Probing the Effect of the Outer Saccharide Resides of N-Linked Glycans on Peptide Conformation

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Asparagine-linked glycosylation, a co-translational protein modification reaction, has the capacity to influence the protein folding process.1 Within the extracellular domain of the α-subunit of the nicotinic acetylcholine receptor (nAChR), an N-linked glycosylation site at Asn141 has been demonstrated to be essential for the folding and assembly of the multimeric nAChR complex.2 The disulfide bond between residues Cys128 and Cys142 that closes this conserved extracellular loop is also critical for the structural integrity of the receptor complex; mutation of the cysteine residues3 or expression of the protein in the presence of reducing agents4 severely impacts the oligomerization of the protein complex. In addition, this loop includes a conserved proline residue at position 136 that is required for nAChR folding and assembly.5

In previous studies of this extracellular loop peptide (peptide 1), a simple chitobiosyl derivative, representing a truncated analogue of the saccharide attached in vivo, at Asn141 was found to influence the free energy of disulfide bond formation and the cis/trans isomerization of the proline peptide bond. Since these processes represent slow steps in the protein-folding process, this observation may provide insight into why N-linked glycosylation is required for the folding and oligomerization of the nAChR. The purpose of this report is to examine the structural effects that larger, mature oligosaccharides exert on the structure of 1. Although it is known that outer saccharide residues play critical roles in glycoprotein function,6 studies to establish how larger saccharides impact peptide and protein conformation have been limited due to the difficulties associated with the preparation of sufficient quantities of homogeneous samples of complex glycopeptides to allow detailed spectroscopic analysis. Since disulfide bond formation and proline isomerization can be assessed quantitatively, it was envisioned that these aspects of peptide structure could be used to accurately measure the conformational effects of the outer saccharides.

Glycosylated analogues of peptide 1 derivatized with a monosaccharide (2), and with a disaccharide (3), were synthesized using previously published methods.7,8 Glycopeptide 4, an analogue derivatized with a complex-type, biantennary saccharide at Asn141, was prepared via a powerful chemoenzymatic method.9 Specifically, glycopeptide 2 was subjected to treatment with Endo-M, an endoglycosidase enzyme that catalyzes a transglycosylation reaction between the synthetic glycopeptide and a proteolytic fragment of the glycoprotein transferrin to produce glycopeptide 4 in good yield. Additionally, the outer sialic acid and galactose residues were enzymatically cleaved from 4 to generate glycopeptides 5 and 6 (see Figure 1), respectively.10

The solution structures of peptides were investigated using standard homonuclear 2D NMR techniques. An NOE-restrained simulated annealing protocol revealed that the peptides adopted a turn structure at the central residues of the loop (His134 and Phe135).11 Although inspection of the NOE data revealed no major structural changes between the nonglycosylated and glycosylated peptides, the glycosylated peptides exhibited a number of spectroscopic characteristics that together reveal a modest, but detectable effect upon modification with larger saccharide derivatives.

NMR studies reveal several weak NOE signals between the asparagine and proximal N-acetylglucosamine in glycopeptide 4, but these NOE signals were not observed for peptides 2 and 3, suggesting that the outer saccharide may conformationally stabilize the core N-acetyl glucosamine residues. The absence of these NOE signals could also be due to the lower molecular weight of 3; however, examination of a series of reference cross-peaks of glycopeptide 3 and 4 revealed that the change in molecular weight did not impact the relative NOE intensities of these peptides. It is noteworthy that neither glycopeptide 5 or 6 exhibited any significant changes in the NOE spectra relative to glycopeptide 4.

Supporting Information for full details.

References and Notes


(9) ES MS for 1 (C16H19NO5S2): Calcd 2098.36; Obsd [MH]+ 2099. (10) ES MS for Glycopeptide 3 (C110H159N25O38S2): Calcd 2504.7; Obsd [MH]+ 2505. See Supporting Information.


Table 1. Percentage of Prolyl–Peptidyl Bond cis Isomer at pH 7.7, 65 °C; Rates of cis/trans Isomerization at pH 7.7, 65 °C

<table>
<thead>
<tr>
<th>Peptide</th>
<th>% cis</th>
<th>k_{cis-trans} (s^{-1})</th>
<th>k_{trans-cis} (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>14.7 ± 0.2</td>
<td>23.0 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>7.26 ± 0.1</td>
<td>16.2 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>7.10 ± 0.2</td>
<td>17.4 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>5.33 ± 0.5</td>
<td>15.2 ± 0.5</td>
</tr>
</tbody>
</table>

Figure 2. Exchange (NOESY) spectra of peptide 1 7 °C (left) 65 °C (right).

Adoption of the correct prolyl isomer is often a slow step in the protein-folding process due to the relatively high activation energy required for isomerization (20 kcal/mol). The saccharide could slow the rate of peptide bond isomerization by more effectively solvating the proline residue; desolvation of the proline amide bond has been proposed to favor the neutral transition state of the isomerization process. However, it is unlikely that the smaller saccharides that decorate peptides 2 and 3 would be able to directly interact with the proline peptide bond. Alternatively, the saccharide may decrease the flexibility at the glycosylation site; numerous examples have been described where glycosylation rigidifies or stabilizes the structure of the peptide or protein. A decrease in flexibility of the peptide backbone may result in a decrease in the rate of cis–trans interconversion of the proline amide bond.

This study suggests that addition of the N-linked saccharide affects certain key steps of protein folding, namely disulfide bond formation and cis/trans proline isomerization. Herein we directly compare the conformational effect of truncated saccharides with larger oligosaccharide structures found in vivo. Although the first two N-acetylglucosamine residues mediate the bulk of the conformational effect, the addition of the outer saccharide residues also appear to influence the peptide structure, indicating that nature may also utilize these outer residues to favorably affect peptide structure. The spectroscopic studies presented suggest that the primary conformational effect of the N-linked glycan is derived from the first two saccharide residues; the lack of observed NOE signals between saccharide and peptide suggests that the saccharide rigidifies the local structure of the peptide and does not interact directly with specific residues (such as proline) in the peptide backbone.

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Supporting Information Available: Details of glycopeptide synthesis, NMR studies, determination of K_{on} values and chemical shift assignments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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