Solid Phase Synthesis of a Fluorescent Peptide: Comparison of Fmoc-Lys(5-FAM)-Resin and Fmoc-Lys[5-FAM(Trt)]-Resin

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Introduction

Many FRET (Fluorescent Resonance Energy Transfer) peptides, as well as non-FRET peptides, requiring C-terminal fluorescent labels can be accomplished using either solution phase or solid phase synthesis. In solid phase synthesis, the use of the fluorophore, 5-carboxyfluorescein (5-FAM) generates stable amide bonds. In order to help facilitate the synthesis of C-terminal fluorescent peptides, we have prepared and performed comparison studies using two kinds of resins, Fmoc-Lys(5-FAM)-Rink Amide resin (**I**) and Fmoc-Lys[5-FAM(trt)]-Rink Amide resin (**II**), the latter contains a phenolic hydroxyl group protected with a trityl group.[1] The reason for introducing the protecting group was to prevent the phenolic hydroxyl groups from reacting with the activated amino acids during peptide synthesis. EREQTVDLSVKRPRTGRKKRRQRRRK(5-FAM)-NH_2, a fluorescent peptide was synthesized using these 2 resins. Syntheses were carried out under the same standard conditions and the peptides obtained showed no significant difference in purity. The results of these studies showed that resin (**I**) is adequate for synthesis of C-terminal fluorescent labeled peptide.

Results and Discussion

Preparation of Resins (I) and (II)

Fmoc-Rink amide resin was deprotected with 20% piperidine in DMF and reacted with Fmoc-Lys(Mtt)-OH [Resin:Fmoc-Lys(Mtt)-OH:HBTU:HOBt:DIEA, 1:3:3:3:6]. After 1h, the resin was thoroughly washed and the Mtt group deprotected with TFA/TIS/DCM (1:2:97). Resin was washed with DCM (twice), MeOH (twice), DCM (twice), 1% DIEA in DMF (twice) and DMF (twice) successively. Fmoc-Lys-Rink amide resin was reacted with 5-FAM [Resin: 5-FAM:DIC:HOBt, 1:2:2:2] in DMF. After the reaction is completed, washed and dried, resin (**I**) was obtained. Resin (**I**) was treated twice with Trt-Cl [resin:Trt-Cl:DIEA, 1:9:9] in DCM for 16 h, then washed and dried to obtain resin (**II**).

Synthesis of EREQTVDLSVKRPRTGRKKRRQRRRK(5-FAM)-NH_2

Synthesis was performed on a SYMPHONY/Multiplex peptide synthesizer (Protein Technologies, Tucson, AZ). Peptide was synthesized using solid phase peptide synthesis employing Fmoc chemistry using resin (**I**) or (**II**). Side chain protecting groups for the amino acids are: t-butyl for Asp, Glu, Ser, Thr, Tyr; trityl for Asn, Cys, Gln, His; Pbf for Arg and Boc for Lys. Couplings were performed with sixfold excess of activated amino acids. Fmoc-amino acids were activated using Fmoc-amino acid: HBTU:HOBt:DIEA, 1:1:1:2, with NMP as the coupling medium. Fmoc protecting groups were removed using 20% piperidine/NMP.

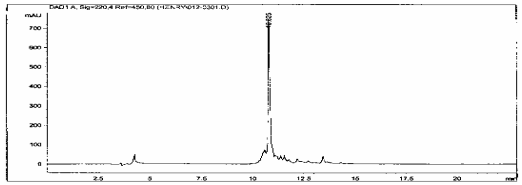

Figure 1. *HPLC profile of crude EREQTVDLSVKRPRTGRKKRRQRRRK(5-FAM)-NH_2 using resin **(II)**. Analysis: A-0.1% TFA/H_2O, B-0.09% TFA/MeCN; 1mL/min, 5-65% B for 20 min.*

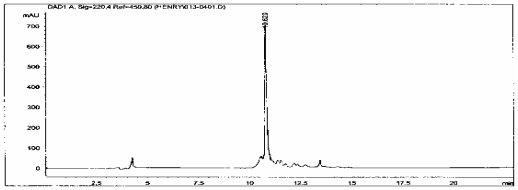

Figure 2. *HPLC profile of crude EREQTVDLSVKRPRTGRKKRRQRRRK(5-FAM)-NH2 using resin **(I)**. Analysis: A-0.1% TFA/H_2O, B-0.09% TFA/MeCN; 1mL/min, 5-65% B for 20 min.*

Upon completion of the chain assembly, the peptide-resin was cleaved with TFA:thioanisole:water:phenol:EDT for 4h. Crude peptides were checked by RP-HPLC (Figure 1-2) and MALDI TOF MS (Figure 3, Theoretical [M+H] 3938.5).

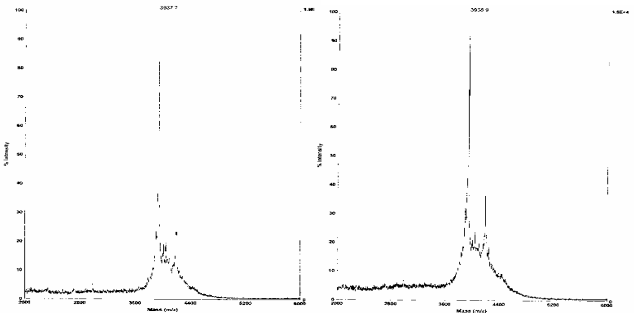

Figure 3. *MALDI-TOF-MS of the crude peptide: (A) Resin **(II)**; (B) Resin **(I)**.*

Conclusion

From the HPLC profiles, the crude peptides obtained with resin (**I**) and resin (**II**) showed no significant difference in purity (Fig. 1 and 2). The crude peptides also showed similar MS data having the correct major MS peak. From these results, the protecting phenolic hydroxyl group with a trityl group in resin (**II**) does not seem to have any apparent advantages; and the unprotected resin (**I**) is adequate for synthesis of C-terminal fluorescent peptide under normal coupling condition.

Reference

1. Fischer, R. et al. *Bioconjugate Chem.* **2003**, *14*, 653-660.