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Peptide ligands for a sugar-binding protein isolated from a random peptide library

(peptide diversity/fd bacteriophage/concanavalin A/affinity panning)

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ABSTRACT **Peptide ligands for the carbohydrate-binding protein concanavalin A (Con A) have been identified by screening a large, diverse peptide library expressed on the surface of filamentous phage. A dodecapeptide containing the consensus sequence Tyr-Pro-Tyr was found to bind Con A with an affinity (dissociation constant, K_d) of 46 μM, comparable to that of a known carbohydrate ligand, methyl α-D-mannopyranoside (K_d of 89 μM). In addition the peptide inhibited precipitation of the α-glucan dextran 1355 by Con A. Given the complexity of oligosaccharide synthesis, the prospect of finding peptides that competitively inhibit carbohydrate-specific receptors may simplify the development of new therapeutic agents.**

Carbohydrate–protein interactions form the basis of a host of biological processes (1, 2). For example, the periplasmic monosaccharide-binding proteins of Gram-negative bacteria serve as receptors for transport and chemotaxis (3), while cell-surface lectins mediate such processes as cell–cell adhesion (4, 5) and lymphocyte migration through lymphoid tissues (6). Carbohydrate recognition is central to the enzymatic synthesis and degradation of polysaccharides, glycoproteins, and glycolipids that play essential roles in metabolism and in the maintenance of cellular structures. Carbohydrate–protein associations are also critical in certain cycles of viral infection. For example, binding of the hemagglutinin protein of human influenza virus to sialic acid residues on erythrocyte cell-surface glycoproteins represents the initial step in influenza infection (7).

Consequently, the development of potent inhibitors of carbohydrate-specific proteins may be of considerable importance in the generation of new therapeutic agents. However, the synthesis of complex carbohydrate ligands and analogs often requires many time-consuming, low-yielding steps. Chemical synthesis of oligosaccharides requires sophisticated strategies for protecting/deprotecting and assembling sugar monomers and for controlling product regiochemistry and stereochemistry (8–10). Enzymatic synthesis using glycosyltransferases has emerged as a useful alternative to chemical synthesis (11, 12). However, this approach is limited by the availability of enzymes with the appropriate specificities.

An alternative approach to the synthesis of polysaccharide ligands for carbohydrate-specific receptors is to ask whether peptides can be found that bind these proteins with high affinities. We describe a strategy for identifying novel peptide ligands for carbohydrate-binding proteins based on the screening of a large, highly diverse peptide library expressed on the surface of filamentous phage fd (13–16). The library consists of phage bearing random octapeptides fused to the amino terminus of the minor coat protein, pIII, and was screened by affinity purification on immobilized receptor. We chose as a model system the lectin Con A from jack bean, whose physicochemical properties have been extensively studied (17). Con A is a tetramer composed of four identical polypeptide chains consisting of 237 residues each. Con A, which interacts preferentially with oligosaccharides bearing terminal α-linked mannose or glucose residues, is frequently employed in the purification and structural characterization of carbohydrates and glycoproteins and is also a lymphocyte mitogen. We report the isolation of peptide ligands for Con A that prevent binding of known monosaccharide ligands of the lectin and that inhibit Con A-dependent precipitation of polysaccharides. The prospect that specific ligands for carbohydrate-binding proteins can be assembled by coupling amino acid building blocks (rather than sugars) should greatly reduce the synthetic difficulties associated with inhibitor design.

MATERIALS AND METHODS

Reagents and Peptides. Con A was obtained from Sigma or Calbiochem, biotinylated Con A from Boehringer Mannheim, and Con A-Sepharose from Pharmacia. Restriction enzymes, T4 DNA ligase, and T4 kinase were obtained from New England Biolabs or GIBCO/BRL. All other chemicals were obtained from Sigma. Oligonucleotides were synthesized with an Applied Biosystems PCR-mate and gel-purified. Peptides were synthesized on a MilliGen (Bedford, MA) synthesizer using fluorenylmethoxycarbonyl and *t*-butyl protecting groups and trifluoroacetic acid deprotection and were purified to >95% purity by reverse-phase HPLC using water/acetonitrile/0.1% trifluoroacetic acid as eluant. Composition of the purified peptides was confirmed by amino acid analysis and mass spectrometry. UV–visible spectra were recorded on a Cary 3 spectrophotometer. Equilibrium dialysis was performed with a Spectrum equilibrium dialysis chamber.

Construction of a Random Octapeptide Library in fd Phage. The random octamer library, constructed according to the protocol of Cwirla *et al.* (16), was kindly provided by W. J. Dower. Briefly, a collection of oligonucleotides encoding all possible octapeptides fused to the spacer peptide Ala-Ser-Gly-Ser-Ala (ASGSA) was synthesized as described (16) by using the nucleotide sequence 5′-C-TCT-CAC-TCC-NNK-NNK-NNK-NNK-NNK-NNK-NNK-NNK-GCA-AGT-GGC-TCT-GCT-ACT-GTT-GAA-AGT-TGT-3′ (oligonucleotide ON-141), where N is A, C, G, or T (equimolar) and K is G or T (40:60). Ten electrotransformations, each using 13.5 μg of ligated vector DNA and 200 μl of electrocompetent *Escherichia coli* MC1061 cells yielded, after 1 hr of nonse-

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lective outgrowth, a library of 1.4×10^9 transformants (75% producing infective phage). The cells were grown under tetracycline selection in 3 liters of L broth to amplify the library, and the phage were isolated as described (16).

Affinity Purification. Affinity purification was performed utilizing two different attachment strategies for the Con A. In the first, biotinylated Con A [20 μg/ml in Con A buffer (CAB: 50 mM NaCl/20 mM Mops, pH 6.8/2 mM $MgCl_2$/2 mM $CaCl_2$/0.2 mM EDTA)] was bound in 96-well microtiter plates coated with streptavidin (20 μg/ml in phosphate-buffered saline). Unbound Con A was removed by three 5-min washes with CAB. In the second strategy, commercially available Con A covalently attached to cyanogen bromide-activated Sepharose was used. Phage ($\approx 10^{10}$ infectious particles) were added to each well of the microtiter plate or to 20 μl of Con A-Sepharose in a microcentrifuge tube, in a final volume of 200 μl of CAB. Phage were allowed to bind for 1 hr at room temperature on a rocking platform. Unbound phage were removed by three 5-min washes with CAB (1 ml). Adherent phage were eluted with either 200 mM methyl α-D-mannopyranoside, 1% yeast mannan, or 100 mM citrate buffer (pH 3.0) for 30 min at room temperature. Eluted phage were amplified as described (16). This panning protocol was then repeated twice.

After three rounds of panning individual amplified phage clones were tested for their ability to specifically bind Con A as determined by ELISA. Biotinylated Con A was bound in the presence of bovine serum albumin (20 μg/ml in CAB) to microtiter plate wells as described above. Phage ($\approx 10^{10}$ transforming units) were added to each well and incubated for 1 hr. As a negative control, phage were added to identically treated wells from which Con A had been omitted. Unbound phage were removed by three washes with CAB. Bound phage were detected in a sandwich assay with rabbit anti-fd phage antiserum and a horseradish peroxidase-coupled goat anti-rabbit antibody (R. W. Barrett, S. E. Cwirla, M. S. Ackerman, A. M. Olson, E. A. Peters, and W. J. Dower, personal communication). Phage clones that displayed specific binding to Con A were amplified and their DNA was sequenced as described (16).

UV Difference Spectroscopy. Dissociation constants for peptide binding to Con A were determined at 20°C by the competition UV difference spectrophotometric method of Bessler *et al.* (18). Con A was 0.72 mg/ml in CAB and the competition difference spectra were recorded at 0.1 and 0.2 mM *p*-nitrophenyl α-D-mannopyranoside. Absorbance changes were measured 90 min after addition of peptide to ensure equilibration with the chromogenic sugar. The absorbance change (ΔA) at 317 nm was plotted in the form $[(\Delta A_{max}/\Delta A) - 1][D]$ vs. [L] where ΔA_{max} is the absorbance change when the protein is saturated with *p*-nitrophenyl α-D-mannopyranoside, [L] is the concentration of the competing ligand, and [D] is the concentration of free chromogenic ligand. The competition dissociation constant K_d is given by the product of the dissociation constant of the chromogenic sugar and the reciprocal slope of this linear representation of the data.

Equilibrium Dialysis. The peptide DVFYPYPYASGS was N-methylated with [^{14}C]formaldehyde (19). The HPLC-purified peptide had a specific activity of 1.5 mCi/mmol (1 mCi = 37 MBq). Equilibrium dialysis was performed using microcells in the Spectrum equilibrium dialysis chamber. Con A (200 μl, 0.1 mg/ml) was placed in one chamber and peptide (200 μl) was placed in the other. The chambers were separated by a SpectraPor 4 membrane with a molecular weight cutoff of 12,000–14,000. Dialysis was performed for 20 hr at 4°C. Samples from each chamber were removed and 50-μl aliquots were combined with 5 ml of Ready Scint (Beckman) for measurement of radioactivity in a Beckman model LS 6000TA scintillation counter.

Inhibition of Dextran Precipitation. The ability of peptide to inhibit the precipitation of Con A was assayed in the dextran 1355 system (20). Briefly, the precipitation reactions were conducted in plastic vials (1.5 ml) containing 18 μg of Con A and various amounts (5–20 μg) of dextran B-1355S in 100 μl of phosphate-buffered saline containing 1 M NaCl. The reaction mixtures were incubated for 48 hr, centrifuged for 10 min at 10,000 × *g*, and washed three times with phosphate-buffered saline containing 1 M NaCl. The protein content of the precipitate in each vial was determined by the method of Lowry with bovine serum albumin as the standard. Stock solutions of the sugar standard methyl α-D-mannopyranoside and all peptides were prepared in phosphate-buffered saline or doubly distilled water. Inhibition of the precipitation was performed by addition of various amounts of sugar or peptide to the reaction mixture containing Con A (18 μg) and dextran B-1355S (15 μg).

RESULTS

The peptide library was constructed as previously described (16) except that a variable octapeptide region was fused to the amino terminus of the phage coat protein pIII via a spacer peptide, ASGSA. This spacer is expected to reduce steric interference of the phage surface with bound receptor. The library, consisting of 1.4×10^9 independent phage recombinants, was screened in three cycles of panning, elution, and amplification against Con A immobilized either covalently on Sepharose beads or noncovalently on streptavidin-coated microtiter plates. Affinity purification was carried out in the presence of 2 mM $CaCl_2$ because saccharide binding by Con A is calcium-dependent (17). In one set of experiments, adherent phage were eluted with buffer containing methyl α-D-mannopyranoside or yeast mannan at concentrations sufficient to block rebinding of peptide at the sugar binding site. Increasing percentages of the input phage (0.039%, 6.7%, and 11%) were recovered in rounds 1, 2, and 3 of screening, suggesting that selective phage enrichment was occurring. In a second series of experiments, phage were eluted with a low-pH citrate buffer (pH 3.0) to denature the receptor–ligand interaction [note that at neutral pH Con A exists as a tetramer of identical 26.5-kDa subunits and undergoes reversible dissociation below pH 5.6 to give a dimeric species (21)]. Similar levels of phage recovery were also observed through three rounds of acid elution.

After the third round of panning, 19 of 19 randomly selected individual phage isolates eluted with carbohydrate and 14 of 15 phage eluted with citrate displayed specific binding to Con A as determined by ELISA (data not shown). DNA from specific binding clones was sequenced and the deduced peptide sequences are shown in Fig. 1. The peptides eluted with methyl α-D-mannopyranoside can be separated into three groups. The first contains eight different sequences, one of which appears in four separate phage clones examined. These peptides are grouped in Fig. 1*a* to show a distinctive consensus sequence Tyr-Pro-Tyr, with additional preferences for Val-Trp/Phe and Pro/Gly in the amino and carboxyl flanking sites, respectively. Note that the conserved motif is duplicated in the replicate clones. The second group includes two members that each contain a pair of cysteine residues separated by four intervening amino acids (raising the possibility of a conformational constraint imposed by a disulfide bond). There is no obvious homology among the third group of sequences, but they all appear very rich in charged or polar residues.

Phage recovered by low-pH elution include several identical clones and the majority bear a close resemblance to the first group of peptides eluted with methyl α-D-mannopyranoside (see Fig. 1*b*). The motif Tyr-Xaa-Tyr replaces the strict consensus Tyr-Pro-Tyr sequence from the previous experi-

A		B
YPYPVFVH	YNMYSTAA	YNYGWVEF
YPYYQYFM	AVNGCRHD	YNYGWVEF
YPYGSYWA	SYTHVASS	YNYGWVEF
VWYPYGAG	KGQAELLR	YNYGWVEF
VWYKYPGW	VFTDQKAQ	YNYGWVEF
DVFYPYPY	LNDNSAGY	YRYDIFRE
DVFYPYPY	WARNTSHS	YDYGSFSK
DVFYPYPY		YSYPYYHL
DVFYPYPY		NYDYMGIW
NRVWYPYG		YPYAYIWT
		VVFYDYGS
NCQGTACS		YEAHYQYG
LRCGWGVC		
		GTWFTNFR
		SRCGLLVE

FIG. 1. Amino acid sequences (deduced from DNA sequence) of amino-terminal octapeptides of pIII from phage eluted with 200 mM methyl α-D-mannopyranoside (*A*) or with citrate buffer (pH 3.0) (*B*).

ment, though this latter triplet does occur in two of the acid-eluted clones. In several clones the amino acid substitutions at the flanking sites follow the preferences noted above. Two phage clones with no clear homology to previously observed sequences were also found. The binding site(s) on Con A for the peptide sequences we have isolated that do not conform to the consensus motif are unknown.

The peptide DVFYPYPYASGS was chemically synthesized and its binding interaction with Con A was evaluated in two separate assays. This sequence was selected because it contained a tandem repeat of the consensus motif from the mannoside elutions and appeared in multiple phage isolates after three rounds of panning. The tetrapeptide ASGS (from the spacer region of the pIII fusion protein) was appended to the octamer sequence to enhance its solubility. The ability of this dodecapeptide to displace a chromogenic ligand, *p*-nitrophenyl α-D-mannopyranoside from Con A was determined by UV difference spectrophotometric assay (18). Fig. 2*A* shows the effect of peptide concentration on receptor occupancy by *p*-nitrophenyl α-D-mannopyranoside at two fixed concentrations of the chromogenic sugar. The data from these two experiments can be fitted by the same line giving a K_d of 46 μM for DVFYPYPYASGS at 20°C (Fig. 2*B*). This compares with a K_d of 89 μM for methyl α-D-mannopyranoside under the same experimental conditions [literature values of 120 μM and 67 μM for methyl and *p*-nitrophenyl α-D-mannopyranosides, respectively, have been reported with this assay at 27°C (18)]. While displacement of the chromogenic sugar from Con A by methyl α-D-mannopyranoside was complete within seconds of mixing, binding of the dodecapeptide required as long as 90 min to attain equilibrium. The reason for this slow approach to equilibrium has not been determined. Reversible binding of the peptide by Con A was confirmed by separating the peptide from the lectin by passage through a dialysis membrane with a 10-kDa cutoff.

The peptide DVFYPYPYASGS was N-methylated with [^{14}C]formaldehyde and its affinity for Con A was determined by equilibrium dialysis at 4°C. Scatchard analysis (Fig. 3) showed the peptide to bind at a stoichiometry of ≈1 per Con A subunit, with a K_d of 70 μM, in reasonable agreement with the UV difference data at room temperature. So and Goldstein (22) measured a K_d of 48 μM at 2°C for methyl α-D-mannopyranoside by equilibrium dialysis.

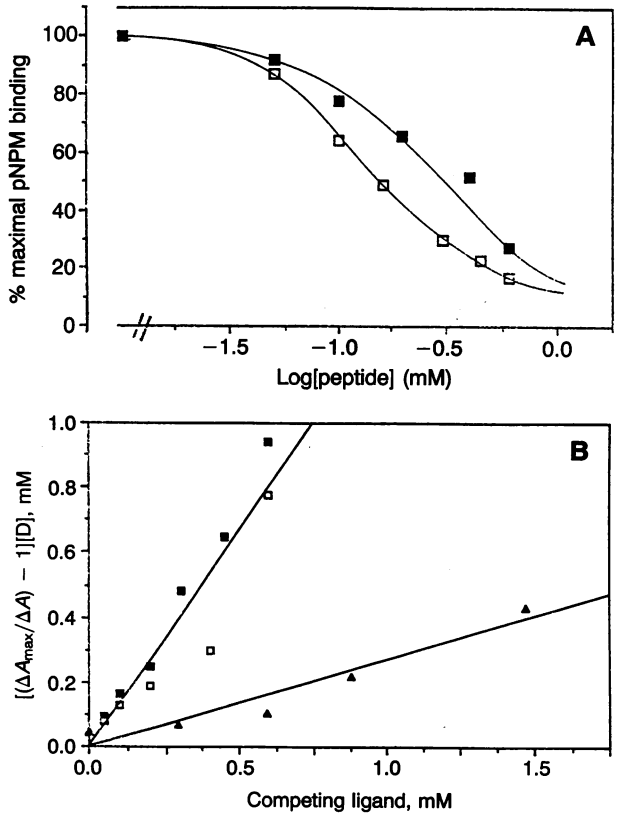

FIG. 2. (*A*) Effect of concentration of peptide DVFYPYPYASGS on binding of *p*-nitrophenyl α-D-mannopyranoside (pNPM) at 0.1 mM (□) or 0.2 mM (■) to Con A (27 μM binding sites). (*B*) Determination of dissociation constants (K_d) of peptide ligands for Con A by competition with pNPM. ■, DVFYPYPYASGS, 0.1 mM pNPM; □, DVFYPYPYASGS, 0.2 mM pNPM; ▲, RVWYPYGSYLTASGS, 0.1 mM pNPM. See *Materials and Methods* for explanation of ordinate label.

The octapeptide YRYDIFRE was selected as a representative Tyr-Xaa-Tyr-containing sequence from the clones eluted at low pH and was chemically synthesized. At concentrations as high as 10 mM, this peptide was unable to inhibit binding of *p*-nitrophenyl α-D-mannopyranoside to Con A (data not shown). Similarly, the tripeptide carboxamides YPY and YNY did not displace bound chromogenic sugar at 10 mM. To determine the ability of the consensus Val-(Trp/Phe)-Tyr-Pro-Tyr-(Pro/Gly) motif to confer Con A binding

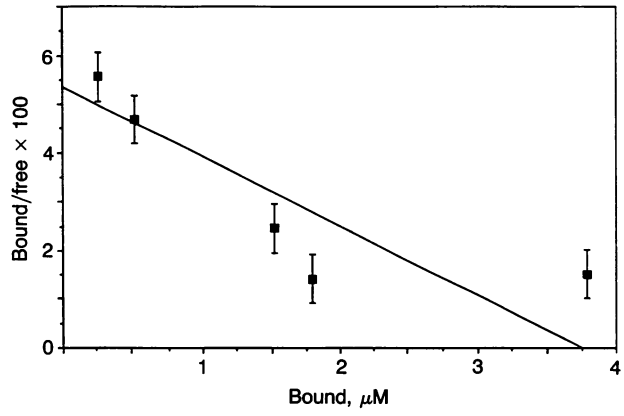

FIG. 3. Scatchard analysis of binding of N-[^{14}C]methylated DVFYPYPYASGS to Con A determined by equilibrium dialysis.

on a longer peptide, we tested the pentadecapeptide RVWYPYGSYLTASGS in the spectrophotometric assay. This peptide inhibited sugar binding with an apparent K_d of 230 μM, higher than that of the dodecapeptide (Fig. 2*B*).

Precipitation of polysaccharides and glycoconjugates by lectins has been extensively documented (23). Branched polysaccharides containing multiple terminal α-glucopyranosyl, α-mannopyranosyl, or fructofuranosyl groups are precipitated by Con A. In Fig. 4 the ability of peptides DVFYPYPYASGS and RVWYPYGSYLTASGS to block precipitation of the α-glucan dextran 1355 by Con A is contrasted with inhibition by methyl α-D-glycosides. For the dodecapeptide an IC_{50} value of 2.6 mM and for the pentadecapeptide an IC_{30} of 5 mM compare with IC_{50} values of 0.35 mM and 1.20 mM for methyl α-D-mannopyranoside and methyl α-D-glucopyranoside, respectively. Neither the octapeptides YRYDIFRE and YNYGWEVF nor the tripeptide carboxamide YPY displayed any inhibitory effects at 8 mM on dextran precipitation.

DISCUSSION

A more detailed understanding of the molecular nature of protein–saccharide interactions should facilitate the design of inhibitors of carbohydrate-binding proteins and could influence the development of new therapeutic agents. For example, inhibitors of neuroaminidases or hemagglutinins might be useful in controlling viral infections, while antagonists of the selectin receptors ELAM1 or CD62 could prove effective in controlling inflammation and tissue damage (24). Although an endogeneous ligand, sialyl-Lewis X (SLe^x) [NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAc], for ELAM1 and CD62 has been synthesized (25–27), the synthesis of a series of carbohydrate analogues (28) is considerably more difficult than, for example, the synthesis of a series of analogues of a peptide hormone or protease substrate. Consequently, the identification of peptide antagonists of carbohydrate–receptor interactions should considerably simplify the synthesis of more potent or selective inhibitors.

A number of naturally occurring proteinaceous inhibitors have been isolated that compete for the binding sites of carbohydrate-specific proteins. For example, the protein tendamistat binds the enzyme α-amylase with a K_d of 9 pM (29–31). Interaction of the tripeptide epitope Trp-Arg-Tyr of tendamistat with the carbohydrate binding site is thought to play a critical role in complex formation (29, 32). These studies suggested that peptides might be found that competitively inhibit other classes of carbohydrate-binding proteins such as lectins or viral receptors. To test this notion we have screened a large library of peptide sequences expressed on the surface of filamentous phage for their ability to bind the lectin Con A.

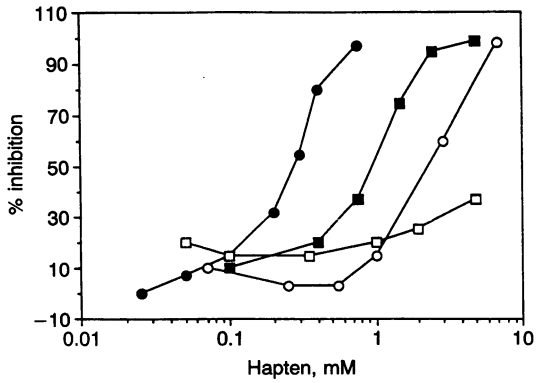

FIG. 4. Inhibition of the precipitation reaction between Con A and dextran B-1355S with methyl α-D-mannopyranoside (●), methyl α-D-glucopyranoside (■), and the synthetic peptides DVFYPYPYASGS (○) and RVWYPYGSYLTASGS (□) as haptens.

An amino-terminal library of random octapeptides consisting of $\approx 1.4 \times 10^9$ members ($\approx$5% of all possible octamers) was affinity-purified against immobilized Con A. When phage were eluted by a low-pH citrate buffer to dissociate the phage–Con A complex, the sequence Tyr-Xaa-Tyr (where Xaa is predominantly a hydrophilic amino acid) was found in 12 of 14 clones recovered. When the adherent phage were repeatedly eluted with buffer containing methyl α-D-mannopyranoside at a concentration sufficient to saturate the carbohydrate binding sites, proline was frequently observed in this triplet sequence. An additional preference for incorporation of Val-Trp/Phe and Pro/Gly residues at the sites flanking the triplet sequence was also apparent. A search of the GenBank database (January 1992) did not identify any known saccharide-binding proteins having a high degree of homology with this consensus sequence, although a highly homologous sequence (VWYPFPY) was found in the fimbrial assembly gene product (FimB) of several strains of the Gram-negative species *Bacteroides nodosus* (33). The fimbrial subunit proteins (*fimA* gene products) of many bacteria have lectin-like properties and mediate adhesion to eukaryotic cells through carbohydrate structures on the extracellular surface of the membrane. Interestingly, the cell-surface receptors for many bacteria are mannose-containing oligosaccharides (34). While adherence by the organism *B. nodosus* is not believed to be mannose-dependent, the structure of its eukaryotic receptor is unknown (33).

Two peptides containing the motif Val-(Trp/Phe)-Tyr-Pro-Tyr-(Pro/Gly) were synthesized and assayed for their ability to inhibit binding by Con A of known monosaccharide and polysaccharide ligands. The K_d values for the dodecapeptide DVFYPYPYASGS were determined to be 46 μM and 70 μM, when measured by UV difference spectroscopy (20°C) and equilibrium dialysis (4°C), respectively. These values compare closely with the binding affinities of two monosaccharide ligands, methyl and *p*-nitrophenyl α-D-mannopyranosides [K_d of 120 and 67 μM at 27°C (18)]. The affinity of the pentadecapeptide RVWYPYGSYLTASGS was lower by a factor of $\approx$5 (K_d of 230 μM). These peptides also inhibited Con A-dependent precipitation of dextran 1355 with IC_{50} values of 2.6 mM and $\approx$15 mM, respectively. The higher inhibitory constants found in the precipitation assay presumably result from the multivalency of the lectin–dextran interaction. However, the 5-fold difference in affinity of the 12-mer and 15-mer peptides for Con A correlates well with the difference in their respective IC_{50} values in the precipitation assay.

Although a number of hydrophobic binding sites exist on the Con A surface, including sites for fluorescent dansyl derivatives (35, 36), tryptophan or indoleacetic acid (37), and benzoic acid derivatives (38), a number of studies indicate that the chromogenic ligand *p*-nitrophenyl α-D-mannopyranoside binds at the saccharide binding site (18, 39, 40). The fact that the potency of the dodecapeptide in the UV difference assay is dependent on the concentration of the chromogenic mannoside, and that the peptide binds with a stoichiometry of $\approx$1 per Con A subunit and also inhibits dextran precipitation, suggests that this peptide also binds at the saccharide binding site (or an overlapping site). A less likely alternative is that this ligand binds a second site, whose occupation induces a conformational change in the protein that abolishes binding of saccharides to Con A.

The saccharide binding site of each Con A subunit has been shown by x-ray crystallography to be a shallow crevice near the protein surface (41). Hydrogen bonds and van der Waals interactions are the predominant forces involved in binding of methyl α-D-mannopyranoside, as is the case with most carbohydrate-binding proteins (1, 42). The sugar interacts with

the lectin through a network of seven hydrogen bonds that connect four oxygens of the ligand with five amino acid residues of the protein. A hydrophobic patch formed by C-5 and C-6 of the saccharide makes van der Waals contacts with the ring atoms of Tyr^{12} and Tyr^{100}. Given the hydrophobic nature of the dodecapeptide sequence, one possible binding motif might involve hydrophobic stacking of the peptide tyrosine side chains against Tyr^{12} or Tyr^{100} in the binding site. Interestingly, the conserved epitope of the proteinaceous inhibitors of α-amylase (Trp-Arg-Tyr) also consists of two aromatic residues separated by a single amino acid. The low affinity (>10 mM) of the tripeptide carboxamides YPY and YNY indicates that other interactions between Con A and the dodecapeptide contribute to its affinity for the lectin and/or that the surrounding amino acids influence the conformation of the YXY sequence. Studies of α-linked mannose oligosaccharides suggest an extended sugar binding site in Con A (43, 44) that could make additional contacts with the dodecapeptide.

The fact that phage bearing the moderately low-affinity peptides which we have characterized should survive the extensive washing employed in the panning protocol undoubtedly reflects a multivalent binding interaction between the phage (which carries three to five copies of the pIII protein) and one or more molecules of the tetravalent lectin. High-avidity interactions between phage particles and immobilized receptors have been attributed to polyvalent binding in previous studies (ref. 16; R. W. Barrett, personal communication). Affinity purification of phage bearing single copies of a random peptide on its surface (45) could lead to the isolation of higher-affinity peptides. Moreover, random mutagenesis of the dodecapeptide sequence might also provide higher-affinity peptides.

Earlier studies have shown that libraries of random peptides expressed on filamentous phage are a source of peptide ligands for biological receptors. The deduced peptide sequences of phage clones isolated by affinity purification with anti-myohemerythrin and anti-β-endorphin monoclonal antibodies were closely related to the known epitopes (14, 16). By screening a library expressing random 15-mer peptides, Devlin *et al.* (15) identified a series of streptavidin-binding phage that contained a consensus tripeptide sequence, His-Pro-Gln. Binding of these clones was inhibited in the presence of 1 μM biotin, suggesting that the peptides bound at or near the biotin binding site. This report further illustrates that peptide display vectors may be used to find novel ligands for biological receptors without regard for the structure of the binding site or the "natural" ligand. We expect that this approach should successfully identify peptide antagonists of other carbohydrate-binding proteins. Indeed, using the *Griffonia simplicifolia* lectin GS $I\text{-}B_4$ in a second model study, we identified in a series of phage clones affinity-purified from our octapeptide library the consensus sequence Trp-Xaa-Xaa-Trp-Zaa, where Xaa is a small aliphatic or polar residue and Zaa is a branched aliphatic amino acid (unpublished work). Large random peptide libraries constructed by either molecular biological or synthetic chemical techniques (46–48) should continue to provide a powerful approach to ligand discovery and optimization.

We thank Bill Dower for providing the octapeptide library and for many helpful discussions. We thank Lois Aldwin and Dave King for synthesizing peptides and acknowledge valuable discussions with Ron Barrett and Peter Schatz.

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