The Stabilizing Effects of O-glycosylation on the Secondary Structural Integrity of the Designed α-loop-α motif by Molecular Dynamics Simulations

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Abstract

In this study, various 400 ps molecular dynamics simulations were conducted to determine the stabilizing effect of O-glycosylation on the secondary structural integrity of the design α-loop-α motif, which has the optimal loop length of 7 Gly residues (denoted as N-A1G3-A1G5-C). In general, O-glycosylation stabilizes the structural integrity of the model peptide regardless of the length and position of glycosylation sites because it decreases the opportunity for water molecules to compete for the intramolecular hydrogen bonds. The designed peptide exhibits the highest helicity when residues 11 and 31 are replaced with Ser residues followed by O-linked with 3 galactose residues, representing the “face-to-face” glycosylation near the loop. In this case, the loop exhibits an extended conformation and several new hydrogen bonds are observed between the main chain of the loop and the galactose residues, resulting in decreasing the fluctuation and increasing the stability of the entire peptide. When the glycosylation are made close to the loop, the secondary structural integrity of the α-loop-α motif increases with the number of galactose residues. In addition, “face-to-face” glycosylation increases the structural integrity of this motif to a greater extent than “back-to-back” glycosylation. However, when the glycosylation are created away from the loop and near the N- and C-termini, no general rule is found for the stabilizing effect.

Key words: Molecular dynamics simulations, O-glycosylation, α-loop-α, Hydrogen bond, Galactose.

Introduction

The de novo design of peptides and proteins has emerged as an important approach for investigating protein structure/function relationships. It also provides a powerful methodology for investigating protein folding (1). Considerable effort is now being directed to the de novo prediction of protein conformations with the aid of computational methods (2, 3). A variety of unique structural motifs, such as coiled coils, four-helix bundles, β-sheet and mixed α-β structures, and α-loop-α, have been designed and reported to fold in solution as determined by nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopy (4-6). Among these, α-loop-α motif has provided model systems for dissecting and quantifying the multiple interactions that stabilize secondary structure formation and for designing site-specific reactivity of functionalized polypeptides (7, 8), such as site-selective glycosylation (9). In addition, it also represents one of the best recognized and fundamental building blocks used in the construction of functional proteins. Usually, several strategies are used to create a stable α-loop-α motif, including (i) incorporating many helix-stabilizing Ala residues for each α-helix (10); (ii) adding salt bridges between residues separated by one helical turn (11); (iii) introducing covalent macrocycles (12); and (iv) incorporating glycosylations, which can be accomplished through interactions with surrounding carbohydrate residues introduced with an experimental (13) or computational (14) combinatorial approach.
The best characterized examples of protein-oligosaccharide bonds in glycoproteins are the N- and O-glycosidic linkages of Asn, Ser, or Thr side chains (15). Glycosylations of proteins have been previously achieved by glycosylation engineering, enzymatic synthesis, or chemical synthesis (15). Furthermore, the site-selective incorporation of carbohydrates into folded peptides in a one-step reaction in aqueous solution and room temperature has also been reported (9). The results from both NMR and CD spectroscopy showed that the incorporation of the galactose residue stabilizes the folded structure of the designed α-loop-α motif. Therefore, it may be of general use in the study of structure/function relationships of proteins in central areas of glycobiology.

As mentioned above, the α-loop-α motif allows polypeptide chains to form stable α-helices, which often present recognition sequences in biological processes (16). In addition, the de novo construction of a compact α-loop-α peptide, in which the amino acid residues essential for the molecular recognition could be embedded, provides an approach to mimetic and antagonist design. Although the basic principles for designing α-helices with desired structural properties have been intensively studied, the connecting loop between two α-helices is less well-defined since it usually plays a minor role on the folding processes (17). However, recent study has shown that the effect of the loop is an important consideration for folding of a stable α-loop-α motif (18). In our previous study, various molecular dynamics (MD) simulations were conducted to determine the optimal length of the Gly loop in the α-loop-α motif (19). The results revealed that the length of the loop significantly affects the stability of the two α-helices. In addition, the optimal loop length to maintain the highest helicity of the α-loop-α motif was found to be the one corresponding to 7 Gly residues.

As protein glycosylation is a common posttranslational modification that produces glycoproteins that are highly complex in terms of both their structure and function, systematic structure/function studies of such glycosylations based on synthetic or computational methods are highly desired. In this study, a set of MD simulations was performed to investigate the stabilizing effects of various types of O-glycosylation on the secondary structural integrity of the designed α-loop-α motif with the optimal loop length of 7 Gly residues (19).

**Methods**

The template peptide with amino acid sequence of N-A_{16}G_{17}A_{16}-C, where the subscript numbers represent the number of repeating residues, was built by the Biopolymer module of the Insight II program (Accelrys, San Diego, CA). The α-loop-α motif was designed based on the structural properties of intermolecular antiparallel coiled-coils (20, 21). Residue pairs [4, 38] and [11, 31] of the template peptide were replaced with Ser residues, following by O-linked with 0, 1, 2, or 3 galactoses to generate 8 model peptides representing the “face-to-face” glycosylations away from and close to the loop, respectively (Fig. 1). In contrast, residue pairs [6, 36] and [13, 29] of the template peptide were replaced with Ser residues, following by O-linked with 0, 1, 2, or 3 galactoses to generate 8 model peptides representing the “back-to-back” glycosylations away from and close to the loop, respectively (Fig. 1). These synthesized peptides were modeled in the SGI O200 workstation (64-bit MIPS R12000 270 MHz CPU and PMC-Sierra RM7000A 350 MHz processor; Silicon Graphics, Inc., Mountain View, CA, USA)-Insight II system with the force field Discover CVFF (consistent valence force field) (22).

The template and model peptides were submitted to 5,000 energy minimization iterations using conjugate gradient method and then used as the initial structures for subsequent MD simulations. Each of the energy-minimized peptides were placed in the center of a pseudo unit cell with the size of 50 × 60 × 70 Å^3 soaked with water molecules. In order to arrange the soaked water molecules randomly, solvent
molecules alone were submitted to 10,000 iterations by conjugate gradient mini-
mization, keeping the peptide atoms fixed to avoid the change of the initial struc-
ture during the solvent randomization step. The systems composed of the mini-
mized water molecules and the peptide were used as the starting images since the
solvent molecules and peptide have both achieved in their well energy-minimized
structures. These systems were then submitted to 400 ps MD simulations after
equilibrating for about 10 ps at 300K, using the Discover module of the Insight II
program. The temperature was maintained for each MD simulation by weak cou-
gling the system to a heat bath according to the method described by Berendsen et
al. (23). The minimum image periodic boundary conditions (PBC) were used to
keep the constant volume during the MD courses. The range of cut-off radius was
set as 14 Å for both non-bonded electrostatic and van der Waals interactions. The
time-step of the MD simulations was 1 fs. The trajectories and coordinates were
saved every 2 ps for further analysis. Secondary structures were predicted based
on the dictionary of secondary structure prediction (DSSP) (24), in which pattern
recognition of hydrogen-bonded was correlated to the geometrical features.

![Diagram](image)

**Figure 1**: Cartoon representation of the designed α-loop-α motif with various O-glycosylations. Naming of each peptide is according to the following rules: Gal represents O-glycosylation by galactose; the 1st and the 2nd numbers represent the sites of O-glycosylation, and the 3rd number represents the number of galactose residues attached to each of the O-glycosylation sites.

**Results and Discussion**

Previous study has shown that the incorporation of galactose residues stabilizes the
tertiary structure of the folded α-loop-α polypeptide motif by NMR and CD spec-
troscopy (9). This interesting result has allowed us to systematically conduct a set
of MD simulations to investigate the stabilizing effects of various types of O-gly-
cosylation on the secondary structural integrity of the designed α-loop-α motif, which
has the optimal loop length of 7 Gly residues (19). Figure 2 shows the sec-
dondary structure propensity of the designed α-loop-α motif predicted according to
DSSP (24) as a function of simulation time. In addition, the helicity of each structure
was plotted as a function of simulation time as shown in Figure 3. The aver-
age helicities of these structures during the entire MD courses are further summarized in Table I. In general, O-glycosylation increases the secondary structural integrity of the designed α-loop-α motif, particularly for the case of Gal_{11,31,3}, where residue pairs [11, 31] of the template peptide were replaced with Ser residues, following by O-linked with 3 galactose residues. This structure, representing the “face-to-face” glycosylation close to the loop, exhibits the highest averaged helicity of 86% (Table I). In addition, the stabilizing effect of O-glycosylation on the secondary structural integrity of the designed α-loop-α motif is more significant when the sites of O-glycosylation were made close to the loop. In the cases of “face-to-face” glycosylations close to the loop, the averaged helicity of the α-loop-α motif increases with the number of galactose O-linked to the glycosylation sites. In contrast, in the cases of “back-to-back” glycosylations close to the loop, the averaged helicity of the α-loop-α motif decreases with the number of galactose attached to the glycosylation sites. However, when the glycosylations were made away from the loop, the stabilizing effect is not directly related to the number of galactose in the glycosylation sites.

Figure 3: Helicity as a function of MD simulation time for the α-loop-α motif with various O-glycosylations.

Figure 4: Snapshots of (A) Gal_{11,31}; (B) Gal_{11,31,3}; and (C) Gal_{13,29,3} during 400 ps MD simulations. Peptides and sugars are shown in ribbon and Corey-Pauling-Koltun (CPK), respectively.

Figure 5: Top and side views of (A) Gal_{4,38}; (B) Gal_{11,31,2}; (C) Gal_{4,38,3}; and (D) Gal_{11,31,3} at the end of 400 ps MD simulations. Peptides and sugars are shown in ribbon and CPK, respectively.

Figure 2: Secondary structures as a function of MD simulation time for the α-loop-α motif with 0, 1, 2, and 3 galactoses O-linked to the Ser pairs of (A) 4 and 38; (B) 11 and 31; (C) 6 and 36; and (D) 13 and 29. The numbers shown on the y-axis in each plot indicate the number of amino acid residues. α-Helix, turn, and coil estimated according to DSSP (24) are shown in red, blue, and green, respectively.
Table 1
Averaged helicity of the designed α-loop-α structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>Averaged helicity (%)</th>
<th>N-helix</th>
<th>C-helix</th>
<th>Total</th>
</tr>
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<tr>
<td>Gal_4_38</td>
<td>54.3</td>
<td>72.3</td>
<td>62.3</td>
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<tr>
<td>Gal_4_38_1</td>
<td>84.0</td>
<td>74.2</td>
<td>79.1</td>
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<tr>
<td>Gal_4_38_2</td>
<td>40.1</td>
<td>81.5</td>
<td>61.0</td>
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</tr>
<tr>
<td>Gal_4_38_3</td>
<td>79.8</td>
<td>44.2</td>
<td>62.0</td>
<td></td>
</tr>
<tr>
<td>Gal_6_36</td>
<td>64.3</td>
<td>82.0</td>
<td>73.2</td>
<td></td>
</tr>
<tr>
<td>Gal_6_36_1</td>
<td>45.4</td>
<td>90.0</td>
<td>68.0</td>
<td></td>
</tr>
<tr>
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<td>80.0</td>
<td>42.0</td>
<td>61.0</td>
<td></td>
</tr>
<tr>
<td>Gal_6_36_3</td>
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<td>59.6</td>
<td>64.0</td>
<td></td>
</tr>
<tr>
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<td>59.2</td>
<td>46.1</td>
<td></td>
</tr>
<tr>
<td>Gal_11_31_1</td>
<td>90.3</td>
<td>40.4</td>
<td>65.4</td>
<td></td>
</tr>
<tr>
<td>Gal_11_31_2</td>
<td>95.0</td>
<td>42.0</td>
<td>69.0</td>
<td></td>
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<tr>
<td>Gal_11_31_3</td>
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<td>81.7</td>
<td>86.0</td>
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<tr>
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<td>46.0</td>
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<tr>
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<td>70.3</td>
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<tr>
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<td>60.6</td>
<td>63.1</td>
<td>62.0</td>
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<td>50.0</td>
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</tbody>
</table>

The snapshots of Gal_11_31, Gal_11_31_3, and Gal_13_29_3 during the 400 ps MD simulations are shown in Figure 4A, B, and C, respectively. It is obvious that the designed α-loop-α motif lost its structural integrity very quickly when it is not O-glycosylated, probably due to the high dielectric constant of the surrounding water molecules, which results in increasing the possibility of hydrogen bonding between amide protons and surrounding solvent molecules and simultaneously promotes the intermolecular hydrogen bonding between the α-loop-α motif and surrounding water molecules and therefore destabilizes the secondary structure of the designed α-loop-α motif. In contrast, the secondary structural integrity is higher when the designed α-loop-α motif was O-glycosylated for both “face-to-face” and “back-to-back” cases. It is possibly attributed to the reason that the hydroxyl groups of the galactose residues can compete against the amide protons for forming hydrogen bonding with the surrounding water molecules. However, the “face-to-face” glycosylation exhibits higher stabilizing effect than the “back-to-back” glycosylation, probably due to that those galactose residues form sugar cluster, thus reduces the structural flexibility of the entire structure.

In order to elucidate the effect of the distance between O-glycosylation and the loop on the structural integrity of the designed α-loop-α motif, the snapshots of Gal_4_38_2, Gal_11_31_2, Gal_4_38_3, and Gal_11_31_3 at the end of 400 ps MD simulations are compared. Figure 5 shows the top and side views of the snapshots of these structures. In the case of Gal_4_38_2 (Fig. 5A), the “face-to-face” glycosylation was made far away from the loop. It is obvious that these galactose residues tend to form sugar cluster, therefore the loop with 7 Gly residues prefers to twist and forms several hydrogen bonds with the N-terminal helix (Table II). These newly formed hydrogen bonds further destabilize the intramolecular hydrogen bonding patterns in the N-terminal helix, thus reducing its secondary structural integrity. In the case of Gal_11_31_3 (Fig. 5D), the “face-to-face” glycosylation was made close to the loop. The sugar cluster formed by these galactose residues forms an additional hydrogen bond with the loop (Table II). In addition, the length of the sugar bridge is approximately similar to the length of the extended loop, which further reduces the flexibility of the loop. Therefore, Gal_11_31_3 exhibits the highest helical content among these designed α-loop-α motifs with various O-glycosylations.

In conclusion, the results from this study strongly demonstrate that O-glycosylation can stabilize the structural integrity of the designed α-loop-α motif because it decreases the opportunity for water molecules to compete for the main chain hydrogen bonds. The designed peptide exhibits the highest helicity when residues 11 and
31 were replaced with Ser residues followed by O-linked with 3 galactose residues, which represents the "face-to-face" glycosylation near the loop. In this case, the loop was extended and an additional hydrogen bond was observed between the loop and the galactose residues, resulting in decreasing the fluctuation and increasing the stability of the entire peptide. When the glycosylation was made close to the loop, the secondary structural integrity of the \( \alpha \)-loop-\( \alpha \) motif increased with the number of galactose residues. In addition, "face-to-face" glycosylation increased the structural integrity of this motif to a greater extent than "back-to-back" glycosylation. However, no general rule was found for the stabilizing effect when the glycosylation was made away from the loop. This study provides interesting results for further de novo design of the \( \alpha \)-loop-\( \alpha \) motif with higher secondary structural integrity in water.

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**References and Footnotes**


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