Synthesis and Antibody Binding Study of MUC1 Mucin Peptides with Unnatural Backbones

Ryan D. Swenson and Thao Yang ❖ Celebration of Excellence in Research and Creative Activity, May 3-4, 2017 ❖ Eau Claire, WI

Department of Chemistry, University of Wisconsin – Eau Claire (supported by the National Science Foundation (NSF) REU grant award #1460728)

Introduction

The MUC-1 mucin is a heavily glycosylated transmembrane protein found on the apical surface of epithelial cells with a short cytoplasmic end and a longer extracellular domain consisting of multiple 20-amino acid tandem repeats. The known roles of mucin protein include cell to cell interactions, protection of the cell, and lubrication of the cell's surface. In tumor cells the protein has alteration of its carbohydrate chains, thus exposing the core protein to the immune system, appearing as a foreign entity (1,2). This study is focused on synthesis of mucin peptides that have binding properties to the monoclonal antibody produced against the mucin from tumor cells.

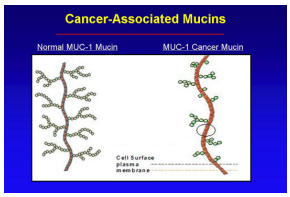

Figure 1. Extracellular domain glycosylation pattern difference between cancer-associated mucin proteins (right) and normal mucin proteins (left). Red circles illustrate the core protein extracellular domain while green circles illustrate the sugar units. http://cancerpace.org/?tag/2007/01/21/mission-in-mcfc-summary/

Aim

In a previous study it has been determined that the shortened mucin peptide with sequence GVTSAPD possesses SM3 monoclonal antibody binding capability, suggesting that this antibody may not be as selective as previously thought as its normal recognition site was accepted to be PDTRP. The results of that study suggest flexibility in terms of the antigen composition and structure. In an attempt to engineer an optimal peptide antigen we synthesized and studied monoclonal antibody binding ability of two mimotopes, GVTSA-4-Aminobenzoic Acid-D and GVTSA-Cyclohexylalanine-D, of the peptide epitope.

References

1. Grinstead, J.S., Kogerty, R.R., Krantz, M.J., Longenecker, M.B., and Campbell, P.A. (2002) *Biochemistry*, 41, 9946-9961.
2. Singh, R. and Bandyopadhyay, D. (2007) *Cancer. Biol. Ther.*, 6, 481-486.
3. Her, C., and Yang, T., (2012) Antibody Binding Study of Mucin Peptide Epitopes. Division of Biological Chemistry, 243th ACS meeting, San Diego, CA., March 25-29.
4. Chan, W. C. and White, P. D., (ed.), (2000) in "Fmoc Solid Phase Peptide Synthesis, A Practical Approach," RP-HPLC using lipophilic chromatography probes, Oxford University Press, p. 269 - 276.
5. Mayer, M. and Meyer, B. (2001) Group Epitope Mapping by Saturation Transfer Difference NMR To Identify Segment of a Ligand in Direct Contact with a Protein Receptor. *J. Am. Chem. Soc.*, 123, 6108 – 6117.

Method

Synthesis of Peptide

- Synthesized peptide through Solid-Phase Peptide Synthesis (SPPS) using Fmoc-Chemistry and Wang resin (3).
- Analyzed peptide using LC-MS (absorbance readings of 220 and 280 nm), followed by purification with HPLC (C_{18} column)
- ^{1}H assignment achieved by 2D NMR (TOCSY, 400 MHz Bruker Spectrometer) at 3-5mg peptide in 20 mM phosphate, 5mM NaCl, pH5 and 7°C.
- STD-NMR analysis for antibody binding experiment (3,5).

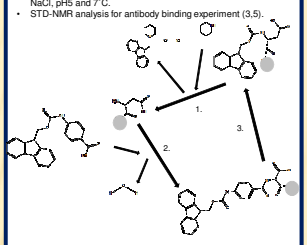

Figure 2. Steps involved in SPPS of linear GVTSA-4Abz-D peptide using Fmoc-Chemistry. 1) Remove Fmoc (20% piperidine). Rinse with DMF (3 x 0.5 min), DCM (3 x 0.5 min), DMF (3 x 0.5 min). Activate new Fmoc-aa with HBTU/HOBt + DIPEA. 2) Coupling reaction (1 hr). Rinse with DMF (2 x 0.5), DCM (3 x 0.5 min), DMF (2 x 0.5). 3) Repeat steps.

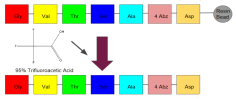

Figure 3. Cleavage of resin bead with 95% Trifluoroacetic Acid.

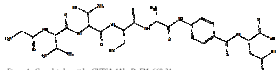

Figure 4. Completed peptide- GVTSA-4Abz-D, FM: 668.31 g

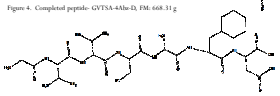

Figure 5. Completed peptide- GVTSA-Cha-D, FM 702.36 g

HPLC Purification

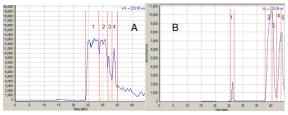

Figure 6. HPLC purification chromatograms. (A) shows peptide epitope GVTSA-4Abz-D with the analyte found in peak 2. (B) shows peptide epitope GVTSA-Cha-D with the analyte found in peak 5 and truncated peptide epitope VTSA-Cha-D found in peak 2.

Post Purification LC-MS

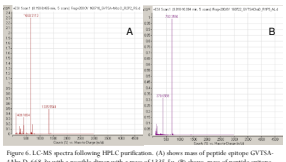

Figure 6. LC-MS spectra following HPLC purification. (A) shows mass of peptide epitope GVTSA-4Abz-D, 668. 3u with a possible dimer with a mass of 1335.3u. (B) shows mass of peptide epitope GVTSA-Cha-D, 702 3u.

Results

NMR Spectroscopy

Protons were assigned by 2D TOCSY and ROESY ^{1}H NMR.

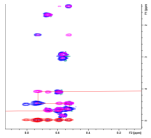

Figure 7. GVTSA4AbzD TOCSY-ROESY Overlap NMR of H-NH Cross Peak Region.

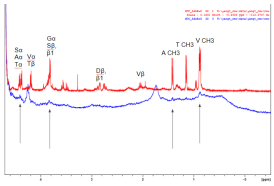

Figure 8. ^{1}H NMR spectra (1–5 ppm) of peptide GVTSA-4Abz-D in the presence of monoclonal antibody SM3 (blue) and reference ^{1}H NMR spectra (red). Arrows indicate STD effect.

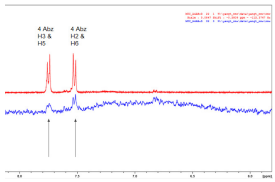

Figure 9. ^{1}H NMR spectra (5–8 ppm) of peptide GVTSA-4Abz-D in the presence of monoclonal antibody SM3 (blue) and reference ^{1}H NMR spectra (red). Arrows indicate STD effect.

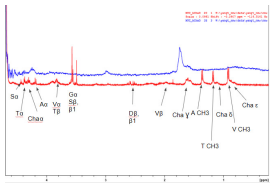

Figure 10. ^{1}H NMR spectra of peptide GVTSA-Cha-D in the presence of monoclonal antibody SM3 (blue) and reference ^{1}H NMR spectra (red). No STD effect observed.

Conclusions and Future Work

- GVTSA4AbzD showed saturation transfer effects at the methyl groups of Alanine and Valine, the hydrogens on the aromatic ring of 4-aminobenzoic acid, as well as ST effects present at about 3.9 ppm, near the Glycine alpha and Serine betas
- GVTSAChaD showed no significant ST effects
- Purify GVTSAPhgD and run antibody-binding test
- Try different residues on GVTSAPD epitope
- Study glycosylated linear and cyclic dimer mucin peptides

Acknowledgments

This research is supported by the National Science Foundation (NSF) REU grant award #1460728. We also acknowledge the UWEC Chemistry Dept. for support of instruments, the UWEC Office of Research and Sponsored Programs (ORSP) for prior supports leading to this research, and the Blugold Commitment for printing this poster.

University of Wisconsin Eau Claire