A Branched Fluorescent Peptide Probe for Real-time Imaging of Activated Human Platelets Infused into a Mouse Model

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Background

Ultra Violet light can be used to irradiate platelets and activate chemically-mediated pathogen reduction, which has been shown to effectively reduce transfusion-associated infections. Yet UV irradiation can cause activation of platelets which may lead to adverse events such as transfusion related acute lung injury. Glycoprotein IIb/IIIa is a surface receptor found on platelets that bind to soluble ligands, including fibrinogen, upon activation as a result of conformational changes. The Pro-X1-X2-X3(Gly)-Asp-X4 sequence was reported as a newly discovered peptide motif that binds to activated platelets.[1] A branched peptide based on this motif has potential for *in vivo* imaging of activated platelets.[2] This imaging probe will enable the study of activated platelet participation in cellular and molecular mechanisms of severe adverse events associated with platelet transfusions in an animal model.

Chemistry

Branched peptides of interest (FP) and control (CP) were synthesized on a Liberty microwave peptide synthesizer from CEM using standard Fmoc chemistry (Figure 1). Fmoc-Lys-Fmoc was used to create the branch point and 6-aminohexanoic acid was used as a spacer. A Cysteine residue at the *N*-terminal was used for the conjugation with Cy5.5 dye.

Experimental

General.

Protected amino acids and *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were purchased from CEM (Matthews, NC) and Fmoc-Lys-Fmoc, 6-

aminohexanoic acid and Rink amide resin from EMD Biosciences (San Diego, CA). The Cy5.5 maleimide was purchased from GE Healthcare (Piscataway, NJ).

Synthesis of FP and CP. Fmoc-protected amino acids (0.5 mmol) were sequentially coupled to Rink Amide resin (167.0 mg) at a 0.6 mmol/g substitution level using HBTU as a peptide coupling reagent. For cleavage of the peptide from the resin, a cleavage cocktail (4.5 mL trifluoroacetic acid, 0.125 mL water, 0.125 mL thioanisole, 0.125 mL liquefied phenol, 0.125 mL of trispropylsilane) was added. The reaction mixture was stirred at room temperature for 2 hours and then filtered. The filtrate was taken to dryness *in vacuo*. Cold diethyl ether was added to the residue to precipitate the product peptide.

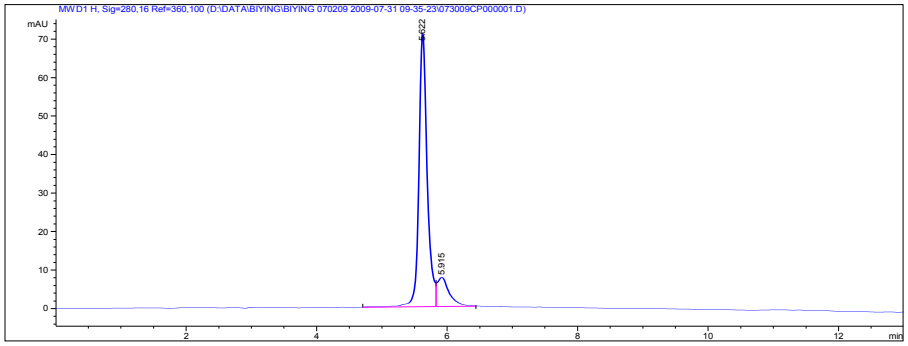
The crude peptide was purified by reversed-phase chromatography on an Agilent HPLC system using a Vydac protein & peptide C_{18} column (4.6×250 mm). The solvent system consisted of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) with a linear gradient of solvent B from 20-65% over 10 minutes at a 1 mL/minute flow rate. The purified CP and FP have retention times of 5.622 minutes and 5.619 minutes, respectively (Figure 2 and 3). The final deprotected peptide was lyophilized to obtain a white solid. MALDI-TOF-MS of CP: $[M+H]^+$ 1838.9, calculated: 1837.9. MALDI-TOF-MS of FP: $[M+H]^+$ 1811.8, calculated: 1809.9.

Figure 1. Structures of CP and FP

CP: Pro-Ser-Pro-Gly-Glu-Trp-Aha
|
Pro-Ser-Pro-Gly-Glu-Trp-Aha-Lys-Gly-Cys-NH_2

FP: Pro-Ser-Pro-Gly-Asp-Trp-Aha
|
Pro-Ser-Pro-Gly-Asp-Trp-Aha-Lys-Gly-Cys-NH_2

Figure 2. HPLC trace and MALDI analysis of purified CP



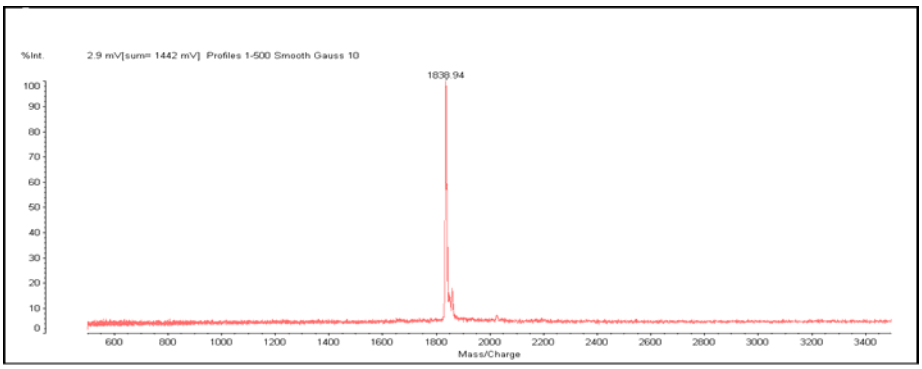
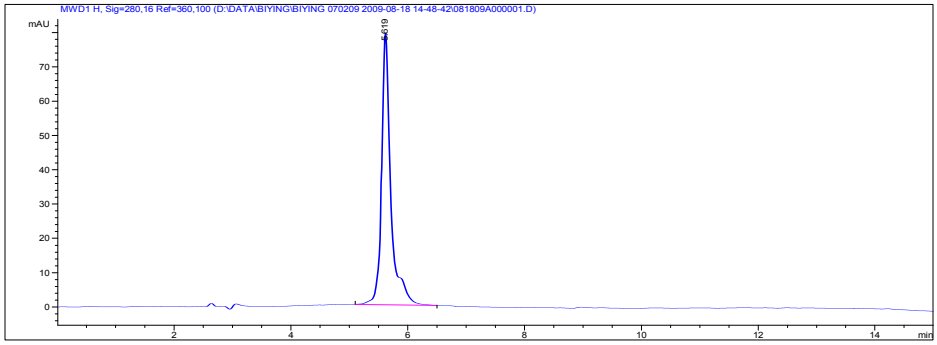
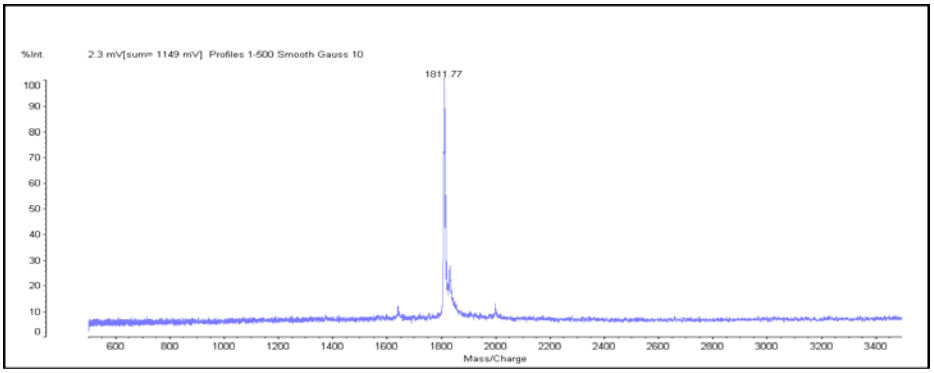


Figure 3. HPLC trace and MALDI analysis of purified FP

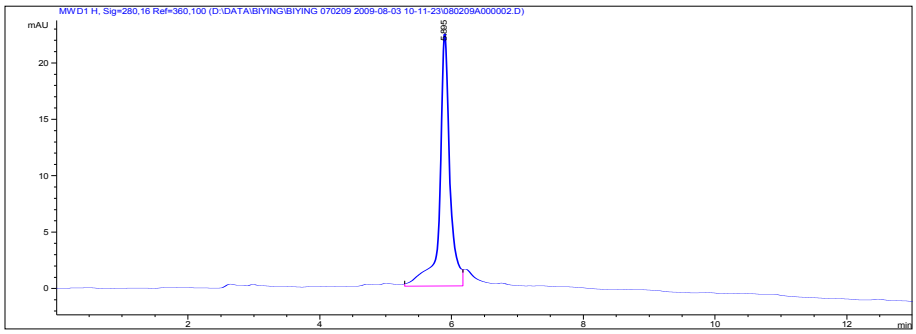




Conjugation of Cy5.5 to peptides. Peptides were dissolved in degassed PBS (pH = 7.4) with a final concentration of 1 mg/ml. 10 μl of TCEP (20 mg/ml) was added and the reaction allowed to proceed at room temperature for 10 minutes. Cy5.5 (5 mg) was dissolved in 50 μl of anhydrous DMF, flushed with argon, and 25 μl of this solution was added to the TCEP-treated peptide mixture. The reaction was allowed to proceed at room temperature for 2 hours and then left at 4°C overnight.

Cy5.5 conjugated peptides were purified by reverse-phase chromatography on an Agilent HPLC system using a Vydac protein & peptide C_{18} column (4.6×250 mm). The solvent system consisted of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) with a linear gradient of solvent B from 20-65% over 10 minutes at a 1 mL/minute flow rate. The purified CP and FP have retention times of 5.895 minutes and 5.835 minutes, respectively. (Figures 4 and 5). The purified Cy5.5-conjugated peptides were lyophilized to produce blue solids. MALDI-TOF-MS of CP: $[M+H]^+$ 2883.54, calculated: 2878. MALDI-TOF-MS of FP: $[M+H]^+$ 2850.39, calculated: 2850.

Figure 4. HPLC trace and MALDI analysis of purified Cy5.5-CP



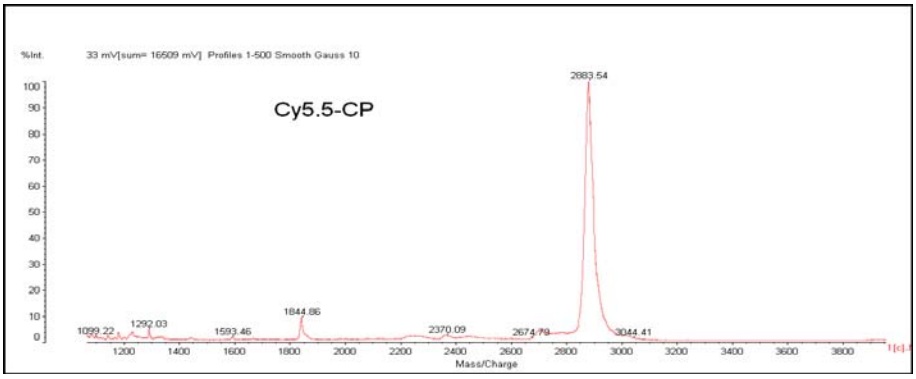
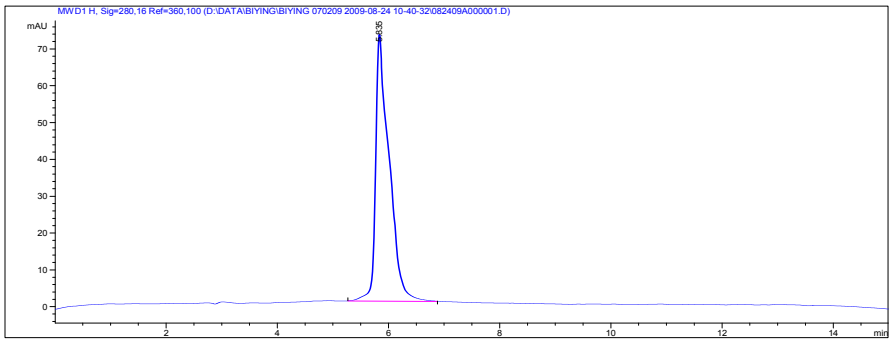
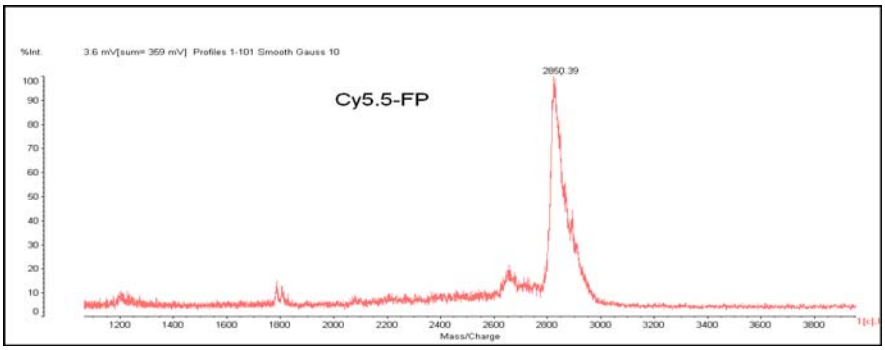


Figure 5. HPLC trace and MALDI analysis of purified Cy5.5-FP





References

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