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Metal Ion-Assisted Peptide Cyclization

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Abstract: We describe an efficient chemoselective method for Ag^+ ion-assisted lactamization or lactonization of linear peptides to obtain end-to-end and sidechain-to-end cyclic peptides in aqueous buffered solutions. © 1997 Elsevier Science Ltd.

For the past two decades, cyclic peptides have been of great interest as synthetic targets both as potential drug leads and as models for conformational analysis.[1-2] The general procedure available for peptide cyclization typically involves a fully protected linear precursor and its cyclization in organic solvents either in solution or on a solid support.[3-5] These methods require strong activation of an acyl moiety by a coupling reagent and the reaction is usually performed in high dilution to avoid intermolecular oligomerization.

Recently, our laboratory has developed new methods for peptide cyclization that differ from conventional schemes in two respects.[6-7] First, unprotected peptide precursors are used. Second, ring-chain tautomerization is exploited to favor the formation of monomeric cyclic peptides and to avoid high dilution. As a concept, reversible ring-chain tautomerization derived from a precursor containing two reactive groups at the N- and C-termini provides a novel approach to obtain entropically favored cyclic peptide monomers. This concept has been successfully demonstrated for cyclization of N-terminal cysteinyl peptides.[6-7] To explore further the potential of ring-chain tautomerization in cyclization of non-cysteinyl peptides, we propose that thiophilic metal ions might coordinate the reactive functionalities of the N- and C-termini of a flexible linear peptide thioester to a cyclic intermediate, thus facilitating the intramolecular cyclization reaction through entropic activation. Thus, the metal ion-assisted ring

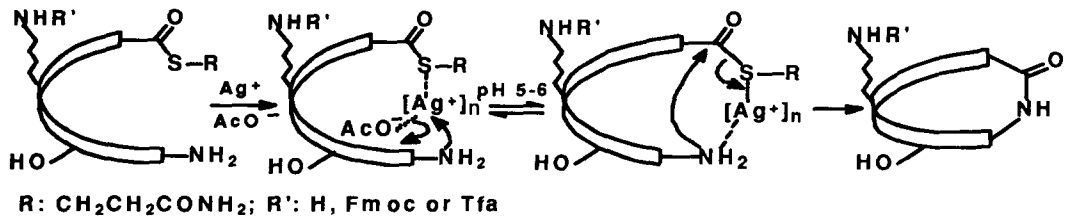

Figure 1. Proposed Scheme for Ag^+ Ion-assisted Peptide Cyclization

formation for the linear precursor could be considered a non-classical ring-chain tautomerization (Fig. 1). Furthermore, thiophilic metal ions play an additional role in the enthalpic activation of C-terminal carbonyl to accelerate the amide bond formation. Based on the work of Schwyzer *et al*,[8] we have found that Ag^+ ion meets our requirement of fulfilling the dual roles of enthalpic and entropic activations by a soft metal ion. Here we report the development of Ag^+ ion-assisted cyclization of minimally protected peptide thioesters.

All Ag^+ ion-assisted cyclizations of minimally protected peptides were performed in aqueous acetate-buffered solutions at pH 5-6 for two reasons. First, the conditions allow ring-chain tautomeric equilibrium mediated by Ag^+ ion through coordination among heteroatomic functional groups in the peptide. Because the affinity of Ag^+ ion is known to be in the order: S>>N>O, the likely coordination of one or more Ag^+ ions between the nitrogen of the α-amino group and the sulfur of the α-thioester could provide the envisioned cyclic intermediate (Fig. 1). Second, under aqueous buffered conditions at slightly acidic pH, hydrolysis assisted by Ag^+ ion is slow, as shown by Schwyzer *et al*,[8] but selectivity for aminolysis is high.

To test our proposal and to determine the chemoselectivity of lactam and lactone formation, we used a free peptide, Ala-Lys-Tyr-Gly-Gly-Phe-Leu-$SCH_2CH_2CONH_2$ **1**, as a model which contains three internal nucleophiles, α- and sidechain amines and a phenolic hydroxyl of Tyr. Cyclization of **1** in 0.2 M acetate buffer at pH 5.4 after 4 h resulted in three compounds as detected by RP-HPLC. Two peptides were identified as lactams of end-to-end (55%) and sidechain-to-end cyclic peptides (17%), respectively, by MALDI-MS and end-group analysis using Sanger's reagent. The third compound was found to be a peptide lactone (22%). As expected, hydrolysis was insignificant (<5%). The molar ratios of these three cyclic peptides were strongly influenced by varying the reaction buffer pH and adding DMSO as a cosolvent. For example, the end-to-end cyclized peptide was obtained as a major product in a yield of 67% in 5 hr when the reaction pH was increased to 5.7 and DMSO was added. At this pH, the

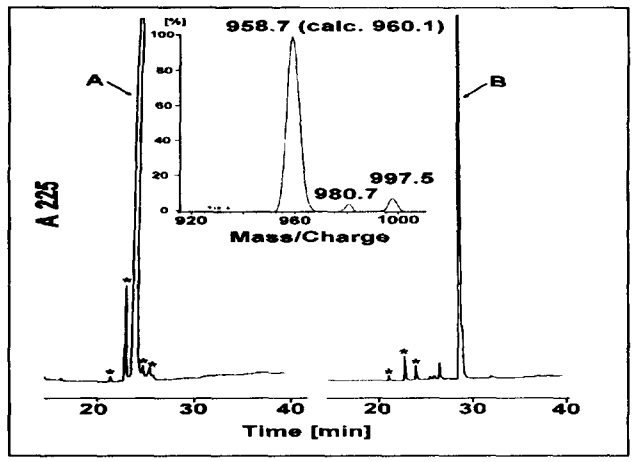

Figure 2. Analytical HPLC of cyclization of Ala-Lys(Fmoc)-Tyr-Gly-Gly-Phe-Leu-$S(CH_2)_2CONHCH_2CO_2H$ **2**. Left panel: starting materiel used without purification. Right panel: cyclization after 2 h. Insert: MALDI-MS of the cyclic product. Calcd for $[M+H]^+$=960.1; found: 958.7. HPLC was performed on a Vydac column (250 x 4.6 mm) at a flow rate of 1 mL/min (buffer A, 0.05% TFA in H_2O; buffer B, 0.04% TFA in 60% CH_3CN in H_2O. Gradient: 1 min, 30% B, isocratic; 30% to 100% B within 30 min; 5 min, 100% B, isocratic. A: **2**. B: cyclo(Ala-Lys-Tyr-Gly-Gly-Phe-Leu). *: impurities from the starting material.

ϵ-amine of the side chain of Lys is protected by protonation, and the α-amine is more nucleophilic than the phenolic hydroxyl of Tyr. Lactonization was greatly suppressed to <2%. Addition of DMSO to the reaction medium changed the apparent pH and favored the formation of the Ag^+-amine complex. However, when the pH was lowered to 5 in the absence of DMSO, the cyclic product via lactonization was obtained in a yield of 60%. No dimerization or oligomerization was detected. These results confirm the usefulness of ring-chain tautomerization in suppressing unwanted competing oligomerization and the need to protect sidechain amines for Ag^+ ion-assisted cyclization.

Based on these results, amines were temporarily masked with Tfa or Fmoc protecting groups which were readily removed by 0.2 M piperidine aqueous solution. Cyclization of crude Ala-Lys(Fmoc)-Tyr-Gly-Gly-Phe-Leu-$S(CH_2)_2CONHCH_2CO_2H$ **2** obtained directly from solid-phase synthesis proceeded cleanly in a yield of 92% as determined by RP-HPLC (Fig. 2). Either end-to-end or sidechain-to-end cyclized peptides were obtained in yields ranging from 70% to 94% (Table 1), demonstrating the high efficiency of this method. Again, no peptide dimerization was detected except in the pentapeptide in which about 5% of the starting material dimerized. The cyclic pentapeptide was obtained in a yield of 76%.

The stoichiometry of Ag^+ ion required in the reaction was determined using **2** as a model. Cyclization was performed in a concentration of 1 mM in 0.2 M acetate buffer (pH 5.5)/DMSO (1:1). The time course of the reaction showed that the cyclization was complete in 2 hr. When 1 or 2 equivalents of Ag^+ ion were used, 48 and 87% of cyclic peptides were obtained. A longer reaction time did not affect the yield. With 3 equivalents of Ag^+ ion, the yield increased to 94%. Thus, at least two equivalent Ag^+ ions were required and three or more equivalent to achieve complete cyclization. Other thiophilic metal ions such as Hg^{2+}, Pb^{2+}, Cu^+, Cu^{2+} were also tested for the cyclization, and only Hg^{2+} was found to be useful, but slower when compared to Ag^+ ion in our model peptides.

Peptide thioesters have been previously used for cyclization of peptides without any metal ion assistance, but those approaches are in general rather inefficient.[10-11] Their use for the orthogonal coupling of unprotected peptides has been successfully demonstrated by Dawson et al.[12] and our laboratory.[13] Cyclization of linear peptide esters can be also achieved using an enzyme-derived ligase, subtiligase,[14] or even catalytic antibodies.[15] Minimally protected peptide thioesters have also found application for segment condensation by their conversion into the corresponding hydroxysuccinimide active esters in the presence of Ag^+ ion.[16] ω-Hydroxycarboxylic thioesters are also useful

Table 1. Conditions and Yields of Ag^+-ion Assisted Cyclization of Peptide Thioesters[9]

Peptide Thioester	Time (hr)	Yield (%)	Epimer (%)
Phe-Gly-Gly-Phe-Leu	4	76	<1
Tyr-Gly-Gly-Phe-Leu	4	70	<1
Ser-Phe-Gly-Gly-Phe-Leu	4	73	<3
Gly-Phe-Gly-Gly-Phe-Leu	4	87	<1
Tfa-Lys-Tyr-Gly-Gly-Phe-Leu	4	75	<2
Lys(Tfa)-Tyr-Gly-Gly-Phe-Leu	4	82	<2
Ala-Lys(Fmoc)-Tyr-Gly-Gly-Phe-Leu	2	92	<1
Gly-Lys(Fmoc)-Tyr-Gly-Gly-Phe-Leu	2	94	<1
Fmoc-Ala-Lys-Tyr-Gly-Gly-Phe-Leu	4	70	<2
Fmoc-Gly-Lys-Tyr-Gly-Gly-Phe-Leu	4	72	<2

macrolide precursors [17] and ring closure is greatly improved by adding soft metal ions such as Ag^+, Hg^{2+} ions as templates to the reaction.[18-19] It is important to point out that these reactions are performed in nonaqueous organic solutions and differ significantly from our proposed conditions. In a slightly acidic solution, Ag^+ ions have higher affinity for the nitrogen and the sulfur of two reactive functionalities, α-amino group and thioester moiety, than for the hydroxy groups on the sidechain, resulting in a non-classical ring-chain tautomerization. As a result of this entropic activation, intramolecular acylation is favored over intermolecular acylation. Furthermore, by controlling the pH of the reaction media, both acylations, lactamization and lactonization, can be achieved, thus allowing a synthetic scheme to generate molecular diversity for a cyclic peptide library. Finally, we envision that this chemistry could be applied to the synthesis of naturally occurring cyclic peptides such as cyclotheonamides and microcystins, and could be extended to the cyclization of peptide thioesters on the solid support.

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9. Cyclizations were carried out in 0.2 M sodium acetate buffer (pH = 5.5). Typically, three equivalents of AgTfa dissolved in DMSO (4.4 mg/1 mL) were added to the thioester peptide to be cyclized in 0.2 M acetate buffer. The reactions were allowed to vortex for 4 h and monitored by RP-HPLC. Usually, cyclizations were completed at this time point. After completion of the reactions, NaCl solution was added to precipitate the excess Ag^+ ion. Silver precipitation was removed by centrifugation. The reported percent yields represent the integrated areas at 225 nm of the product peaks after the disappearance of the starting peptide precursors. The identity and purity of all cyclic products were confirmed by MALDI-MS.
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