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A New approach for the synthesis of peptides as thioesters

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Abstract Peptide thioesters are the key building blocks for many convergent peptide synthesis strategies, including the well-known native chemical ligation [1]. Compared with conventional stepwise solid-phase peptide synthesis, the native chemical ligation is found to be a good technique for the synthesis of proteins with high molecular weights [2]. But the synthesis of peptide thioesters in good and purity is a difficult task. The Fmoc solid phase peptide thioester synthesis using two linkers gives good result. With the help of RP-HPLC and MALDI-TOF MS, the purity and mass of the peptide thioesters can be analysed.

Keywords-Native chemical ligation, Solid phase peptide synthesis, RP-HPLC, MALDI-TOF MS.

I. INTRODUCTION

The synthesis and semi-synthesis of proteins often rely on techniques by which peptides and proteins can be ligated under mild conditions[3]. Introduction of native chemical ligation method by Kent opened a new era in the field of synthesis of proteins. Native chemical ligation is a widely used technique for the synthesis of long chain proteins from peptide fragments[4]. The conventional ligation method requires minimum two peptide fragments, one is a peptide amide and the other is a peptide thioesters. A C-terminal peptide thioester undergoes a rearrangement with an N-terminal cysteine residue peptide amide to form a long chain polypeptides or proteins through S to N acyl shift[5].

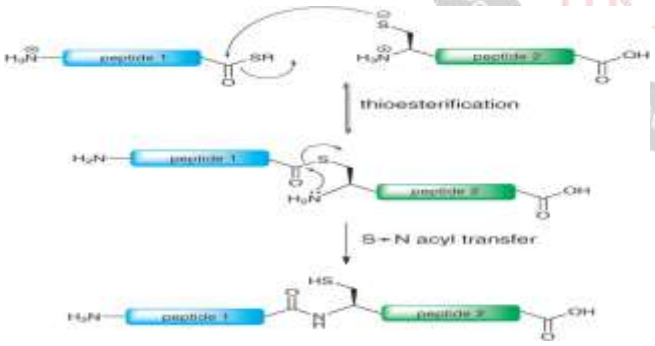

Fig . 1 Polypeptides

Conventional Fmoc solid phase peptide synthesis gives peptide amides in good yield and purity. The Fmoc solid-phase method requires neither the repetitive use of trifluoroacetic acid nor strong acid treatment. Thus, the direct preparation of peptide thioesters by an Fmoc solid-phase method will bring many advantages to peptide synthesis. If this can be realized, it will be possible to prepare partially protected peptide thioesters directly from adequately protected peptide resins by treating the resins with an acid[6]. But conventional method has limited success in the case of peptide thioesters because thioesters are not generally stable to the basic reaction conditions that are commonly employed for the removal of Fmoc group[7].

II. MATERIAL AND METHODS

All amino acids, resin and linker are purchased from Peptide International, USA. 1-Hydroxybenzotriazole (HOBt), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU), Thioanisol, ethanedithiol, piperidine and diisopropylethylamine (DIEA) were purchased from Sigma Aldrich. All other chemicals and HPLC grade solvents were purchased from Merck (India). RP-HPLC was performed on a Pharmacia Akta purifier instrument using reverse-phase semi preparative column.

Fmoc solid phase synthesis of peptide

Peptides were synthesized on a polymer resin having amino groups as the active functional group, was taken in a 15 mL glass peptide synthesiser containing a sintered ware filter on one side and a receiving adaptor fitted with a calcium chloride guard tube on the other. It was allowed to swell in DMF for 12 hours. To the swelled resin, rink amide linker was attached with the help of HOBt, HBTU and DIEA. The first Fmoc-amino acid was added after the attachment of sulphonamide linker to the linker attached to the resin. The coupling of first amino acid was carried out at -4^0C for 6h. Double coupling was done for the effective attachment of the aminoacid. Fmoc group was removed by using DBU in DMF. Total solid phase peptide synthesis involves a sequence of reactions such as removal of Fmoc group and coupling reaction of amino acids.

Removal of peptide from the resin

When all the amino acids were attached, the Fmoc removed peptidyl resin was activated with iodoacetonitrile that makes the cleavage of peptide- thioester from the resin easier. It was then treated with thiophenol and benzyl

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mercaptan before the treatment of cleavage cocktail. Finally 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. It was then dried under vacuum and lyophilized.

Purification of Peptide

The lyophilized peptide was purified by RP-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 and lyophilized to obtain the pure powder form of the peptide. The mass was confirmed by MALDI-TOF-MS.

III. RESULT AND DISCUSSION

Peptide thioesters were synthesised by solid phase peptide synthesis. Fmoc amino acids were used for the synthesis in order to avoid the formation of side products i.e, unexpected peptides. In conventional methods, piperidine is used for the removal of Fmoc group. But in this case, Fmoc group was removed by using DBU because the thioester linkage is highly unstable towards the piperidine. The rink amide linker helps to cleave the peptide from the polymer resin and the sulphonamide linker helps in the formation of peptides as peptide thioesters. In conventional methods, there is no activation step involved. But in this method activation step is necessary for the easy removal of peptide thioesters from the solid support. For the maximum precipitation of the peptide thioesters, ice cold ether was used as the precipitating agent. The resultant peptide thioester was dried and lyophilized.

The amino acid sequence of the peptide synthesised was GPIQ GPCT GQHL G. The resultant peptide thioester was dissolved in suitable buffer and checked the purity by RP-HPLC. It was performed on Pharmacia Akta purifier system with 214-nm UV detection, using a C-18 analytical column (4.6X250 mm) at a flow rate of 1 mL/min, a sempreparative column (10X250 mm) at a flow rate of 4 mL/min, or a preparative column (25X250 mm) at a flow rate 20 mL/min. All runs used linear gradients of 10-50% buffer B in A (A) water containing 0.1% TFA, B) acetonitrile containing 0.1% TFA) over 50min. The RP-HPLC profile shows that the synthesised peptide thioester is about 60-65% pure.

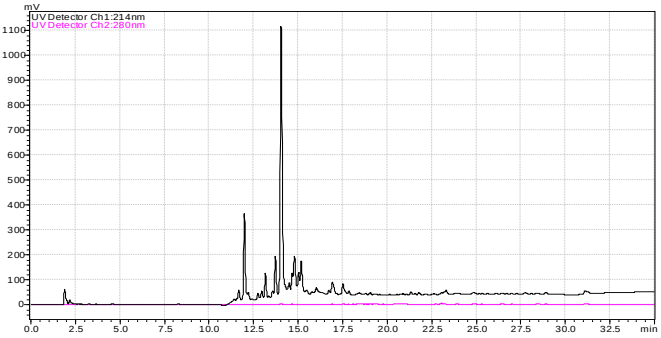

Fig 2 RP-HPLC profile of the peptide thioester

The mass was confirmed by MALDI-TOF-MS. The observed mass is 1396.99 Da. Theoretically the mass was calculated by peptide mass calculator, it was 1396.61 Da. From these it is clear that the observed mass showed a good agreement with the theoretical mass.

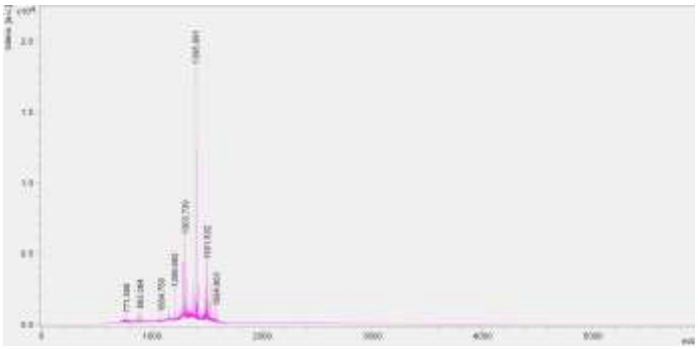

Fig 3 MALDI-TOF MS of the synthesised peptide thioester

IV. CONCLUTION

Solid phase peptide synthesis of peptide thioesters with the help of two linkers gives a good result in comparison with other conventional methods. This new approach of synthesis could overcome the limitations of conventional methods in some extent. The RP-HPLC & MALDI-TOF MS results supported the above statement. RP-HPLC result showed that the peptide thioester is about 60-65% pure. The mass observed from MALDI-TOF MS is almost equal to the theoretical mass.

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