MUC1 Tandem Repeat Structural Studies Using Nuclear Magnetic Resonance Spectroscopy

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Abstract

In this project we employed the Solid Phase Peptide Synthesis Method to synthesize mucin peptides. The methodology for the synthesis of linear and cyclic mucin peptides are presented here. The amino acid sequence of the mucin peptides were derived from the tandem repeat of the mucin-1 (MUC-1) protein. 2D ^{1}H NMR is employed to study the structures of these peptides. Subsequent studies will focus on the binding properties of mucin peptides to monoclonal antibody expressed against tandem repeat of the MUC-1 protein.

Introduction

Mucin proteins are membrane-associated glycosylated proteins expressed by several types of normal epithelial cells. MUC-1 is normally expressed on the luminal surface of breast and ovarian epithelial cells, but in carcinomas MUC-1 is upregulated and aberrantly glycosylated.[2] Like other mucins, MUC-1 is responsible for the lubrication of epithelial surfaces, protection against dehydration, and constitutes a barrier to infection.[4] The tumor-associated MUC-1 contains sugar side chains that include a sialic acid, which terminates chain extension. This is due to the over expression of sialyltransferase (ST3Gal I). The shorter sugar side chain exposes the peptide backbone, which can be recognized by the tumor specific antibody SM3.[2]

The structure of MUC-1 consists of an extracellular domain (N-terminal domain), a transmembrane domain, and a cytoplasmic domain (C-terminal domain). A mid region located on the extracellular domain is termed the "tandem repeat domain" because it consists of a 20-amino acid sequence, which is repeated multiple times. The primary structure of the tandem repeat domain of MUC-1 mucin is rich in proline (Pro), serine (Ser) and threonine (Thr) and contains several O-glycosylation sites at the Ser and Thr residues.[1] The tandem repeat sequence that we propose to base our peptide sequence on is found on human mammary tumor MUC-1 mucin, which has the 20-amino acid tandem repeat being GVTSAPDTRPAPGSTAPPAH.[3] It is believed that the reduction of glycosylation in cancer MUC-1 mucin results in exposure of the core peptide sequence that defines the cryptic epitope region that elicit cellular immune responses.[2] Thus, the amino acid sequence in the tandem repeat region is believed to be one of the sequences that are critical for inducing cellular immune responses against certain types of carcinomas. In this project we present the synthesis of linear and cyclic MUC1 peptides derived from the tandem repeat domain having the amino acid sequence GVTSAPD. The synthesis of this peptide was carried out manually by the Solid Phase Peptide Synthesis Method (SPPS), employing the Wang resin.

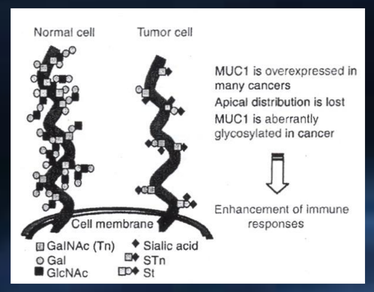

Figure 1.) Modified MUC-1 glycosylation by tumor cells compared to glycosylation of a normal cell.

Procedure

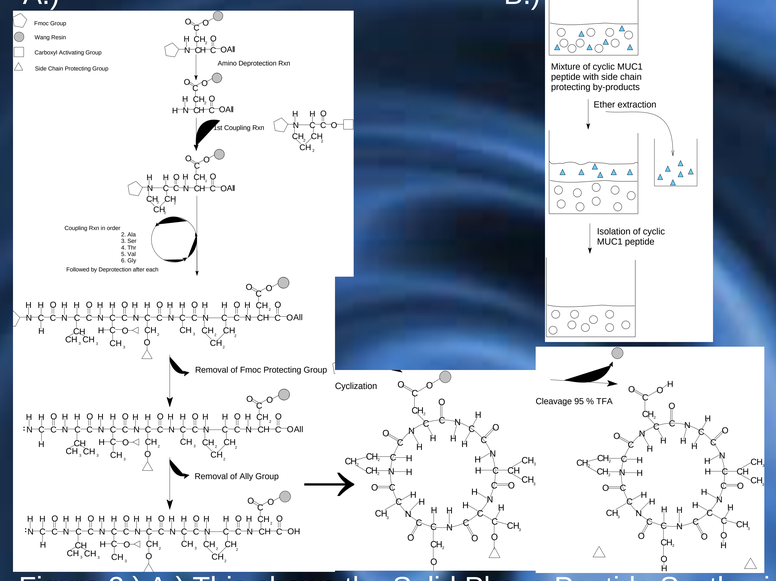

Figure 2.) A.) This shows the Solid Phase Peptide Synthesis of the mucin peptide. Several coupling and deprotection steps were used in the synthesis. First a linear peptide is formed on the resin, then a head-to-tail cyclization method was used to form the cyclic peptide. 95% TFA was added to cleave off the cyclic peptide from the resin. B.) Then, the peptide was extracted by Ether and freeze-dried.

LC/MS Results

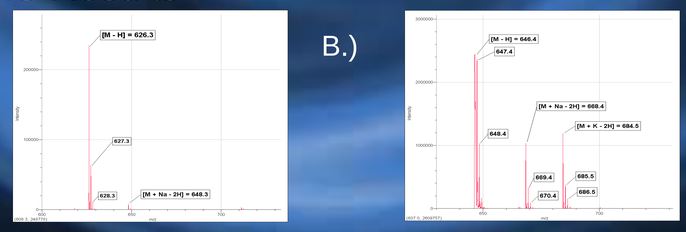

Figure 3.) A.) LC/MS data for cyclic MUC1 peptide showing the parent ion at m/z of 626.3 amu plus its isotopic ions and the parent ion with a Na^+ at m/z value of 648.3 amu. B.) LC/MS data for Linear MUC1 peptide showing the parent ion at m/z of 646.4 amu plus its isotopic ions. The ions [M+Na^+-2H] and [M+K^+-2H] are also observed, at 668.5 amu and 684.5 amu, respectively.

NMR Results

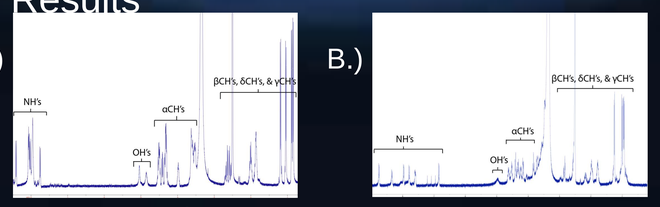

Figure 4.) A.) 1D ^{1}H NMR spectrum for the linear GVTSAPD peptide, and B.) 1D ^{1}H NMR spectrum for cyclic GVTSAPD peptide showing the different spectral characteristics of the spectra.

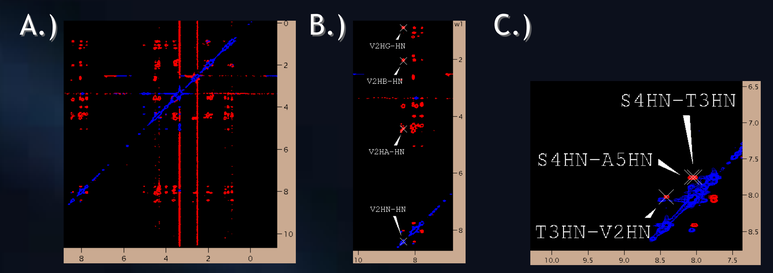

Figure 5.) A.) 2D ROESY ^{1}H NMR spectrum for linear peptide. B.) Example of a spin system determined from the 2D ROESY for valine. C.) ROE peaks in the NH region which provide insight about the 3D structure of the peptide. Complete ^{1}H assignments for all amino acid residues were accomplished using the TOCSY data.

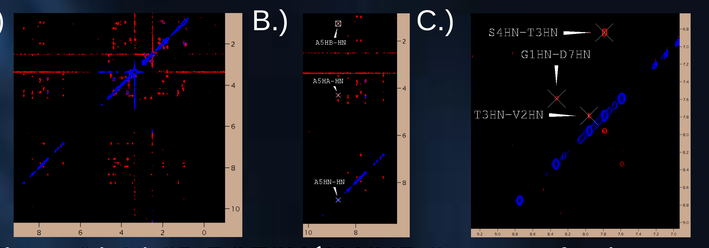

Figure 6.) A.) 2D ROESY ^{1}H NMR Spectrum for the cyclic GVTSAPD peptide. B.) Example of a spin system determined from the 2D ROESY for alanine. C.) ROE peaks in the NH region which provide information about the 3D structure of the peptide.

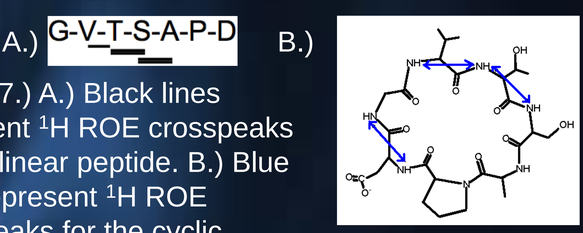

Figure 7.) A.) Black lines represent ^{1}H ROE crosspeaks for the linear peptide. B.) Blue lines represent ^{1}H ROE crosspeaks for the cyclic peptide.

Conclusions

1. For the linear MUC1 peptide, there are three NOEs observed along the backbone of the linear MUC1 peptide at Val_2-Thr_3, Thr_3-Ser_4, and Ser_4-Ala_5 in the NH-NH region.
2. For the cyclic MUC1 peptide, also three NOEs are detected along the peptide backbone centered at Val_2-Thr_3, Thr_3-Ser_4, and Asp_7-Gly_1 in the NH-NH region.
3. The NOEs observed in the NH-NH region indicated that the peptide backbone of both linear and cyclic MUC1 peptides have certain unique three-dimensional structure in dimethyl sulfoxide.

References

1.) Acres, Bruce, and Jean-Marc Limacher. "MUC1 as a Target Antigen for Cancer Immunotherapy." Future Drugs 4 (2005): 493-502.

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3.) Ramanathan, R. K., K. M. Lee, J. McKolanis, E. Hitbold, W. Schraut, A. J. Moser, E. Warnick, T. Whiteside, J. Osborne, H. Kim, R. Day, M. Troetschel, and O. J. Finn. "Phase I Study of a MUC1 Vaccine Composed of Different Doses of MUC1 Peptide with SB-AS2 Adjuvant in Resected and Locally Advanced Pancreatic Cancer." Cancer Immunological Immunotherapy 54 (2005): 254-264.

4.) Schofield, D. P., M. S. Simms, and M. C. Bishop. "MUC1 Mucin in Urological Malignancy." British Journal of Urology International 91 (2003): 560-566.

Acknowledgements

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