

Synthesis of Cyclic Mucin Peptide Derived from the Tandem Repeat Domain of MUC-1 Mucin

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Abstract

Mucin proteins are membrane-associated glycosylated proteins expressed by normal vertebrate epithelial cells. Specifically, the MUC-1 members serve to lubricate the epithelial surfaces, protect from dehydration, and serve as a barrier to infection in ovarian, airway, gastrointestinal tract, and pancreatic cells lining the ducts. Overexpression and abnormal glycosylation of MUC-1 proteins are found in carcinoma cells and allow for specific adhesive capabilities. The Solid-Phase Peptide Synthesis method was used to synthesize the cyclic mucin peptide TSAPDTRPAP, a portion of the Variable Number Tandem Repeat domain (VNTR) of the MUC-1 protein. In this poster, the reaction mechanisms of peptide synthesis using Fmoc- amino acids and coupling reagents (HBTu and HOBt) will be described. Preliminary characterization of the peptide by One-Dimensional Nuclear Magnetic Resonance Spectroscopy (1D-NMR) and Liquid Chromatography-Mass Spectrometry (LC-MS) will be presented.

Introduction

The mucin protein family consists of membrane-associated glycosylated proteins expressed on normal epithelial cells. Specifically, MUC-1, a member of the mucin family, is normally located on the apical surface of the epithelium in the breast, airway, gastrointestinal tract, pancreas, and among many others near the lumen surface.¹ In these areas MUC-1 functions to lubricate the epithelial surface, protect from dehydration, alter cellular adhesion properties, function in signal transduction, and serve as a barrier to infection.¹ Due to the diversity of carbohydrates present on mucins' surface, harmful foreign objects may bind mucin. Following this the mucin peptide can be cleaved from the epithelium surface and properly disposed of.⁴ Despite MUC-1's conventional and imperative role, a certain form of cancer, breast cancer, is linked to the overexpression and improper glycosylation of this protein.²

Traditionally, MUC-1 consists of a cytosolic, transmembrane, and extracellular domain, with the extracellular domain of most concern in cancers.

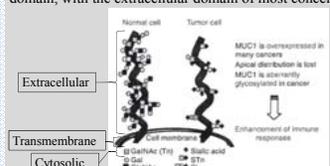


Figure 1. Picture of the MUC-1 peptide displaying the differences in glycosylation between normal and tumor cells.

The extracellular domain possesses a backbone with a Variable Number Tandem Repeat (VNTR) of amino acids that is highly glycosylated. Normally, this VNTR extracellular domain has long length O-linked glycans at the hydroxyl ends of serine and threonine amino acids. However, in breast cancer the extracellular domain has improper and decreased glycosylation, exposing the VNTR backbone. The shorter O-linked glycan side chains result because of the addition of sialic acid by the enzyme sialic acid transferase which terminates the glycan side chain. Overexpression of the enzyme sialic acid transferase correlates with cancer formation. The exposure of the VNTR region in carcinoma epithelia is thought to elicit an immune response that leads to the production of antibodies.² Furthermore, the extracellular portion of mucin may be cleaved from the epithelium and circulate in the blood. As a result, individuals with cancer can be identified by detecting antibody/MUC-1 antigen complexes in the blood using assays that add the MUC-1 peptide to the blood sample and test for the presence of MUC-1 antibodies.⁵

Therefore, the difference in glycosylation of the VNTR backbone region of the MUC-1 plays a crucial role in differentiating cancerous and noncancerous cells. A portion of the fundamental amino acid sequence encoding the VNTR peptide backbone, TSAPDTRPAP, was synthesized in this project to begin investigating its properties. The peptide was synthesized using the Solid-Phase Peptide Synthesis method, using the Wang resin. This poster will present the synthesis methodology of this peptide and preliminary characterization results of the peptide by LC-MS and NMR spectroscopy.

Resources

1. Bardsch, Michael D. et al. "Oligosaccharides Expressed on MUC1 Produced by Pancreatic and Colon Tumor Cell Lines." *Journal of Biological Chemistry*, 272 (1997): 24198-24202.
2. Burchell, Joy et al. "An α2,3 sialyltransferase (ST3Gal) is elevated in primary breast carcinomas." *Glycobiology*, 9 (1999):1307-1311.
3. Habermann, Dr. Nooshin. "Solid Phase Peptide Synthesis." *Wright College*. Retrieved from <http://web.whitaker.edu/people/webpages/personal/webpages/Habermann/Solid%20Phase%20Peptide%20Synthesis.pdf>
4. McAuley, Julie L. et al. "MUC1 cell surface mucin is a critical element of the mucosal barrier to infection." *Journal of Clinical Investigation*, 117 (2007): 2313-2324.
5. Menshoff-Pouilly, S. von et al. "Survival in Early Breast Cancer Patients Is Favorably Influenced by a Natural Humoral Immune Response to Polymorphic Epithelial Mucin." *Journal of Clinical Oncology*, 18 (2000): 574-583.
6. Sigma Aldrich, Co. "Solid Phase Peptide Synthesis Scheme." *Sigma Life Science*. (2009). <http://www.sigmaaldrich.com/life-science/custom-oligos/custom-peptides/learning-center/solid-phase-synthesis.html>

Methods

1. Fmoc Removal (Deblocking) Mechanism

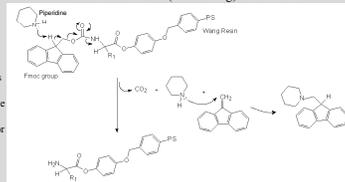


Figure 2. The Fmoc protecting group on the amino acid (a.a.) N-terminus is removed using piperidine. In this process CO₂ is released. Following the Fmoc removal, the growing peptide chain, attached to the resin, is ready for the addition of another a.a.

General Solid-Phase Peptide Synthesis Scheme

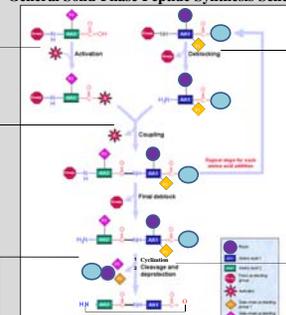


Figure 3. General Solid-Phase Peptide Synthesis Scheme Retrieved from <http://www.sigmaaldrich.com/life-science/custom-oligos/custom-peptides/learning-center/solid-phase-synthesis.html>.

2. Amino Acid Activation and Coupling Mechanism

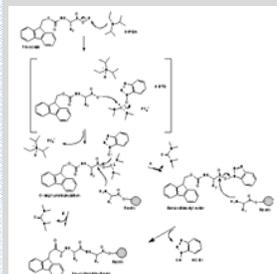


Figure 4. To prepare for a.a. coupling, DIPEA assists in HBTu's activation of the C-terminus. A.a. coupling then occurs at a faster rate to extend the peptide each round.

4. Head-Tail Cyclization Mechanism

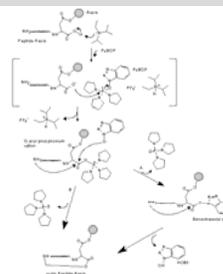


Figure 5. PyBOP and HOBt are used to perform the cyclization between the first and last a.a. Following cyclization the peptide is ready to be cleaved from the resin.

3. Removal of ODMab α-Carboxyl Protecting Group Mechanism

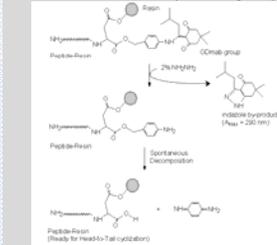


Figure 6. The ODMab group present on a.a. #1's C-terminus must be removed to later make a cyclic peptide. NH₂NH₂ is used to remove the C-protecting group. Following this the peptide is ready for Head-to-Tail cyclization.

Results

The following observations were made using LC-MS and 1D-NMR.

LC-MS

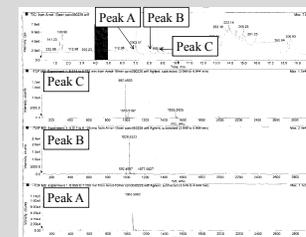


Figure 7. LC-MS spectrum of the crude peptide, exhibiting three ions with m/z of 1063.57 amu, 1028.5 amu, and 992.5 amu. The ion with a m/z ratio of 992.5 has a mass similar to the theoretical mass of the intended cyclic peptide that is approximately 993.1 amu.

1D-NMR



Figure 8. 1D-NMR spectrum of the crude peptide in 100% D₂O. This spectrum lacks the NH's present in Figure 9 because the H's exchange with the D's in D₂O; however, some NH's appear due to the presence of a small amount of H₂O in D₂O.

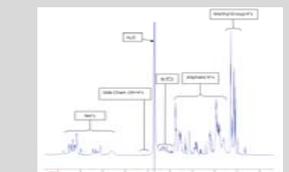


Figure 9. This figure more clearly shows the 1D-NMR spectrum of the crude peptide in 90% H₂O/10% D₂O. It displays the typical characteristics of a peptide NMR spectrum, showing the NH's, α-H's, aliphatic H's (β-H's, γ-H's, and δ-H's), methyl groups, and side chain OH groups.

Conclusions

1. It is highly likely that the peptide synthesized was the expected TSAPDTRPAP cyclic mucin peptide.
 - Peak 'C' in LC-MS Figure 7 possesses the expected m/z ratio of approximately 992.5 amu.
 - 1D-NMR spectra in Figures 8 and 9 display dispersed NH's, suggesting a cyclic peptide.
 - 1D-NMR spectra show all the H's (i.e. α-H, β-H, γ-H, δ-H) typical of a peptide.
 - The Figure 9, 1D-NMR spectrum hints at the presence of sidechain OH groups of threonine and serine studding the peptide, which is consistent with the amino acids incorporated.
2. Future work will include purifications and 2D-NMR to validate the intended peptide synthesized.

Acknowledgements

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