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Polypeptide synthesis using an expressed peptide as a building block via the thioester method

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Abstract

An expressed peptide was proven to be useful as a building block in the synthesis of a polypeptide via the thioester method. A partially protected peptide segment, [Lys(Boc)^{15,25,31,48,57,68,80,95}]-Max(14–101), was prepared via transamination of an N-terminal amino group from p21Max(13–101), which was obtained by expression using *Escherichia coli*. This peptide was utilized in the synthesis of [Ser(PO_3H_2)^{2,11}]-p21Max(1–101) via condensation with the peptide thioester, Boc-[Ser(PO_3H_2)^{2,11}]-p21Max(1–13)-SCH_2CH_2CO-Leu-OH.

Keywords: peptides and polypeptides; peptide building blocks; thioesters; condensations.

The use of bacterially expressed peptide segments in combination with chemically synthesized ones as building blocks offers great potential as a route to the synthesis of a wide variety of polypeptides. Recent efforts in our laboratory have been directed at developing a method for polypeptide synthesis using *S*-alkyl peptide thioesters (peptide thioesters) as building blocks.[1] Here we report a method for the preparation of a partially protected peptide segment, as a building block for the thioester method, from an expressed peptide, and its condensation with a peptide thioester which contains phosphoserine residues.

A method for the removal of the N-terminal residue of a protein via transamination, which was developed by Dixon et al.,[2] has been successfully applied to the removal of the N-terminal extra methionine residue of a recombinant human growth hormone by Nishimura et al.[3] Dixon's method involves several reaction steps, in which the stable intermediate, N^{α}-α-oxoacyl peptide **2**, is produced from a free peptide **1**. If we could introduce a Boc group to the side chain amino group of peptide **2**, and then could safely remove the α-oxoacyl group, a selectively protected C-terminal building block **4** would be obtained as shown in Scheme 1. In order to evaluate the usefulness of this strategy, we synthesized

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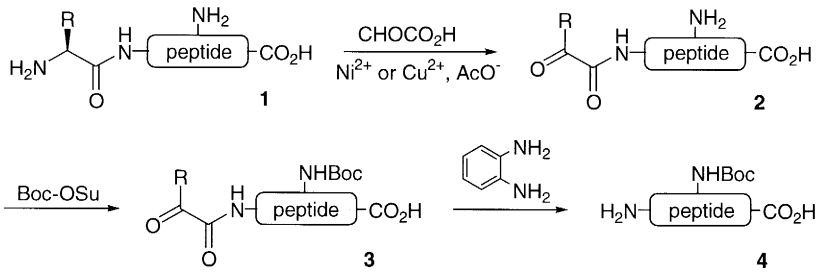
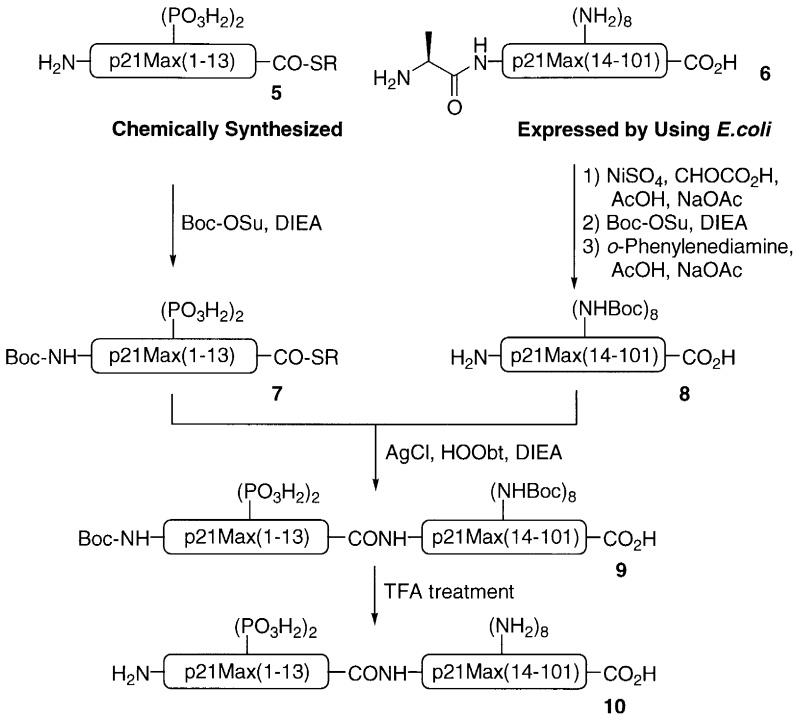
a partial sequence of the phosphorylated p21Max protein.[4] Max protein is known to specifically bind to duplex DNA by forming *homo*- and *hetero*-complexes with the Myc family proteins. Its binding activity is reduced by phosphorylation. This synthesis will greatly contribute to understanding of the structural bases of the regulation mechanism of Max protein.



Scheme 1. Strategy for the preparation of partially protected peptide segments from free peptides

The sequence of the target peptide, [Ser(PO_3H_2)^{2,11}]-p21Max(1–101), is as follows: M*pS*DNDDIEVE*pS*DA↓DKRAHHNALERKRRDHIKDSFHSLRDSVPSLQGEKASRAQILDKATEYI-QYMRRKNHTHQQDIDDLKRQNALLEQQVRALEKARSSAQ (**10**), in which *pS* represents a phosphoserine residue and the arrow, ↓, indicates the coupling site. A strategy for the synthesis of peptide **10** is shown in Scheme 2. For synthetic purposes, two peptide segments, Boc-[Ser(PO_3H_2)^{2,11}]-p21Max(1–13)-SCH_2CH_2CO-Leu-OH (**7**) and [Lys(Boc)^{15,25,31,48,57,68,80,95}]-p21Max(14–101) (**8**), were prepared, based on a chemical and a biological approach, respectively.



Scheme 2. Strategy for the synthesis of [Ser(PO_3H_2)^{2,11})-p21Max(1–101) (**10**). -SR=-SCH_2CH_2CO-Leu-OH

Ala-p21Max(14–101) (**6**), which was prepared by expression using *E. coli*,[4b] was transaminated by treatment with glyoxylic acid (0.5 M) and nickel(II) sulfate (5 mM) in an acetate buffer (2 M acetic

acid and 1 M sodium acetate).[2] The reaction was monitored by ion-exchange chromatography using HiTrap™ SP (1 mL) (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and reached completion in 2 h. After the addition of ethylenediaminetetraacetic acid to inactivate nickel ions, the N^{α}-pyruvoyl product, CH_3COCO-p21Max(14–101) (**11**), was partially purified by reversed-phase (RP) HPLC using a C4 column (Cosmosil $5C_4$ AR-300, 4.6×150 mm, Nacalai Tesque, Kyoto, Japan) to remove excess reagents. The amino groups of the lysine residues in peptide **11** were then protected via the introduction of Boc groups using *N*-*t*-butoxycarbonyloxysuccinimide (Boc-OSu) by the usual method,[1] to give CH_3COCO-[Lys(Boc)^{15,25,31,48,57,68,80,95}]-p21Max(14–101) (**12**). To remove the pyruvoyl group, peptide **12** was treated with *o*-phenylenediamine (25 mM) in an acetate buffer (1 M acetic acid and 1 M sodium acetate)[2,3] containing 30% DMF for 48 h to give [Lys(Boc)^{15,25,31,48,57,68,80,95}]-p21Max(14–101) (**8**) in 34% yield based on peptide **6** after purification by RP-HPLC using a C4 column (Fig. 1(A)). The mass number of peptide **8** was in good agreement with the calculated value (found: *m*/*z* 11 215; calcd for $[M+H]^+$: 11 214).

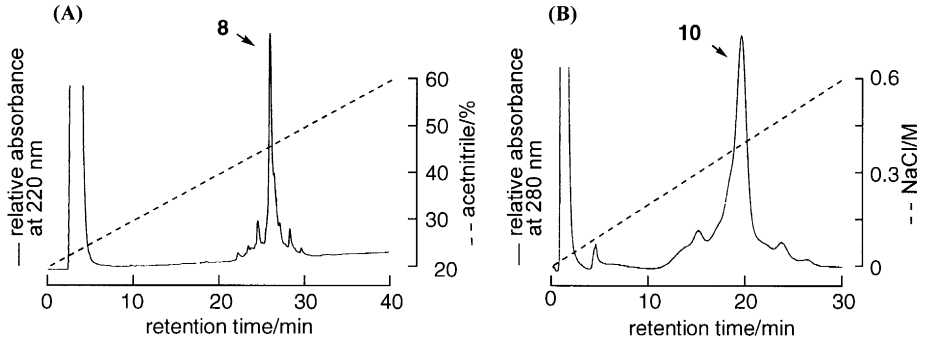


Fig. 1. HPLC elution profiles. (A) RP-HPLC of crude [Lys(Boc)^{15,25,31,48,57,68,80,95}]-p21Max(14–101) (**8**). Column: Cosmosil $5C_4$AR-300 (4.6×150 mm) (Nacalai tesque, Kyoto, Japan); eluent: 0.1% TFA in aqueous acetonitrile using a linear gradient indicated on the chromatogram (1.0 mL/min). (B) Ion-exchange chromatography of crude [Ser$(PO_3H_2)^{2,11}$]-p21Max(1–101) (**10**). Column: HiTrap™ SP (1 mL) (Amersham Pharmacia Biotech AB, Uppsala, Sweden); eluent: 50 mM sodium phosphate buffer containing 8 M urea (pH 5.1) using a linear gradient of sodium chloride concentration indicated on the chromatogram (1.0 mL/min)

In initial experiments on the transamination of peptide **6**, when copper(II) sulfate was used instead of nickel(II) sulfate, the desired product **11** rapidly formed, but was then gradually destroyed under the same conditions as were used for its formation. Although the use of nickel(II) sulfate resulted in a slower reaction, the product was stable and the reaction was more easily controlled.

This peptide segment **8** was condensed with a phosphorylated peptide thioester, Boc-[Ser$(PO_3H_2)^{2,11}$]-p21Max(1–13)-SCH_2CH_2CO-Leu-OH (**7**),[1c,5] by treatment with silver chloride in the presence of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOObt) and *N*,*N*-diisopropylethylamine (DIEA) in DMSO for 24 h,[1] to give Boc-[Lys(Boc)^{15,25,31,48,57,68,80,95},Ser$(PO_3H_2)^{2,11}$]-p21Max(1–101) (**9**). After washing the reaction mixture with ether, peptide **9** was treated with trifluoroacetic acid (TFA) containing 5% water to afford [Ser$(PO_3H_2)^{2,11}$]-p21Max(1–101) (**10**), which was purified by ion-exchange chromatography (Fig. 1(B)), followed by RP-HPLC on a C4 column, in 32% yield based on the C-terminal peptide **8**. The mass number and amino acid analysis of peptide **10** were in good agreement with calculated values (found: *m*/*z* 11 994; calcd for $[M+H]^+$: 11 995). Amino acid analysis: $Asp_{17}Thr_{2.2}Ser_{7.8}Glu_{18}Pro_{0.99}Gly_{1.7}Ala_{10}Val_{3.8}Met_{0.48}Ile_{5.0}Leu_{8.3}Tyr_{1.1}Phe_{0.94}Lys_{7.8}His_{5.9}Arg_{10}$. The epimerization of the Ala residue at the condensation site was less than 14%. An examination of coupling conditions for the peptide thioesters wherein epimerization can be suppressed is now in progress.

In conclusion, expressed peptides can be utilized as building blocks to polypeptide synthesis using the thioester method. This methodology can be applied to the synthesis of a wide variety of polypeptides, including segmentally isotope-labeled proteins.

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