Synthesis of Protected Peptides Containing Phosphoserine with Oxime Resin

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A protected peptide containing phosphoserine was successfully synthesized by the solid-phase method using the oxime resin. The protected phosphopeptide was utilized to further elongation to a longer peptide and conjugation to a template molecule.

Much attention has been attracted to phosphorylated peptides and proteins, because such peptides have been elucidated to have significant roles in the regulation of protein-protein interactions, enzyme activities, and antigen-antibody recognition.[1] Therefore, considerable effort has been devoted to the development of convenient synthetic methods for phophopeptides.[2] On the other hand, the solid-phase synthesis (SPS) using the oxime resin is known to be useful to obtain a variety of protected peptides with *t*-butyloxycarbonyl (Boc) chemistry.[3] Protected peptides are applicable to further elongation of peptides and conjugation with non-peptidyl compounds. When phosphopeptides would be efficiently synthesized by the oxime resin SPS, the method could afford a versatility to the synthesis of the modified peptides with various structures. In this study, we attempted to synthesize protected peptides containing phosphoserine (PSer) corresponding to a B-cell epitope sequence in αS1-casein[4] by the oxime resin SPS (Figure 1).

Boc-Val-Pro-Asn-PSer(OR)$_2$-Ala-Glu(OBzl)-Glu(OBzl)-OH **1**

Boc-Val-Pro-Asn-PSer(OR)$_2$-Ala-Glu(OBzl)-Glu(OBzl)-
-Arg(Mts)-Leu-His(Bom)-Ser(Bzl)-Met-Lys(ClZ)-Glu(OBzl)-OH **2**

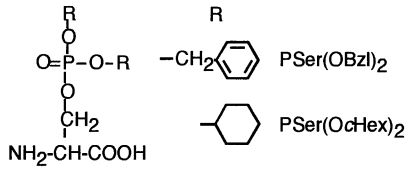

Figure 1. Protected PSer-peptides; **1**, αS1-casein(112-118); **2**, αS1-casein(112-125). Bzl, benzyl; Bom, benzyloxymethyl; *c*Hex, cyclohexyl; ClZ, 2-chlorobenzyloxycarbonyl; Mts, mesitylenesulfonyl.

At first, protections of phosphate in the PSer residue [benzyl (Bzl) and cyclohexyl (*c*Hex)][2] were examined. Two different coupling methods, BOP-HOBt and DCC-HOBt,[3] were also compared [BOP, benzotriazole-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole]. The first amino acid, Boc-Glu(OBzl), was introduced in *p*-nitrobenzophenone oxime resin with *N,N'*-dicyclohexylcarbodiimide (DCC) in dichloromethane (DCM) (0.50 mmol/g

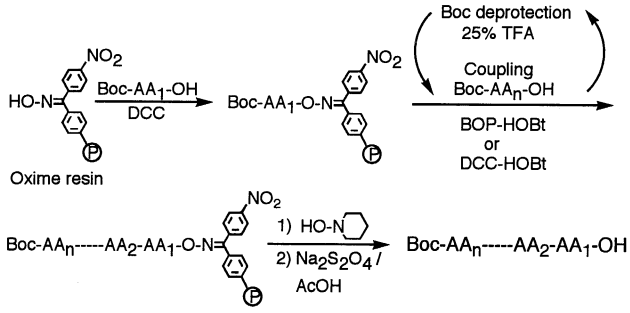

Figure 2. Synthesis of a protected peptide by the oxime resin SPS.

resin). The stepwise elongation of Boc-amino acids (3 equiv.) was carried out by manual SPS (Figure 2). Trifluoroacetic acid (TFA) (25%) in DCM was used for the deprotection of the Boc groups. The BOP-HOBt (3 equiv.) method was performed in the presence of diisopropylethylamine (5 equiv.). In the case of the DCC-HOBt method, the BOP-HOBt method was also used until Boc-PSer(OR)$_2$ was introduced, and then Boc-PSer(OR)$_2$ (3 equiv.) and the remaining amino acids were condensed using DCC-HOBt. The protected peptide was cleaved from the resin with 1-hydroxypiperidine (4 equiv.) and the piperidine group was removed with sodium dithionite (5 equiv.) in acetic acid (AcOH). The product was identified by FAB-MS.[5] As a result, only the use of DCC-HOBt method gave a satisfactory result in combination with the *c*Hex protection (70% yield, 90% purity on HPLC[6]). When the Bzl protection was used, peptide **1** was obtained in poor yield (5% in 85% purity) by the BOP-HOBt method. Though the DCC-HOBt method with the Bzl protection gave a crude product in 31% yield, the purity was not good (42%; three major peaks on HPLC). These were probably due to the fact that the Bzl group was labile in the TFA treatments during SPS.[2] However, even though PSer was protected with the acid stable *c*Hex groups, the BOP-HOBt method did not give satisfactory yield and purity of the peptide **1** (26% yield, 47% purity). This method is known to give a ΔAla-peptide formed by the β-elimination of *O*-phosphono moiety in a PSer-peptide and its derivatives.[7] Combination of the DCC-HOBt method and the acid stable *c*Hex protection gave a good result for the synthesis of the protected PSer-peptide on the oxime resin.

Because the protected oligopeptide containing a PSer residue was obtained by the oxime resin SPS, the peptide **1** was utilized for further elongation by the segment condensation in solution (Figure 3). Peptides **1** and **3** synthesized individually by the oxime resin SPS were successfully condensed with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) and HOBt in dimethylformamide (DMF). The piperidine group was

successively removed with sodium dithionite in AcOH/DMF (1/1, v/v) to give the protected tetradecapeptide **2** (91% yield, 95% purity). The protecting groups in **2** were successfully removed with 1 mol dm^{-3} trifluoromethanesulfonic acid (TFMSA)[8] in TFA in the presence of thioanisole and *m*-cresol at r.t. for 4 h. The crude peptide was purified on HPLC[9] to give the deprotected PSer-peptide **4** in good yield (52% yield, >95% purity).[10] The deprotection with trimethylsilyl trifluoromethanesulfonate[11] gave a different compound probably modified with trimethylsilyl groups. The treatment with anhydrous HF did not afford the product **4**.

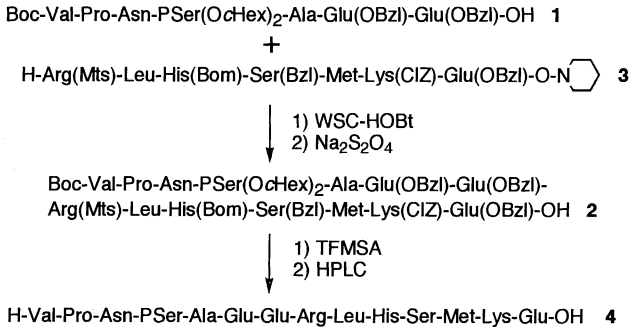

Figure 3. Segment condensation of the PSer-peptide **1**.

The protected PSer-peptide **2** was conjugated with a template composed of Lys, β-Ala, and ethylenediamine to give a multi-antigenic peptide **5** (Figure 4).[12] The peptide **2** was condensed with the template by the WSC-HOBt method. The protecting groups were removed with TFMSA and purification with Sephadex G-50 gave the conjugate **5** (35% yield). The antigenic activities of the peptides **4** and **5** against serum from patients with milk allergy are under investigation.

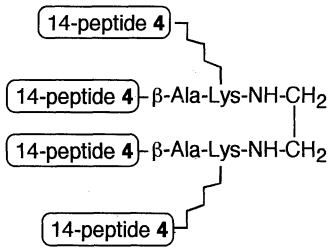

Figure 4. Conjugation of the PSer-peptide **4** with a template.

Thus, the protected phosphopeptides were synthesized by the oxime resin SPS. The protected peptides can be utilized to further elongation of peptide segments and the condensation with other non-peptide compounds. The synthetic method provides a versatility in the synthesis of phosphopeptides which could improve the studies on related fields. Syntheses of other phosphopeptides including PThr and PTyr are in progress.

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References and Notes

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5 FAB-MS: **1** *m/z* 1269 $(M+H)^+$, 1291 $(M+Na)^+$, 1307 $(M+K)^+$.
6 Wakosil 5C4 column (4.6 x 150 mm) with a linear gradient of 30-100% acetonitrile/0.1% TFA over 30 min. Peaks were detected at 220 nm and purity of peptides was determined with the integral values.
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9 YMC-Pack ODS-A323 column (10 x 250 mm) with a linear gradient of 15-45% acetonitrile/0.1% TFA over 30 min. Peaks were detected at 220 nm.
10 FAB-MS: *m/z* 1706 $(M+H)^+$, 1728 $(M+Na)^+$, 1744 $(M+K)^+$.
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