SOLID PHASE SYNTHESIS OF C-TERMINAL PEGYLATED PEPTIDES FOR THERAPEUTIC APPLICATIONS

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Introduction

PEGylation of peptides and proteins have been afforded as a potential tool for enhancing the therapeutic properties of this biomolecules (1). The chemistry for the pegylation of peptides has the principal advantages that the peptide can be modified with PEG on the solid phase during the synthesis using appropriate orthogonal protection scheme for the selectivity pegylation. In the present work, a new PEG-PS support was design to obtain C-terminal pegylated peptide using PEG of molecular weight 1500 and 2000 Da for provide not only the adequate physicochemical properties needy for peptide synthesis also the adequate molecular weight of PEG for enhancing the therapeutic properties of PEG-peptide conjugate. The PEG-PS support was used to obtain pegylated peptide derived from the LBP protein as attractive candidate for sepsis treatment and other diseases associate to lipopolysaccharide (LPS). Peptides were synthesized manually using the Fmoc/tBu strategy, purified by RP-HPLC and characterized by ESI-MS and ^{1}H-NMR. The RP-HPLC profile showed a broad peak due to the PEG polydispersity, the mass spectrum showed good correspondence between theoretical and experimental mass and the ^{1}HNMR showed the signal of PEG at 3.6 ppm. Pegylation did not affect the binding ability of the peptide to LPS and PEG-peptide conjugate showed higher inhibitory effect. Novel PEG-PS support was design and synthesized to obtain C-terminal Pegylated peptide with enhanced therapeutic properties as attractive candidate for treatment of sepsis.

Methods

Synthesis of PEG-PS suport: Fmoc-AM-OH handle was coupled to MBHA resin using DIC/HOBt activation. The Fmoc group was removed by treatment with 20 % piperidine/DMF. The Fmoc-βAla-OH was coupled to the resin. Succinic anhydride and DIEA were added to the resin and reacted during 30 min. Finally, poly (propylene glycol-b-ethylene glycol-b-propylene glycol) bis(2-aminopropyl ether (1500 Da or 2000 Da), PyBOP and DIEA were added and the reaction was shaken for 16 hours.

Peptide Synthesis: The peptides were synthesized manually using the Fmoc/tBu (2) chemistry on PEG-PS resin.

PEG-PS support design

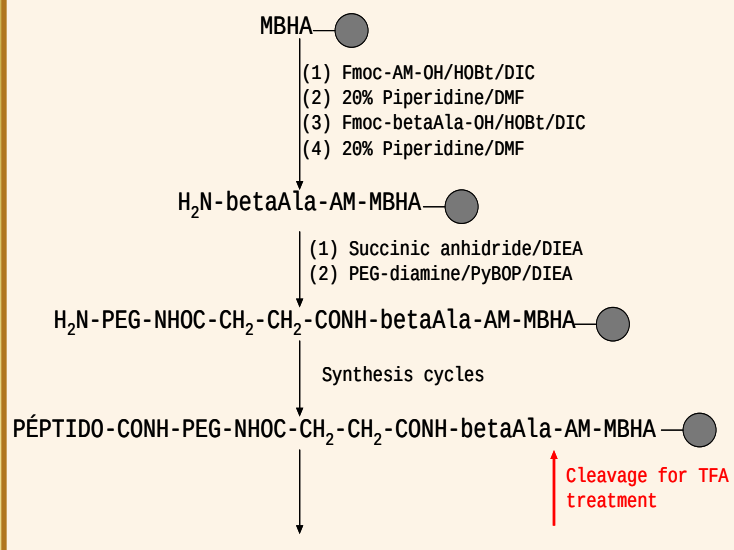


Figure 1. Methodology designed for obtaining the PEG-PS support for synthesis of C-terminal pegylated peptides.

Results

Solid Phase Synthesis of LBPK95A peptide pegylated on C-terminal

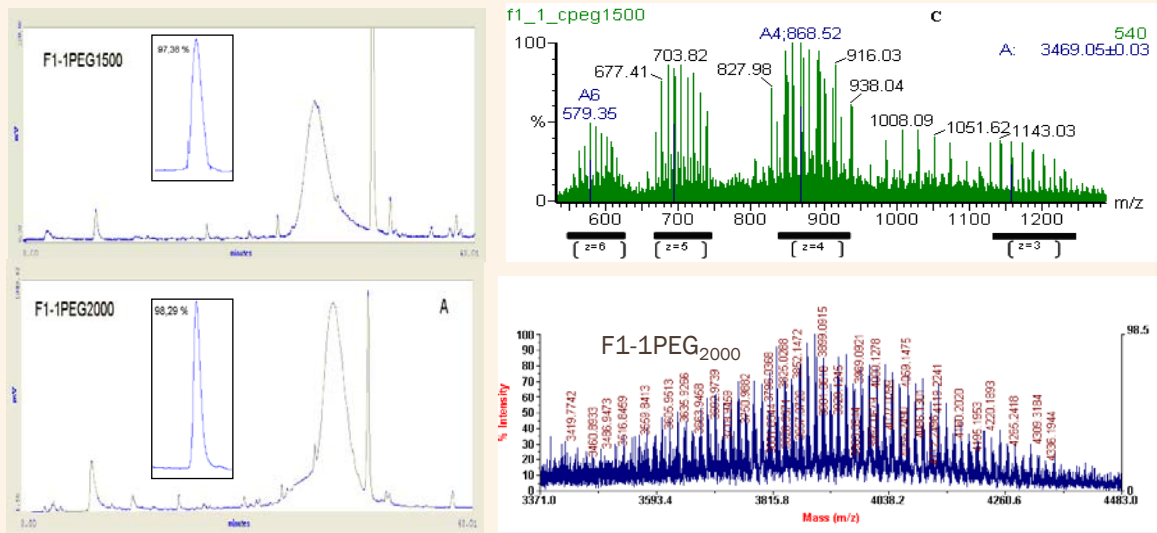


Figure 3: RP-HPLC profile and MS spectra of peptides F1-1PEG$_{1500}$ y F1-1PEG$_{2000}$. Gradient: 5-60% of Buffer B during 35 min. Buffer A: 0,1 TFA/H_2O, Buffer B: 0,05 % TFA/CH_3CN, λ=226 nm. Flow:0,8 mL/min.

The purity grade of each MAP was higher than 95 %. The Mass spectrometry spectrum of each monomer MAP showed good agreement between experimental and theoretical mass

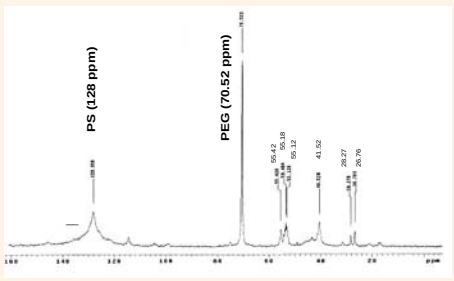


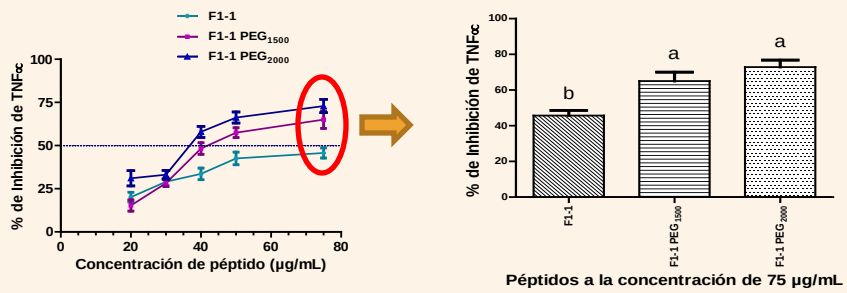
Figure 2. ^{13}C-NMR in gel phase of PEG-PS support. Signal to 128 ppm corresponds to aromatics carbon of PS and signal to 70.52 ppm corresponds to carbons of metilene groups of PEG.

Table 1. Yields and substitution of PEG-PS support

Solid Support	Yield	Substitution
MBHA resin	1 g	0,54 mmol/g
PEG$_{1500}$-PS resin	1,49 g	0,12 mmol/g
PEG$_{2000}$-PS resin	1,66 g	0,11 mmol/g

Low substitution level of PEG-PS resins is due to the PEG crosslinking on the resins during the PEG coupling

Inhibition of LPS-induced TNF production in human whole blood by E1-1 and F1-1 PEGylated peptides



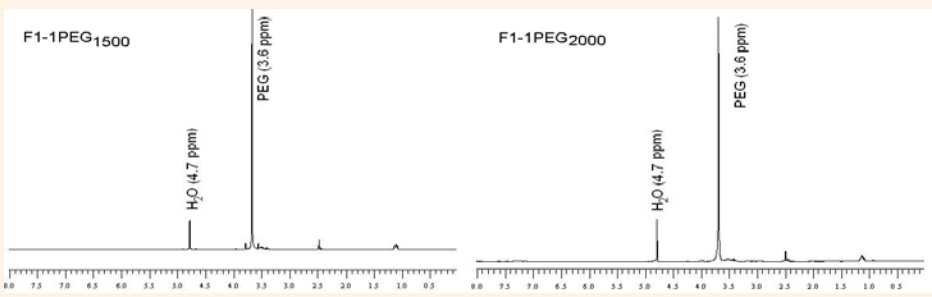


Figure 4. ^{1}H-NMR spectra of peptides of peptides F1-1PEG$_{1500}$ y F1-1PEG$_{2000}$. Signal to 3,6 ppm corresponds to protons of metilene groups of PEG.

C-terminal PEGylation of peptides with PEG$_{1500}$ and PEG$_{2000}$ did not affect the capacity of peptides to inhibit the toxic effect of LPS. The capacity of E1-1PEG$_{2000}$ and F1-1PEG$_{2000}$ peptides to inhibit the toxic effect of LPS was higher than the capacity of unmodified peptides at 75 µg/mL.

References

1. Veronese, F. M. y Pasut, G. (2005). PEGylation, successful approach to drug delivery. Drug Discov. Today 10, 1451-1458.
2. Atherton, E., Fox, H., Harkiss, D., Logan, C. J., Sheppard, R. C. y Williams, B. J. (1978). J. Chem. Soc. Chem. Commun. 537-539.

Conclusions

1. PEG-PS supports were designed and synthesized to obtain C-terminal PEGylated peptides with PEG of molecular weight 1500 and 2000 Da.
2. PEGylation did not affect the capacity of peptides to inhibit the toxic effect of LPS.

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