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Biochemistry

A family of concanavalin A-binding peptides from a hexapeptide epitope library

(lectin/carbohydrate binding site/peptide mimics/peptide library/filamentous bacteriophage)

JAMIE K. SCOTT*†, DURAIKKANNU LOGANATHAN‡, R. BLAINE EASLEY*, XIAOFEN GONG*, AND IRWIN J. GOLDSTEIN‡

*Division of Biological Sciences, University of Missouri, Columbia, MO 65211; and ‡Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

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ABSTRACT **The lectin concanavalin A (Con A) binds methyl α-D-mannopyranoside (MeαMan) as well as α-D-mannosyl groups at the nonreducing terminus of oligosaccharides. Ligand peptides that mimic the binding of MeαMan to Con A were identified from screening an epitope library composed of filamentous phage displaying random hexapeptides. A consensus sequence was identified among affinity-purified phage; Con A binds phage bearing this sequence and is inhibited from doing so by MeαMan. When tested for binding against a panel of lectins, phage bearing this sequence bind only weakly to a closely related D-mannose-binding lectin, indicating that binding to Con A is highly selective. A synthetic peptide bearing the consensus sequence blocks the precipitation of Con A by dextran with an inhibition strength equivalent to that of methyl α-D-glucopyranoside. These results demonstrate that the specificity of Con A is not limited to carbohydrates and that highly selective sugar-mimics for lectins of plant, animal, or bacterial origin may be identified from epitope libraries.**

The lectin Con A comprises a significant fraction of the protein in the jack bean (*Canavalia ensiformis*). It is a tetramer, consisting of four identical subunits that bind with moderate affinity (K_d 120–500 μM) to the α anomers of D-mannose and D-glucose (1). When oligosaccharides containing these sugars are displayed on a cell surface, Con A binds with high avidity as a result of multivalent interactions. Because of this high avidity and its specificity for particular sugars, the function of Con A is presumed to involve binding to D-mannosyl-containing oligosaccharides as part of a specific cell recognition process. In an effort toward developing specific target-ligands for lectins, we have discovered a set of related peptides that bind at or near the sugar-binding site of Con A with affinity equivalent to that of methyl α-D-glucopyranoside (MeαGlc). These peptides were obtained from screening a hexapeptide epitope library with Con A.

The epitope library consists of 200 million filamentous phage clones that display about 70% of all possible hexapeptides (2). The use of epitope libraries in identifying ligand peptides has been demonstrated for protein-binding antibodies (2–4), as well as for the biotin-binding site of streptavidin (5). The latter result was the first demonstration that epitope libraries can be used to discover peptides that mimic the binding of nonpeptide ligands.

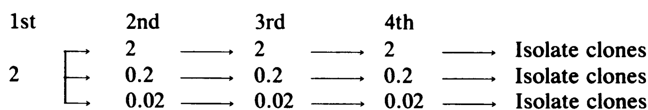
Phage bearing Con A-binding sequences were affinity-enriched from the library and identified by (*i*) exhibiting a consensus sequence that is shared among randomly chosen phage and by their ability (*ii*) to selectively bind to Con A in ELISA, (*iii*) to be significantly retained by immobilized Con A, and (*iv*) to be specifically inhibited from binding and enrichment on Con A by methyl α-D-mannopyranoside (MeαMan). Furthermore, hexapeptide bearing the consensus sequence was shown to bind directly to Con A and to inhibit the precipitation of Con A by dextran with a strength equivalent to that of MeαGlc, whereas peptides bearing selected alanine replacements in the sequence inhibited binding to a lesser extent. Binding of the consensus sequence to Con A is selective, as phage bearing this sequence bind weakly to only one of several MeαMan-binding lectins that share structural homology with Con A and not at all to lectins that recognize other sugars. Further, the consensus sequence was not isolated in side-by-side screenings of the epitope library with Con A and several of these MeαMan-binding lectins.

The specificity of peptide recognition by Con A is demonstrated by the ability of the consensus sequence to directly bind Con A and to be inhibited from binding by MeαMan. In addition, the remarkable selectivity of this sequence for Con A is shown by its inability to bind strongly to other D-mannose-binding lectins. That we have identified peptides with these properties indicates that epitope libraries may be valuable in probing the specificity of both plant and animal lectins and more generally, in identifying lead compounds for development of receptor-targeted drugs.

MATERIALS AND METHODS

Isolation of Ligand Phage from the Hexapeptide Epitope Library. Phage from the hexapeptide library were isolated by "biopanning" (2, 6). About 10^{12} phage particles were incubated overnight with biotinylated Con A (bio-Con A) at 2 μM in Tris-buffered saline (TBS) containing 10 nM $MnCl_2$ and 100 nM $CaCl_2$ and adjusted to pH 7.0–7.2 (TBS/MnCa); soluble epidermal growth factor receptor (a gift of M. Das, University of Pennsylvania) was also present at half the molar concentration of the Con A. The reactions mixtures were incubated for 20 min on streptavidin-coated polystyrene Petri dishes. Unbound phage were removed by extensive washing in TBS/MnCa containing 0.5% Tween 20 (TBS/MnCa/Tween), and the remaining phage were eluted with acid. The eluates were neutralized and used to infect cells.

The strategy used in the series of four biopannings with three different concentrations of bio-Con A (2, 0.2, and 0.02 μM) is shown below.



Scheme I

Abbreviations: bio-Con A, biotinylated Con A; MeαGlc, methyl α-D-glucopyranoside; MeαMan, methyl α-D-mannopyranoside; NphαMan, *p*-nitrophenyl α-D-mannopyranoside.

†To whom reprint requests should be sent at present address: c/o Dr. Elizabeth Getzoff, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

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A high concentration of bio-Con A (2 μM) was used in the first round of biopanning to maximize the yield of ligand phage. Three samples of amplified phage from the first round of biopanning were subjected to a second round of biopanning at 2, 0.2, or 0.02 μM bio-Con A, followed by amplification of the enriched phage. Two more rounds of biopanning at the three lectin concentrations and amplification were carried out in series, for a total of four rounds of biopanning. Clones from the fourth round of biopanning at each concentration of bio-Con A were chosen at random and propagated, and their DNA was sequenced in the epitope region (2).

Assessment of Phage Binding by Micropanning. Phage clones were propagated in *Escherichia coli* K91 cells and partially purified by two precipitations with polyethylene glycol (2), followed by pelleting by centrifugation at 58,000 rpm for 70 min at 15°C in a Beckman TLA rotor and resuspension in 1 ml of TBS/0.02% NaN_3. Virion concentrations were estimated from agarose gel electrophoresis (7). Lectins used in micropanning assays include bio-Con A and the biotinylated forms of *Lens culinaris* agglutinin, *Pisum sativum* agglutinin, *Sophora japonica* agglutinin, and *Griffonia simplicifolia* lectin (Vector Laboratories). Polystyrene microtiter wells were coated with 1 μg of streptavidin in 0.1 M $NaHCO_3$ at 4°C overnight or at 37°C for 4 hr, washed with TBS, and then incubated with about 50 pmol of biotinylated lectin in TBS/MnCa at 4°C overnight or at room temperature for 6 hr. Wells were washed six times by filling with TBS/MnCa/Tween, then 30 μl of TBS/MnCa/Tween or 5 mM MeαMan in TBS/MnCa/Tween was added to each well and incubated for 30 min at 4°C. About 10^8 phage particles in 5 μl of TBS/MnCa/Tween were added to each well and incubated at 4°C for 4 hr. The incubation mixtures were removed and the wells were washed 10 times (5 min per wash) with TBS/MnCa/Tween at 4°C. Retained phage were eluted from the wells by incubation for 10 min in 30 μl of 0.1 M HCl, pH 2.2 with glycine, containing bovine serum albumin at 1 μg/ml and then were transferred to microcentrifuge tubes containing 3.6 μl of 0.5 M Tris base. Phage from the reactions and the eluates were titered on *E. coli* K91 cells (8). Percent yields were estimated from the number of transforming units in the eluates divided by those in the reactions.

Detection of Binding of Biotinylated Lectin to Phage by ELISA. Microtiter wells were coated overnight at 4°C with about 10^{11} CsCl-purified (6) virions ($\approx$700 fmol equivalents of phage-borne peptide), 700 fmol of horse gamma globulin, or 1 mg of crystallized bovine serum albumin (Pentex, Kankakee, IL) in 30 μl of 0.1 M $NaHCO_3$ or were incubated with buffer alone. Wells were washed three times in TBS/Tween, and then 30 μl of 10 nM biotinylated lectin in TBS/MnCa/Tween with or without 20 mM MeαMan was added to appropriate wells and incubated overnight at 4°C. Plates were developed with an ABC Elite kit (Vector Laboratories). Wells were washed seven times with cold TBS/MnCa/Tween and once with cold TBS/MnCa, then 30 μl of ABC solution was added to each well and incubated at room temperature for 30 min. Wells were washed seven times with cold TBS/MnCa/Tween, and 30 μl of developing solution was added to each well and incubated at room temperature for 1–2 hr. Solute optical density in each well was measured at 405 and 490 nm by a microplate reader.

Synthetic Peptides. About 30 μg of hexapeptide was synthesized on each polyethylene pin (see ref. 9; a generous gift of H. M. Geysen, Chiron Mimotopes, Victoria, Australia). Pins were cleaned before each use (9), dried, and pretested by incubation in ABC solution and development as described below. Then they were cleaned again and tested for bio-Con A binding. First, the pins were blocked by incubation for 4 hr at room temperature in microtiter wells with 0.5% nonfat dry milk/0.1% Tween 20/TBS, then washed in TBS for 5 min, transferred to microtiter wells containing 130 μl of 10 nM bio-Con A/0.05% dialyzed bovine serum albumin/TBS/MnCa with or without 5 mM MeαMan, and incubated at 4°C for 4–16 hr. Pins were washed four times in TBS/MnCa/Tween, transferred to wells containing 130 μl of ABC solution and incubated at room temperature for 30–45 min. The pins were washed four times, transferred to wells containing developing solution, and incubated for 45–75 min. The pins were removed, and the optical density of each well was measured.

The peptides MYWYPY, MAWAPA, and VGRAFS (kindly provided by T. Leung and W. Mandecki, Abbott) were synthesized on a 430A peptide synthesizer (Applied Biosystems), purified by reversed-phase HPLC in a gradient of acetonitrile in 0.1% trifluoroacetic acid, and lyophilized. The sequences of purified peptides were confirmed by mass spectroscopic and amino acid analysis, and in select cases, peptide concentrations were verified by amino acid analysis.

The synthetic peptides, MeαMan, and MeαGlc were tested for their ability to inhibit the precipitation of Con A by dextran (10). Stock solutions of MeαMan (10 mM), MeαGlc (10 mM), MAWAPA (4.9 mM), and VGRAFS (5.4 mM) were prepared both in water and in 10% (vol/vol) dioxane, whereas MYWYPY (1.62 mM) was soluble only in 10% dioxane. Reaction mixtures (100 or 200 μl) contained 18 μg of Con A, 15 μg of dextran B-1355, and sugar or peptide inhibitor in 1 M NaCl/10 mM phosphate, pH 7.2; when present, dioxane was at a final concentration of 2%. Reaction mixtures were incubated at room temperature for 48 hr. After microcentrifugation for 10 min, the pellets were washed with buffer and centrifuged three times; protein content in pellets was measured by the method of Lowry.

The peptide VGRAFS was tested for its ability to compete with the chromogenic ligand *p*-nitrophenyl α-D-mannopyranoside (NphαMan) for the sugar-binding site of Con A. For this assay, difference spectra (range, 400–290 nm) were recorded on a Varian Cary 219 dual-beam spectrophotometer at 25°C with partitioned (Yankeelov, Beckman Instruments Fullerton, CA) cuvettes (11). Briefly, to one side of two matched cuvettes was added 1 ml of 80 μM Con A binding sites in phosphate-buffered saline, pH 7.2; to the other side, 1 ml of NphαMan in the same buffer was added. Baseline was recorded for the two cuvettes, and then the sample cuvette was mixed and the difference spectrum was recorded, noting the absorbance maximum (ΔA) at 317 nm. The reference cuvette was then mixed to restore the baseline. Concentrations of 20–240 μM NphαMan mixed with Con A in buffer were used to obtain the value ΔA_m, the change in absorbance corresponding to complete protein saturation; then cuvettes containing Con A in one side and 200 μM NphαMan plus 0.2–1.5 mM peptide or 50–600 μM MeαMan in the other side were mixed to obtain difference spectra. The inhibitor K_d values were calculated from absorbance data (11).

RESULTS

A Sequence Motif Is Shared Among Phage Isolated with bio-Con A. Phage clones were randomly isolated after the final round of each series of biopannings, and their epitope regions were sequenced. Table 1 shows these sequences arranged into four groups, A–D. All of the sequences in group A share a single motif, YPY. The frequency with which phage bearing the YPY motif, including those bearing the sequence MYWYPY, occur increases concomitantly with the decrease in the concentration of bio-Con A used in biopanning. This result indicates that these sequences were affinity-selected and that they are among the tightest-binding sequences available in this epitope library, as their occurrence increases with stringency of selection. The sequences flanking the random hexapeptide region may also be involved in the selection of YPY by Con A, as the consensus sequence

Table 1. Epitope sequences of phage isolated after four rounds of biopanning with bio-Con A

	2 μM	0.2 μM	0.02 μM
Group A	MYWYPY (2)	MYWYPY (8)	MYWYPY (11)
	IPWYPY (2)	IPWYPY (7)	
	LYWYPY (6)	LYWYPY	
	RIFYPY (2)	IAWYPY (3)	YTWYPY
	PIFYPY	IFWYPY	PYWYPY
	LPFYPY	VWWYPY (2)	
	FYWYPY		
	YVYYPY (5)		
	FYYYPY		
Group B	PLFVRY	TAFQLS	VGRAFS
	TYSATV	HRVGGT	VSWYIS
	WFSFMS (2)		
	ENGRKS		
	SSSGFW		
	VPGVSF		
	ARRYSR		
	HSSYFF		
Group C	RRHHHH (2)	CACRLK	
	-LHHHH		
	HWLVHH		
	HRHKHQ		
Group D	WPDMVR		RAAGIV
	FNAAVL		

Sequences are shown below the concentration bio-Con A used for the last three rounds of biopanning. Parenthetic numbers signify the number of clones identified with the stated sequence. All repeatedly isolated sequences are encoded by identical nucleotide sequences, indicating that they derive from a single clone.

occurs in only one register, at the last three residues of the hexapeptide variable region. Moreover, an aromatic residue (W, F, or Y) always precedes the YPY consensus sequence and is most likely also involved in the selection process. To confirm our results, this biopanning experiment was repeated with minor changes (including the absence of soluble epidermal growth factor receptor). Once again, the YPY motif was observed in 11 of 20 clones, with 17 of the 20 having YXY and 15 of these 17 having an aromatic residue preceding the YXY sequence (data not shown). Furthermore, side-by-side biopannings with two other MeαMan-binding lectins (*L. culinaris* agglutinin and *P. sativum* agglutinin) that accompanied the repeat Con A biopanning failed to select this or any sequence motif (data not shown).

In loose accordance with group A, each sequence in group B includes at least one hydroxylated amino acid (S, T, or Y) as well as an aromatic residue. Group C comprises residues that could potentially interact with the transition metal that is located in the sugar-binding site: these are H and C. Interestingly, all of the group C sequences can potentially achieve stable interactions with Con A by forming bidentate chelates with Mn^{2+} (12). Group D includes the remaining sequences that were identified. The sequence data indicate that epitopes in groups B and C probably bind Con A, but with lower affinities than those in group A, since none of these sequences share a strict consensus, and their relative frequencies of occurrence decline as the stringency of selection increases among the parallel biopannings.

Phage-Borne Epitopes Bind Con A. Representative phage selected from groups A–C were tested by two assays for direct binding to Con A. Binding of bio-Con A to CsCl-purified, plate-immobilized phage was tested by ELISA (Table 2). Phage bearing WYPY bound bio-Con A well above background, as did those displaying RRHHHH. Phage-borne VGRAFS also bound lectin above background, but to a lesser degree. Binding to phage—as well as background binding to CsCl-purified fl phage, to albumin-coated wells, and to uncoated wells—was inhibited in the presence of MeαMan. Direct binding of affinity-selected phage to plate-immobilized Con A was also tested by micropanning assays (Table 2) in which the yield of phage obtained in a miniaturized biopanning procedure was compared with that of control phage bearing a non-binding sequence. Phage bearing the WYPY sequence motif exhibited high yields on micropanning, with MYWYPY producing the highest yield, indicating that phage bearing this sequence bind most tightly to Con A. Each of the affinity-selected phage clones tested bound Con A with some specificity, as the phage yield from all these clones was significantly decreased when panning was done in the presence of MeαMan. In contrast, the yield of phage bearing the control sequence DFLEKI increased under this condition.

Synthetic Peptides Bind Con A. The peptides MYWYPY, LYWYPY, and IAWYPY were each synthesized on two pins and assayed for binding to Con A in the absence or presence of 5 mM MeαMan. The pins were cleaned and pretested for background binding, then tested for their ability to bind Con A (Table 3). The peptide MYWYPY gave the strongest signal, with the other WYPY-bearing peptides giving relatively high signals compared with background. This binding was specific, as it was significantly decreased in the presence of MeαMan.

To determine relative affinity compared with MeαMan, the hexapeptides MYWYPY (from group A), MAWAPA (a control peptide), and VGRAFS (from group B) were synthesized

Table 2. Binding of bio-Con A to affinity-purified phage

Epitope sequence	ELISA with immobilized phage*: No MeαMan	ELISA with immobilized phage*: 20 mM MeαMan	Micropanning with immobilized bio-Con A†: Input TU	Output TU: No MeαMan	Output TU: 5 mM MeαMan	% inhibition
MYWYPY	1015	39	4.1×10^7	2.6×10^7	1.1×10^5	100
LYWYPY	1067	38	6.0×10^7	5.1×10^6	7.0×10^5	86
IAWYPY	1039	62	4.0×10^7	5.6×10^6	5.4×10^5	90
VGRAFS	829	29	8.2×10^7	6500	510	92
RRHHHH	1000	37	1.6×10^7	4.4×10^4	2600	94
DFLEKI	—	—	7.5×10^6	1900	6000	−220
fl phage	529	25	—	—	—	—
Horse γG	1412	84	—	—	—	—
Albumin	683	18	—	—	—	—
Blank	585	18	—	—	—	—

Horse γG, horse gamma globulin; albumin, bovine serum albumin.
*Data were calculated as the average of two replicates [$(OD_{405} - OD_{490}) \times 10^3$].
†TU, transforming units. Percent inhibition was calculated as [(no MeαMan TU − 5 mM MeαMan TU)/no MeαMan TU] × 100.

Table 3. Binding of bio-Con A to peptides on pins

Epitope sequence	First pin		Second pin	
	Pretest	bio-Con A	Pretest	bio-Con A, MeαMan
MYWYPY	41	1069	46	325 (73)*
LYWYPY	43	682	46	204 (75)
IAWYPY	52	1108	52	444 (63)
Background*	—	43	—	44

The bio-Con A concentration was 10 nM, and MeαMan was 5 mM. Values are ($OD_{405} - OD_{490}$) × 10^3. Percent inhibition was calculated as {1 − [bio-Con A, 5 mM MeαMan signal − pretest signal)/(bio-Con A signal − pretest signal)]} × 100 and is given in parentheses.
*Average of four wells that did not incubate a pin.

and tested for their ability to inhibit the precipitation of Con A by dextran (Fig. 1). The peptide MYWYPY inhibited precipitation with an IC_{50} of 490 μM, whereas the IC_{50} values of MeαMan and MeαGlc were 120 μM and 480 μM, respectively. The inhibitory strength of the control peptide MAWAPA was about half that of the parent sequence, indicating that tyrosine side chains contribute to binding. As expected, the group B peptide VGRAFS produced weaker inhibition that was similar to that of the control peptide MAWAPA.

The peptide VGRAFS was also tested for its ability to displace the chromagenic ligand NphαMan from the sugar-binding site of Con A. (Because of relative insolubility and insufficient quantity, the other peptides were not tested.) The plots of NphαMan displacement vs. competitor concentration are linear for VGRAFS and MeαMan (Fig. 2), indicating that both bind Con A competitively (11). The data produced K_d values of 80 μM for MeαMan and 1.62 mM for the peptide; these values are in rough agreement with the IC_{50} values calculated from the precipitation inhibition assays. By comparison, a K_d of 800 μM was estimated for the binding of MYWYPY to Con A.

Specificity of Phage Bearing Con A-Binding Epitopes. In micropanning experiments in which phage were panned on a panel of closely related MeαMan-binding lectins, including Con A, *L. culinaris* agglutinin and *P. sativum* agglutinin (13), the highest yields by far were obtained when phage bearing the WYPY motif were panned on Con A; a significant yield was also seen when phage bearing MYWYPY were panned on *P. sativum* agglutinin (Table 4). When immobilized phage were tested for binding to the above lectins by ELISA, again the strongest binding was seen with Con A, and weaker, but detectable binding was obtained for all of the peptides with pea lectin, but not with lentil lectin (Table 4). When *G. simplicifolia* I lectin and *S. japonica* agglutinin were tested by micropanning assays and ELISA, these lectins, which do not bind MeαMan, showed no binding to the phage (data not shown).

DISCUSSION

This work demonstrates that two very different molecules, carbohydrate and peptide, are recognized by Con A with similar affinities and that, most likely, the ligand peptides described herein competitively inhibit Con A–MeαMan interaction by binding at or near the sugar-binding site. MYWYPY, the most promising peptide isolated from the hexapeptide epitope library, binds Con A with a K_d of about 800 μM, equivalent to that of MeαGlc, yet, surprisingly, is highly selective, as it is able to distinguish between closely related lectins that bind MeαMan.

Recent crystallographic studies of the structure of Con A in complex with MeαMan show that the sugar is bound in the C1 chair conformation via a network of seven hydrogen bonds that link oxygen atoms O-3, O-4, O-5, and O-6 of the sugar to residues Asn-14, Leu-99, Tyr-100, Asp-208, and Arg-228, as well as by hydrophobic interactions between C-5 and C-6 of the sugar and the ring atoms of Tyr-12 and Tyr-100 (14). It is tempting to speculate that epitopes bearing the WYPY motif bind as a result of both hydrogen bonding with the hydroxyl groups of tyrosine residues (which mimic sugar oxygens) and hydrophobic interactions with carbons on the aromatic side chain (which mimic sugar carbons). In support of this, replacement of tyrosine in the consensus sequence with alanine resulted in an ≈50% reduction in inhibitory strength (Fig. 1). Furthermore, all of the group B sequences have at least one residue with a hydroxylated side chain (Y, T, or S), and all but one have at least one aromatic residue. In keeping with this, the group B peptide we tested,

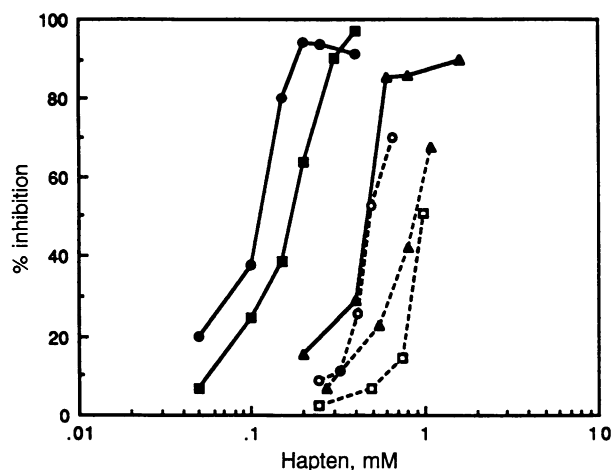

FIG. 1. Inhibition of the precipitation reaction between Con A and dextran B-1355-S by MeαMan (●), aqueous MeαMan (■), MeαGlc (▲), and the synthetic peptides MYWYPY (○), MAWAPA (□), and VGRAFS (△). IC_{50} values are as follows: MeαMan, 0.12 mM; aqueous MeαMan, 0.18 mM; MeαGlc, 0.48 mM; MYWYPY, 0.49 mM; MAWAPA, 0.98 mM; and VGRAFS, 0.9 mM. Vials contained 2% dioxane except for a control experiment done in the absence of dioxane (aqueous MeαMan).

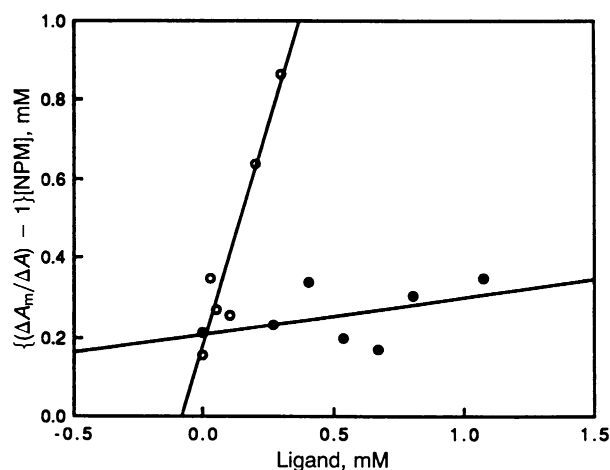

FIG. 2. Displacement of NphαMan from Con A by competing ligands (○, MeαMan; ●, peptide VGRAFS). ΔA_m is the change in absorbance corresponding to complete saturation of Con A with NphαMan, ΔA the change in absorbance for every addition of the competing ligand, and [NPM] the concentration of free NphαMan. Concentration of total NphαMan was 200 μM and that of Con A was 20 μM, corresponding to 40 μM combining sites. Dissociation constants, K_d, determined from this plot for MeαMan and VGRAFS are 0.08 mM and 1.62 mM, respectively.

Table 4. Binding of biotinylated lectins to affinity-purified phage

Epitope sequence	MeαMan	Micropanning with immobilized lectin*				ELISA with immobilized phage†		
		bio-Con A	bio-LCA‡	bio-PSA	Buffer	bio-Con A	bio-LCA	bio-PSA
MYWYPY	−	1733	2.0	60	0.7	982	23	50
	+	73	0.7	0.7	1.3	71	18	19
LYWYPY	−	600	3.0	1.0	1.0	845	20	52
	+	55	0.5	0.3	1.0	80	17	17
VGRAFS	−	1.4	1.2	2.0	0.2	574	19	42
	+	0.8	2.0	1.6	2.0	40	19	18
RRHHHH	−	2.3	3.0	0.3	1.0	840	27	68
	+	2.0	1.0	0.8	1.0	50	17	17
DFLEKI	−	<1.0	>1.0	2.5	<1.0	—	—	—
	+	<1.0	1.0	<1.0	1.0	—	—	—
f1 phage	−	—	—	—	—	352	21	27
	+	—	—	—	—	94	19	18
Fetuin	−	—	—	—	—	1561	562	435
	+	—	—	—	—	53	86	24

Biotinylated lectins included *L. culinaris* agglutinin (bio-LCA) and *P. sativum* agglutinin (bio-PSA).
*Micropanning data are relative yields and are calculated as percent yield obtained from micropanning phage on lectin-coated wells divided by the average percent yield from two wells that were treated with buffer alone.
†ELISA data are the average ($OD_{405} - OD_{490} \times 10^3$) from two wells.

VGRAFS, bound less tightly than MYWYPY but bound specifically to Con A (Figs. 1 and 2; Table 2).

Why do not other, closely related MeαMan-binding lectins recognize Con A-binding peptides? In general, the structure of the sugar-binding sites of these lectins is highly conserved, however, the sugar-contact residues Arg-228, Leu-99 and Tyr-100 of Con A are replaced by Gly-99, Ala-210 and Glu-211 in favin, pea, and lentil lectins (15). These differences may be involved in the observed binding specificity for the ligand peptides that Con A selected from the epitope library. However, the binding of peptide to Con A probably involves more than the residues known to contact MeαMan, as the consensus sequence is four residues long; these as yet undefined contacts may be responsible for the selective binding to Con A. In support of this, phenyl α-D-mannopyranoside is known to bind Con A, favin, pea, and lentil lectins with higher affinity than MeαMan, indicating that there are other conserved residues in the sugar-binding site that are available for hydrophobic interaction (13, 16). It should be possible to determine which residues contribute to binding by studying the crystallographic structure of peptide in complex with Con A (14).

The approach described here may be applied to the discovery of ligand peptides that specifically block the binding of biomedically significant animal lectins to their biological targets (e.g., the vascular E-selectin, ELAM-1, which is thought to target neutrophils and T cells to areas of inflammation; ref. 17). Although, in general, peptides themselves make poor drug candidates, they may serve as lead compounds, providing chemical information that would be used to develop pharmacologically active agents (18); selectivity of binding is a key characteristic of good lead compounds. Our results are particularly encouraging for the use of epitope libraries in identifying lead compounds, as the ligand peptides described here have high selectivity, albeit like their sugar counterparts, moderate affinity.

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