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Rational Design and Synthesis of Peptide Ligands for an Anti-Carbohydrate Antibody and Their Immunochemical Characterization

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Abstract—Molecular mimics of carbohydrates present an alternative source of compounds to target pathways involving protein–carbohydrate interactions. Certain peptides act as molecular mimics of carbohydrates in binding to anti-carbohydrate antibodies. A series of potential peptide ligands for the anti-carbohydrate antibody SYA/J6, directed against *Shigella flexneri* Y, was designed by molecular modeling based on a crystal structure of the antibody complex with a carbohydrate-mimetic peptide. These octapeptides were synthesized using solid-phase peptide synthesis, and their recognition by the antibody was investigated. The results shed light on the nature of peptide–carbohydrate mimicry.

Introduction

Shigella flexneri Y is a Gram-negative enterobacterium that causes bacillary dysentery by invading the colonic mucosa.[1] The *O*-polysaccharide found on its cell surface, consisting of the repeating unit →2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→ (Fig. 1),[2,3] provides a suitable vaccine candidate. However, the development of vaccines based on polysaccharides may be complicated by several factors, including poor immunogenicity, and poor response in infants.[4] In several cases, these factors have been circumvented, with considerable success, by conjugation of polysaccharides to proteins.[4] An alternative strategy is the use of carbohydrate-mimetic peptides, which have demonstrated immunogenic potential.[5]

Carbohydrate-mimetic peptide ligands have been identified for a wide range of anti-carbohydrate antibodies, and for a limited number of other carbohydrate-binding proteins.[5] Significantly, immunological studies showed that in some cases, these peptides could not only bind to anti-carbohydrate antibodies, but could induce an immune response against the original carbohydrate.[5]

As part of our program to study the nature and origin of peptide–carbohydrate mimicry and to develop peptide-based vaccines, we report herein the design of peptide ligands for the monoclonal antibody SYA/J6,[3] directed against the *Shigella flexneri* Y O-polysaccharide, based on molecular modeling from a crystal structure of the antibody complexed to a carbohydrate-mimetic peptide. The synthesis of these peptide candidates and their evaluation as surrogate ligands for the antibody are also described.

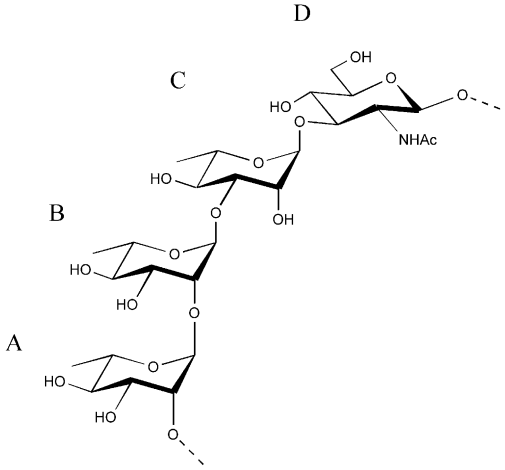

Figure 1. Repeating unit of the *Shigella flexneri* Y O-polysaccharide.

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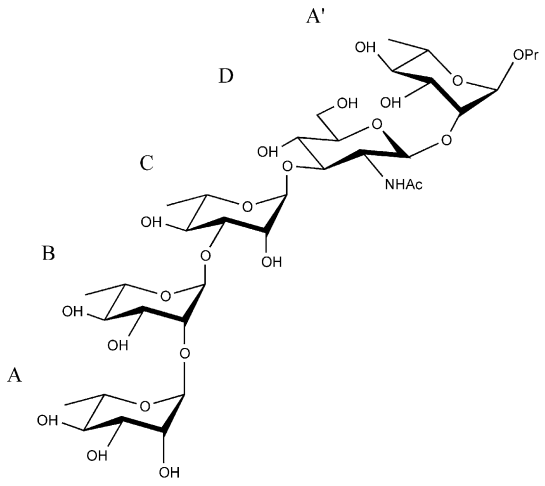

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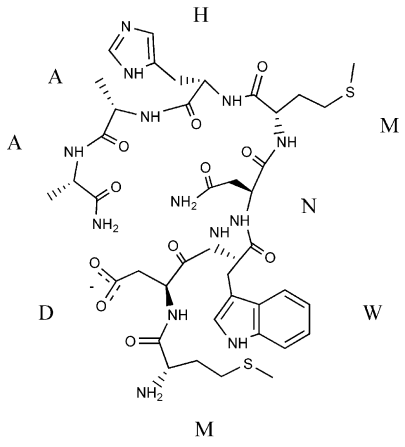

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Chart 1.

Results and Discussion

Harris et al.[6] identified the octapeptide MDWNMHAA (2, Chart 1) as a ligand for the monoclonal antibody (mAb) SYA/J6 through the screening of a phage-displayed library of peptides with the antibody. Comparison of the thermodynamics of binding of the antibody to the octapeptide to that of its binding to the native pentasaccharide (**1**, Chart 1) present on the cell surface of *Shigella flexneri* Y revealed that the enthalpy (ΔH) of binding to the octapeptide was favorable, but this was offset by an unfavorable entropy (ΔS) of binding (Table 1).[7]

Table 1. Comparison of the binding thermodynamics of SYA/J6 to pentasaccharide (**1**) and MDWNMHAA (**2**)[7]

	Pentasaccharide (**1**)	Octapeptide, MDWNMHAA (**2**)
K_A	$(2.5\pm2.5)\times10^5$	$(5.7\pm0.3)\times10^5$
ΔG (kcal mol^{-1})	-7.4 ± 0.6	-7.85 ± 0.05
ΔH (kcal mol^{-1})	-1.5 ± 2.8	-16.9 ± 0.8
$-T\Delta S$ (kcal mol^{-1})	-5.9 ± 3.4	9.04 ± 0.9

The crystal structure of the SYA/J6 Fab fragment in complex with the octapeptide **2** has been determined[7] and provided a starting point for the rational design of modified peptides as potential new ligands and mimotopes. Residues buried deeply within the combining site and involved in many intermolecular interactions were maintained, as they are likely to be essential for complex formation. Therefore, the key positions Trp-3 and Asn-4 were not varied.

However, the 1- and 5-positions of the peptide offer possibilities for modification to improve the binding affinity. The Met-1 side chain is somewhat solvent-exposed, contacting the edge of the binding groove and having an intramolecular interaction with Trp-3. The Met-5 residue forms several key interactions with the combining site but also offers possibilities for modification.[7] Its side chain stacks closely against the aromatic side chain of Trp H33, while the amide group forms a hydrogen bond to a water molecule, which in turn is hydrogen-bonded to the Gly H96 carbonyl oxygen. The presence of tightly bound water molecules within the complex is one possible reason for the unfavorable entropy of association;[8] another possible contributing factor is the conformational entropy loss on binding.[9]

Molecular modeling techniques were employed for the rational design of peptides with modifications at the Met-1 and Met-5 positions. Peptides were modified within the binding site, beginning from coordinates of the **2**–antibody complex, and subjected to energy minimization. The calculated intermolecular potential energies (van der Waals and electrostatic components) were used to indicate the quality of the resulting bound complexes, with reference to the values calculated for the native peptide **2** and for other modified peptides.

Molecular modeling showed that a modified peptide with replacement of Met-5 by Pro could bind within the site, maintaining hydrophobic interactions with Trp H33. In the predicted complex (Fig. 2a), the C-δ of Pro would occupy the space filled by a water molecule in the parent peptide–Ab complex. Therefore, two intermolecular hydrogen bonds would be lost. However, we reasoned that the reduced conformational freedom on introduction of the cyclic Pro residue, and the loss of one immobilized water molecule, might produce a compensatory decrease of the unfavorable entropy term. In addition, the 5-position of this octapeptide is located at the beginning of a turn of α-helix. The replacement of Met-5 by Pro might increase the population of the α-helical conformation in the free peptide, as Pro residues are favored in the first residue of an α-helix.[10]

Further modification to (4*R*)-4-hydroxyproline at the 5-position would have similar advantages in reducing

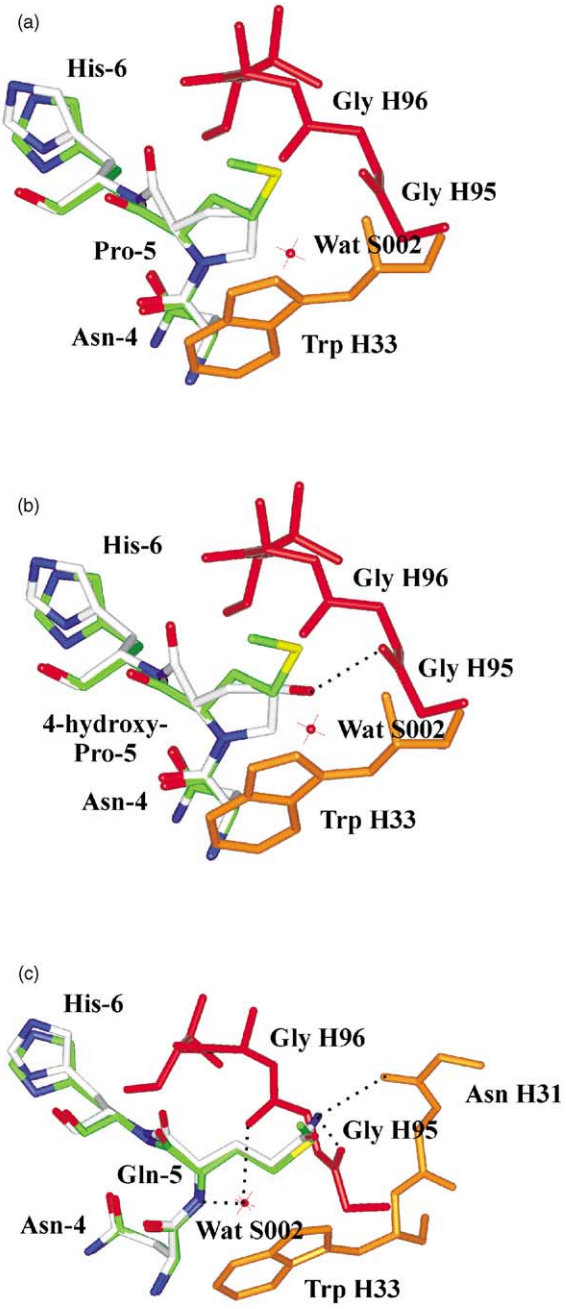


Figure 2. Views of predicted complexes of modified octapeptides, with the parent octapeptide **2** shown for reference. For clarity, residues 4–6 of the peptides are shown, as a close-up view of the site of substitution. The carbon atoms of the modified octapeptides are colored white, while those of the parent octapeptide are colored green. Selected residues of the binding site are shown in orange and red. Residues of the modified octapeptides and of the binding site are labeled. The water molecule S002, observed in the parent octapeptide–antibody complex,[7] and which would be displaced by the introduction of the Pro residues, is shown in red. Intermolecular hydrogen bonds predicted for the modified octapeptide–antibody complexes are indicated as dashed lines. (a) Met-5→Pro; (b) Met-5→4-hydroxy-Pro; (c) Met-5→Gln.

the entropy term, while the 4-OH group could form a hydrogen bond to the carbonyl oxygen atom of Gly H95 (Fig. 2b).

A substitution of Met-5 by glutamine was predicted to allow hydrophobic contact along the Trp H33 ring, as in the known complex, while the amide group of glutamine could form two new hydrogen bonds (Fig. 2c), to the carbonyl oxygen atoms of Asn H31 and Gly H95.

In order to provide a reference point to judge the value of the calculated interaction energies, the 1-position of the peptide was also targeted for modification. Previous work[11] in which peptides were synthesized on pins[12] had indicated that the substitutions Met-1→Thr and Met-1→Ile did not affect binding to the antibody adversely. These two peptides were predicted to bind well, having similar intermolecular potential energies to the parent octapeptide **2** (Table 2). As an additional reference point, several other modified peptides, that had been shown *not* to bind in pin synthesis,[11] were also evaluated by molecular modeling. For example, the substitutions Trp-3→His, Trp-3→Phe, and Trp-3→Gln, with minimal binding in the pin ELISA, had van der Waals energies less favorable than **2** by 12, 6, and 10 kcal mol^{-1}, respectively. Changes in the electrostatic energy were smaller for the first two substitutions, while Trp-3→Gln had a *more* favorable electrostatic energy (Table 2); this indicated that the intermolecular van der Waals energy was likely to provide a more accurate estimate of the actual binding affinity. The steric effects resulting from clashes between the ligand and binding site are also reflected in the van der Waals energy. For example, there is no room in the binding site at the 4-position of the peptide for a larger residue than Asn; this side chain forms a key hydrogen bond to Thr L91 O. Molecular modeling of the substitution Asn-4→Tyr produced a highly distorted complex, with the van der Waals energy increased by 25 kcal mol^{-1} (to −57 kcal mol^{-1}, Table 2). For these reasons, modified peptides with intermolecular van der Waals energies of greater than −80 kcal mol^{-1} were not considered for synthesis, while electrostatic energy was used as a secondary criterion.

Many other substitutions were considered, and subjected to the molecular modeling protocol. The steric and electrostatic demands of complementarity with the binding site are very stringent, and therefore, many of these proved unfavorable; representative examples are provided in Table 2. The four modified peptides (**3**–**7**, Chart 2) described above, with van der Waals and overall intermolecular energies comparable to the parent octapeptide, were selected for synthesis and testing.

Synthesis

The octapeptides **2**–**7** were synthesized using solid-phase synthesis techniques.[13] Since an amide functionality was required at the C-termini of the peptides, the synthesis was accomplished on Rink amide MBHA resin.[14] Rink amide MBHA resin, being more acid-stable than Rink amide and Rink amide AM resin, results in less contamination of the peptides by colored impurities. Classical Fmoc chemistry[15] was employed in the synthesis with *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) employed as an in situ activator[16] of the Fmoc-amino acid. Fmoc-amino acid derivatives with acid-labile side chain protecting groups were utilized so that simultaneous side chain deprotection

Table 2. Calculated intermolecular potential energies in complexes of the octapeptides **2**–**7** with mAb SYA/J6

Octapeptide	Intermolecular potential energy (kcal mol^{-1})	van der Waals energy (kcal mol^{-1})	Electrostatic energy (kcal mol^{-1})
2	−247	−82	−165
3	−245	−80	−164
4	−247	−82	−165
5	−256	−85	−171
6	−244	−84	−160
7	−244	−84	−160
Modifications with no binding/greatly reduced binding in previous pin synthesis[11]			
W3→H	−231	−70	−161
W3→F	−239	−76	−164
W3→Q	−244	−72	−172
N4→Y	−222	−57	−166
Examples of modifications not chosen for synthesis			
M5→K	−253	−73	−180
M5→AIB	−237	−78	−159
M5→(4*S*)-4hyP	−234	−73	−161

NH_2-Met-Asp-Trp-Asn-Met-His-Ala-Ala-$CONH_2$ (**2**)

NH_2-Thr-Asp-Trp-Asn-Met-His-Ala-Ala-$CONH_2$ (**3**)

NH_2-Ile-Asp-Trp-Asn-Met-His-Ala-Ala-$CONH_2$ (**4**)

NH_2-Met-Asp-Trp-Asn-Gln-His-Ala-Ala-$CONH_2$ (**5**)

NH_2-Met-Asp-Trp-Asn-Pro-His-Ala-Ala-$CONH_2$ (**6**)

NH_2-Met-Asp-Trp-Asn-4-HyP-His-Ala-Ala-$CONH_2$ (**7**)

4-HyP = 4-hydroxyproline

Chart 2.

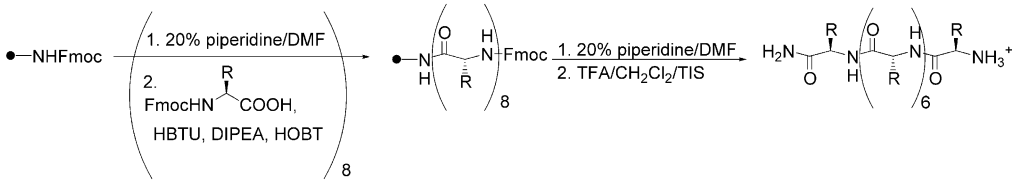
and cleavage of the peptide from the resin could be achieved by treatment with an acid. The Fmoc-amino acid derivatives utilized in the synthesis are: Fmoc-Met-OH, Fmoc-Asp(O-t-Bu)-OH, Fmoc-Trp-OH, Fmoc-Asn-OH, Fmoc-His(Trt)-OH, Fmoc-Ala-OH, Fmoc-Thr(O-t-Bu)-OH, Fmoc-Ile-OH, Fmoc-Gln-OH, Fmoc-Pro-OH and Fmoc-4-HyP(O-t-Bu)-OH. Successive deprotection of the Fmoc group and coupling of the appropriate amino acid derivatives then yielded the desired resin-bound octapeptide sequences (Scheme 1). After removal of the N-terminal Fmoc group, the resin was treated with trifluoroacetic acid/dichloromethane/triisopropylsilane (7:7:1) to release the free peptide into solution. Triisopropylsilane serves as a cation scavenger and prevents reattachment of the peptide to the resin-bound cation through nucleophilic side chains such as tryptophan or methionine.[13] After isolation and HPLC purification on a C_{18} reverse-phase column (gradient elution CH_3CN/H_2O), the octapeptides were obtained as white solids, in yields ranging from 35 to 48%. The octapeptides were characterized by 1D and 2D (TOCSY and ROESY) NMR spectroscopy, and MALDI and high resolution FAB mass spectrometry.

Immunochemistry

The octapeptides **2**–**7** were screened by ELISA for their ability to compete with *Shigella flexneri* Y lipopolysaccharide (LPS), as the solid phase antigen, for binding to the antibody SYA/J6. Samples of the antibody were allowed to equilibrate with varying concentrations of each octapeptide. These solutions were then added to a polystyrene plate coated with *Shigella flexneri* Y lipopolysaccharide. Competition for binding was indicated by a reduction in the amount of antibody bound to the solid phase (measured using a secondary antibody–peroxidase conjugate). Figure 3 shows the results of the assay, and the IC_{50} values are given in Table 3.

The results indicate that the octapeptides **3** and **4**, in which Met-1 of the parent octapeptide **2** was substituted with Thr and Ile, respectively, bind to the antibody with affinities similar to that of the parent octapeptide **2**. Therefore, interactions between the 1-position of the peptide and residues at the edge of the combining site (e.g., His H58) or with the rest of the peptide (Trp-3) are relatively unimportant.

The modified peptides **5** and **6**, with substitution at Met-5 of the parent octapeptide by Gln and Pro, respectively, were also recognized by the antibody. However,



Scheme 1.

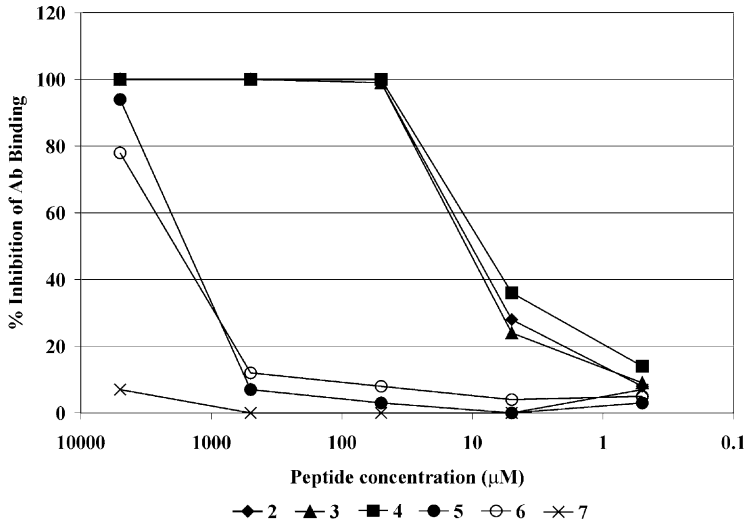


Figure 3. Results of the competitive ELISA binding assay, showing the inhibition of antibody binding to *Shigella flexneri* Y LPS by octapeptides **2**–**7**.

Table 3. IC_{50} values for the inhibition of binding of mAb SYA/J6 to *S. flexneri* Y LPS by octopeptides **2**–**7**

Octapeptide	IC_{50} values
2	19 μM
3	21 μM
4	15 μM
5	2.7 mM
6	3.1 mM
7	—[a]

[a]50% inhibition was not attained.

the binding affinity decreased by roughly 150-fold with respect to the parent octapeptide, corresponding to a difference in free energy of binding, $\Delta\Delta G \cong 3$ kcal mol^{-1}. Therefore, any benefit gained by a reduction in conformational freedom and bound water upon introduction of a Pro residue may have been offset by the loss of two hydrogen bonds. In the case of the Gln substitution (**5**), the water molecule would have been retained (Fig. 2C); therefore, the only change would be the interactions with Trp H33 in the site. One concludes that the hydrophobic Met-5/Trp H33 interaction is important.

The octapeptide **7**, with substitution of Met-5 by 4-HyP, did not bind. The objective of introducing an additional hydrogen bond to a combining site residue not contacted by the natural ligands was completely unsuccessful in this case. However, the strategy of maintaining several key interactions while varying others has been successful in other cases with smaller ligands.[18] In our case, it appears that the large number of intermolecular interactions and large number of functional groups on the ligand make it difficult to determine from structure alone which interactions are important. Nevertheless, these functional data help to reveal the requirements for the peptide–antibody interaction and may be used as a guide in further design strategies.

Conclusions

We have designed and synthesized a series of peptide ligands for the anti-carbohydrate antibody SYA/J6 and demonstrated the limited success of rational design. Only some of the peptide ligands were recognized and bound by the antibody, and those that bound did so with similar or lower affinities compared to the parent peptide. Nonetheless, the results provide some insight into the requirements for peptide–carbohydrate mimicry and validate the concept that peptides may be used as surrogate haptens to bind to an anti-carbohydrate antibody.

Experimental

General methods

The octapeptides were prepared on Rink Amide MBHA resin (Novabiochem) using standard Fmoc chemistry.[16] DMF was freed of amines by concentrating under high vacuum and the amine was collected in a dry-ice-cooled trap. The DMF was then distilled and stored over molecular sieves. Both the Fmoc deprotection and Fmoc-amino acid coupling reactions were monitored by a ninhydrin assay (Kaiser test: 5% ninhydrin in ethanol, 80% phenol in ethanol and 2% 0.001 M aqueous KCN in pyridine). 1D NMR spectra were recorded on a Bruker AMX400 spectrometer, while 2D NMR spectra were recorded on a Bruker AMX600 spectrometer in 10% D_2O in water. Chemical shifts were referred to external DSS (3-(trimethylsilyl)-1-propanesulfonic acid). MALDI-TOF mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix using a PerSeptive Biosystems Voyager-DE instrument. High resolution mass spectra were LSIMS (FAB), run on a Kratos Concept H double focusing mass spectrometer at 10,000 RP.

General procedure for synthesis of octapeptides

Rink amide MBHA resin (200 mg, loading = 0.73 mmol g^{-1}) was placed in a flask and DMF (15 mL) was added.

The suspension was allowed to stand for 1 h to allow the resin to swell. The suspension was filtered and the resin was washed with DMF (3×15 mL). A solution of 20% piperidine in DMF (15 mL) was then added to the flask. The flask was stoppered and shaken for 30 min with a mechanical shaker. The suspension was then filtered and the resin was washed with DMF (3×15 mL). A solution of Fmoc–Ala–OH (140 mg, 0.45 mmol) preactivated with HBTU (160 mg, 0.42 mmol) for 15 min and HOBT (61 mg, 0.45 mmol) in DMF (15 mL) was then added to the resin. The flask was stoppered and shaken with a mechanical shaker. The coupling reaction was monitored by withdrawing a sample of the resin, washing it with DMF and carrying out a Kaiser test. After the completion of the reaction, the suspension was filtered and the resin was washed with DMF (3×15 mL). Deprotection of the Fmoc group with 20% piperidine in DMF and coupling of the appropriate Fmoc-amino acid derivative was successively repeated until the desired octapeptide sequence was obtained. After removal of the N-terminal Fmoc group (20% piperidine in DMF), the resin was filtered and washed successively with DMF (3×15 mL) and dichloromethane (3×15 mL). A solution of trifluoroacetic acid/dichloromethane/triisopropylsilane (7:7:1) (15 mL) was then added to the resin. The suspension was shaken with a mechanical shaker for 2 h after which it was filtered. The resin was then washed with fresh 95% TFA. The filtrates were combined and concentrated. The residue was then purified by reverse-phase HPLC with a Waters liquid chromatograph (gradient elution, CH_3CN/H_2O) on a C_{18} column. The appropriate fractions were combined, concentrated under reduced pressure until all acetonitrile was removed and the remaining solution was then lyophilized to afford the octapeptides as white solids.

NH_2-Met-Asp-Trp-Asn-Met-His-Ala-Ala-$CONH_2$ (2). White solid (68 mg, 48% yield). ^{1}H NMR (90% H_2O/10% D_2O) δ (ppm): 10.15 (br, s, Trp ring NH); 8.77 (d, J=7.4 Hz, Asp NH); 8.59 (s, His H-2); 8.41 (d, J=7.6 Hz, His NH); 8.36 (d, J=6.5 Hz, Trp NH); 8.32 (d, J=5.9 Hz, Ala-8 NH); 8.25 (d, J=5.8 Hz, Ala-7 NH); 8.19 (d, J=7.5 Hz, Asn NH); 8.05 (d, J=6.8 Hz, Met-5 NH); 7.63 (d, J=8.0 Hz, Trp H-4); 7.53 (br, s, C-terminal Ala amide NH); 7.51 (br, s, Asn side chain NH); 7.50 (d, J=8.1 Hz, Trp H-7); 7.28 (2H, s, His H-4 and Trp H-2); 7.24 (dt, J=7.1 and 0.9 Hz, Trp H-6); 7.15 (dt, J=7.1 and 0.8 Hz, Trp H-5); 7.05 (br, s, C-terminal Ala amide NH); 6.87 (br, s, Asn side chain NH); 4.28 (3H, m, Ala α-H, Ala α-H, Met-5 α-H); 4.03 (t, J=6.8 Hz, Met-1 α-H); 3.32 (dd, J=14.8 and 6.6 Hz, Trp β-H); 3.27 (dd, J=15.5 and 5.9 Hz, His β-H); 3.22 (dd, J=14.9 and 7.8 Hz, Trp β-H); 3.15 (dd, J=15.5 and 8.8 Hz, His β-H); 2.91 (dd, J=17.0 and 6.6 Hz, Asp β-H); 2.79 (dd, J=17.0 and 7.3 Hz, Asp β-H); 2.67 (dd, J=15.8 and 6.8 Hz, Asn β-H); 2.58 (dd, J=15.8 and 6.9 Hz, Asn β-H); 2.48 (ddd, J=13.7, 8.1 and 5.5 Hz, Met-5 γ-H); 2.43–2.36 (2H, m, Met-5 γ-H and Met-1 γ-H); 2.27 (td, J=13.7 and 6.8 Hz, Met-1 γ-H); 2.07 (3H, s, Met-5 ε-CH_3); 2.03–1.84 (4H, m, Met-5 β-CH_2 and Met-1 β-CH_2); 1.96 (3H, s, Met-1 ε-CH_3); 1.41 (3H, d, J=7.1 Hz, Ala-8 β-CH_3); 1.38 (3H, d, J=7.3 Hz, Ala-7 β-CH_3). MALDI-TOF MS: (m/z) calcd for $C_{41}H_{59}N_{13}O_{11}S_2$: 975.13 [$M^+$+H]. Found: 975.25 [$M^+$+H]. HRMS: ($m/z$) calcd for $C_{41}H_{59}N_{13}O_{11}S_2$: 974.3977 [$M^+$+H]. Found: 974.3967 [$M^+$+H].

NH_2-Thr-Asp-Trp-Asn-Met-His-Ala-Ala-$CONH_2$ (3). White solid (48 mg, 35% yield). ^{1}H NMR (90% H_2O/10% D_2O) δ (ppm): 10.15 (br, s, Trp ring NH); 8.86 (d, J=7.4 Hz, Asp NH); 8.60 (s, His H-2); 8.41 (d, J=7.3 Hz, His NH); 8.32 (2H, d, J=5.8 Hz, Trp NH and Ala-8 NH); 8.25 (d, J=5.9 Hz, Ala-7 NH); 8.16 (d, J=7.5 Hz, Asn NH); 8.04 (d, J=6.4 Hz, Met NH); 7.63 (d, J=8.0 Hz, Trp H-4); 7.53 (br, s, C-terminal Ala amide NH); 7.51 (d, J=8.1 Hz, Trp H-7); 7.51 (br, s, Asn side chain NH); 7.28 (s, His H-4); 7.27 (s, Trp H-2); 7.25 (t, J=7.3 Hz, Trp H-6); 7.16 (t, J=7.0 Hz, Trp H-5); 7.05 (br, s, C-terminal Ala amide NH); 6.88 (br, s, Asn side chain NH); 4.28 (3H, m, Ala α-H, Ala α-H, Met α-H); 3.86 (dq, J=12.4 and 6.3 Hz, Thr β-H); 3.76 (d, J=6.4 Hz, Thr α-H); 3.32 (dd, J=15.3 and 6.0 Hz, Trp β-H); 3.27 (dd, J=15.5 and 6.2 Hz, His β-H); 3.21 (dd, J=15.1 and 8.0 Hz, Trp β-H); 3.16 (dd, J=15.4 and 8.9 Hz, His β-H); 2.92 (dd, J=17.2 and 6.7 Hz, Asp β-H); 2.80 (dd, J=17.2 and 7.1 Hz, Asp β-H); 2.67 (dd, J=15.9 and 6.9 Hz, Asn β-H); 2.57 (dd, J=15.9 and 6.8 Hz, Asn β-H); 2.48 (ddd, J=13.6, 7.9 and 5.8 Hz, Met γ-H); 2.40 (td, J=13.5 and 7.6 Hz, Met γ-H); 2.07 (3H, s, Met ε-CH_3); 1.98 (m, Met β-H); 1.90 (m, Met β-H); 1.41 (3H, d, J=7.3 Hz, Ala-8 β-CH_3); 1.37 (3H, d, J=7.1 Hz, Ala-7 β-CH_3); 0.99 (3H, d, J=6.3 Hz, Thr γ-CH_3). MALDI-TOF MS: (m/z) calcd for $C_{40}H_{57}N_{13}O_{12}S$: 945.03 [$M^+$+H]. Found: 945.13 [$M^+$+H]. HRMS: ($m/z$) calcd for $C_{40}H_{57}N_{13}O_{12}S$: 944.4049 [$M^+$+H]. Found: 944.4034 [$M^+$+H].

NH_2-Ile-Asp-Trp-Asn-Met-His-Ala-Ala-$CONH_2$ (4). White solid (65 mg, 47% yield). ^{1}H NMR (90% H_2O/10% D_2O) δ (ppm): 10.15 (br, s, Trp ring NH); 8.69 (d, J=7.6 Hz, Asp NH); 8.59 (s, His H-2); 8.41 (d, J=7.5 Hz, His NH); 8.36 (d, J=6.4 Hz, Trp NH); 8.32 (d, J=5.9 Hz, Ala-8 NH); 8.25 (d, J=5.8 Hz, Ala-7 NH); 8.18 (d, J=7.4 Hz, Asn NH); 8.03 (d, J=6.8 Hz, Met NH); 7.63 (d, J=7.9 Hz, Trp H-4); 7.53 (br, s, C-terminal Ala amide NH); 7.51 (br, s, Asn side chain NH); 7.49 (d, J=8.1 Hz, Trp H-7); 7.28 (2H, s, His H-4 and Trp H-2); 7.24 (t, J=7.1 Hz, Trp H-6); 7.15 (t, J=7.8 Hz, Trp H-5); 7.05 (br, s, C-terminal Ala amide NH); 6.88 (br, s, Asn side chain NH); 4.28 (3H, m, Ala α-H, Ala α-H, Met α-H); 3.72 (d, J=6.0 Hz, Ile α-H); 3.32 (dd, J=14.9 and 8.3 Hz, Trp β-H); 3.27 (dd, J=15.4 and 5.8 Hz, His β-H); 3.20 (dd, J=14.8 and 8.3 Hz, Trp β-H); 3.15 (dd, J=15.5 and 8.6 Hz, His β-H); 2.94 (dd, J=17.2 and 7.1 Hz, Asp β-H); 2.80 (dd, J=17.2 and 7.1 Hz, Asp β-H); 2.69 (dd, J=15.9 and 6.8 Hz, Asn β-H); 2.58 (dd, J=15.9 and 7.0 Hz, Asn β-H); 2.47 (ddd, J=13.5, 8.1 and 5.9 Hz, Met γ-H); 2.40 (td, J=13.4 and 7.6 Hz, Met γ-H); 2.07 (3H, s, Met ε-CH_3); 1.98 (m, Met β-H); 1.89 (m, Met β-H); 1.73 (br, m, Ile β-H); 1.40 (3H, d, J=7.3 Hz, Ala-8 β-CH_3); 1.37 (3H, d, J=7.3 Hz, Ala-7 β-CH_3); 1.35 (m, Ile γ-H); 1.04 (m, Ile γ-H); 0.78 (3H, t, J=7.4 Hz, Ile δ-CH_3); 0.66 (3H, d, J=6.9 Hz, Ile γ-CH_3). MALDI-TOF MS: (m/z) calcd for $C_{42}H_{61}N_{13}O_{11}S$: 957.09 [$M^+$+H]. Found: 957.09

$[M^+ + H]$. HRMS: (m/z) calcd for $C_{42}H_{61}N_{13}O_{11}S$: 956.4412 $[M^+ + H]$. Found: 956.4389 $[M^+ + H]$.

NH_2-Met-Asp-Trp-Asn-Gln-His-Ala-Ala-$CONH_2$ (5). White solid (56 mg, 40% yield). ^1H NMR (90% H_2O/10% D_2O) δ (ppm): 10.15 (br, s, Trp ring NH); 8.80 (d, $J=7.5$ Hz, Asp NH); 8.59 (s, His H-2); 8.43 (d, $J=7.5$ Hz, His NH); 8.39 (d, $J=6.4$ Hz, Trp NH); 8.32 (d, $J=5.7$ Hz, Ala-8 NH); 8.28 (d, $J=5.7$ Hz, Ala-7 NH); 8.18 (d, $J=7.5$ Hz, Asn NH); 8.07 (d, $J=6.5$ Hz, Gln NH); 7.60 (d, $J=7.9$ Hz, Trp H-4); 7.53 (br, s, C-terminal Ala amide NH); 7.50 (d, $J=8.2$ Hz, Trp H-7); 7.50 (br, s, Asn side chain NH); 7.47 (br, s, Gln side chain NH); 7.28 (2H, s, His H-4 and Trp H-2); 7.24 (t, $J=7.5$ Hz, Trp H-6); 7.16 (t, $J=7.4$ Hz, Trp H-5); 7.05 (br, s, C-terminal Ala amide NH); 6.87 (br, s, Asn side chain NH); 6.85 (br, s, Gln side chain NH); 4.28 (2H, m, Ala α-H, Ala α-H); 4.13 (dd, $J=13.9$ and 6.0 Hz, Gln α-H); 4.04 (t, $J=6.5$ Hz, Met α-H); 3.33 (dd, $J=14.8$ and 6.3 Hz, Trp β-H); 3.27 (dd, $J=15.3$ and 5.7 Hz, His β-H); 3.24 (dd, $J=14.6$ and 7.4 Hz, Trp β-H); 3.15 (dd, $J=15.6$, 8.7, His β-H); 2.92 (dd, $J=17.2$ and 6.6 Hz, Asp β-H); 2.80 (dd, $J=17.1$ and 7.4 Hz, Asp β-H); 2.64 (dd, $J=15.6$ and 6.3 Hz, Asn β-H); 2.58 (dd, $J=15.9$ and 7.0 Hz, Asn β-H); 2.40 (m, Met γ-H); 2.30 (m, Met γ-H); 2.22 (2H, m, Gln γ-CH_2); 2.02–1.92 (6H, m, Met ε-CH_3, Met β-CH_2, Gln β-H); 1.87 (m, Gln β-H); 1.41 (3H, d, $J=7.3$ Hz, Ala-8 β-CH_3); 1.37 (3H, d, $J=7.1$ Hz, Ala-7 β-CH_3). MALDI-TOF MS: (m/z) calcd for $C_{41}H_{58}N_{14}O_{12}S$: 972.06 $[M^+ + H]$. Found: 972.01 $[M^+ + H]$. HRMS: (m/z) calcd for $C_{41}H_{58}N_{14}O_{12}S$: 971.4158 $[M^+ + H]$. Found: 971.4145 $[M^+ + H]$.

NH_2-Met-Asp-Trp-Asn-Pro-His-Ala-Ala-$CONH_2$ (6). White solid (60 mg, 44% yield). ^1H NMR (90% H_2O/10% D_2O) δ (ppm): 10.17 (br, s, Trp ring NH); 8.84 (d, $J=7.3$ Hz, Asp NH); 8.61 (d, $J=1.4$ Hz, His H-2); 8.38 (d, $J=6.9$ Hz, Trp NH); 8.28 (d, $J=6.2$ Hz, Ala-8 NH); 8.27 (d, $J=8.0$ Hz, His NH); 8.13 (d, $J=8.1$ Hz, Asn NH); 7.99 (d, $J=5.8$ Hz, Ala-7 NH); 7.62 (d, $J=7.9$ Hz, Trp H-4); 7.57 (br, s, Asn side chain NH); 7.52 (br, s, C-terminal Ala amide NH); 7.50 (d, $J=8.2$ Hz, Trp H-7); 7.31 (s, His H-4); 7.25 (d, $J=2.22$ Hz, Trp H-2); 7.25 (dt, $J=7.1$ and 1.0 Hz, Trp H-6); 7.16 (dt, $J=7.0$ and 1.0 Hz, Trp H-5); 7.05 (br, s, C-terminal Ala amide NH); 6.94 (br, s, Asn side chain NH); 4.28 (dq, $J=7.3$ and 3.0 Hz, Ala α-H); 4.27 (dq, $J=7.2$ and 2.5 Hz, Ala α-H); 4.12 (dd, $J=8.7$ and 3.8 Hz, Pro α-H); 4.08 (t, $J=6.7$ Hz, Met α-H); 3.58 (ddd, $J=9.7$, 7.4 and 5.0 Hz, Pro δ-H); 3.31–3.25 (3H, m, Pro δ-H, Trp β-H and His β-H); 3.21 (dd, $J=14.5$ and 6.9 Hz, Trp β-H); 3.14 (dd, $J=15.5$ and 9.4 Hz, His β-H); 2.90 (dd, $J=17.0$ and 6.3 Hz, Asp β-H); 2.79 (dd, $J=17.0$ and 7.8 Hz, Asp β-H); 2.74 (dd, $J=15.8$ and 8.4 Hz, Asn β-H); 2.55 (dd, $J=15.8$ and 6.2 Hz, Asn β-H); 2.48 (ddd, $J=13.7$, 7.8 and 6.7 Hz, Met γ-H); 2.39 (ddd, $J=13.7$, 7.5 and 6.7 Hz, Met γ-H); 2.14 (br, m, Pro β-H); 2.10–2.01 (2H, m, Met β-CH_2); 2.02 (3H, s, Met ε-CH_3); 1.84 (br, m, Pro β-H); 1.72 (2H, m, Pro γ-CH_2); 1.41 (3H, d, $J=7.3$ Hz, Ala-8 β-CH_3); 1.38 (3H, d, $J=7.1$ Hz, Ala-7 β-CH_3). MALDI-TOF MS: (m/z) calcd for $C_{41}H_{57}N_{13}O_{11}S$: 941.05 $[M^+ + H]$. Found: 941.06 $[M^+ + H]$. HRMS: (m/z) calcd for $C_{41}H_{57}N_{13}O_{11}S$: 940.4099 $[M^+ + H]$. Found: 940.4108 $[M^+ + H]$.

NH_2-Met-Asp-Trp-Asn-4-HyP-His-Ala-Ala-$CONH_2$ (7). White solid (57 mg, 41% yield). ^1H NMR (90% H_2O/10% D_2O) δ (ppm): 10.12 (br, s, Trp ring NH); 8.74 (d, $J=7.5$ Hz, Asp NH); 8.59 (d, $J=1.4$ Hz, His H-2); 8.50 (d, $J=7.6$ Hz, His NH); 8.37 (d, $J=7.3$ Hz, Trp NH); 8.31 (d, $J=5.9$ Hz, Ala-8 NH); 8.19 (d, $J=7.9$ Hz, Asn NH); 8.17 (d, $J=5.9$ Hz, Ala-7 NH); 7.66 (d, $J=8.0$ Hz, Trp H-4); 7.58 (br, s, Asn side chain NH); 7.53 (br, s, C-terminal Ala amide NH); 7.50 (d, $J=8.1$ Hz, Trp H-7); 7.33 (s, His H-4); 7.25 (dt, $J=7.1$ and 0.8 Hz, Trp H-6); 7.25 (s, Trp, H-2) 7.17 (dt, $J=7.1$ and 0.9 Hz, Trp H-5); 7.05 (br, s, C-terminal Ala amide NH); 6.94 (br, s, Asn side chain NH); 4.51 (br, s, Hyp γ-H); 4.43 (t, $J=8.0$ Hz, Hyp α-H); 4.29 (q, $J=7.1$ Hz, Ala α-H); 4.27 (q, $J=7.1$ Hz, Ala α-H); 4.04 (t, $J=6.8$ Hz, Met α-H); 3.82 (dd, $J=11.4$ and 4.4 Hz, Hyp δ-H); 3.72 (d, $J=11.4$ Hz, Hyp δ-H); 3.32–3.27 (2H, m, Trp β-H and His β-H); 3.22–3.16 (2H, m, Trp β-H and His β-H); 2.85 (dd, $J=16.8$ and 6.5 Hz, Asp β-H); 2.73 (2H, dd, $J=16.5$ and 7.5 Hz, Asp β-H and Asn β-H); 2.57 (dd, $J=16.4$ and 7.0 Hz, Asn β-H); 2.41 (td, $J=13.9$ and 7.6 Hz, Met γ-H); 2.30 (td, $J=13.9$ and 7.0 Hz, Met γ-H); 2.28 (m, Hyp β-H); 2.03–1.92 (6H, m, Met ε-CH_3, Met β-CH_2, Hyp β-H); 1.41 (3H, d, $J=7.3$ Hz, Ala-8 β-CH_3); 1.38 (3H, d, $J=7.1$ Hz, Ala-7 β-CH_3). MALDI-TOF MS: (m/z) calcd for $C_{41}H_{57}N_{13}O_{12}S$: 957.04 $[M^+ + H]$. Found: 957.40 $[M^+ + H]$. HRMS: (m/z) calcd for $C_{41}H_{57}N_{13}O_{12}S$: 956.4049 $[M^+ + H]$. Found: 956.4055 $[M^+ + H]$.

Molecular modeling

Calculations were performed using the CVFF force field[17] within InsightII/Discover (Accelrys, Inc.). The coordinates of the crystal structure of mAb SYA/J6 in complex with the native octapeptide **2** were used as a starting point. Modified peptides were optimized by energy minimization within the binding site (200 steps, using the conjugate gradient method) while heavy atoms of the binding site were held fixed, followed by manual repositioning and repeated energy minimization, if necessary. Intermolecular van der Waals and electrostatic energies were calculated, and used to evaluate the quality of the resulting complexes.

Competitive ELISA binding assay

A 96-well polystyrene plate (Fisher) was coated with *S. flexneri* Y LPS (1 μg/mL in Tris-buffered saline solution (TBS; 50 mM Tris, 150 mM NaCl, pH 7.5)) by incubation overnight at 4 °C with shaking. After washing 4 times with TBS/0.1% Tween 20, wells were blocked with skim milk solution (5% skim milk powder in TBS) for 20 min at 37 °C with shaking, then washed another 4 times before addition of antibody/peptide solutions. The mAb SYA/J6 (60 nM in skim milk solution) and peptide (0.5 μM–5 mM in skim milk solution, in 10-fold dilution increments) were allowed to equilibrate by incubation overnight at 4 °C with shaking. These solutions were then added to the LPS-coated plate, and

incubated again overnight at 4 °C with shaking. The wells were washed six times with TBS/0.1% Tween 20. The secondary antibody (Pierce, ImmunoPure goat anti-mouse IgG (H + L), conjugated to horseradish peroxidase, diluted 1:1000 in skim milk solution) was added and the plate was incubated for 20 min at 37 °C with shaking. The plate was washed six times with TBS/0.1% Tween 20. The concentration of bound antibody was measured by a colorimetric assay for horseradish peroxidase, as follows. To the washed plate was added a freshly prepared solution of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (0.51 mM) (ABTS; Sigma) in citric acid–phosphate buffer (61.4 mM citric acid, 77.2 mM disodium hydrogen phosphate, pH 4) containing 0.03% H_2O_2. The color was allowed to develop and was measured as the difference between absorbance at 405 and 490 nm (A_{405}–A_{490}); the values measured at 25 min, corresponding to maximum intensity, were used.

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