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Disulfide Cyclization of Protected Peptide Assembled on Oxime Resin

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Abstract: *After the assembly of protected peptide sequences containing a pair of cysteinyl groups on oxime resin, the disulfide bond was directly formed on the resin. The subsequent cleavage gave an oxytocin precursor and a protected cyclic fragment (1-10) of salmon calcitonin in good yields..*

Kaiser's oxime resin has been demonstrated to be a useful anchor for solid-phase-synthesis (SPS) of protected oligopeptide intermediates.[1] The intermediates could be condensed with others in solution phase or solid-phase in the synthesis of longer polypeptides.[2] Furthermore, convenient preparations of cyclic peptides have been also established by the application of the advantage of the anchoring moiety in oxime resin as an active ester.[3] However, such SPS and cyclization-cleavage (CC) method is limited for only formation of peptide (acid amide) bond. For the cyclization with disulfide bond, which is another important loop formation often found in naturally occurring peptides, the oxime resin has not been applied to the type of solid-phase-cyclization.

Recently, Albericio *et al.* reported the cyclization of disulfide containing peptides in solid-phase synthesis.[4] They finally demonstrated the successful preparation of oxytocin in various assembly chemistry (Boc- (*t*-butyloxycarbonyl) and Fmoc- (9-fluorenylmethyloxycarbonyl) strategies) and on various resins containing linkers such as 4-methylbenzhydrylamide and tris(alkoxy)benzylamide. The disulfide loop was formed on the resin under pseudo-dilution condition,[4] then oxytocin molecule was cleaved by the treatment

with anhydrous HF simultaneously accompanied by side chain deprotection. This method has an advantage in avoiding the high dilution condition in cyclization and in directly giving the final product. However, it seems difficult to prepare the protected cyclic intermediates.

Since Kaiser's oxime resin was developed for the convenient preparation of protected peptide intermediates, the cyclization with disulfide bond on the oxime resin was expected to bring a great advantage in the preparation of valuable hormonal peptides containing disulfide bond(s) such as calcitonin and endothelin. Therefore, we attempted the direct cyclization of protected oxytocin sequence assembled on the oxime resin. The decapeptide fragment of salmon calcitonin (1-10) with the disulfide loop was also prepared on the resin and successfully cleaved as a useful protected intermediate.

The synthesis of protected oxytocin was carried out as shown in Fig. 1. Starting with Boc-Gly-oxime resin (2.0 g, substitution level, 0.47 mmol/g), Boc-amino acid (3.0 equiv.) was coupled 8 times on the amino acid resin (1.0 equiv.) after the deprotection with 25% CF_3COOH by the aid of BOP (benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate) (3.0 equiv.), HOBt (1-hydroxybenzotriazole) (3.0 equiv.), and *N,N*-diisopropylethylamine (5.0 equiv.) in *N,N*-dimethylformamide (DMF). After each coupling, the completion was confirmed by the Kaiser test.[5] The thorough washing with appropriate solvents and drying *in vacuo* gave 3.2 g of peptidyl resin (95% from the weight increase). The half amount (1.6 g) of the resin was suspended with DMF (15 ml) and treated with I_2 (1.2 g, 10 equiv.) for 1 h at room temperature.[6] After washing with DMF, the resin was treated with ammonium acetate (10 equiv.) in DMF (15 ml) overnight. After the resin suspension was filtrated and washed with DMF, the combined solution of the filtrate and washing was concentrated and then the desired cyclic peptide was precipitated with H_2O; yield, 480 mg (80%); FAB-MS *m/z* 1265 $(M+H)^+$. Another half was treated with ammonium acetate as described above to give the linear protected oxytocin peptide, which was collected in the same manner; yield, 530 mg (79%); FAB-MS *m/z* 1410 $(M+H)^+$. The amount of the linear peptide was treated in DMF (14 ml) with I_2 (1.2 g, 12 equiv.) as a reference

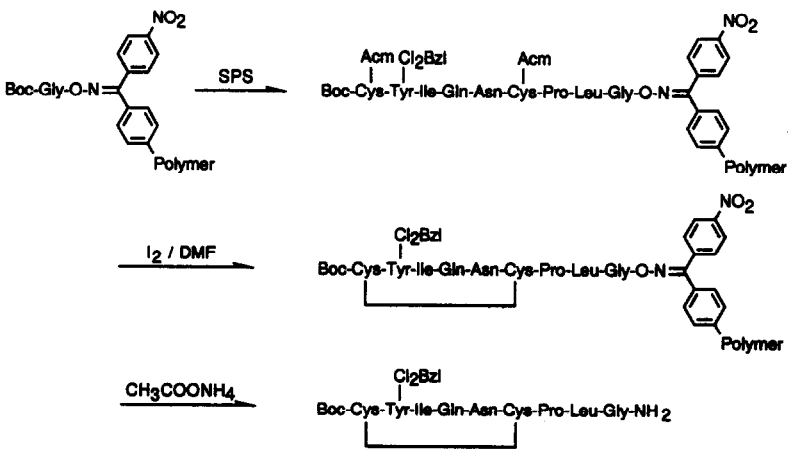

Fig. 1. Synthesis of protected oxytocin *via* disulfide cyclization on the oxime resin. Acm, acetamidomethyl; Cl_2Bzl, 2,6-dichlorobenzyl.

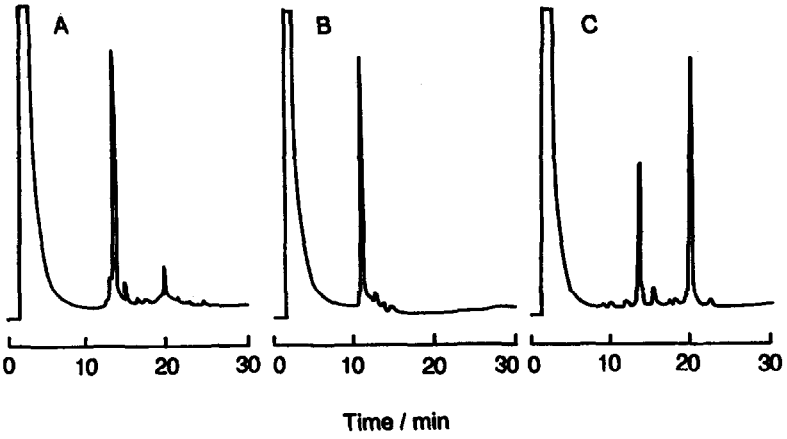

Fig. 2. HPLC of protected oxytocin peptides. (A) cyclized with I_2 in DMF and then cleaved with 10 equiv. ammonium acetate from the resin. (B) linear peptide cleaved as above without cyclization. (C) after solution-phase cyclization of purified protected linear peptide. HPLC condition; Wakosil C4 (4.5 x 150 mm) with a linear gradient of 37-100% CH_3CN/0.1% CF_3COOH over 30 min. Detected at 220 nm. Flow rate, 1.0 ml/min.

experiment to evaluate the efficiency of the pseudo-high dilution cyclization on oxime resin. The excess I_2 was quenched with ascorbic acid and the crude cyclic protected oxytocin was obtained; yield, 370 mg (78%). The HPLC analysis of these three products are shown in Fig. 2. The monomeric cyclization (80%) was predominant in the disulfide formation by solid-phase. The yield of the dimer (retention time, 20.1 min) was depressed down to 20%, though the dimerizations in parallel and antiparallel are not distinguished at present. The cyclization of linear peptide in solution-phase mainly gave the dimer (60%), because the concentration was as high as 3×10^{-2} M. The reference experiment clearly emphasized that the disulfide cyclization on the oxime resin is an efficient strategy to synthesize the protected cyclic peptides with a disulfide loop.

The usefulness of disulfide cyclization on oxime resin would be demonstrated when it is successfully applied to the preparation of the synthetic intermediate with a disulfide loop for a longer valuable hormonal peptide. Therefore, the *N*-terminal peptide fragment of salmon calcitonin was assembled and cyclized on the oxime resin as shown in Fig. 3. Though Kaiser's

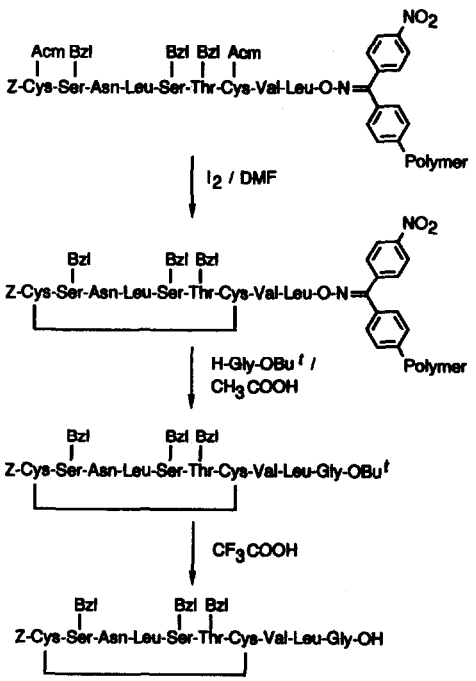

Fig. 3. Synthetic scheme for the useful intermediate for calcitonin with a disulfide loop and side chain protections. Bzl, benzyl.

original procedure includes the cleavage with *N*-hydroxypiperidine and then treatment with Zn/AcOH to give protected peptide acid, the latter procedure could not be applied in this case, because the disulfide bond is simultaneously reduced. Thus, we assembled nonapeptide with Z (benzyloxycarbonyl) group as α-amino-protection, cyclized, and then cleaved with H-Gly-OBu^t (t-butyl) to give the fully protected cyclic decapeptide. Starting from Boc-Leu-oxime resin (2.0 g, 0.92 mmol), 1.2 g (85%) of the desired intermediate [FAB-MS *m/z* 1455 $(M+H)^+$] with 82% purity on HPLC was obtained [including the dimer (15%)]. The treatment with TFA may produce the protected peptide acid for further condensation with another *C*-terminal fragment of calcitonin. When the cyclization was carried out from the linear protected peptide in solution, the dimer was mainly obtained in 65% yield. Recently, Barlos *et al.* reported the excellent synthesis of protected cyclic decapeptide acid corresponding to salmon calcitonin (1-10) by Fmoc chemistry on 2-chlorotrityl resin.[7] This peptide can be used as a intermediate for the synthesis of the hormone. Though their procedure produces only protected peptide acid, the oxime resin method can give the cyclic peptides useful for not only acid components, but also amine components of fragment condensation by the combination of protecting groups such as Z and Fmoc.

In conclusion, peptide sequences containing two protected cysteinyl residues were assembled on oxime resin by SPS, and then disulfide cyclization was conveniently carried out to produce cyclic protected peptides, which are useful as intermediates in further synthesis of valuable peptides. This success in preparation of cyclic peptides with a disulfide loop on the oxime resin in good yield will give further expansion of its utility in the practical synthesis of hormonal peptides.

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