Structural and Functional Consequences of Peptide-Carbohydrate Mimicry

CRystal Structure of a CARBOHYDRATE-Mimicking Peptide Bound to CONCANAVALIN A*

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The functional consequences of peptide-carbohydrate mimicry were analyzed on the basis of the crystal structure of concanavalin A (ConA) in complex with a carbohydrate-mimicking peptide, DVFYPYPYASGS. The peptide binds to the non-crystallographically related monomers of two independent dimers of ConA in two different modes, in slightly different conformations, demonstrating structural adaptability in ConA-peptide recognition. In one mode, the peptide has maximum interactions with ConA, and in the other, it shows relatively fewer contacts within this site but significant contacts with the symmetry-related subunit. Neither of the peptide binding sites overlaps with the structurally characterized mannose and trimannose binding sites on ConA. Despite this, the functional mimicry between the peptide and carbohydrate ligands was evident. The peptide-inhibited ConA induced T cell proliferation in a dose-dependent manner. The effect of the designed analogs of the peptide on ConA-induced T cell proliferation and their recognition by the antibody response against α-D-mannopyranoside indicate a role for aromatic residues in functional mimicry. Although the functional mimicry was observed between the peptide and carbohydrate moieties, the crystal structure of the ConA-peptide complex revealed that the two peptide binding sites are independent of the methyl α-D-mannopyranoside binding site.

Molecular recognition mediates purposeful communication in living processes in a predesigned manner. Since specificity of recognition is defined by the structural properties common to all molecular processes, it is possible to encounter systemic dysfunction due to unrelated molecules sharing common structural features. Autoimmune diseases are a manifestation of such an aberration of normal function. Since many microbial agents share common determinants with host proteins, the immune response mounted by the host against a specific determinant of a microbial agent may cross-react with the mimicking host determinant, leading to autoimmunity (1, 2). On the other hand, functional mimicry also occurs by design as an effective control in several regulatory mechanisms that include transcription and cellular signaling (3, 4). Molecular mimicry has implications in rational drug design as well. Design and development of nonpeptidyl drugs, mimicking therapeutically important bioactive peptides and proteins, provide increased selectivity, improved bioavailability, and prolonged activity (5, 6). Therefore, understanding the structural principles governing molecular mimicry could be important in its successful exploitation.

In an attempt to systematically delineate the structural relationship between two chemically dissimilar but functionally equivalent molecular structures, we had earlier compared the antibody responses and the topological features of a peptide and a carbohydrate moiety, which recognize a common receptor, ConA* (7). ConA is a lectin specific for binding to mannose-containing carbohydrates on the cell surface (8). It is also a lymphocyte mitogen and a useful reagent for differentiating normal from malignant cells (9, 10). Peptide ligands of ConA were identified by screening a large and diverse peptide library expressed on the surface of a filamentous phage (11, 12). The peptide, DVFYPYPYASGS (12-mer), was shown to bind to ConA with an affinity comparable with that of the methyl α-D-mannopyranoside (11). We have earlier shown that the murine pAbs raised against this peptide bind to α-D-mannopyranoside, and similarly, the pAbs raised against the sugar react with 12-mer (7). It was apparent that the ConA binding activities of various analogs correlate well with the topological relationship between the peptide and the carbohydrate ligands. Here we report the crystal structure of a carbohydrate-mimicking peptide bound to ConA and analyze its functional implications.

EXPERIMENTAL PROCEDURES

Peptide Synthesis and Purification—The peptides were synthesized on an automated peptide synthesizer, as described earlier (7), using solid phase 9-fluorenylmethyl oxycarbonyl chemistry. The crude peptides were purified by reverse phase high pressure liquid chromatography (Waters) using water, acetonitrile, 0.1% trifluoroacetic acid as eluant. The peptides were characterized for their purity by molecular mass determination using a single quadruple mass analyzer (Fisons Instruments).

Crystallization and Data Collection—ConA (Sigma) at a concentration of 0.32 mg/ml was co-crystallized with a 20-fold excess of peptide using the vapor diffusion method with 1 mM ammonium sulfate in 50 mM Tris buffer (pH 9.0). The x-ray intensity data were collected on an image plate detector (Marresearch, Germany) installed on a rotating anode x-ray source (Rigaku, Japan) operated at 40 kV and 70 mA (CuKα radiation). The crystal-to-detector distance was 295 mm, and 0.25° oscillation frames were recorded. The diffraction data were collected from two different crystals, processed separately using the DENZO (13) suite of programs, and subsequently merged using SCALEPACK. The data collection and refinement statistics are given in Table I. The crystallization of ConA in complex with methyl α-D-mannopyranoside was reported in this space group earlier, although atomic resolution structural analysis was not carried out due to disorder in the crystals (14).

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1 The abbreviations used are: ConA, concanavalin A; pAb, polyclonal antibody.
**Structure Determination and Refinement**—Molecular replacement calculations were carried out using the program AMoRe (15). The dimer of the tetrameric ConA in complex with methyl α-D-mannopyranoside (16, 17) was used as a model for rotation/translation function calculations between 10- and 4-Å resolution. The initial model was subjected to rigid body refinement in X-PLOR (18), first treating dimer as a rigid body and then each monomer independently. This was followed by several cycles of positional refinement. Higher resolution data, up to 2.75 Å, were added in a stepwise fashion. Both conventional R-factor (R_{cryst}) and the free R-value (R_{free}) were used to monitor the progress of refinement. The electron density maps were calculated using the CCP4 package (19) and were displayed with the help of O (20) on INDIGO (Silicon Graphics Inc.). Iterative rebuilding of the models was based on 2F_{o} – F_{c} as well as F_{c} – F_{o} maps alternatively. Non-crystallographic symmetry restraints with a weight of 120 kCal mol⁻¹ Å⁻² were used throughout the refinement. Water molecules were included in the model using 3o in F_{c} – F_{o}, and 1o in 2F_{o} – F_{c}, electron density cut-off if they made reasonable hydrogen bonding contacts.

**Table I**

| Parameter                  | Value |
|---------------------------|-------|
| Ligand                    | DFYPYYPYASGS |
| Cell constants (Å)        | a = 102.5; b = 118.3; c = 253.6 |
| Space group               | C222,1 |
| Maximum resolution (Å)    | 2.75 |
| Completeness (%)          | 86.4 |
| No. of observed reflections| 47255 |
| No. of independent reflections | 34787 |
| Multiplicity              | 6.0 |
| Average (I)/(S|SigI) | 67.5 |
| Completeness in last shell| (2.75–3.07 Å) (%) |
| R_{cryst} (%)             | 6.7 |
| No. of solvent atoms      | 289 |
| r.m.s. deviation bond length (Å) | 0.009 |
| r.m.s. deviation bond angles (°) | 1.632 |
| R_{cryst} (%)             | 19.2 |
| R_{free} (%) (10% of the data) | 26.5 |

* r.m.s., root mean square.

All experiments were performed in triplicate, and results are presented as counts/min as a function of peptide concentration.

**Immunization and Antibody Binding Assays**—The p-aminophenyl α-D-mannopyranoside (Sigma) and 12-mer were conjugated to bovine serum albumin/diptheria toxoid using the glutaraldehyde method, as described previously (7). The sugar-bovine serum albumin conjugate was used for raising the polyclonal antisera in female BALB/c mice. Antibody binding was assayed by enzyme-linked immunosorbent assay. The 12-mer-diptheria toxoid was adsorbed on 96-well Maxisorp Nunc plates at a concentration of 2 µg/well in 50 mM sodium carbonate buffer, pH 9.6, overnight at 4 °C. Wells with adsorbed antigens were blocked with 1% gelatin in PBS before adding serial dilutions of anti-sugar pAbs. Horseradish peroxidase-labeled goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc.) was used as the secondary antibody. The peroxidase substrate o-phenylenediamine was added finally to detect binding of the pAb. For the competitive assay, constant amounts of anti-sugar pAbs were incubated with equal volumes of increasing concentrations of peptides. The level of inhibition was calculated by comparison of the absorbance (at 490 nm) from the wells reacted with the antibody in the presence of the soluble competitor and those reacted with the antibody in the absence of the competitor.

**RESULTS**

**Overall Structure**—The asymmetric unit of ConA-peptide complex crystals contains two dimers with each monomer comprising of 237 residues of ConA, two metal ions, Mn^{2+} and Ca^{2+}, and one 12-mer peptide. The two dimers, defined by monomers A and B and monomers C and D respectively, are positioned such that the ConA tetramers ABA’B’ and CDC’D’ are generated by the two independent crystallographic 2-fold axes. A’B’ is a dimer related to AB by a crystallographic 2-fold axis, and C’D’ is a dimer related to CD by another independent crystallographic 2-fold axis. The structures of the two independent dimers AB and CD are almost exactly identical. However, the two subunits within each of the dimers, namely A and B (or C and D), have certain regions significantly different from each other. Particularly, the loop incorporating residues 200–206 has different conformation in the two subunits. In addition, residues 116–123, which comprise a loop between the adjacent β-strands, are poorly defined in the electron density map of both the subunits. The root mean square deviation between the two subunits for all of the backbone atoms is 0.36 Å, and that for the loop 200–206 is 2.5 Å.

**Peptide Conformation and Its Interaction with ConA**—All of the residues of the peptide molecule could be traced in the electron density map. A representative F_{o} – F_{c} map of the 12-mer peptide bound to ConA is shown in Fig. 1. The peptide binds to ConA in two different modes. The overall conformation is somewhat similar in these modes with differences primarily in the side chain orientations. Superimposition of the two con-
formations gives a root mean square deviation of 2.27 Å for the C-α trace, although their conformational energies are comparable. The main chain conformation of the peptide in both of the modes is extended with a hairpin formed by residues Tyr⁶, Pro⁷, and Tyr⁸, resulting in the amino and the carboxyl termini coming in close proximity. All of the four peptide molecules associated with the four subunits of ConA were found to be within the energetically favored regions of the Ramachandran plot. A hydrogen bond stabilizing the loop conformation is found in the peptide bound to the A (and D) subunit between the terminal carboxyl group of the peptide and the Tyr⁴-OH.

In the P1 mode, the peptide interacts with subunit A (and D) in a shallow crevice, and it is largely exposed to the solvent from one side (Fig. 2A). The peptide shows extensive interactions with ConA and binds to residues Tyr²², Pro²³, Lys³⁸–Asp⁴⁴, Lys⁴⁶, Lys²⁰⁰, Ser²⁰¹, and Ser²⁰⁴–Pro²⁰⁶. We shall refer to this as the primary peptide binding site. In this mode, the only contacts of the 12-mer peptide with the symmetry-related molecule are through Pro⁶ and Tyr⁸. Distinct hydrogen bonds observed between the peptide and ConA are listed in Table II. On the other hand, in P2 mode, the peptide is sandwiched between two crystallographically related ConA subunits. Here, the peptide binds to subunit B (and C) in a site roughly corresponding to the primary binding site of the subunit A (Fig. 2B). The residues of ConA in contact with the peptide in this mode are Thr¹¹, Pro¹³, Met¹⁶–Lys⁴⁶, Lys²⁰⁰, Ser²⁰¹, and Pro²⁰⁶. The peptide in P2 mode exhibits significant contacts with the crystallographic equivalent of subunit A, defining the secondary peptide binding site in that subunit. The protein residues involved in defining this site are Thr¹¹, Thr¹⁵, Ser²¹, Pro²³, Lys³⁹, His²⁰⁵, and Pro²⁰⁶. Thus, the P2 binding mode and the peptide conformation appear to be influenced by the symmetry contacts. There are five hydrogen bonds between the peptide and the protein, out of which two are with the symmetry-related subunit (Table II).

Functional Consequences of Peptide-Carbohydrate Mimicry—While exploring the structural basis of peptide-carbohydrate mimicry, it is pertinent to address its implications vis-à-vis the biological function. ConA is a known mitogenic stimulant of T lymphocytes (9). The binding of ConA to the T cell surface glycoproteins through carbohydrate moieties is implicated in this function. It was observed that the normal proliferative effect of ConA is inhibited in a dose-dependent manner in the presence of 12-mer (Fig. 3A). Inhibition of cell
proliferation could be observed at a 31 μM concentration of 12-mer, and any further increase in the concentration resulted in a steady decline in proliferation. At a peptide concentration of about 100 μM, 50% inhibition of the ConA-induced T-cell proliferation is achieved, which is comparable with its dissociation constant \((K_d)\). Thus, the inhibition of T cell proliferation correlates well with the affinity of the native peptide toward ConA. Also, the control experiment carried out by adding varying concentrations of the 12-mer in the absence of ConA to splenic cells showed no effect on the inhibition. This implies that the interaction of ConA with the cell surface mannone-rich oligosaccharides was indeed blocked by this peptide. T cell proliferation was also analyzed in the presence of various analogs of the 12-mer where Tyr\(^6\) of the YPY motif, a consensus sequence among the ConA binding peptides, was replaced by another aromatic residue, Phe (DVFPFPYASGS) and a non-aromatic polar residue Ser (DVFPFPYASGS). As seen in Fig. 3A, the inhibition of proliferation is differentially affected by these analogs with Phe-substituted analog showing relatively higher inhibitory effect than the Ser-substituted analog.

Substitutions at Tyr also affect the carbohydrate mimicking property of the peptide. Analogs of the 10-mer peptide, MYWYPYASGS, which showed better cross-reactivity with the anti-α-d-mannopyranoside pAbs (7) in comparison with the 12-mer, were synthesized by substituting Tyr\(^4\) with Phe (MYWFPYASGS) and Ser (MYWSPYASGS) and analyzed for their carbohydrate-mimicking activity. Binding activities of the Tyr\(^4\)-substituted analogs with anti-α-d-mannopyranoside pAbs were analyzed by enzyme-linked immunosorbent assay (Fig. 3B). Substitution analysis showed that replacement of Tyr\(^4\) by Phe had no effect, whereas Ser substitution at the same position decreases its cross-reactivity with anti-α-d-mannopyranoside pAbs. Similar results were also obtained in the case of the analogs where Tyr\(^6\) was changed to Phe and Ser, respectively.

**DISCUSSION**

Functional mimicry involving apparently unrelated molecules may not necessarily imply a structural relationship. Indeed, molecules with no obvious shape similarity have been shown to induce the same signal (6, 21). However, in several instances shared surface features at the functional site have been suggested to be critical in eliciting mimicry (22). Structural similarities leading to functional mimicry have important consequences in immune recognition. We had earlier shown that the antibody responses against a carbohydrate moiety could distinguish structural features within the carbohydrate-mimicking peptide (7). It was therefore anticipated that the crystal structure of the ConA-12-mer complex would provide finer details of the structural relationship between the carbohydrate and the peptide ligands.

The backbone conformation of the peptide-bound form of ConA closely resembles that of ConA in its native form as well as in the form complexed with various carbohydrate ligands (23–25), the only exception being for the loop incorporating residues 200–206. The conformation of this loop in the case of the B subunit is similar to that in native ConA. However, this is not the case for the A subunit. This loop has been reported to be poorly defined in trimannose complex (24) but was well ordered in the peptide-ConA complex. In addition, significant changes in side chain conformations in the binding site occur due to ConA-peptide interaction. It has been observed that the interactions of ConA with different ligands influence crystal packing, since the ligand participates in stabilization of the

| Peptide | ConA | Distance | A |
|---------|------|----------|---|
| P1 conformation | | | |
| Asp\(^1\) OD2 | Lys\(^{200}\) NZ | 2.8 | |
| Val\(^2\) O | Lys\(^{26}\) NZ | 3.3 | |
| Tyr\(^4\) N | Ser\(^{204}\) O | 3.4 | |
| Tyr\(^6\) OH | Pro\(^{26}\) N | 3.4 | |
| Ser\(^{22}\) O | Glu\(^{3}\) NE2 | 3.3 | |
| Pro\(^{23}\) O | (Lys\(^{26}\)) NZ | 2.6 | |
| Tyr\(^6\) OH | (Asn\(^{21}\)) OD1 | 3.0 | |

**TABLE II**

**Residues in parentheses correspond to the symmetry-related molecule.**

**Fig. 3.** Functional significance of ConA-peptide binding, A, effect of 12-mer and its analogs on ConA-induced T cell proliferation. The control contains only peptide. The counts/min value for cells in the absence of ConA alone is 9 × 10\(^4\). B, inhibition of anti-α-d-mannopyranoside-bovine serum albumin pAbs binding to solid phase 12-mer diptheria toxoid by soluble 10-mer and its analogs in a competitive enzyme-linked immunosorbent assay.

**Fig. 4.** Stereoview of the surface representation of the A subunit of ConA showing the bound peptide in the primary peptide binding site (PPBS) and the symmetry-related peptide in the secondary peptide binding site (SPBS). Comparison of the two sites has been made with respect to the trisaccharide binding site (TSBS) on ConA. The peptide molecules at the primary site (orange) and at the secondary site (pink) and trimannose (blue) are shown as stick drawings.
crystal contacts (24–26). Molecular packing in the crystals of the ConA-peptide complex is such that the crystallographically characterized mannose/trimannose binding sites are not accessible to the peptide in any of the four subunits. A primary peptide binding site could be defined independent of the sugar binding site in all four subunits. On the other hand, a secondary peptide binding site is generated as a result of molecular packing in only two of the four subunits.

The 12-mer peptide interacts with ConA in two different ways. The contacts of the peptide that binds only to the primary binding site are different from those of the peptide that binds both at the primary and the secondary binding sites. An interesting feature of the structure is that the peptide shows conformational adaptability while binding at the primary binding site in the two different modes, although crystal packing facilitates this variation. The secondary peptide binding site, which is generated by the interaction of symmetry-related molecule, may also be of physiological consequence. It is located between the mannose binding site and the primary peptide binding site (Fig. 4). The interactions in this case are dominantly hydrophobic, involving aromatic residues in addition to hydrogen bonds. However, the peptide binding sites do not correspond to the hydrophobic sites earlier identified in ConA (27, 28). There are stacking interactions between the aromatic residues of the peptide and the protein. The two different modes of peptide-ConA interaction observed here conform with the multiple ways of oligosaccharide binding to ConA (26).

The functional mimicry between the sugar and the peptide that was earlier suggested (7, 11, 12) has been further established. It has been known that ConA induces mitogenicity by the mannose moieties of the complex carbohydrates of cell surfaces (29, 30). The 12-mer inhibits ConA-induced T-cell proliferation, implying that the peptide could share the binding sites of mannose moieties of the complex carbohydrates of cell surface in solution. Moreover, as suggested earlier (7, 11, 12), the YPY region of the peptide appears to be critical for ConA binding. The substitution of Tyr by Phe in YPY motif shows marginal effect on the inhibition of ConA-induced T cell proliferation; on the other hand, a Ser substitution shows significant loss of inhibition. Also, we had shown that the pAbs raised against α-D-mannopyranoside cross-react with the peptide and that the anti-peptide pAbs cross-react with the sugar (7). The effect of designed analogs of the carbohydrate-mimicking peptide on anti-sugar pAb cross-reactivity also implies that the YPY region of the peptide is indeed sensitive to the antibody cross-reactivity. The substitution of Tyr by Phe in the YPY motif shows no effect on cross-reactivity with anti-sugar pAbs. On the other hand, a Ser substitution shows significant loss in the activity. Thus, the aromatic residues contribute to the inhibition of ConA-induced T cell proliferation as well as the carbohydrate mimicry as observed in terms of the immune response. The aromatic nature of residues involved in carbohydrate-peptide mimicry, observed here, is not entirely unprecedented. There are other reports where aromatic amino acids contribute toward the functional mimicry through carbohydrate resemblance (31–33).

The comparison of peptide and sugar complexes of the lectin does not indicate any overlap between the two ligands. However, the peptide binding sites are in close proximity to the mannose and trimannose binding sites (Fig. 4). Only the recently reported structure of an extended pentasaccharide bound to ConA (34) shows some overlap of the terminal sugar of the pentasaccharide with the peptide binding site. The possibility that the peptide also binds at the monosaccharide binding site in solution cannot be completely excluded. However, crystal packing might have prevented this mode of peptide binding. The comparison of the peptide-ConA and sugar-ConA interactions highlights the independent nature of binding sites of the two ligands, although the solution studies imply the existence of the functional mimicry between the carbohydrate and peptide moieties. It was hoped that the crystal structure of ConA bound to peptide would shed light on the structural basis for the peptide carbohydrate mimicry. However, at least for the time being, it remains a mystery.

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