Structural Properties of Substituted Mucin Peptides in Solution by 2D NMR

Cheng Her ❖ Thao Yang ❖ Chemistry Department ❖ University of Wisconsin-Eau Claire

Abstract

The short mucin peptide with the sequence Glycine-Valine-Threonine-Serine-Alanine-Proline-Aspartic acid has been shown to have significant interactions with specific monoclonal antibody. Its proline residue appears to be required for the binding. To explore the function of proline further, we studied the structures of three substituted mucin peptides. The three substituted peptides have their proline residues replaced by phenylalanine, aspartic acid, and leucine. We employed two-dimensional nuclear magnetic resonance (2D NMR) to study the structures of these peptides in aqueous solution. The Nuclear Overhauser Effect (NOE) and temperature coefficients of the amide hydrogens were used to evaluate for structural properties. The structure of the peptide will help us understand the function of the different groups on the peptide, thus better assist us in the analysis of the binding experiments that will be carried out in the future. This poster will present structural properties of the three peptides in aqueous buffer in the presence and absence of Sodium Dodecyl Sulfate (SDS). Results showed that there are more NOE's on the peptide structures in buffer with SDS than those in buffer without SDS.

Background

Mucins are high molecular weight transmembrane glycoproteins found on epithelial cells (1). The functions of mucins varies greatly from protection against pathogenic infections to regulation of cell signaling (4). The gene MUC-1 codes for a type of mucin known as MUC-1 mucin. The mucin has tandem repeat domains of 20-mino acid sequence (GVTSAPDT RPPGSTAPPAH) found on the extracellular extension of the protein (3). In normal epithelial cells, MUC-1 mucins are intensively glycosylated and primarily found on the apical surface. The heavy glycosylation gives optimal conditions for hydration, lubrication and protection against pathogens (4). Tumor mucins, on the other hand, are expressed on multiple cell surfaces. Additionally, the glycosylation of tumor MUC-1 mucins is abnormal, resulting in fewer carbohydrate chains and a less complex structure. This simpler and greatly shortened glycosylation makes the cells more vulnerable to infections and leads to the exposure of MUC-1 mucin epitopes that induce low level of immune responses (2). The combination of the exposure of MUC-1 epitopes and the immune system's response to it, suggests an opportunity to utilize mucin-based vaccine for immunotherapy of tumors.

Objectives

- Do mucin peptides possess unique 3D structures indicative of the active structure in solution?
- NOE will be used to evaluate for unique structures of the peptides.

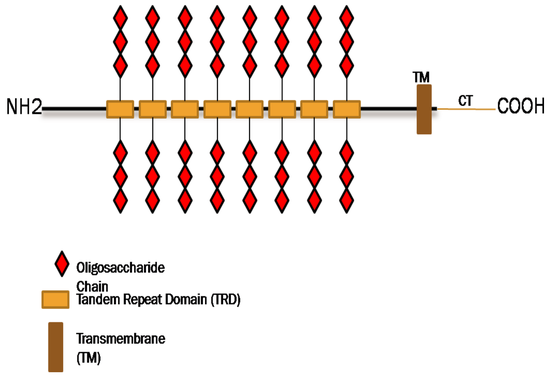

Figure 1. General structure of MUC-1 mucin protein. The extracellular domain consists of heavily glycosylated tandem repeats of the 20- amino acid sequence. There is also a hydrophobic transmembrane domain and a cytosolic tail.

Methods

- Run 1D NMR spectra and assess for quality spectra.
- Run 2D TOCSY experiments for making sequential proton assignments.
- Run 2D ROESY or NOESY experiments for making proton-proton spatial assignment.
- Experiments were carried out in aqueous buffer pH 5 at 7 °C, 25 °C and in SDS micelles at 25 °C.

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Results

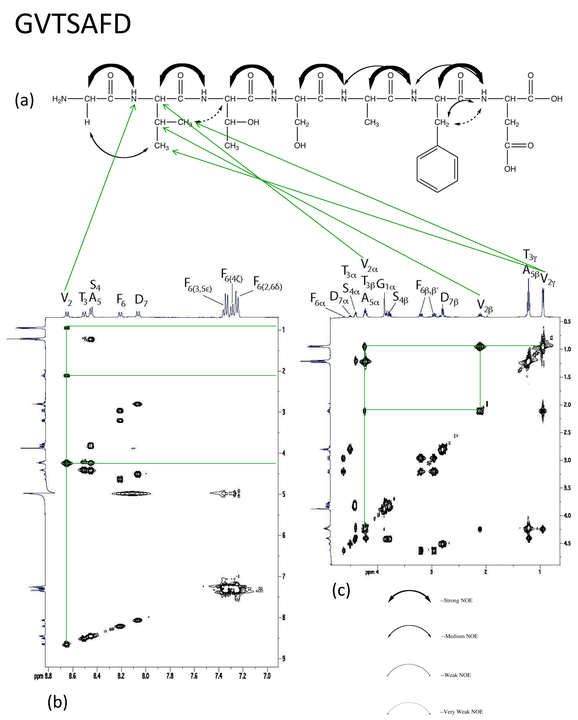


Figure 2. (a) Linear peptide model showing the observed NOE's (curved arrows) of mucin peptide GVTSAFD in 7 °C water. (b) NH region of 2D TOCSY spectrum of GVTSAFD in 7 °C water. Green arrows connect NMR peaks to corresponding protons on the peptide chain. Green lines in the 2D NMR spectrum show an example of a spin system of Valine; the rest of the protons on other amino acids are similarly assigned.

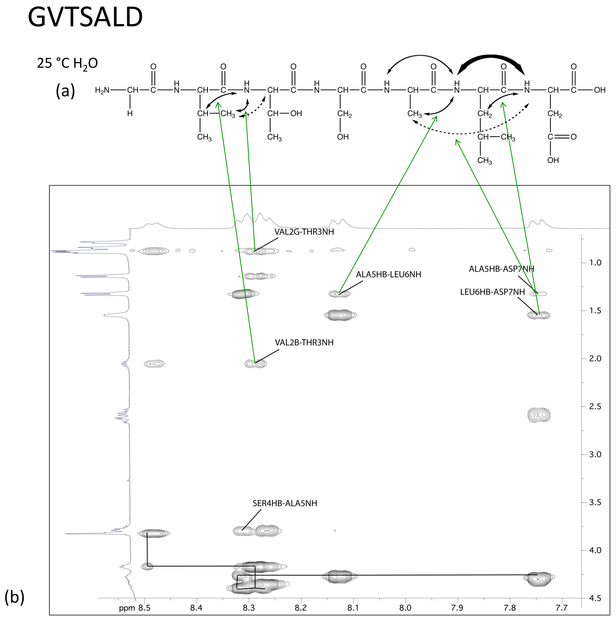


Figure 3. (a) Linear peptide model showing the observed NOE's (curved arrows) for GVTSALD in 25 °C water. (b) 2D ROESY spectrum of peptide GVTSALD in 25 °C water, showing the NOE's for $\alpha H_i - NH_{(i+1)}$ sequential assignments along the peptide backbone. Green arrows connect NOE's observed in the 2D NMR data to the corresponding two H's that gave rise to those particular NOE's.

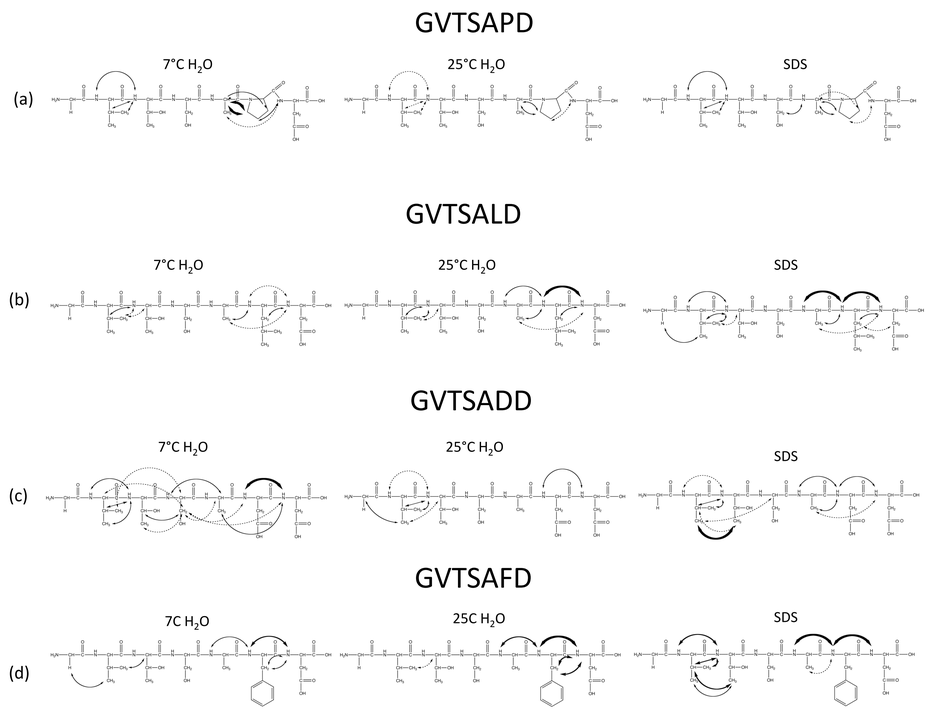
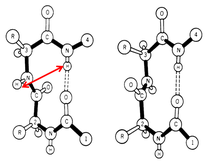


Figure 4. Linear peptide model showing NOE of (a) GVTSAPD in 7° C water, 25° C water, and SDS, (b) GVTSALD in 7° C water, 25° C water, and SDS, (c) GVTSALD in 7° C water, 25° C water and SDS, and (d) GVTSAFD in 7° C water, 25° C water and SDS.

Table 1. Temperature coefficients (ppm/°C) of the last two residues in each peptide in water and SDS. Values multiplied by -10^3.

Temp. Coeff. in water	GVTSALD	GVTSADD	GVTSAFD	Temp. Coeff. in SDS	GVTSALD	GVTSADD	GVTSAFD
NH of residue 6	7.3	5.3	6.3	NH of residue 6	6.6	5.6	3.3
NH of residue 7	4.7	5.6	4.1	NH of residue 7	4.4	4.3	3.1



Ideal β-turn

Discussion

In an ideal β-turn there is a hydrogen bond between the $CO_{(i)} - NH_{(i+3)}$ which makes the distance between $NH_{(i+2)}$ - $NH_{(i+3)}$ to be less than 5 Å, indicated by the red arrows on the β-turn (5). The low temperature coefficient of the NH of the last residue in our data indicates the presence of a hydrogen bond between the Asp7-NH and the Ser4-CO. Furthermore, the NH NOE of the 6th and 7th residues is relatively strong, indicating their close proximity (similar to the red arrows on the β-turn). These two observations are consistent with the properties of a beta-turn structure.

Conclusion

•The low NH temperature coefficient of the last residue suggests the presence of hydrogen bond with that NH.
•There are strong NH-NH NOE interactions of the last three residues.
•These two observations suggest the presence of a beta turn structure at the C-terminal.
•For GVTSALD and GVTSAFD, the NH-NH NOE interactions of the last three residues are stronger in SDS solution than in water.
•All substituted peptides studied show properties indicative of a β-turn structure at the C-terminal.

References

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