Abstract

Post-translational modification of peptides plays an important role in various protein-protein interactions. One such modification is the sulfation of tyrosine residues on the extracellular face of transmembrane proteins. The sulfation of these proteins can alter the binding affinity between the extracellular portion of the protein and its respective ligand. C-C chemokine receptor type 5 (CCR5), an HIV-1 co-receptor found on white blood cells, is a transmembrane protein that interacts with chemokines, and also with the gp120 envelope glycoprotein on HIV-1. The affinity of chemokines to CCR5 is affected by the different sulfation patterns on the extracellular portion of the receptor. Isolating the CCR5 protein is inefficient for studying the effects of different sulfation patterns on its affinity because the expression of the protein leads to a heterogeneous mixture of sulfation patterns. Instead, a region of the extracellular N-terminal portion of the protein, Nt-CCR5(8-20), was chemically synthesized using solid phase sulfation, with sulfation of the tyrosine 14 residue. After synthesizing the desired peptide, and studying the deprotection of the neopentyl ether protecting group, the crude peptide was purified using reverse phase HPLC. The availability of this sulfated N-terminal receptor peptide will enable future experiments to study the interaction between this purified peptide and chemokines.

Objectives

- Tyrosine sulfation is a common post translational modification that may affect the protein’s structure, stability, and binding affinity. The tyrosine residues on the extracellular portion of the CCR5 receptor undergo different patterns of sulfation by tyrosine sulfotransferases.
- In order to access specifically sulfated versions of CCR5 to study the effect of sulfation on its interactions with chemokines, residues 8-20 of the extracellular portion of the receptor, Nt-CCR5(8-20), was synthesized using solid-phase peptide synthesis (SPPS).
- In this experiment, Nt-CCR5(8-20) was synthesized with a sulfate group on the tyrosine 14 residue.
- In order to preserve the stability of the sulfate group on tyrosine during acidolytic cleavage from the resin, a neopentyl protecting group was used. The neopentyl group was then removed through deprotection with ammonium acetate. Finally, the resulting crude peptide was purified using reverse phase HPLC.

Methods

Solid-Phase Peptide Synthesis

Figure 4: Chemical synthesis begins at the carboxyl terminus, which is attached to a solid resin support (polystyrene beads). It consists of a series of activation, coupling and deprotection reactions until the desired peptide is acquired. The amino terminal is protected with a Fmoc protecting group to allow selective activation of the carboxyl end of the incoming amino acid with the amino end of the peptide attached to the resin.

Cleavage, Deprotection, and Purification

Figure 5: After the synthesis of the desired sulfopeptide, acidic cleavage conditions are used to release the desired sulfopeptide from the solid support resin. Although the sulfotyrosine residue is labile which may lead to a loss of the acid labile sulfonate moiety, this is prevented by the addition of an orthogonal protecting group on the sulfate. The acid-stable sulfonate protect group used is a neopentyl (np). After the cleavage, deprotection of this protecting group was done using a small molecule reagent, such as ammonium acetate. After deprotection, the desired sulfopeptide can be purified to homogeneity via High Performance Liquid Chromatography (HPLC), performed using gradients of ammonium acetate and acetonitrile to avoid acid promoted hydrolysis of the sulfate esters.

Results

Preparative HPLC Data

Figure 6: Multiple purifications were performed on 4 mg quantities of crude Nt-CCR5(8-20) sulfated on tyrosine 14. It was solubilized in a 1:1 ratio of milliQ water & a 1:3 ratio of ACN to Water using 3.33ml of ACN (no TFA) and 2.66ml of water (no TFA). It was sonicated twice to allow it to better dissolve. A preparative HPLC was used to separate the components of the sample. This is used to purify sufficient quantities of a substance for further use or analysis. The gradient used was 10%-30% of ACN with no TFA in 51 minutes. The solvents that were used were ACN (no TFA) and 0.1M Ammonium Acetate pH8. A C18 reverse phase column was used for the analysis.

Analytical HPLC Data

Figure 7: Shown above is a single chromatograph of our crude sample using an analytical HPLC. An analytical HPLC is used to identify as many constituents of a sample and to determine their concentrations. The crude sample had a purity of 74.65%. The gradient used was 15%-60% ACN with 0.1% TFA in 20 minutes. The column used was 4.6 x 150mm, with detection being at 220 nm and the temperature set at 40ºC.

Figure 8: HPLC of Nt-CCR5(8-20) sulfated on tyrosine 14 purified by Preparative HPLC. The analysis is of fractions with retention times of 30.8min, 32.4 min that had been combined. The resulting material had a purity of 94.65%. The gradient used was 15%-60% ACN with 0.1% TFA in 20 minutes. The column used was 4.6 x 150mm, with detection being at 220 nm and the temperature set at 40ºC.

Conclusions

- Peptides with sulfation on the tyrosine 14 residue have successfully been synthesized, cleaved, and deprotected without the loss of the sulfate group.
- Using reverse phase HPLC, the crude peptide was purified. This product can now be used for future studies to test the effects of sulfation on binding affinity.
- Different sulfation patterns of the peptide can also be synthesized and prepared in a similar manner to study binding affinity.

References

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Background

- Chemokines are small signaling proteins that induce the recruitment of lymphocytes, upon binding to a chemokine receptor.
- One such chemokine receptor is CCR5, which also binds to gp120 on the HIV-1 viral envelope. HIV-1 facilitates entry into a white blood cell by binding to CD4 and its co-receptor, CCR5.
- Studying the interactions between CCR5 and chemokines can help develop ways to prevent the binding of HIV.

Conclusion

- Post-translational modification of peptides plays an important role in various protein-protein interactions. One such modification is the sulfation of tyrosine residues on the extracellular face of transmembrane proteins. The sulfation of these proteins can alter the binding affinity between the extracellular portion of the protein and its respective ligand.