International Journal of Advanced and Innovative Research (2278-7844)/

Volume 7 Issue 7

SYNTHESIS OF PEPTIDE-THIOESTERS

Preethi.P.C
Department of chemistry, Christian College, Kattakada, India-695572
preethipc02@gmail.com

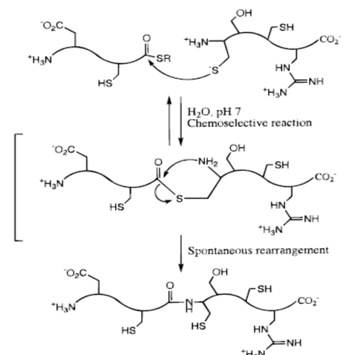
***Abstract*- Native chemical ligation is a widely used technique for the synthesis of long chain peptides or proteins. The essential requirements for this process are peptide thioesters and peptide amides. The low yield and purity are the challenging factors in the synthesis of peptide thioesters. The synthesis of peptide thioesters using double linker strategy on a solid support gives better yield and purity. The quality of the product was analysed using RP-HPLC and MALDI-TOF MS.**

***Keywords*- Native chemical ligation, peptide thioester, HPLC, MALDI-TOF MS**

INTRODUCTION

Proteins and peptides are essential for life, being involved in several biological processes [1]. Homogeneous and structurally defined glycoproteins can in principle be obtained by total chemical synthesis [2]. The synthesis and semi-synthesis of proteins often rely on techniques by which peptides and proteins can be ligated under mild condition. Small proteins can be synthesised through conventional solid phase peptide synthesis. But in the case of long chain proteins, the conventional solid phase peptide synthesis has limited success. The linear assembly of peptides larger than about 60 amino acids is compromised by the accumulation of resin-bound by-products [3]. In order to overcome these limitations, Kent and co-workers developed a new method known as chemical ligation [4]. It is a powerful tool for the synthesis of proteins or long chain peptides by the combination of peptide fragments. In this reaction, an unprotected C-terminal peptide thioester reacts with an N-terminal cysteine residue of another unprotected peptide segment to yield a native amide bond in the resultant product [5].

Despite being versatile, the synthesis of peptide thioester is challenging, time consuming and poor yield. In earlier years peptide thioesters were synthesised by Boc chemistry. In order to overcome the limitations of this method Fmoc-SPPS was introduced [6]. The direct syntheses of peptide thioesters by Fmoc amino acids have been attempted also have limited success because of the repeated removal of Fmoc group under basic conditions. The conversion of peptide amide to corresponding thioesters is a chemoselective reaction, which occurs in the final stage of solid phase synthesis. In order to obtain a thioester of sufficient stability, a spacer was introduced in between the resin and alkane sulphonamide.



MATERIALS AND METHODS

1. *Chemicals*

1-Hydroxybenzotriazole (HOBt), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU), Fmoc-amino acids, were purchased from Novabiochem Ltd (UK). Thioanisol, ethanedithiol, piperidine and diisopropylethylamine (DIEA) were purchased from Sigma. All other chemicals and solvents (HPLC-grade) were purchased from Merck (India). FT-IR spectra were recorded on a Bomem MB-series spectrometer using KBr pellets. HPLC was performed on a Pharmacia Akta purifier instrument using C_{18} Sephasil peptide reverse-phase semi preparative column.

2. *Synthesis of peptide thioester by double linker strategy*

Peptides were synthesized manually on DMF-swollen, C-terminal amino acid-incorporated, amino-methyl resin in a silanized 15-mL glass reaction vessel containing a sintered ware filter on one side and a receiving adaptor fitted with a calcium chloride guard tube on the other. The first amino acid was added after the attachment of 3-carboxypropane sulfonamide to the linker attached to the resin. The coupling of first amino acid was carried out at -4^{0}c for 6h. Fmoc deprotection was carried out using 20% DBU in DMF. A sequence of operations such as deprotection and coupling reaction was carried out for incorporating the respective amino acid residues.

After the attachment of all the amino acid, the deprotected peptidyl resin was activated with iodoacetonitrile for the ease of cleavage of peptide- thioester from the resin. The activated peptidyl resin was treated with thiophenol and benzyl mercaptan before the treatment with cleavage cocktail. Finally,

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the peptide thioester was precipitated with ice cold t- butyl methyl ether. The precipitated peptide was washed with ice- cold ether in order to remove the scavengers, dried and lyophilized.

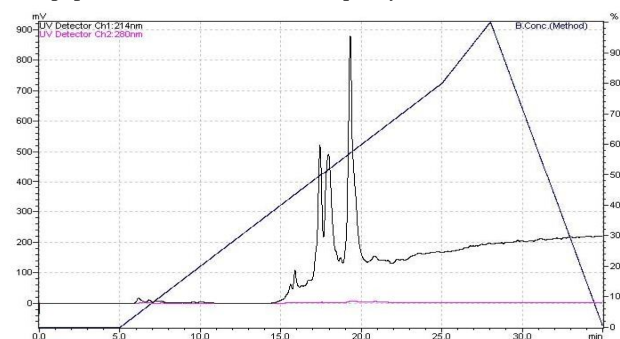
2. *Purification of Peptide*

The cleaved peptide was purified by HPLC using a C_{18} reverse phase column. The solvent systems used were 80% Acetonitrile, CH_3CN/ 20% H_2O (0.1% TFA) and 100% water (0.1% TFA). The gradient used was 5 - 45% CH_3CN/H_2O in 40 mins. The major peak was collected, the solvent evaporated from it and lyophilized to obtain the pure peptide in the form of a powder and was confirmed by MALDI-TOF MS.

RESULT AND DISCUSSION

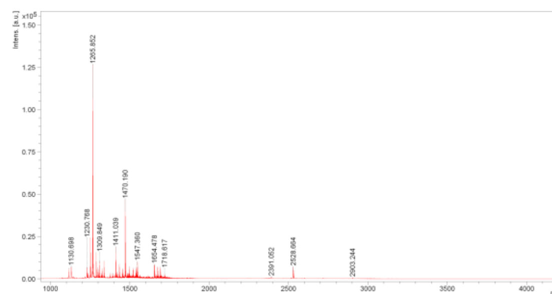
C-terminal peptide thioesters were synthesized for chemical ligation, through double linker strategy method on solid phase synthesis. In order to avoid the formation of side products Fmoc amino acids were used. Conventionally pieridine is used for the removal of Fmoc group. In this case, the removal of Fmoc group was carried out using DBU because the thioester linkage is highly unstable towards the piperidine. The rink amide linker and 3-carboxy propane sulfonamide were used as the two linkers. The rink amide linker makes the cleavage process of peptide thioester from the solid support easier and the sulfonamide linker gives the peptide as peptide thioester. After the attachment of all the amino acids, activation with iodoacetonitrile was carried out for the ease of cleavage of peptide thioesters form the solid support. TFA is used for the cleavage process. The cleaved peptide thioester was precipitated with ice- cold ether and lyophilized. The amino acid sequence was AGRQICVSAIHL.

The purity was checked by RP-HPLC and was confirmed by MALDI-TOF MS. It was very clear that the peptide thioester shows about 75% purity. Compared with other methods this peptide thioester shows better purity.



RP-HPLC profile of the synthesised peptide thioester

The mass of the peptide thioester was calculated by peptide mass calculator. The mass is 1266.67DA. The experimental mass showed a good agreement with the theoretical mass. The MALDI-TOF MS gives 1265.85DA as the mass.



MALDI-TOF MS of the peptide thioester

CONCLUTION

A good quality peptide thioester was synthesised by double linker strategic method. The relatively high purity of the obtained peptide thioester was confirmed by RP-HPLC and MALDI-TOF MS. Based on these, we can conclude that the double linker strategic method is very effective for the synthesis of peptide thioesters compared to other conventional methods.

ACKNOWLEDGMENT

The author is thankful to our college Principal and management for giving me the lab to conduct this investigation.

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