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# Tentacle type peptides as artificial lectins against sulfated Lewis X and A

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ABSTRACT

An effort has generated peptides from a phage-displayed library that selectively bind to the sulfated carbohydrates HSO3-LeA and HSO3-LeX. Even though more than six phaged peptides were identified by using the biopanning procedure, only one synthesized peptide displayed a consistently high binding affinity and specificity against the cognate HSO3-LeA. This dimeric, tentacle type peptide has a low micromolar affinity against the cognate sugar, which is even more specific than an antibody (Table 2(b)). Thus, it suggests that tentacle type peptides can be used as alternatives to antibodies to bind to aberrant cell-surface carbohydrates that are either the causes or results of carbohydrate-indicating disease states.



Sulfation and desulfation of hydroxyl functionality are important post-translational modifications that alter the biological function of substances including carbohydrates and phenols.[1] Various glycoproteins, such as mucins, possess sulfated oligosaccharides[2,3] which endow them with special biological properties. Sulfation can mask antigenic and lectin-binding sites, protect them from premature degradation, and regulate the biosynthesis and biological functions of glycoproteins and proteoglycanas. For example, heparin sulfate on the surface of all adherent cells modulates the actions of a large number of extracellular ligands.[4] As sulfated trisaccharides,[5] sulfated Lewis X ($HSO_3$-LeX (**1**)) and sulfated Lewis A ($HSO_3$-LeA (**2**)) (Fig. 1) are widely distributed in tumor mucin. Sulfated Lewis X is known to be the predominant determinant of tumor mucin, as in the LS174T-HM7 human colon carcinoma mucin.[6] Sulfated Lewis A is also found in the respiratory mucins of a secretor patient suffering from chronic bronchitis.[7]

Since sulfated forms of carbohydrates[8] and phenolic compounds are associated with various diseased states,[9] these post-translationally modified substances could serve as markers or targets for the related diseases.[10] The generation of tools for specific recognition of sulfated carbohydrates, therefore, could play a diagnostic or therapeutic role. Phage library systems using antibody gene libraries have been successfully applied to obtain ligands against biologically relevant sulfated species[11] and sulfated carbohydrates.[12] However, these applications require immunization processes to generate biased libraries. The phage displayed random peptide library methodology, which does not need immunization processes, is an excellent alternative for the generation of ligands against target molecules. This system is applicable for the production of antibodies against non-immunogenic or unstable species.

In previous studies, we have shown that multigenic peptides serve as tools for selective recognition of cell surface carbohydrates. Even though the 12-mer peptides originally selected did not have strong affinities, multigenic repeats of the original peptides have specific sub-micromolar affinities against cell surface carbohydrates.[13] Encouraged by these results, we have continued efforts designed to select peptides against $HSO_3$-LeA and $HSO_3$-LeX as baits. Below, we describe the results of this investigation, which has led to (1) the development of a method for multi-step selection of phaged peptides against the sulfated carbohydrates, (2) the measurement of binding affinities of the selected peptides, and (3) a specificity comparison of the selected peptide against various sulfates containing carbohydrates. Importantly, the results show that the $FAA^{di}$ peptide has a micromolar affinity and a reasonable specificity against $HSO_3$-LeA.

In this investigation, a phage-displayed 12-mer peptide library was used for selection against $HSO_3$-LeA and $HSO_3$-LeX. Biotinylated carbohydrates, spaced with polyacrylamide (PAA), were immobilized on solid supports.[14] Five rounds of biopanning procedures were carried out giving selected phages whose DNAs were cloned and sequenced (Table 1). Even though multiplicated clones were obtained, several different sequences from the selected phages were observed. As a result, the phaged peptide that displayed the highest specificity against two sulfated carbohydrates was reselected by using binding tests of each individual phage peptide to the immobilized carbohydrate conjugate. As shown in Figure 2, FAA phaged peptide generated against $HSO_3$LeA after washing and elusion gave more phage counts than RNR. However, phaged

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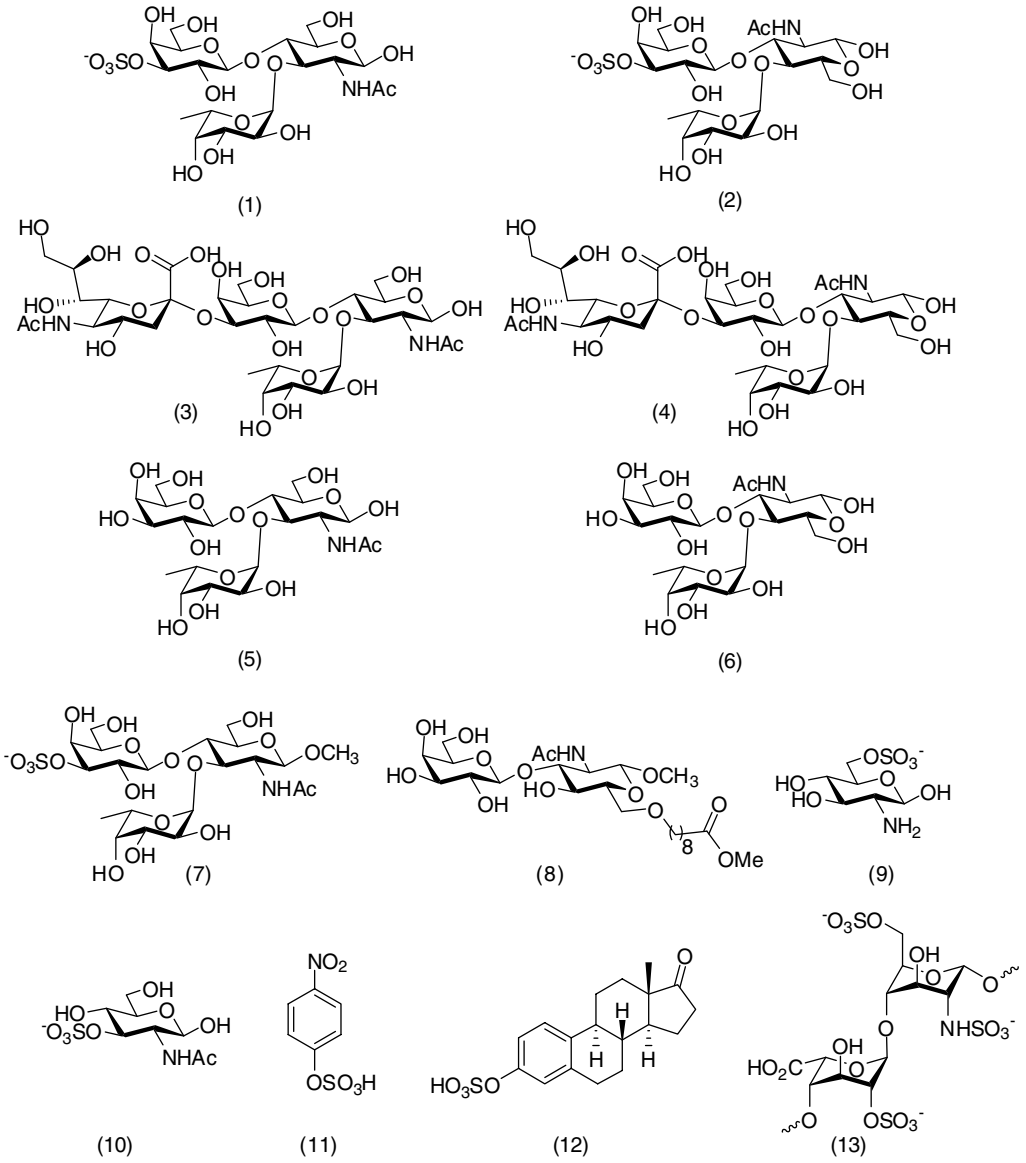


**Figure 1.** Sulfated and related carbohydrates and various sulfated chemicals. (1) $HSO_3$LeX (3-*O*-($SO_3H$)-Galβ1-4(Fucα1-3)GlcNAc) (2) $HSO_3$LeA (3-*O*-($SO_3H$)-Galβ1-3(Fucα1-4)GlcNAc) (3) sLeX (4) sLeA (5) LeX (6) LeA (7) methyl-3-*O*-($SO_3H$)-Galβ1-4(Fucα1-3)GlcNAc (8) 8-methoxy carbonyloctyl 3-*O*-($SO_3H$)-b-D-Galβ1-3GlcNAc (9) D-glucosamine 6-sulfate (10) *N*-acetyl-glucosamine 3-sulfate sodium salt (11) *p*-nitrophenyl sulfate (12) estrone-sulfate (13) Heparin.

**Table 1**
Deduced amino acid sequences of the selected phaged peptides against $HSO_3$LeX and $HSO_3$LeA

| Carbohydrate | Peptide sequence | Abbreviations |
|---|---|---|
| $HSO_3$LeX | APWHLSSQYSRT | APW |
| | NLAPIKVSLTSL | NLA |
| | SNQIPSSARAFI | SNQ |
| | TALATSSTYDPH | TAL |
| $HSO_3$LeA | FAAPMRTVQKID | FAA |
| | RNRRSIQRPMIS | RNR |

peptides produced against $HSO_3$LeX (APW, SNQ, TAL), except for NLA, gave similar counts.

The findings suggest that these peptides have very similar affinities and specificities against the cognate carbohydrate. As a result, the peptides were synthesized and subjected to further investigations. The binding affinities of the peptides, prepared as tentacle type dimers using lysine as a C-terminal residue,[15] were measured against cognate carbohydrate and related carbohydrates by using a surface plasmon resonance technique. Surface-based affinity measurements are particularly applicable to determining carbohydrate recognition, owing to the fact that immobilized carbohydrates effectively mimic cell-surface carbohydrates in which multiple interactions take place.

The binding affinities of the peptides on carbohydrate-immobilized chips, listed in Tables 2a and b, provide interesting preliminary information. Firstly, each dimeric tentacle type peptide shows a sub-millimolar to micromolar binding affinity to its cognate carbohydrate. Second, some of the peptides display a high level of specificity for sulfated carbohydrates. For example, the FAA $^{di}$ peptide has at least a 5-fold stronger affinity against the cognate $HSO_3$LeA than it does against either sLeA (**4**) or LeA (**6**). However, the peptides selected against $HSO_3$LeX, have weaker and less specific binding affinities than the FAA $^{di}$ peptide as was expected based on the results of the phage count experiments (Fig. 2a and b). For examples, the SNQ$^{di}$ peptide binds selectively but only weakly to $HSO_3$-LeX. The TAL$^{di}$ peptide binds to the cognate carbohydrate

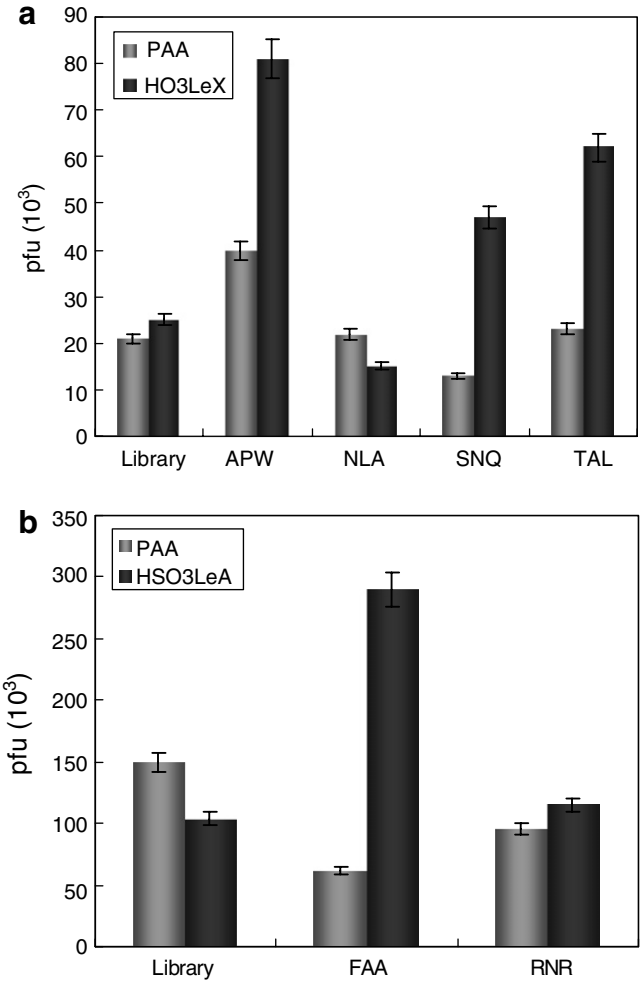


**Figure 2.** A selected phage-displayed peptide shows specific binding to a carbohydrate, $HSO_3$-LeX, $HSO_3$-LeA not to PAA-supported polymer. Carbohydrate-PPA-biotin (black bar) and $HOCH_2(HOCH)_4CH_2NH$-PAA-biotin (gray bar) were immobilized on a streptavidin-coated plate. Library phage and selected phage solution ($2.5 \times 10^{10}$ plaques forming unit; pfu) were transferred to each well. After a short incubation period of 30 min at room temperature and washing, the bound phage was eluted and titered. Values indicate average and a standard deviation of at least three experiments.

**Table 2**
Apparent $K_D$ values of tentacle type dimer peptides[a]

| Peptides | $HSO_3$LeX (μM) | sLeX (μM) | LeX (μM) |
|---|---|---|---|
| *(a) LeX series* | | | |
| APW$^{di}$ | NB | 67 | NB |
| SNQ$^{di}$ | 2800 | NB | NB |
| TAL$^{di}$ | 160 | 4.0 | NC |
| LeX antibody[b] | 140 | NC | 0.00033 |
| sLeX antibody[b] | NC | 0.0018 | NC |
| *(b) LeA series* | | | |
| | $HSO_3$LeA (μM) | sLeA (μM) | LeA(μM) |
| FAA$^{di}$ | 9.8 | 40 | 43 |
| LeA antibody[b] | 0.0069 | 0.012 | 0.20 |

Various concentrations of peptides in PBS were injected over the surface for 120 s and dissociation for 240 s. Each dimeric tentacle type peptide shows sub-millimolar to micromolar binding affinity to its cognate carbohydrate. NB, not binding; NC, binding affinities could not be calculated because of inconsistent RU values in various concentrations of peptides injected.

[a] Apparent $K_D$ values of injecting peptides on carbohydrate-immobilized chips were determined by using BIAcore 3000.

[b] Antibodies were purchased from Calbiochem-Novabiochem International, San Diego, CA. Values indicate apparent binding constants obtained by 1:1 Langmuir binding model using BIAevaluation program. Values of $\chi^2$ below 10 and T-values greater than 10 are accepted.[17]

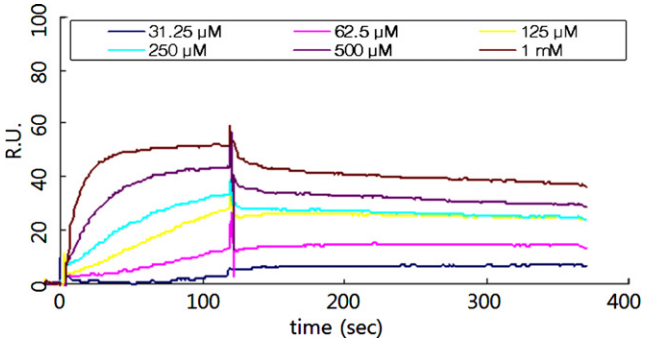


**Figure 3.** Representative SPR sensorgram of FAA$^{di}$ peptide to $HSO_3$LeA using BIAcore 3000. Various concentrations of the dimer peptide were injected (31.25, 62.5, 125, 250, 500, 1000 μM). R.U., response unit.

with sub-millimolar affinity but it also binds to sLeX (**5**) with a similar affinity (Fig. 3).

Even though the phaged-based selection process provided several candidate peptides against both sulfated LeA and LeX, only FAA $^{di}$ displayed a consistent binding affinity and specificity in both its phaged and its synthesized peptide forms against sulfated LeA. BLAST search of the FAA peptide indicates that it is highly homologous with several sulfatase and desulfurase peptides (Table 3). This observation suggests that the peptide might contain a direct recognition site for sulfates.

Since sulfated carbohydrates are recognized by the FAA$^{di}$ peptide with reasonable affinity, it is necessary to define minimal recognition motifs in carbohydrates that are required for binding to the peptide. For this determination, various substances containing sulfates, such as methyl-3-*O*-($SO_3H$)-Galβ1-4(Fucα1-3)GlcNAc (**7**), 8-methoxycarbonyloctyl-3-*O*-($SO_3H$)-β-D-Galβ1- 3GlcNAc (**8**),[16] D-glucosamine 6-sulfate (**9**), *N*-acetyl-glucosamine 3-sulfate (**10**), *p*-nitrophenyl sulfate (**11**), estrone-sulfate (**12**), and heparin (**13**), were eluted on the peptide-immobilized chip using an SPR technique. Surprisingly, only the cognate carbohydrate showed reasonable binding. Substance containing sulfated groups but no sugar moieties (**11**, **12**) or sulfated groups with one or two pyranosides residues (**8**, **9**, **10**) did not bind to the peptide. Even heparin (**13**) showed no significant binding to FAA$^{di}$, suggesting that the presence of at least three pyranoside units and sulfates groups is essential for binding to this peptide.

Lastly, the specificity of the FAA$^{di}$ peptide was compared with that of the AWH$^{di}$ peptide, which was previously generated by selection as a specific artificial lectin against sLeX (**5**)[13] by using an SPR technique. As shown by the data given in Table 4, each of two peptides has tighter binding affinities against the cognate carbohydrates. The data suggest that both peptides recognize three pyranosides that have very strong dipoles, which can be a consequence of the presence of S–O or C–O bonds in the respective sulfated and sialyl carbohydrates. If this analysis is correct, the peptides are able to differentiate an S–O from a C–O dipole via a 3- to 4-fold difference in binding affinities between sulfated and sialyl carbohydrates. The FAA$^{di}$ peptide even has a tighter and more specific binding to the cognate sugar compared to that of AHW$^{di}$.

In summary, this effort has generated peptides from a phage-displayed library that selectively bind to the sulfated carbohydrates $HSO_3$-LeA and $HSO_3$-LeX. Even though more than six phaged peptides were identified by using the biopanning procedure, only one synthesized peptide displayed a consistently high binding affinity and specificity against the cognate $HSO_3$-LeA. This dimeric, tentacle type peptide has a low micromolar affinity against the cognate sugar, which is even more specific than an antibody (Table 2(b)). Thus, it suggests that tentacle type peptides can be used as alternatives to antibodies to bind to aberrant cell-surface carbohy-

**Table 3**
Homologous proteins of carbohydrate-specific peptides arising by a BLAST search

| Carbohydrate | Sequence | Homologous proteins | Identities (%)/Positives (%) |
|---|---|---|---|
| $HSO_3$Le A | FAAPMRTVQKID | Cysteine desulfurase,[8] SufS subfamily [Nitrosospira multiformis ATCC 25196] | 72/81 |
| | | *N*-acetylglucosamine-6-sulfatase precursor [*Tenacibaculum* sp. MED152][a] | 47/64 |

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

**Table 4**
Cross-activity between the FAA dimer and AHW dimer peptides [a]

| | $HSO_3$LeA (μM) | sLeX (μM) |
|---|---|---|
| $FAA^{di}$ | 9.8 | 40 |
| $AHW^{di}$ | 200 | 67 |

[a] Apparent binding affinities were measured by using BIAcore 3000. Values indicate apparent binding constants obtained by 1:1 Langmuir binding model using BIAevaluation program. Values of $\chi^2$ below 10 and *T*-values greater than 10 are accepted.[17]

drates that are either the causes or the results of carbohydrate-indicating disease states.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.003.

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