Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 511–517
Bioorganic & Medicinal Chemistry

Selection and syntheses of tentacle type peptides as 'artificial' lectins against various cell-surface carbohydrates

Soonsil Hyun, Jiyoung Kim, Miyun Kwon and Jaehoon Yu*

Department of Chemistry and Education, Seoul National University, Seoul 151-742, Republic of Korea

Received 30 June 2006; revised 18 September 2006; accepted 19 September 2006
Available online 10 October 2006

Abstract—Sialyl Lewis X and its derivatives are cell-surface carbohydrates that are involved in cell–cell recognition by carbohydrate-mediated interactions. Unfortunately, owing to the similarities between carbohydrates only a limited number of tools are available for their differentiation. In this study, we prepared a selected phage-displayed peptide library against LeX (**2**), SLN (**3**), or LN (**4**), which compared to sLeX (**1**) lack sialic acid, fucose, and both sialic acid and fucose from constituents, respectively. Sequences of the selected peptides, prepared as tentacle type dimeric peptides, were prepared and shown to have micromolar affinities for the cognate carbohydrates. The specificities displayed by these 'artificial' lectins overwhelm those of natural lectins. These results suggest that they can serve as useful tools to detect changes in the terminal monosaccharide of cell-surface carbohydrates.

1. Introduction

Cell-surface carbohydrates, composed of several different kinds of mono-saccharides, interact with many types of lectins as part of a communication signaling network between cells.[1] Since the biogenesis of the cell-surface carbohydrates is tightly regulated, alterations in their structures result in the dysfunction of processing enzymes, which is the foundation of many human diseases.[2,3] Alterations in glycosylation patterns play key roles in governing the invasive and metastasis potentials of many types of cancers.[4] Viral virulence as well as bacterial and parasite infections are also mediated by host-cell surface carbohydrates, especially their terminal saccharides, which are recognized by invading pathogens.[5,6]

The loss of terminal carbohydrate fragments from normal cell-surface carbohydrates may be governed by natural lectins that have reduced affinities to altered forms. Although early detection of changes in terminal carbohydrate may uncover pathogenic markers for the identification of glycosyltransferase dysfunction, to date specific tools to detect these altered cell-surface carbohydrates are few in number. Moreover, the identification of these altered cell-surface carbohydrates remains a complex and laborious task as a result of the structural diversity of carbohydrates.[7] Therefore, the development of new methods for facile and easy detection of changes in terminal carbohydrate is a significant goal. An immunological approach to this problem seems ideal at first site, but owing to the poor immunogenicity of carbohydrates,[8] only limited number of antibodies for cell-surface carbohydrates are available.

Recently, we have carried out experiments in which a 12-mer peptide, serving as an 'artificial' lectin, was selected using a phage-displayed library against sialyl Lewis X (sLeX, **1** in Fig. 1) as an 'artificial' lectin.[9] To induce multiple binding sites, the selected peptide was synthesized as tentacle type peptide.[10] Synthetic peptides showed sub-micromolar affinities as well as high specificity for the cognate carbohydrate.

Encouraged by this initial observation, a more thorough study was designed to select peptides against a more diverse array of carbohydrates. Several carbohydrates that lack terminal saccharides from sLeX were chosen for this effort. By using a phage-displayed 12-mer peptide library, peptides selected against LeX (**2**), sialyl lactosamine (SLN, **3**), and *N*-acetyllactosamine (LN, **4**) were identified. Then, syntheses of selected sequences were performed as tentacle-type peptides. The peptides, obtained in this manner, not only had reasonable micromolar binding affinities to the cognate carbohydrates.

Keywords: Artificial lectin; Cell-surface carbohydrates; Phage-displayed peptide library; Tentacle peptide; Specificity; Lectin-chip.
* Corresponding author. Tel.: +82 2 880 7761; fax: +82 2 889 0749; e-mail: jhoonyu@snu.ac.kr

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.09.035

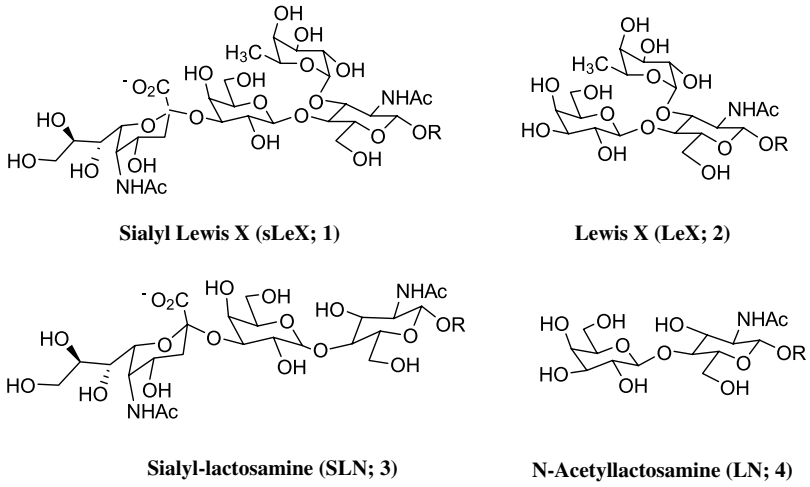

Figure 1. Structures of carbohydrates.

More significantly, the peptides displayed specificities that are superior to the natural lectins, even though their binding affinities are at least an order of magnitude weaker than those of natural lectins.

2. Results and discussion

2.1. Selection of peptides from phage-displayed library against carbohydrates

A Phage-displayed 12-mer peptide library (New England Biolabs, USA) was used for selection against LeX (**2**), SLN (**3**), or LN (**4**), which compared to sLeX (**1**) lack sialic acid, fucose, and both sialic acid and fucose from constituents, respectively. The carbohydrates were used in their polyacrylamide (PAA)-biotinylated forms for attachment to a solid support.[11] Four or five rounds of biopanning were performed until the selected phages were cloned and sequenced (Table 1).[9] From the initially selected phages, the most carbohydrate-specific phage was reselected by using carbohydrate-PAA-biotin and PAA-biotin with streptavidin-coated plates. For example, anti-LeX-1 phage showed more specific binding than anti-LeX-2 phage (Fig. 2). These selected phages were subjected to further investigation.

Homology searches were then carried out with deduced sequences of the selected peptides using a BLAST search program.[12] As expected, the selected peptide sequences have high homologies with a variety of carbohydrate-related proteins (Table 2). Interestingly, many of the homologous proteins contain similar tandem repeat sequences.[13] This is especially true for *trans*-sialidase, which is found as a high homology protein in both sLeX and SLN-specific peptides.[6] One of the functions of tandem repeat peptides might be the recognition of carbohydrates on the surfaces of host cells and tandem repeat peptides may be used as a general carbohydrate-recognition tool in nature. Hemagglutinin[14] and lectin-like proteins were also found to be highly homologous protein, suggesting that all of the selected peptides are likely to specifically recognize cognate carbohydrates.

Table 1. Deduced amino acid sequences against various carbohydrates

Carbohydrate	Peptide	Peptide sequence[a]
sLeX	Anti-sLeX-1	AHWIPRYSSPAT (6)
	Anti-sLeX-2	SHWDQPRPGLKP (1)
	Anti-sLeX-3	GTHLIAGGASHL (1)
	Anti-sLeX-4	QFTSAPPSLLQL (1)
	Anti-sLeX-5	QISQRSLLDPLL (1)
LeX	Anti-LeX-1	YNPLPQPSVTTS (8)
	Anti-LeX-2	HAHSWPPAHQLH (2)
SLN	Anti-SLN-1	LYMPPGSIYSLN (11)
	Anti-SLN-2	HSTLDRRSTPPI (2)
	Anti-SLN-3	LNASQYALSSMQ (2)
LN	Anti-LN-1	NFMESLPRLGMH (17)
	Anti-LN-2	SISWQSGHPMSL (3)

[a] Numbers in parenthesis are numbers of multiple detected sequences.

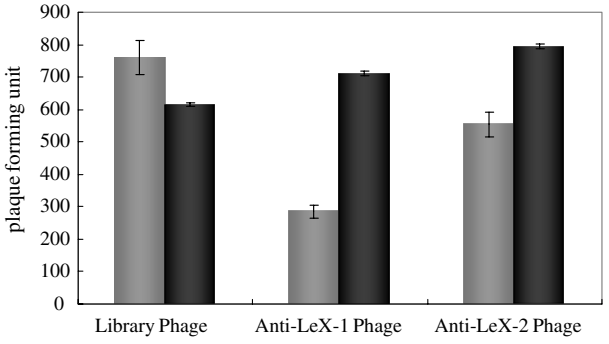

Figure 2. The selected phage-displayed peptide showed specific binding to a carbohydrate, LeX, not to PAA-supported polymer. LeX-PPA-biotin (black bar) and $HOCH_2(HOCH)_4CH_2NH$-PAA-biotin (gray bar) were immobilized to a streptavidin-coated plate. Library phage and selected phage solution (1×10^{10} plaques forming unit; pfu) were transferred to each well. After a short incubation for 20 min at room temperature and washing, the bound phage was eluted and titered. Experiments were triplicated and averaged.

Table 2. Homologous proteins of carbohydrate-specific peptides by BLAST search

Carbohydrate	Sequence	Homologous proteins	Identities (%)/positives (%)
Sialyl-Lewis X	AHWIPRYSSPAT	*trans*-Sialidase [*Trypanosoma cruzi*][a 13a]	34/48
	SHWDQPRPGLKP	Adhesin-related protein P30 [*Mycoplasma pneumoniae*][a 13b]	30/49
Lewis X	YNPLPQPSVTTS	Flagellum-adhesion glycoprotein, putative [*Trypanosoma cruzi*][a 13c]	30/50
		Mucin [*Homo sapiens*][a 13d]	36/53
Sialyl-LN	LYMPPGSIYSLN	Receptor lectin protein kinase-like [*Cucumis melo*][26]	58/75
	HSTLDRRSTPPI	Hemagglutinin [Influenza A virus][27]	72/90
		trans-Sialidase [*Trypanosoma cruzi*][a 13a]	30/38
N-Acetyl LN	NFMESLPRLGMH	AmpC beta-lactamase [*Hafnia alvei*][28]	42/50
		Uvomorulin (E-cadherin) [*Homo sapiens*][a 13e]	35/51
	SISWQSGHPMSL	Novel protein with lectin C-type domains [*Danio rerio*]	50/75

[a] This part of protein is composed of tandem repeat peptide.

2.2. Syntheses of selected peptides as tentacle type multimers

In order to confirm the binding affinities and specificities of the selected peptides, syntheses of the selected sequences of each were carried out. We expected that linear 12-mer peptides would possess low affinities to the cognate carbohydrates.[15] To overcome these low affinities, we prepared the selected peptides as dimeric tentacle type peptides in order to create multiple binding sites with the carbohydrates. Solid-phase syntheses of peptides were carried out starting with Lys, which contains two amino groups that can be used to link two identical linear sequences. A standard solid phase chemistry protocol, using Fmoc-protected amino acids and a purification by HPLC, was employed. Unfortunately, it was not possible to synthesize all of the tentacle type tetramer peptides probably due to the steric hindrance. In addition, studies on synthetic peptides (anti-sLeX2, anti-SLN1) could not be carried out owing to their poor solubility in aqueous media.

2.3. Affinities and specificities of the carbohydrate binding peptides

Evaluation of the binding affinity of each peptide was carried out against carbohydrates-immobilized on a solid-supported sensor chip by using a surface plasmon resonance (SPR) technique. Surface-based affinity measurements are particularly applicable to carbohydrate-recognition studies, because immobilized carbohydrates effectively mimic cell-surface carbohydrates, where multiple interactions take place. Moreover, the SPR method is easily adapted to specificity studies and high-throughput screening.[16] Recent investigations have shown that the use of carbohydrate chip based recognition has advantages over conventional affinity measurements.[17]

Since binding affinities of linear monomer peptides are expected to be in millimolar ranges, their specificities are difficult to be compared. Comprehensive binding affinities and their comparisons were done with tentacle dimer peptides, which were synthesized in reasonable yields. Binding affinities of injecting peptides on carbohydrate-immobilized chips determined by using the SPR technique are listed in Table 3. In cases where there were no increases of RU values by injecting peptides and not consistent RU values due to the poor binding affinities, they were reported as not binding (N.B.) and not calculable (N.C.) binding constant, respectively.

The data reveal several important observations. First, each dimeric tentacle type peptide shows a micromolar binding affinity to its cognate carbohydrate. These affinities are substantially stronger than normally seen for monomeric carbohydrate-recognition due to the presence of multiple binding sites.[18] Second and most importantly, each dimer peptide has an extremely high level of specificity for the cognate carbohydrate. Binding affinities of the synthesized peptides to the cognate carbohydrates are at least 5 -times stronger than to other similar carbohydrates in most cases except anti-LeX peptide. This peptide is the least discriminating one and binds to the cognate carbohydrate with 20 μM of affinity. It binds to sLeX with 2.5 -times weaker affinity than to the cognate carbohydrate, in spite that there is an extra sialic acid moiety in sLeX. Third, peptides generated against relatively small carbohydrates, such as SLN and LN, keenly discriminate cognate small carbohydrates. This fact suggests that specific interactions leading to binding

Table 3. Binding affinities of tentacle type dimer anti-carbohydrate peptides against various carbohydrates[a]

Peptide carbohydrate	AHWIPRYSSPAT (anti-sLeX^{di}-1)	YNPLPQPSVTTS (anti-LeX^{di}-1)	HSTLDRRSTPPI (anti-SLN^{di}-2)	NFMESLPRLGMH (anti-LN^{di}-1)	SISWQSGHPMSL (anti-LN^{di}-2)
Sialyl-Lewis X (sLeX)	0.15	0.05	NC	NC	0.2
Lewis X (LeX)	NB	0.02	NB	NB	NC
Sialyl-lactosaimne	13	NB	0.03	2	14
N-Acetyl LN	0.6	NB	NB	0.3	0.05

[a] Binding affinities (K_d in mM) were measured using BIAcore 3000. NB, not binding; NC, binding affinities could not be calculated because of inconsistent RU values in a variety concentrations of carbohydrates injected.

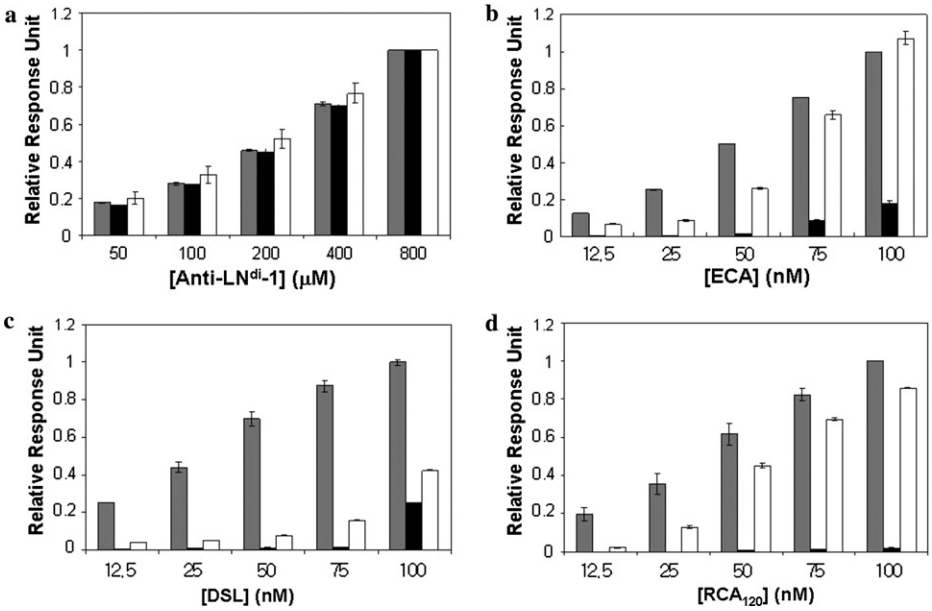

Figure 3. Binding affinity (responsive unit; RU) reductions of anti-LN dimer peptide (a) and 'natural' lectins (b–d) against LN-immobilized chip in the absence and the presence of monosaccharide. Various concentrations of 'artificial' or natural lectin in PBS were injected over the surface for 120 s and dissociation for 240 s. Gray bar, lectin only; black bar, lectin with 5 mM GlcNAc; white bar, lectin with 5 mM Gal. RU values were measured after 115 s from injection start (nearly steady-state). All values were triplicated and averaged.

Table 4. Specificity comparison between 'artificial' and natural lectins against LN[a]

Lectin	K_d wo/monosaccharide (M)	K_d w/GlcNAc (M)	Specificity ratio[b]	K_d w/Gal (M)	Specificity ratio[b]
Anti-LN^{di}-1	3×10^{-4}	6×10^{-4}	2.0	4×10^{-4}	1.3
DSL	4×10^{-7}	7×10^{-6}	18	3×10^{-5}	75
ECA	2×10^{-5}	4×10^{-5}	2.0	6×10^{-5}	3.0
RCA120	3×10^{-7}	1×10^{-6}	3.3	2×10^{-5}	67

[a] K_d values were determined with LN-BSA immobilized chip.
[b] Specificity ratio was defined as K_d with a monosaccharide/K_d without a monosaccharide.

are by the complementarity of peptide sequences against cognate carbohydrates.

In accord with previous observations made in studies with sLeX, tentacle type tetramer peptides constructed in this effort possess stronger affinities (For example, anti-LeX tetramer peptide showed 2.5 μM of K_d value).[9] More tightly binding peptides can be obtained if a larger numbers of lysine residues are incorporated at the C-terminal position at the beginning of the tentacle type peptide synthesis without ruining specificities.[19] But some of tetramers could not be synthesized due to the steric reasons. Systematic comparison of binding affinities and specificities with tetramer peptides could not be carried out.

In order to test the specificities of the anti-carbohydrate peptides, responsive unit (RU) values for binding of both 'artificial' and natural lectins[20] to the immobilized LN in the presence and absence of monosaccharide were determined by using the SPR method. As data in Figure 3, RU values from the 'artificial' lectin remain unchanged (less than 5% reduction) in the presence of monosaccharide (5 mM of galactose or *N*-acetylglucosamine), while those from natural lectins are significantly reduced when the same amount of monosaccharide is present. Thus, binding specificities of the lectins against LN, represented as ratios of K_d values in the presence and absence of a monosaccharide as a competitor, were evaluated (Table 4). The specificity ratios of the anti-LN peptide are near unity, irrespective of monosaccharides, while specificity ratios of natural lectins (*ECA*, *DSL*, and RCA_{120}) were found to be much deviated from unity, in the presence of galactose or *N*-acetylglucosamine. Thus, even though its binding affinity is at least an order of magnitude weaker than the natural lectin, the 'artificial' lectin has a much greater specificity.

2.4. 'Artificial' lectin chip differentiates specific glycoproteins

Since the specificity of the 'artificial' lectin system is superior to the natural one, it should serve as a useful tool to discriminate between glycoproteins. In order to gain support for this proposal, an experiment was carried out with immobilized anti-LN peptide to determine if the cognate LN-attached bovine serum albumin (LN-BSA) can be distinguished from a mixture of other glycoproteins. Biotinylated anti-LN peptide[21] was immobilized to a solid support. A mixture of

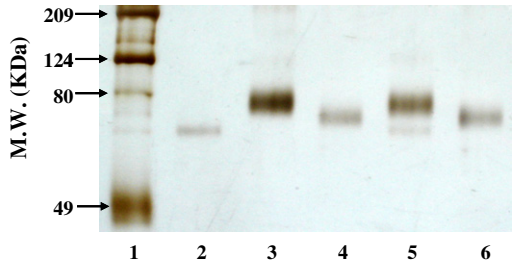

Figure 4. An SDS–polyacrylamide gel showed that a carbohydrate-conjugated protein can be discriminated by anti-carbohydrate peptide-immobilized chip. Lane 1, molecular weight standards (Bio-Rad, myosin, 209 kDa; β-galactosidase, 124 kDa; BSA, 80 kDa; ovalbumin, 49 kDa); lane 2, BSA (Sigma, 66 kDa); lane 3, sLeX-BSA (Calbiochem, 74–76 kDa); lane 4, LN-BSA (Calbiochem, 69–72 kDa); lane 5, first washing fraction with 10 mM Tris–HCl at pH 8.0; lane 6, first elution fraction with elution buffer (50 mM Tris–HCl, 150 mM NaCl at pH 7.5). Glycoprotein in lane 6 was identified as LN-BSA by MALDI-TOF mass.

sLeX-BSA, LN-BSA, and BSA (same molar ratio) was then applied to anti-LN^{tetra} peptide-immobilized matrix. A rapid removal of all weakly binders left only the strongly bound LN-BSA, which was then eluted with elution buffer and characterized by electrophoresis on an SDS–polyacrylamide gel. As shown in Figure 4, a single silver-stained band that was corresponding to LN-BSA by mass analysis (71 kDa) was only eluted glycoprotein.

These results not only demonstrate the superior specificity of this carbohydrate selected peptide system, they also suggest that this 'artificial' lectin system can be employed as an affinity ligand against specific carbohydrates. Since immobilized forms of 'artificial' lectins can discriminate the cognate carbohydrates, the system could serve as the basic strategy for an 'artificial' lectin chip. Specific recognition systems could be designed for partial structures of sLeX that would detect glycosyl transferase-deficient conditions that could be uniquely characteristic of disease or abnormal stages.[22] Although antibodies could serve as an alternative tool to recognize partial structures of cell-surface carbohydrates, their use is limited by high cost and poor immunogenicity against natural polysaccharides. In contrast, selection of a peptide from a phage-displayed peptide library against target carbohydrates and the synthesis of the selected peptide as multimeric form is a relatively economical procedure.

3. Conclusion

Selection of peptides was carried out from a phage-displayed peptide library against target carbohydrate, and then, the selected peptides were synthesized as dimeric forms. These artificial lectins have low-micromolar affinities against cognate carbohydrates. Specificities of these 'artificial' lectins overwhelm that of a natural lectin, even though binding affinities of latter one are much stronger. Immobilized 'artificial' lectins also discriminate cognate glyco-proteins, suggesting that they can be used for discovery of marker glycoproteins due to alterations of carbohydrate structures in diseased conditions. Thus, they make the technique more simple and facile alternatives for the generation of carbohydrate specific antibodies.

4. Experimental

4.1. Biopanning against PAA-biotin-conjugated carbohydrates; cases for Lewis X and SLN

The procedure is a modification of that described in the instruction manual of the PhD-12™ phage display peptide library kit (New England Biolabs, USA). Briefly, 100 μL solution of Lewis X-polyacrylamide (PAA)-biotin (20 μg, GlycoTech, USA) in 1 mL TBS (50 mM tris(hydroxymethyl)aminomethane, 150 mM NaCl at pH 7.5) was added to a well of a streptavidin-coated microplate (High Binding Capacity Coated microplate, company, USA) and incubated for 1 h at room temperature. For reference, same amount of solution of $HOCH_2(HOCH)_4CH_2NH$-PAA-biotin (20 μg, GlycoTech, USA) in 1 mL TBS was added to a well and incubated for 1 h at room temperature. After removal of the supernatant, each well was washed 3 times with 200 μL TBST (50 mM tris(hydroxymethyl)aminomethane, 150 mM NaCl, and 0.1% v/v Tween 20 at pH 7.5). Phage (5×10^{10} pfu) solution in 100 μL of TBS was added to the $HOCH_2(HOCH)_4CH_2NH$-PAA-biotin-coated well and stirred gently for 1 h at room temperature. Then the resulting phage solution was transferred to the LeX-PAA-biotin coated well and incubated for 1 h at room temperature. Non-bound phage was removed by washing 10 times with 200 μL TBST. Bound phage was eluted with 100 μL of elution buffer (0.2 M glycine–HCl, pH 2.2) for 10 min, followed by neutralizing with 15 μL of neutralizing buffer (1 M tris(hydroxymethyl)aminomethane–HCl, pH 9.1). Ten microliters of the phage solution was diluted and was titered by using the known method.[23] The remaining eluted phage was amplified to a 15 mL of grown culture (early-log phase) of *Escherichia coli* (ER2537) with vigorous shaking for 4.5 h at 37 °C. The resulting suspension was centrifuged for 10 min at 10,000 rpm and 4 °C. The supernatant was mixed with 1/6 volume of PEG/NaCl solution (20% w/v polyethylene glycol-8000, 2.5 M NaCl) and stored overnight at 4 °C. The stored solution was centrifuged for 15 min at 10,000 rpm at 4 °C to isolate the amplified phage precipitant as a pellet. The pellet was re-suspended on 1 mL of TBS. Reprecipitation was accomplished by addition of 185 μL PEG/NaCl solution at 0 °C for 1 h. The phage pellet, obtained by centrifugation, was re-suspended in 200 μL TBS (containing 0.02% NaN_3) and was titered. The other round of biopanning was performed by using 5×10^{10} pfu as of the initial input phage. In the washing steps, the concentration of Tween 20 was raised up to 0.5% v/v to remove weak binders. The eluted phage obtained from the 5th round was titered and plaques were used for sequencing.

4.2. Biopanning against carbohydrate-BSA conjugates; cases for sLeX, LN

One hundred and fifty microliters of carbohydrate-BSA (Calbiochem, USA, 10 μg) in 1 mL of binding buffer (0.1 M $NaHCO_3$ at pH 8.6) was added to a 96-well microplate (Costar, USA) and incubated overnight at 4 °C. After removal of the supernatant, 200 μL of blocking buffer (0.1 M $NaHCO_3$, 5 mg/mL BSA, and 0.02% NaN_3 at pH 8.6) was added to each well. After incubation for 2 h at 4 °C, each well was washed 3 times with 250 μL TBST.

4.3. Selection of carbohydrate specific peptides from initially selected phages

Solutions of 20 μg/mL of carbohydrate-PAA-biotin and 20 μg/mL of the background PAA-biotin in TBS were added to a 96-well streptavidin-coated microplate. Each well was brought to a volume of 100 μL and incubated for 1 h at room temperature. After removal of the supernatant, each well was washed 6 times with 250 μL of 0.5% TBST. Selected phage and the library phage (a control) solutions (1×10^{10} pfu) in 100 μL of TBS were transferred to each well. After incubation for 10 min at room temperature with gentle stirring, the plate was washed 10 times with 250 μL of 0.5% TBST. Bound phage was eluted with 100 μL of elution buffer for 10 min, followed by neutralizing with 15 μL of neutralizing buffer. Ten microliters of the phage solution was diluted and was titered as was described. Numbers of plaques were counted by tittering selected phage against carbohydrates or reference polymers. Rest of procedures is same as in previous section.

4.4. Synthesis and purification of selected peptides

Syntheses of tentacle type peptides were carried out by using fluorenylmethoxycarbonyl (Fmoc)-lysine as a branch core. An attachment of Fmoc-lysine to a resin was carried out, followed by sequential addition of amino acids that were deduced from DNA sequences of selected phages. Solid-phase peptide syntheses were carried out by using Fmoc-protected amino acids and Rink amide MBHA resins (Novabiochem, USA). All peptides were synthesized with 30 μmol scale. To the resin were added Fmoc-protected amino acid (12 equiv), benzotriazole-1-yl-oxy-tris-pyrridino-phosphonium hexafluorophosphates (PyBOP, 12 equiv), and *N*,*N*-diisopropylethylamine (DIPEA, 24 equiv) in DMF (1 mL). The suspension was stirred for 4 h at room temperature to complete the reaction, which was monitored by Kaiser test.[24] Resins were filtered and washed with DMF (1 mL × 10 times). Above coupling reactions were repeated until the last amino acid was added. After washing with DMF (1 mL × 5 times) and MeOH (1 mL × 3 times), cleavage of the peptide from the resin was achieved by treatment with a mixture of trifluoroacetic acid (TFA)/thioanisole/water (95:2.5:2.5 by volume) for 2 h at room temperature. The filtrate obtained during removal of resins was treated with a nitrogen stream to remove excess TFA. The residue obtained in this way was triturated with diethyl ether (50 mL). The resulting suspension was centrifuged at 2500 rpm for 5 min at −20 °C giving the solid crude peptide, which was purified by using a semi-preparative HPLC waters 7.8 × 300 mm 125 Å C18 Bondapak® column; linear gradient of 5–95% aqueous CH_3CN (0.1% TFA). MALDI-TOFMS was used for the identification of the purified peptides. Anti-sLeX^{di}-1 ($C_{134}H_{195}O_{33}$ N_{39}) MS ($M+H^+$): 2881.2 (Calcd) 2880.7 (Found), anti-LeX^{di}-1 ($C_{122}H_{191}$ $N_{31}O_{39}$) MS ($M+H^+$): 2716.0 (Calcd) 2716.0 (Found), anti-LeX^{tetra}-1 ($C_{214}H_{375}N_{62}O_{80}$) MS ($M+H^+$): 5096.6. (Calcd) 5096.0 (Found), anti-SLN^{di}-2 ($C_{125}H_{212}O_{38}N_{44}$) MS ($M+H^+$): 2940.3 (Calcd) 2939.0 (Found), anti-LN^{di}-1 ($C_{130}H_{207}O_{33}N_{39}S_4$) MS ($M+H^+$): 2973.5 (Calcd) 2972.9 (Found), anti-LN^{di}-2 ($C_{122}H_{187}O_{35}N_{35}S_2$) MS ($M+H^+$): 2769.1 (Calcd) 2768.5 (Found), anti-LN^{tet}-1-biotin ($C_{282}H_{449}N_{83}O_{70}S_9$) MS ($M^+$): 6410 (Calcd) 6409.0 (Found).

4.5. Affinity and specificity measurement of peptides against carbohydrates using SPR

Solutions of various carbohydrate-PAA-biotin (GlycoTech, USA) were prepared in buffer at a concentration of 0.5 mg/mL. The carboxymethyl groups in a CM5 sensor chip were cleaved by equilibration with PBS buffer and then the free carboxyl groups were activated by treatment with 40 μL of the coupling solution (200 mM *N*-ethyl-*N*′-(dimethylaminopropyl) carbodiimide, 50 mM *N*-hydroxysuccinimide). Thirty microliters of streptavidin solution (0.01 mg/mL in 10 mM NaOAc, pH 5.5) was then injected onto the sensor chip at a flow rate of 5 μL/min. After streptavidin was immobilized, 40 μL of 1.0 M ethanolamine was added. The resulting flow cell was equilibrated with PBS buffer. The same amount of PAA-biotin was immobilized onto another flow cell as a control. Each synthetic peptide was dissolved in water to make 10 mM of stock solution. Injection samples were prepared by serial dilution of the peptide stock solution with the buffer to make appropriate concentrations: dimer, 800, 400, 200, 100, 50, 25, 12.5, and 0 μM. All the samples were injected by using the serial automated method which is comprised of (1) sample injection (60 μL), (2) dissociation (240 s), and (3) regeneration (30 μL of 20 mM NaOH) by using a BIAcore 3000. The signals from the reference were subtracted from those of the carbohydrate-PAA-biotin immobilized surfaces. Each sample was injected at a flow rate of 20 μL/min using the KINECT command. The equilibrium-binding constant (K_d) of each peptide to immobilized carbohydrate-PAA-biotin was calculated by using the steady state affinity data from 7 different peptide concentrations. Binding affinities of peptides to carbohydrates were calculated by using a steady-state kinetic method. For specificity ratios, samples of anti-LN peptide and natural lectins (*DSL, Datura stramonium lectin; ECA, erythrina cristagalli agglutinin; RCA, ricin communis agglutinin* from Sigma) were prepared by serial dilution of stock solution with the buffer to make appropriate concentrations: the peptide, 800, 400, 200, 100, 50, 25, 12.5, and 0 μM; the natural lectin, 150, 100, 75, 50, 25, 12.5, and 0 nM. All samples were incubated with galactose or *N*-acetylglucosamine (5 mM) in PBS for 30 min at room temperature. Each

sample (40 μL) was injected onto the LN-coated-CM5 sensor chip at a flow rate of 20 μL/min.

4.6. Affinity selection of glycoprotein using lectin chip

A solution of biotinylated anti-LN tetramer peptide (200 μg/mL) was added to a Bio-spin disposable column (Bio-Rad) containing 60 μL of streptavidin-immobilized agarose beads (Sigma) and incubated for 30 min. Beads were washed 5 times with 500 μL of 10 mM Tris–HCl (pH 8.0). A mixture of sLeX-BSA, LN-BSA, and BSA (8 μg/mL) in 200 μl of 10 mM Tris–HCl (pH 8.0) was applied to the column. The column was washed with 500 μL of 10 mM Tris–HCl (pH 8.0) to remove weak binders. Specific binder was eluted with 500 μL of 50 mM Tris–HCl (pH 7.5). The resulting aliquot was concentrated and desalted using a centrifugal filter (Amicon). An aliquot (4 μL) was applied for mass analysis using MALDI-TOFMS (Applied Biosystems Voyager-DE STR). Average mass of BSA, sLeX-BSA, and LN-BSA was 66, 75, 71 kDa, respectively. Another aliquot (4 μL) was mixed with same volume of 5× SDS Laemmli sample preparation buffer and denatured by heating 100 °C for 5 min. Sample was electrophoresed on 10% polyacrylamide SDS gel. After electrophoresis, gel was visualized by silver staining (Amersham Pharmacia Biotech AB, Vorum) silver staining protocol.[25]

Acknowledgments

Financial support of this work was provided by KOSEF and by 21C Frontier Program, Center for Biological Modulators. S. H. is a beneficiary of the Brain Korea 21 (BK21) program.

References and notes

1. Hirabayashi, J. *Glycoconjugate J.* **2004**, *21*, 35–40.
2. Furukawa, K.; Okajima, T. *Biochim. Biophys. Acta* **2002**, *1573*, 377–381.
3. Freeze, H. H.; Aebi, M. *Curr. Opin. Struct. Biol.* **2005**, *15*, 490–498.
4. (a) Laidler, P.; Litynska, A.; Hoja-Lukowicz, D.; Labedz, M.; Przybylo, M.; Ciolczyk-Wierzbicka, D.; Pochec, E.; Trebacz, E.; Kremser, E. *Cancer Immunol. Immunother.* **2006**, *55*, 112–118; (b) Bironaite, D.; Nesland, J. M.; Dalen, H.; Risberg, B.; Bryne, M. *Tumour Biol.* **2000**, *21*, 165–175.
5. Inzana, T. J.; Glindemann, G.; Cox, A. D.; Wakarchuk, W.; Howard, M. D. *Infect. Immun.* **2002**, *70*, 4870–4879.
6. Saavedra, E.; Herrera, M.; Gao, W.; Uemura, H.; Pereira, M. A. *J. Exp. Med.* **1999**, *190*, 1825–1836.
7. (a) Peter-Katalinic, J. *Methods Enzymol.* **2005**, *405*, 139–171; (b) Medzihadszky, K. F. *Methods Enzymol.* **2005**, *405*, 116–138.
8. Lesinski, G. B.; Westerink, M. A. *Curr. Drug Targets Infect. Disord.* **2001**, *1*, 325–334.
9. Kwon, M.; Jeong, S.; Lee, K. H.; Park, Y. K.; Yu, J. *J. Am. Chem. Soc.* **2002**, *124*, 13996–13997.
10. Tam, J. P. *J. Immunol. Methods* **1996**, *196*, 17–32.
11. Carbohydrate-PAA-biotins, GlycoTech Corporation, USA, 2005.
12. BLAST search program <www.ncbi.nlm.nih.gov/BLAST/>.
13. (a) Smith, L. E.; Uemura, H.; Eichinger, D. *Mol. Biochem. Parasitol.* **1996**, *79*, 21–33; (b) Layh-Schmitt, G.; Hilbert, H.; Pirkl, E. *J. Bacteriol.* **1995**, *177*, 843–846; (c) El-Sayed, N. M. et al. *Science* **2005**, *309*, 409–415; (d) Gerard, C.; Eddy, R. L., Jr.; Shows, T. B. *J. Clin. Invest.* **1990**, *86*, 1921–1927; (e) Rimm, D. L.; Morrow, J. S. *Biochem. Biophys. Res. Commun.* **1994**, *200*, 1754–1761.
14. Connor, R. J.; Kawaoka, Y.; Webster, R. G.; Paulson, J. C. *Virology* **1994**, *205*, 17–23.
15. Buts, L.; Dao-Thi, M. H.; Loris, R.; Wyns, L.; Etzler, M.; Hamelryck, T. *J. Mol. Biol.* **2001**, *309*, 193–201.
16. Vila-Perello, M.; Gutierrez Gallego, R.; Andreu, D. *Chembiochem* **2005**, *6*, 1831–1838.
17. (a) Shin, I.; Cho, J. W.; Boo, D. W. *Comb. Chem. High Throughput Screen* **2004**, *7*, 565–574; (b) Fukui, S.; Feizi, T.; Galustian, C.; Lawson, A. M.; Chai, W. *Nat. Biotechnol.* **2002**, *20*, 1011–1017.
18. (a) Adler, P.; Wood, S. J.; Lee, Y. C.; Lee, R. T.; Petri, W. A., Jr.; Schnaar, R. L. *J. Biol. Chem.* **1995**, *270*, 5164–5171; (b) Weis, W. I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441–473.
19. Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2795.
20. Three natural lectins, *Datura stramonium lectin (DSL), Erythrina cristagalli agglutinin (ECA), Ricin communis agglutinin (RCA)*, were purchased from Sigma and used without further purification. All of them are known to possess high affinities against *N*-acetyl-*N*-acetylglucosamine and *N*-acetyllactosamine.
21. Solid-phase peptide synthesis using Fmoc-Lys(biotin) as the first amino acid, followed by two Fmoc-Lys for the tetramer tentacle peptide. The synthesized peptides were purified by HPLC and identified by MULDI-TOFMS.
22. Sperandio, M.; Thatte, A.; Foy, D.; Ellies, L. G.; Marth, J. D.; Ley, K. *Blood* **2001**, *97*, 3812–3819.
23. Ph.D. 12 Phage Display Peptide Library Kit, instruction manual, New England BioLabs, USA, 1997.
24. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.
25. Mortz, E.; Krogh, T. N.; Vorum, H.; Gorg, A. *Proteomics* **2001**, *1*, 1359–1363.
26. Van Leeuwen, H.; Garcia-Mas, J.; Coca, M.; Puigdomenech, P.; Monfort, A. *Mol. Genet. Genomics* **2005**, *273*, 240–251.
27. Connor, R. J.; Kawaoka, Y.; Webster, R. G.; Paulson, J. C. *Virology* **1994**, *205*, 17–23.
28. Nadjar, D.; Rouveau, M.; Verdet, C.; Donay, L.; Herrmann, J.; Lagrange, P. H.; Philippon, A.; Arlet, G. *FEMS Microbiol. Lett.* **2000**, *187*, 35–40.