25% TFA in DCM for 30 min and the resin was washed with DCM (x2), isopropyl alcohol (x1), DCM (x3), and DMF (x1). To the resin was added Boc-Asp(OcHex)-OH (3.0 equiv.), BOP (3.0 equiv.) in DMF (30 ml), and then Et_3N (6.5 equiv.). The mixture was shaken for 60 min, and the resin was filtered and washed with DMF (x4) and DCM (x4). The consecutive coupling of Boc-Gly-OH and Boc-Arg(Tos)-OH by the same procedure gave the tetrapeptide resin (sequence **1**).
9. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595-598.
10. HPLC was carried out on a Hitachi L-6200 intelligent pump equipped with a Hitachi L-4200 UV-VIS detector and a Hitachi D-2500 chromato-integrator; column, MS-GEL C18 120Å (4.6 x 150 mm); eluent, linear gradient of 37-100% acetonitrile/0.1% TFA for 30 min; detected at 220 nm. The protected cyclic tetra- and octapeptides were eluted at 16.0 and 17.0 min, respectively.
11. Sakakibara, S; Shimonishi, Y. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 1412.
12. FAB-MS; m/z $(M+H)^+$ = 462 and 923 for the cyclic tetra- and octapeptides, respectively.
13. Kumagai, H.; Tajima, M.; Ueno, Y.; Giga-Hama, Y.; Ohba, M. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 74.

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