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

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Introduction

The MUC1 mucin is a heavily glycosylated transmembrane protein found in the apical surface of epithelial cells and serves as a dynamic scaffold for multiple transmembrane and cytokine receptors. The N-terminal domain of this protein is rich in cysteine residues and is involved in the formation of multiple disulfide bonds that are essential for maintaining the structure of the molecule. The C-terminal domain of the mucin contains a number of sites that can be modified by addition of N-acetylglucosamine (GlcNAc) or N-glycolyl-N-acetylneuraminic acid (Neu5Gc). The addition of these modifications can alter the function of the protein, including its ability to bind to other proteins or to be recognized by the immune system. The monoclonal antibody binding site is located in the N-terminal domain of the mucin and consists of multiple 20-amino acid tandem repeats. These repeats can form intra- and intermolecular disulfide bridges, which can affect the conformation and binding properties of the peptide.

Method

Synthesis of Peptide

Synthesis of peptide libraries was achieved through Solid Phase Peptide Synthesis (SPPS). The peptide was synthesized using Fmoc-Chemistry and Wang resin. The sequence of the peptide was GVTSAPD, which was selected based on previous studies. The peptide was synthesized as a linear tetrapeptide and then modified with 4-aminobenzoic acid at the N-terminus to form the peptide GVTSA-Cha-D.

Results

NMR Spectroscopy

NMR spectra were obtained using 2D TOCSY and ROESY at 400 MHz Bruker Spectrometer. Protons were assigned by 2D TOCSY and ROESY ¹H NMR. STD-NMR analysis was performed using 3-5mg peptide in 20 mM phosphate, 5mM NaCl, pH 5 and 7˚C. Spectra were recorded at 1.8 column. Spectra were recorded at 1.8 column. Spectra were recorded at 1.8 column.

Conclusions and Future Work

The results ofthis study suggest flexibility in terms of the antigen composition and that the antibody may not be as selective as previously thought. Future work could include the study of different residues on the GVTSAPD epitope and the effect of these modifications on the binding properties of the peptide.

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References