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A monosaccharide-modified peptide phage library for screening of ligands to carbohydrate-binding proteins

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ABSTRACT

A monosaccharide-modified β-loop peptide library displayed on phage has been constructed and used for the screening of glycopeptide ligands against a carbohydrate-binding protein. The β-loop peptide library was designed and modified with a mannose derivative on phage. The glycopeptide ligands to concanavalin A (ConA), a mannose-binding protein, were obtained from the mannose-modified peptide phage library. The amino acids neighboring the mannose unit of glycopeptides not only reinforced the binding affinity but also gave diverse binding characteristics.

Interactions between carbohydrates and carbohydrate-binding proteins (CBPs) play important roles in various biological events such as cell–cell communication, tumor metastasis, inflammation and virus infection.[1–5] Therefore, the ligands to CBPs have attracted attention for glyco-microarrays,[6] drugs and drug delivery systems,[7] and molecular probes.[8] In particular, carbohydrate-based molecules are useful to produce ligands due to their specificity for CBPs. However, the complicated procedures of carbohydrate preparation still make diverse carbohydrate molecules difficult to use as ligands to CBPs. On the other hand, ligands for CBPs have also been explored from peptide library, since peptides have advantages in construction of molecules with diverse structures. Phage display is one of useful methods to give peptide library,[9] and several peptide ligands to CBPs have been reported.[10] These peptide ligands can mimic carbohydrate molecules, however, it is thought that only peptide structures are not enough to produce ligands with directional affinity to specific CBPs.

Previously, we have designed monosaccharide-modified peptides as ligands to detect CBPs.[11] A monosaccharide derivative such as mannose, galactose and *N*-acetylglucosamine is arranged at the side chain of specified amino acid, and the amino acids neighboring the monosaccharide unit are varied to produce peptides with diverse sequences. The peptides were conjugated with a fluorophore or gold nanoparticles (GNPs) to produce optical probes for the lectin detection. Target lectins can be detected based on the fluorescent response and color change due to surface plasmon resonance of GNPs, respectively. Glycopeptide ligands show various binding affinity by the cooperative work of the monosaccharide unit and surrounding amino acids. For the next step to improve the diversity of a monosaccharide-modified peptides, we attempt to construct a monosaccharide-modified peptide library using a phage display technique and perform screening of ligands to CBPs.

Concanavalin A (ConA), a mannose-binding protein, was chosen as a model CBP, and a mannose-modified peptide library was designed. Previously, we reported that a β-loop peptide, with a loop structure stabilized by antiparallel β -strands,[12] could be a useful scaffold for a phage displayed peptide library.[13] Four or five amino acids in loop region can be randomized to give the libraries. Thus, we designed a β-loop peptide library, GKITV-$X_1X_2CX_3X_4$-KTYEG, that have 20 kinds of amino acids at X_n position and a fixed cysteine residue at the middle of the loop as a mannose modification site (Fig. 1). The peptide library was displayed on the phage fusing with a Cys-free gene-3-protein (pIII).[14] Then, a mannose-modified peptide library was prepared by introducing a mannose unit into the side chain of the cysteine residue via a disulfide bond using 2-(3-nitropyridyl disulfide ethyl)-mannopyranoside (Man-Npys). The modification of peptides on the phage protein was confirmed by blotting analysis using biotin-Npys (Fig. S1). Infectious ability of phage was maintained before and after the mannose modification (Fig. S2).

An affinity-based screening of mannose-modified peptide against ConA was performed. Biopanning were repeated 5 times with the competitive elution method by methyl-α-D-mannose (Me-Man). After cloning and DNA sequencing of individual clones,

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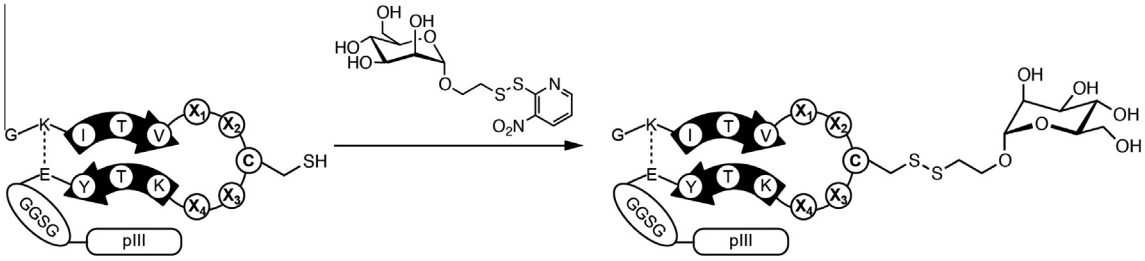

Figure 1. Construction of the mannose-modified peptide library on the Cys-free-pIII phage.

18 different phage clones were identified (Table S1). The 4 phage clones have two cysteine residues, indicating that these clones might be modified with two mannose units and bind to ConA by multivalent effect.[15] Thus, the relative binding amount of 14 phage clones with single cysteine residue to ConA was evaluated by enzyme-linked immunosorbent assays (ELISAs) (Fig. 2). The relative binding amounts of c5 and c13 phages were higher than that of the library phage, indicating that these were selected as phage clones of stronger binders through the biopanning. The competitive phage ELISA experiment using Me-Man revealed that several phage clones including c5 and c13 bound to the binding site of ConA (Fig. S3).

To evaluate the secondary structure and binding affinity of peptides screened by biopanning, peptides were chemically synthesized and modified with a mannose unit. The sequences of c5 and c13 were selected as p5 and p13 peptides, since these phage clones showed higher binding amounts to ConA than the library phage. The G-loop peptide, having a GGCGG sequence in the loop, was also synthesized as a control to evaluate the effect of amino acids neighboring the mannose unit. All peptides were used with and without a mannose modification.

The secondary structure of peptides was analyzed by attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectra (Fig. 3). Both p5 and p13 with and without the mannose unit showed peaks around 1625, 1680 and 1695 cm^{-1}. As these peaks are primarily assigned to β-turn (1660–1700 cm^{-1}), β-sheet (1625–1635 cm^{-1}), antiparallel β-sheet (1685–1695 cm^{-1}),[16] the p5 and p13 peptides formed a β-loop structure. Especially, there was no significant difference in IR spectra between p5 and p5-Man, suggesting that p5 peptide maintains a stable β-loop structure even after the mannose modification. The p13 peptide showed a peak around 1650 cm^{-1} assigned to random coil (1640–1660 cm^{-1}).[15] In addition, a peak around 1625 cm^{-1} of p13-Man was smaller than p13. These results indicate that the β-loop structure of p13 might be slightly less stable than that of p5. On the other hand, G-loop and G-loop-Man contain higher percentages of random coil structures since these peptides showed broad bands around 1635–1680 cm^{-1}. Circular dichroism spectra of these peptides were also measured, supporting the FTIR results (Fig. 4S).

The binding parameters of peptides to ConA were estimated by surface plasmon resonance (SPR). The p5-S-Man peptide with only the loop sequence lacking the antiparallel β-sheet region in p5-Man peptide was also designed and used for the binding analysis. Typical SPR sensorgrams composed of the association and dissociation processes for p5-Man and p13-Man were shown in Figure 4. All mannose-modified peptides showed time dependent binding to ConA-immobilized surface (Fig. 4 and Fig. S5). The response in sensorgram increased according to the increase of peptide concentration. On the other hand, p5, p13 and p5-*S* peptides without the

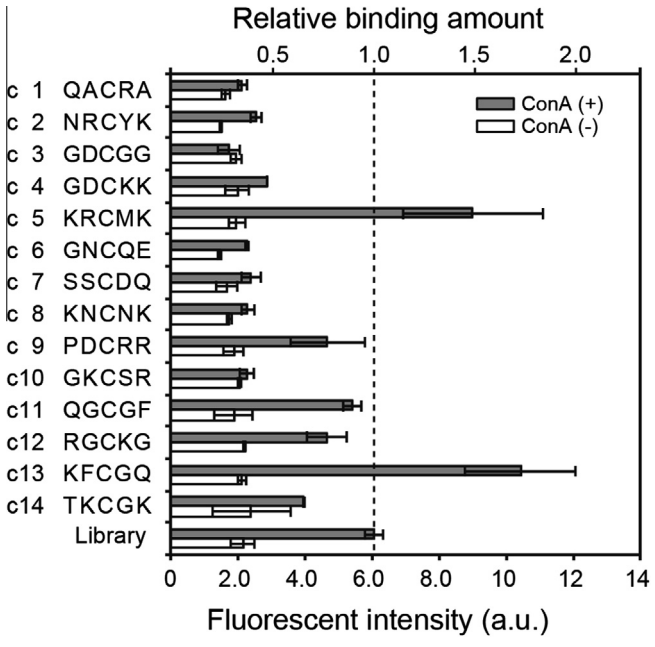

Figure 2. ELISA assays for the amounts of bound phage clones identified from the mannose-modified β-loop peptide library displayed on phage against ConA. The gray and white bars indicate the relative amounts bound to ConA-immobilized and non-immobilized microplates, respectively. The relative binding amounts was estimated based on the fluorescent intensity of library bound to immobilized ConA. ConA was immobilized with the concentration of 10 μg/mL (0.38 μM) and phage concentrations were 0.5 nM. For all samples, $n = 3$. Error bars represent the standard deviation.

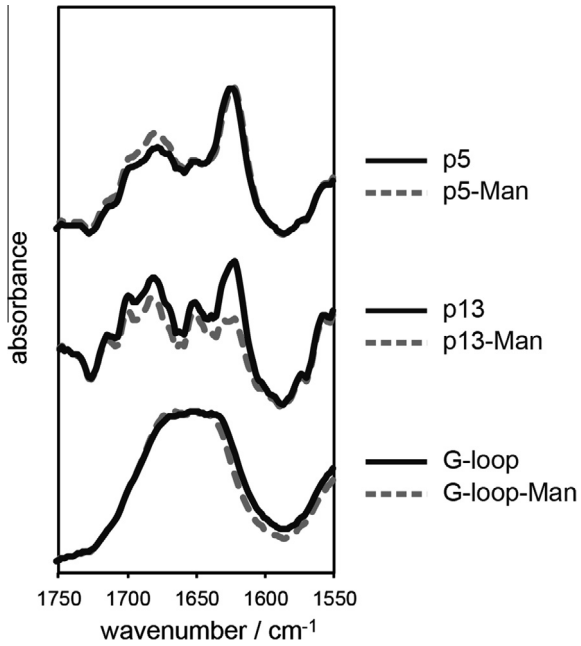

Figure 3. Secondary structural analysis of peptides by ATR-IR spectroscopy. All peptides were measured at 500 μM.