Analysis of the Antibody Binding of Derivative MUC1 Peptides via STD NMR

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Abstract

The binding epitope PDTRP, found within the VNTR domain of MUC1 glycoprotein, is recognized by the immune system and binds mucin monoclonal antibody SM3. This study analyzes the binding ability of the peptide GVTSAPD, an upstream sequence preceding PDTRP, as well as six derivative peptides against specific mouse MUC1 mucin monoclonal antibody (IgG1, 6A4) by Saturation Transfer Difference NMR (STD NMR) to determine the specific residue critical for antibody binding and whether analogous side chain characteristics in the derivative sequences would enhance binding. The STD NMR results indicated that Pro_6 is a critical residue for binding as it displayed greater saturation transfer effects for its side chain protons than did any other residue. Substituting the Pro_6 residue with single hydrophilic or hydrophobic aliphatic residues eliminated all STD effects while substitution of Pro_6 with single hydrophobic aromatic residues produced STD effects at the aromatic protons. Substitution at Ser_4 position for Asn produced STD effects that were similar in pattern and intensity to those of the native sequence. The results indicate that the Pro_6 residue is critical for antibody binding and substitution at this position for aromatic residues conserves binding ability. This suggests that these substituted peptides may possess biological activity.

Introduction

Mucins (shown in **Fig. 1**) are a class of heavily *o*-glycosylated proteins, most commonly found on epithelial surfaces, which provide many protective cellular functions such as the formation of mucosal barriers [1]. The range of human mucins (MUC) spreads from MUC1 to MUC21, however the specific mucin this study is associated with is the MUC1 transmembrane protein. MUC1 has been detected as a carcinoma-linked antigen. This is believed to be caused by a loss in cellular polarity attributed in part to MUC1 protein. This loss of polarity triggers an epithelial-mesenchymal transition (EMT) producing aggressive cancer cells. Additionally, transmembrane mucins have been found to be overexpressed in cancer cells, leading to prolonged EMT activation and eventual malignancy [2,3]. Thus far it has been difficult to create an effective immunotherapy or vaccine for carcinomas as many tumors produce immunosuppressive effects [5,6].

It is believed that using truncated MUC1 peptide sequences could be an effective vaccination against carcinomas due to the nature of the *o*-glycosylation of cancer cell-associated mucins. These mucin proteins are overexpressed and hypoglycosylated, leaving short amino acid sequences exposed to the extracellular environment [4,5,7]. This potentially increases the efficacy of these proteins as antigens for the signaling of cytotoxic T cells if previously exposed to a vaccination of short peptide epitopes of MUC1 [4,5]. Furthermore, evidence shows that using a mimotope, a derivative sequence of the exposed peptide epitope (a substitution of 1 or 2 amino acids), may elevate the immune response when presented with a tumor cell exhibiting the natural epitope [5,6].

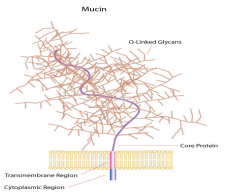

Fig. 1 Transmembrane mucin protein with *o*-glycosylation explicitly shown. This glycosylation is less dense in mucins associated with cancer cell membranes [12].

This study examines the antibody-binding efficacy of 6 of these MUC1 epitope derivatives in addition to the natural epitope that appears in the previously studied tandem repeat GVTSAPDTRPAPGSTAPPAH [7,8]. This was done via saturation transfer difference (STD) NMR with the purpose of determining the specific residues that play an active role in antibody binding. Additionally, the study aims to identify a derivative epitope that would exhibit strong antibody-binding interactions that would eventually elicit a more efficient immune response. Results are shown in **Fig 3:** panels **A** and **B**.

Objectives

Multivalency MUC1 antigens are being sought in the development of cancer vaccine. Substituted mucin peptides such as GVTSAFD and GVTSAWD and their derivatives (cyclic mucin peptides) that have binding ability to MUC1 antibody may provide useful additional epitopes toward designing such agents.

The purpose of the study is to examine the strength of binding interactions between one natural MUC1 epitope and 6 derivative epitopes with monoclonal antibody (mAb) 6A4 via STD NMR. **Fig. 2** shows a representation of the *natural* epitope. Epitopes used are as follows:

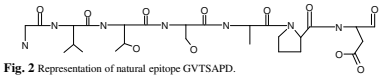

Fig. 2 Representation of natural epitope GVTSAPD.

Natural Peptide Epitope:

GVTSAPD

Derivative peptide epitopes used in this study: *

GVTN**A**PD
GVTSA***D***D
GVTSA***L***D
GVTSA***Y***D
GVTSA***F***D
GVTSA***W***D

*Bolded characters indicate amino acid deviations from the natural epitope.

Methods

Epitope Modification via Peptide Synthesis

All peptides used in this study were manually synthesized in the solid-phase using standard Fmoc-chemistry [9].

HPLC Purification

Purification of synthesized peptides was carried out via reverse phase HPLC with a C_{18} column and water and acetonitrile mobile phases. Isolated peptide fractions were collected for further analyses using a UV/Visible light detection module. Results seen in **Fig. 5**.

Methods

Mass Spectral Analysis

Mass spectra were acquired to begin characterization of obtained peptide fractions. These spectra were compared to the theoretical masses of each peptide epitope used in the study.

NMR Spectroscopy

2D TOCSY and ROESY ^{1}H NMR were used to complete unambiguous proton assignments for all peptide epitopes. STD NMR technique was used to determine peptide-antibody binding. **Fig. 4** below shows one such spectrum off the peptide epitope GVTSAWD. [10,11].

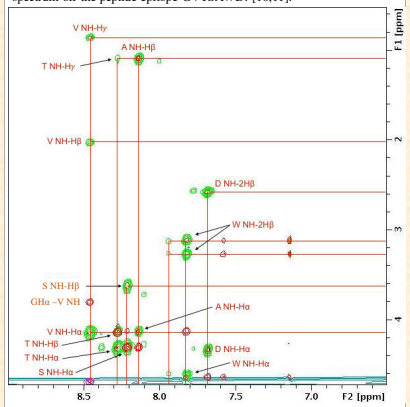

Fig. 4. Overlay of TOCSY (green) and ROESY (red) NMR spectra and spin systems of peptide epitope GVTSAWD.

Results

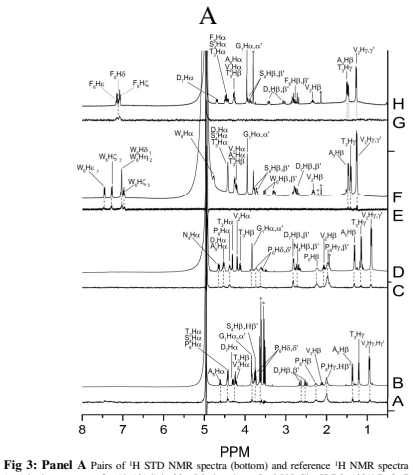

Fig 3: Panel A Pairs of ^{1}H STD NMR spectra (bottom) and reference ^{1}H NMR spectra (top) in the presence of mAb (6A4) in 20 mM phosphate, 5 mM NaCl, pH 7.0, 100% D_2O, 7 °C. Dashed lines connect the protons that experienced STD effect on the STD NMR spectra to the corresponding protons on the ^{1}H NMR spectra. The pairs of spectral traces are: A&B). for GVTSAPD peptide, with highest STD peak intensity located at Pro6Hγ resonance, its intensity was adjusted to the same height as the one in the normal ^{1}H NMR spectrum; note that contaminants at 3.6 ppm were subtracted out in the STD spectra as they do not interact with the mAb; C&D). for GVTNAPD peptide, with similar STD effects to spectrum (A); E&F). for GVTSAWD peptide; G&H). for GVTSAFD peptide. Contaminants are marked by asterisks (*) on both panels. Peptide epitopes GVTSAPD, GVTSAWD, GVTNAPD, and GVTSAFD were all shown to bind mAb (6A4) though at differing intensities.

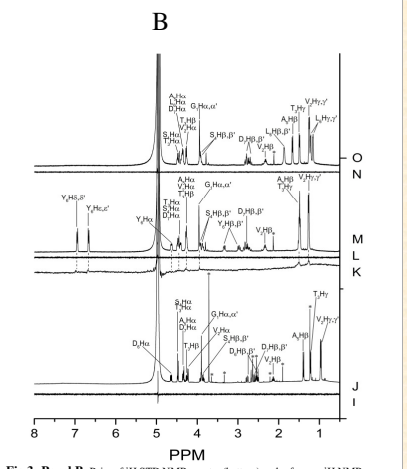

Fig 3: Panel B Pairs of ^{1}H STD NMR spectra (bottom) and reference ^{1}H NMR spectra (top) in the presence of mAb. The pairs of spectral traces are: I&J). for GVTSADD peptide, showing flat line for STD NMR spectrum; K, L&M). for GVTSAYD peptide; (K), same spectrum as L but without protein background subtraction; N&O). for GVTSALD. Peptide epitopes GVTSADD and GVTSALD were not shown to bind mAb (6A4). Peptide epitope GVTSAYD, however, did display limited binding capability.

Results

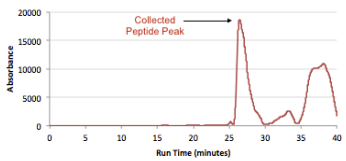

Fig. 5. HPLC chromatogram showing isolated peak of peptide epitope GVTSAWD.

Conclusions

The results indicate that Proline-substituted derivatives GVTSAWD and GVTSAFD retain the ability to bind mAb(6A4), but to a much lesser degree; GVTSALD and GVTSADD do not, however. The normal epitope GVTSAPD, upstream from the main binding site in its domain, retained mAb binding ability as well and was further maintained when Ser_4 was replaced with Asn (GVTNAPD), indicating that Pro_6 is critical for antibody binding. This information indicates that hydrophobic ring structures such as Trp, Phe and Tyr at the Pro position are critical for the binding of antibody.

References

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