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Glyco-helix: parallel lactose-lactose interactions stabilize an α-helical structure of multi-glycosylated peptide

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Glyco-helix is designed as a novel model system to investigate *cis* carbohydrate–carbohydrate interactions. Adhesive Lac–Lac interactions stabilize α-helix of Lac-peptide in the presence of fluorinated alcohols, but no such an interaction was observed in GlcNAc-peptide.

Specific carbohydrate–carbohydrate interactions have been demonstrated to play important roles in a number of biological events. For example, glycosphingolipids (GSLs) on the cell surface aggregate *via* side-by-side (*cis*)- interaction to form "GSL signaling domain" that is associated with signal transduction proteins such as c-Src, FAK and Rho A.[1] Face-to-face (*trans*) -interaction between two GSL signaling domains mediates important biological processes including cell adhesion, signal transduction, and immune responses in often Ca^{2+} dependent manner.[2] Clusters of carbohydrates have also been observed on protein surface, and have been suggested to be involved in a carbohydrate–carbohydrate interaction.[2,13] Strong and specific *trans*-interactions between carbohydrate clusters have been demonstrated by using synthetic polymers, SAMs and gold particles.[3] Schmidt and co-workers have shown that Ca^{2+} induces a cross-shaped structure of a covalently linked Le^x dimer that may be a functional unit for *trans*-interaction.[4] Synthetic model systems can provide valuable mechanistic insights for both *cis*- and *trans*-interactions between carbohydrates. Herein, we wish to report a novel peptide-based system, named "Glyco-helix", in which adhesive lactose–lactose interactions and the subsequent parallel packing were observed.

Glyco-helices are 19-residue peptides having seven *O*-glycosylated Ser residues at (i) and (i + 3) or (i) and (i + 4) positions so that a cluster of 7 carbohydrates would be formed on one side of the helical structure (Figure 1). *Cis*-interactions (adhesive or repulsive) of carbohydrate moieties should affect the stability of glyco-helix, because their packing interactions are energetically coupled with the helix-coil transition of the peptide. β-Lactoside and β-*N*-acetyl-glucosaminide are selected as carbohydrate units in glyco-helices **1** and **2**, respectively, because they are core carbohydrate residues of various GSLs, including GM3 and Le^x.

Glyco-helices (**1** and **2**) and the corresponding non-glycosylated peptide (**3**) were prepared using the standard Fmoc-strategy on the Rink resin. For the coupling of glycosyl-Ser,[5] 1.5 equivalent of amino acid was used with BOP (3.0 eq.), HOBt (3.0 eq.), and *N*-methylmorphorine (4.5 eq.), and the coupling time was elongated to 12 h. After cleavage of the glycopeptide from resin (9 : 1 TFA-water, 1 h), the resulting water-insoluble peptide was treated with sodium methoxide in methanol (pH 9, 1 h) and the subsequent 10% hydrazine in water (1 h) to give the fully deprotected peptide. The constitution of the synthetic glycopeptides was confirmed by ESI-MS that showed tetra-cationic molecular ion ($M + 4H^+$) peaks at 1079.9 (**1**, calc. 1080.0), 868.0 (**2**, calc. 868.1), and 512.5 (**3**, calc. 512.5).

Circular dichroism (CD) spectra showed that all peptides (**1**, **2**, and **3**) are in a disordered conformation in buffer. However, the helical conformation could be induced for two peptides, **1** and **3**, by adding 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) or 2,2,2-trifluoroethanol (TFE) (data not shown). The CD spectra showed a typical coil-to-helix transition with an isodichroic point at around 205 nm for both peptides (Figure 2A). On the other hand, peptide **2** showed a negligible helical content even in the presence of high concentrations of HFIP. Many research groups reported that even single *N*-, *O*-, or *C*-glycosylation results in a dramatic reduction of the helical content of the parent peptides.[6] Multi-glycosylated anti-freeze peptides (*Boreogadus saida*) that contain a repeating glyco-tripeptide sequence, Thr(*O*-β-Gal(1-3)-α-GalNAc)-Ala-Ala, show no α-helical structure even in 100% TFE.[7] Suggested explanations for the helix destabilizing effect of glycosylation include steric interactions and entropic restriction of carbohydrate residue on a rigid helix backbone.[6] In globular proteins, however, a cluster of carbohydrates have been found on both loop and helix regions.[13] Recently, Polt et al. have demonstrated that the

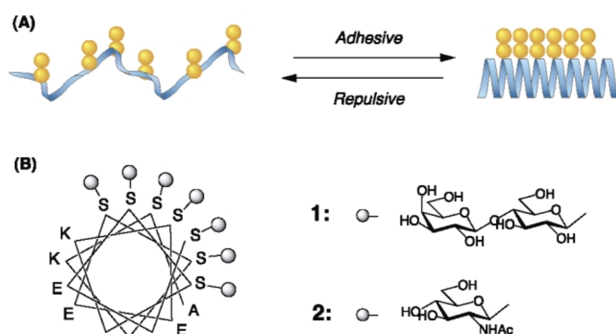

Fig. 1 (A) Dynamic transition of glyco-helix between its random coil and helix conformations. (B) The helix wheel projection of glyco-helices **1** and **2** (Ac–YGG*S*EE*SS*KK*S*EE*SS*KK*S*A-amide), where Y = Tyr, G = Gly, *S* = *O*-β-lactosyl (**1**) or β-*N*-acetyl-glucosaminyl (**2**) Ser, E = Glu, K = Lys, and A = Ala.

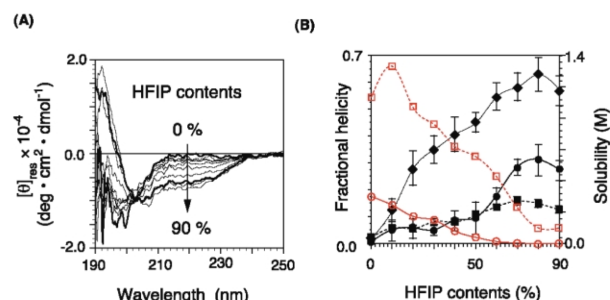

Fig. 2 (A) CD spectra of **1** in HEPES buffer (10 mM, pH 7.0) containing various concentrations of HFIP (0 ~ 90 % v/v). Temp = 20 °C. $[\theta]_{res}$ is the the mean residue ellipticity. Bold curves are CD spectra of **1** under 0 and 90% HFIP. (B) (Left scale) Fractional helicity of **1** (●, solid line), **2** (■, dotted line), and **3** (⧫, thin solid line) in HEPES buffer (10 mM, pH 7.0) containing various contents of HFIP (0 ~ 90 % v/v). Temp = 20 °C. Fractional helicity of the peptides was calculated through the method described in the literature (ref. 9). (Right scale) Solubility of lactose (○, red solid line) and *N*-acetyl-D-glucosamine (□, red dotted line) in aqueous HFIP at 20 °C.

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introduction of *O*-α-mannose residue on Ser can actually *stabilize* the helical conformation in water. Thus, the effect of glycosylation on peptide conformation appears to be dependent on the structure of carbohydrates and their microenvironments.[14] Our CD data of peptide **2** are consistent with the strong helix destabilizing effect of multiple-*O*-glycosylation. However, multi-lactosylated peptide **1** adopts a reasonably stable helical conformation although the helical content is about half of the corresponding non-glycosylated peptide **3**. The remarkable stability of the helical conformation of peptide **1** in the presence of fluorinated alcohols suggests the presence of the adhesive lactose–lactose *cis*-interactions that compensate for the helix-destabilizing effects of glycosylation. The sigmoidal helix induction curve of **1** is quite in contrast to the broad and non-cooperative curve of **3** that is consistent with many other small peptides found in literature.[8] The helix induction of **1** clearly coincides with the loss of solubility of lactose at around 50% HFIP (Figure 2B), suggesting that desolvation and subsequent clustering of the hydrogen-bonded lactose residues promote the formation of the helical structure. On the other hand, considerably higher solubility (1.3×10^{-1} M) of *N*-acetylglucosamine in 80% HFIP aqueous solution indicates that the saccharine units of **2** are well solvated even under high HFIP contents to disrupt the α-helix formation. Molecular mechanics calculation of **1**in vacuo (Figure 3) shows that, in the helical structure, the lactose residues are hydrogen-bonded to each other and packed in a parallel orientation along the helix axis (helical pitch: 5.4 Å). Similar parallel packing is observed in the X-ray crystal structure of lactose, in which each lactose units are packed along the c-axis with a repeat distance of 4.8 Å.[10]

We examined the effect of Ca^{2+} on the conformation of glyco-helix **1** in the presence of 60% HFIP. The addition of Ca^{2+} shifts the helix-coil equilibrium of **1** in favor of a disordered conformation (Figure 4). Na^{+} destabilized the helical conformation of **1** to a lesser extent. Both Ca^{2+} and Na^{+} had negligible effect on the CD spectrum of non-glycosylated peptide **3**. These results clearly show that Ca^{2+}-binding to the lactose units is responsible for the helix destabilization of **1**, and neither increasing ionic strength nor the disruption of internal ion pairs between Lys and Glu residues has considerable effects on the helical stability of the peptides. The estimated binding constants (K_a = 60 ± 11 M^{-1} and 11 ± 4 M^{-1} for Ca^{2+} and Na^{+}, respectively)[11] are too high for monomeric lactose, and comparable to those of a dimeric lactose derivative in methanol,[4] suggesting the formation of a Ca^{2+}-polyvalent lactose complex in glyco-helix **1**. Although more detailed structural studies are required to elucidate the mechanism of the Ca^{2+}-induced helix-to-coil transition of **1**, it is clear that a cluster of such simple carbohydrates as lactose can undergo a drastic conformational transition in the presence of Ca^{2+}.

In conclusion, we described the synthesis and conformational analysis of glyco-helix as a tool to monitor *cis* carbohydrate–carbohydrate interactions. Our data show that the desolvation of the saccharide units as well as Ca^{2+} ion have a dramatic effect on *cis* lactose interactions. With appropriate design modifications, the α-helical content of glyco-helices could be increased in more aqueous environments where direct comparison with native carbohydrate clusters may be possible.

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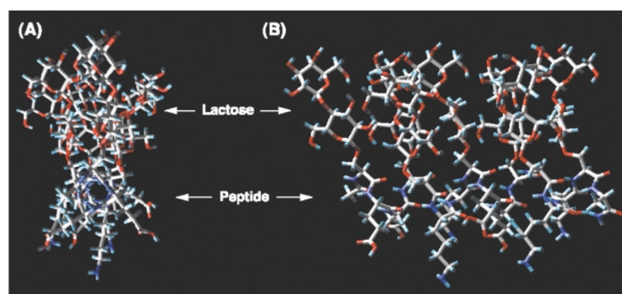

Fig. 3 (A) Top and (B) side views of glyco-helix, **1**, demonstrated by molecular mechanics calculation. The *C*-terminal residues (Ac-Tyr-Gly-Gly) are omitted for clear presentation. Hydrogen, carbon, nitrogen, and oxygen atoms are depicted in turquoise, white, red, and blue, respectively.

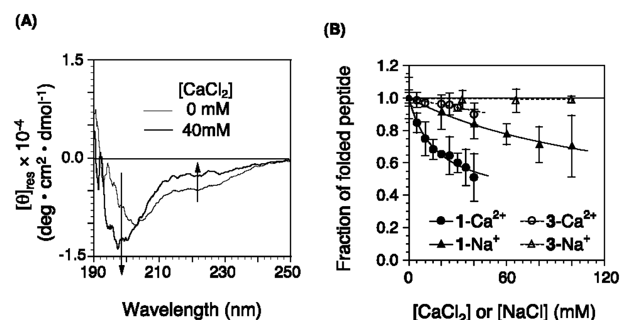

Fig. 4 (A) CD spectra of **1** in HEPES buffer (10 mM, pH 7.0, 60% HFIP) containing 0 and 40 mM $CaCl_2$. Temp = 20 °C. (B) Fraction of folded peptide ($[f]_M/[f]_0$ where $[f]_M$ and $[f]_0$ are the fractional helicities in the presence and absence of metal ion, respectively) of **1** and **3** under various concentrations of calcium- and sodium chloride.

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