Models of binding of 4′-nitrophenyl α-D-mannopyranoside to the lectin concanavalin A

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Molecular models for the complex formed between the lectin concanavalin A (Con A) and the saccharide derivative 4′-nitrophenyl-α-D-mannopyranoside (α-PNM) are presented, combining evidence from ^{1}H-n.m.r. measurements, semi-empirical energy calculations and interactive graphics modelling. The models are in good agreement with the experimental data. Close examination of the models suggests that hydrophobic interactions together with van der Waals interactions and hydrogen bonds contribute to the stability of the complexes. It appears that there is a limited number of possible modes of binding of α-PNM to Con A.

Keywords: Lectin; concanavalin A; saccharide–lectin interaction; model of binding; ^{1}H-n.m.r.; energy calculation; interactive graphics modelling

Introduction

Concanavalin A (Con A) is a representative member of the lectin class of plant proteins[1,2]. Con A has specific biological activities, which depend on its binding to cell surface receptors. This binding can be inhibited by derivatives of glucose and mannose[3,4].

Con A agglutinates cells transformed by oncogenic viruses[5], inhibits growth of malignant cells in animals[6] and exhibits mitogenic activity as a result of its sugar-binding properties[7]. It has also been used in studies about the number and mobility of cell-surface receptors associated with cell–cell interactions[8]. Therefore, it is important to find the modes of binding of carbohydrates to this protein.

At pH values greater than 6, Con A exists as a tetramer[9]. Each subunit consists of 237 residues and contains two metal sites: one (S_1) that binds transition metal ions, and another (S_2) that binds preferentially calcium ions. Evidence suggests that both sites must be occupied for saccharide binding activity to occur[10].

Derivatives of Con A in which Zn^{2+}, Co^{2+}, or Mn^{2+} ions occupy the S_1 site, possess equal abilities to bind sugars. The three-dimensional structure of Con A has been elucidated by X-ray crystallographic studies at 1.75 Å resolution (Ref. 11 and references therein), with the S_1 site occupied by a manganese ion. The main structural feature of the protein is a β-sandwich, formed by two antiparallel β-pleated sheets.

Despite extensive efforts, it has not been possible to solve the structure of Con A–carbohydrate complexes and to determine near atomic resolution the modes of protein–sugar interaction (Ref. 11 and references therein). However, information has been gathered about the nature, the position and the properties of the sugar binding site and possible modes of sugar binding, from a variety of crystallographic, n.m.r. and theoretical studies[11–17].

Most studies agree that the sugar binding site is at a distance of approximately 1.2 nm from the manganese ion. Nevertheless, there are still ambiguities concerning the exact orientation of the bound saccharides in the active site of the protein[15–17].

In this work, we present models of binding of 4′-nitrophenyl-α-D-mannopyranoside to Con A, combining evidence from ^{1}H-n.m.r. measurements, semi-empirical energy calculations and interactive graphics modelling.

Materials and methods

N.m.r. methods

Native Con A and 4′-nitrophenyl-α-D-mannopyranoside (α-PNM) were obtained from Sigma Ltd.

Zn-Con A and Mn-Con A were prepared by demetallizing the native protein under acid conditions and replacing the (S_1) metal by Zn and Mn respectively[19]. The metal content was determined by atomic absorption and e.s.r. spectroscopy after denaturing the metalloprotein. Prior to use, the metalloprotein was enriched in fragment-free molecules[20].

The samples were prepared in a deuterated phosphate buffer 0.1 N, pH 5.6 and ionic strength 1, adjusted with KCl. Concentrations were 9×10^{-5} M for Con A and 2.7×10^{-3} M for the mannoside. Proton spin-lattice relaxation times were measured by the inversion-recovery method on a Varian XL-100 FT spectrometer. The temperature of the probe was 24°C (297 K). The

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intensities of the peaks were subjected to a non-linear least squares fitting.

Energy calculations

Energy calculations and energy minimization were performed by a Fortran 77 computer program, developed in the Astbury Department of Biophysics, University of Leeds, UK, which was modified for use in the CYBER-170/730 CDC computer of the University of Athens. The program was kindly provided by Dr A. J. Geddes. A Lennard-Jones 6-12 potential was employed for attractive and repulsive non-bonded interactions[21] and the hydrogen bond potential of Balasubramanian and Seetharamulu[22] was used. Electrostatic interactions were not taken into account.

Models for α-PNM were calculated with our own Fortran 77 computer program (Hamodrakas, unpublished), which constructs any molecule of known geometry, using the precise data of Brown and Levy[23], for the sugar ring, which was taken to have the expected $^4C_1$ chair conformation, and assuming standard geometry for the remainder of the molecule.

Modelling

The coordinates of the atoms of Con A were obtained (entry 3CNA) from the Protein Data Bank[24].

Modelling was performed by utilizing the interactive computer graphics facilities of the European Molecular Biology Laboratory, Heidelberg. An Evans and Sutherland Multipicture System was used, with colour and black and white displays, 256 kilowords of extended memory, and various input and output devices. The system is hosted by a Digital Equipment Corp. VAX-11/785 computer. The interactive molecular modelling program EMBLFrodo, a descendant of the popular Frodo by T. A. Jones, was employed.

The program Midas of UCSF was also used to test the feasibility of the proposed models on the PS-350 Evans and Sutherland Multipicture System of EMBL Heidelberg.

For the construction of our molecular models we have also used a Basic computer program, written for a Cannon AS100 microcomputer, with 256 kbytes of memory and screen resolution of 640 × 400 pixels, which constructs and manipulates space filling or skeletal models of micro- and macromolecules (Athanasiadis and Hamodrakas, unpublished).

Physical models were built with Pauling and Corey CPK and Kendrew skeletal kits.

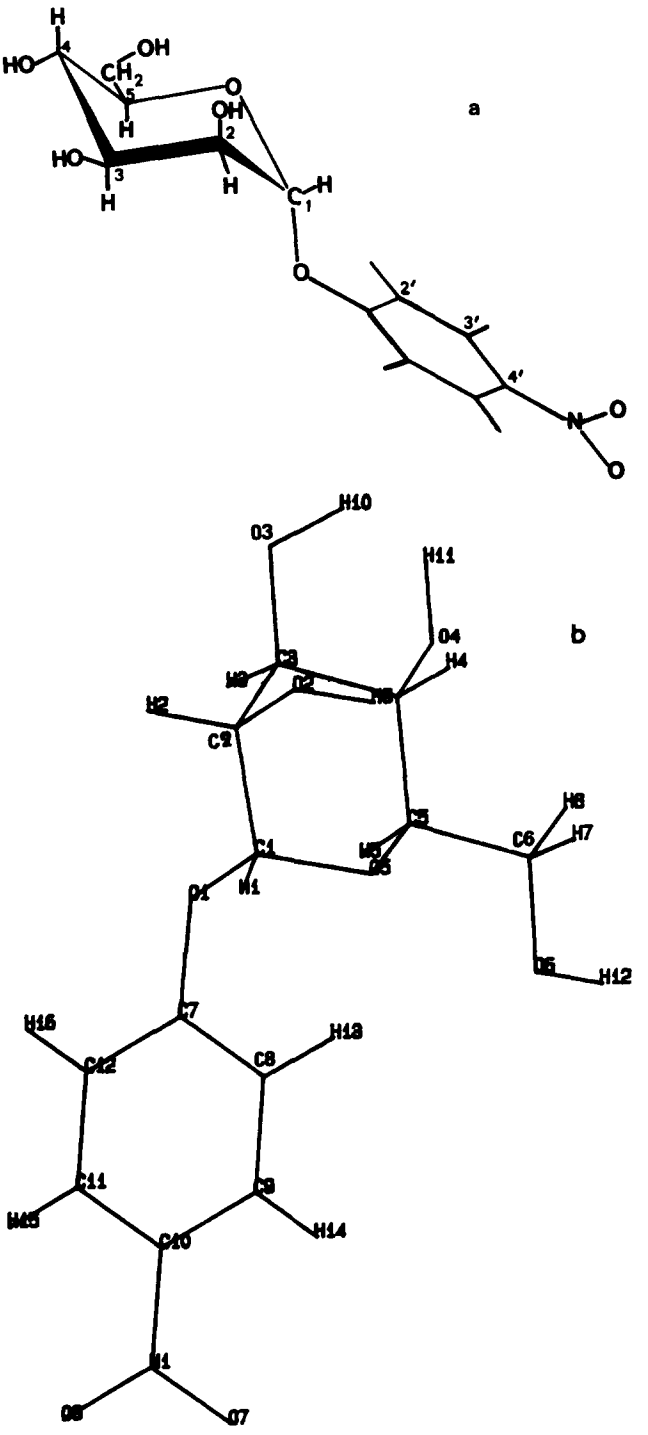

Figure 1 (a) Chemical formula of 4′-nitrophenyl-α-D-mannopyranoside (α-PNM). (b) Atomic numbering scheme of α-PNM

Results

Atomic numbering scheme

The chemical formula of 4′-nitrophenyl-α-D-mannopyranoside (α-PNM) and the numbering scheme used are shown in *Figures 1a* and *1b* respectively. For Con A the numbering scheme of the Protein Data Bank entry 3CNA was used[24].

^{1}H-n.m.r. results

The distances obtained from ^{1}H-n.m.r. experiments are given in *Table 1*. They were calculated from the paramagnetic contribution of the Mn^{++} ion on the α-PNM protons. The Solomon–Bloembergen equation was used, after neglecting the scalar interaction and the terms in $\omega_s\tau_c$ (Ref. 18). The paramagnetic contribution was calculated in each case, by subtracting the observed relaxation rate in the Zn–ConA–mannoside from that in the Mn–ConA–mannoside complex. The relaxation rate of the free mannoside was also taken into consideration.

Table 1 Mean distances $\bar{r}$ from Mn^{2+} of 4′-nitrophenyl-α-D-mannopyranoside protons in its complex with Mn–Con A

Proton	δ_{ppm}	$\bar{r}$ (nm)
2′	8.545	1.384
3′	7.545	1.509

The temperature dependence of the mannoside spectrum showed that at 24°C fast exchange conditions prevail. The value of the correlation time used, $\tau_c = 8 \times 10^{-8}$ s, is consistent with ^{13}C n.O.e. and chemical exchange rates[18].

Stable conformations of α-PNM

Possible stable conformations for α-PNM, corresponding to potential energy minima, were calculated utilizing semi-empirical energy calculations and energy minimization algorithms as described in Methods. A Lennard–Jones 6–12 potential was used to describe non-bonded interactions[21]. Three possible conformations were found, shown in *Table 2*, differing slightly in energy, numbered (and referred to hereafter) I, II and III.

They may represent possible structures of the mannoside when binds to Con A.

Models of binding of α-PNM to Con A

Assuming that α-PNM binds to Con A having adopted one of its stable conformations, it would appear that there is a variety of possible ways of binding. However, this is not true. If the molecule adopts conformations II or III (see *Table 2*), it is very difficult to find favourable modes of binding of these two conformers to Con A, even after modifying considerably the side chain conformations of the binding site residues of Con A: this check was made by the interactive graphics system. The difficulty is mainly due to steric hindrance of the aglycone part of the molecule with the protein. It also appears that the conformation of Leu 99 of Con A would have to change considerably, which might affect drastically the integrity of the binding site.

We have therefore looked for possible modes of binding of conformer I (*Table 2*) with the protein. This search, performed by the interactive graphics system, was guided by three criteria:

(a) to find the best hydrophobic contacts between the molecules and the protein,

(b) to find the maximum number of hydrogen bonds between α-PNM and Con A, and,

(c) to modify as few as possible side chain conformations of the binding site residues (the main chain torsion angles were not varied).

At the same time, care was taken to fix the confirmation of the residues participating in the hydrogen bonding system of the water molecules, which are ligands to the Ca^{2+} and Mn^{2+} ions (e.g. Asn 14). Also, the conformation of Leu 99 and Gly 98 was not changed.

Certain modes of binding of conformer I fulfil these requirements. The first (model 1) is shown in *Figure 2*. Several H-bonds can be formed between the hydroxyl groups of a α-PNM and specific atoms of the binding site:

O6 (α-PNM)–OD1 (Asp 208)
O5 (α-PNM)–NE (Arg 228)
O2 (α-PNM)–NH2 (Arg 228)
O3 (α-PNM)–OH (Tyr 12)
O4 (α-PNM)–OH (Tyr 100)

The hydrophobic groups of H3 and H5 of the pyranose ring are directed towards Leu 99 in an orientation that favours hydrophobic interactions. In addition, several favourable interactions occur between the aglycone part of the molecule and the protein. We note, in particular, a

Table 2 Possible stable conformations of α-PNM, corresponding to potential energy minima, as a function of the torsion angles ϕ (O5-C1-O1-C7) and ψ (C1-O1-C7-C8)[a] (there are three possible conformations designated as I, II and III)

Conformation	ϕ (O5-C1-O1-C7)°	ψ (C1-O1-C7-C8)°	Energy (kcal/mol)
I	69.1	−76.6	−3.7
II	39.6	−127.8	−3.8
III	−174.5	−68.3	−3.4

[a] The recommendations proposed by the Joint Commission on Biochemical Nomenclature (Ref. 31) are used to describe the glycosidic torsion angle ϕ

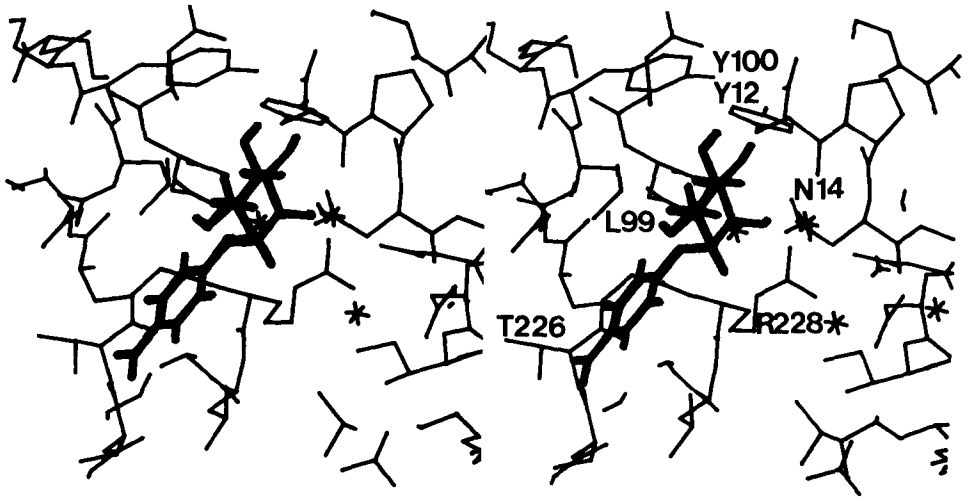

Figure 2 Stereo-pair model of Con A – 4′-nitrophenyl-α-D-mannopyranoside complex in one of the probable modes of binding (Model 1, see also *Table 3a*). Some binding site residues are indicated by the one-letter code

possible hydrogen bond between O7 of the NO_2 group with OG1 of Thr 226.

Conformational changes were necessary for four binding site residues Thr 226, Arg 228, Tyr 12, Tyr 100.

In this model the α-PNM H2′, H3′ distances from Mn^{2+} are, $D(H2'-Mn^{2+})=1.439$ nm, $D(H3'-Mn^{2+})=1.485$ nm in excellent agreement with the distances measured by ^{1}H-n.m.r.

Table 3a shows the x,y,z coordinates of the atoms of α-PNM in this binding mode, in the coordinate system of the protein. It also includes a list of the torsion angles of the residues which were modified.

Another favourable mode of binding of conformer I is shown in *Figure 3*. Possible H-bonds between the hydroxyl groups of α-PNM and specific atoms of the protein in this case are:

O1 (α-PNM)–NE (Arg 228)
O2 (α-PNM)–N (Leu 99)
O3 (α-PNM)–OD2 (Asp 208)
O4 (α-PNM)–ND2 (Asn 14)
O6 (α-PNM)–OH (Tyr 12)

In this model (model 2) the hydrophobic groups H3 and H5 of the pyranose ring of α-PNM are oriented towards Arg 228, whereas the O5 atom of the ring is located close to Leu 99 and is not involved in any H-bond.

Table 3a Atomic coordinates (nm) of 4′-nitrophenyl-α-D-mannopyranoside (α-PNM) in the coordinate system of Con A (Model 1)

Atom	X	Y	Z
C3	2.3497	2.5585	2.7448
C4	2.2541	2.4646	2.6736
C5	2.3259	2.3843	2.5653
C6	2.2300	2.2966	2.4883
O2	2.3277	2.7298	2.5800
O3	2.2791	2.6442	2.8327
O4	2.1956	2.3781	2.7707
O5	2.3864	2.4752	2.4734
O6	2.3009	2.2077	2.4045
H1	2.5190	2.6243	2.4510
H2	2.4985	2.7099	2.6896
H3	2.4229	2.4984	2.8020
H4	2.1755	2.5252	2.6255
H5	2.4037	2.3208	2.6112
H6	2.1684	2.2404	2.5592
H7	2.1634	2.3603	2.4289
H9	2.2576	2.6794	2.5363
H10	2.1838	2.6249	2.8339
H11	2.1480	2.4344	2.8338
H12	2.2468	2.1287	2.3930
C2	2.4229	2.6451	2.6427
C1	2.4855	2.5590	2.5322
O1	2.5923	2.4830	2.5858
C7	2.6819	2.4130	2.4999
C8	2.6419	2.2943	2.4400
C9	2.7289	2.2262	2.3556
C10	2.8559	2.2770	2.3311
C11	2.8960	2.3959	2.3910
C12	2.8089	2.4639	2.4755
H13	2.5504	2.2576	2.4576
H14	2.7000	2.1407	2.3125
H15	2.9873	2.4325	2.3735
H16	2.8377	2.5494	2.5186
N1	2.9467	2.2060	2.2431
O7	2.9092	2.0949	2.1870
O8	3.0655	2.2536	2.2202

A list of the side-chain torsion angles of the binding-site residues which were modified is also included. Angles marked with an asterisk were not changed from crystallographically determined values

Residue	x_1	x_2	x_3	x_4
Tyr 12	−166.0	139.8		
Tyr 100	−42.6	1.0		
Thr 226	9.7			
Arg 228	*	*	71.9	−119.8

Table 3b Atomic coordinates (nm) of 4′-nitrophenyl-α-D-mannopyranoside (α-PNM) in the coordinate system of Con A (Model 2)

Atom	X	Y	Z
C3	2.2147	2.2924	2.3953
C4	2.1780	2.2941	2.5426
C5	2.2655	2.3923	2.6202
C6	2.2353	2.3886	2.7682
O2	2.3920	2.1356	2.4261
O3	2.1451	2.1892	2.3278
O4	2.0401	2.3287	2.5540
O5	2.4023	2.3561	2.6020
O6	2.1199	2.4648	2.7969
H1	2.5502	2.3294	2.4688
H2	2.3965	2.2719	2.2745
H3	2.1886	2.3903	2.3509
H4	2.1945	2.1930	2.5835
H5	2.2490	2.4948	2.5827
H6	2.2203	2.2846	2.7989
H7	2.3217	2.4278	2.8232
H9	2.4224	2.1354	2.5179
H10	2.0845	2.1412	2.3869
H11	2.0277	2.4119	2.5054
H12	2.0872	2.4373	2.8833
C2	2.3642	2.2669	2.3796
C1	2.4461	2.3633	2.4665
O1	2.4341	2.4950	2.4148
C7	2.5195	2.5988	2.4634
C8	2.4950	2.6566	2.5873
C9	2.5780	2.7576	2.6346
C10	2.6856	2.8009	2.5579
C11	2.7101	2.7430	2.4338
C12	2.6270	2.6420	2.3866
H13	2.4176	2.6256	2.6426
H14	2.5603	2.7992	2.7238
H15	2.7874	2.7741	2.3786
H16	2.6447	2.6005	2.2974
N1	2.7721	2.9062	2.6071
O7	2.7491	2.9603	2.7231
O8	2.8727	2.9466	2.5353

A list of the side-chain torsion angles of the binding-site residues which were modified is also included. Angles marked with an asterisk were not changed from crystallographically determined values

Residue	x_1	x_2	x_3	x_4
Tyr 12	−146.4	120.8		
Tyr 100	−74.0	−8.1		
Asp 208	−82.6	140.8		
Thr 226	9.7			
Arg 228	*	*	71.9	−119.8

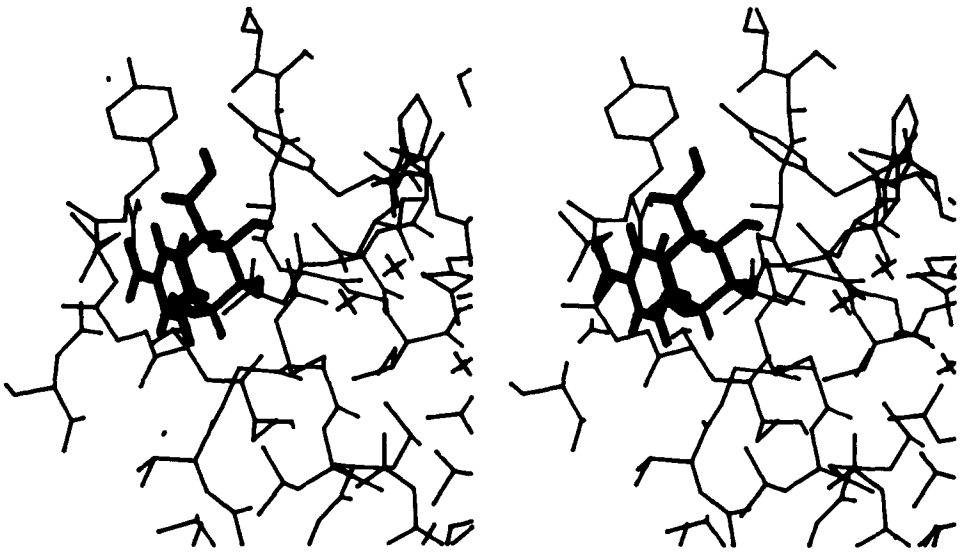

Figure 3 Stereo-pair model of Con A – 4′-nitrophenyl-α-D-mannopyranoside complex in another probable mode of binding (Model 2, see also *Table 3b*)

In order to accommodate the saccharide part of the molecule into the binding site, the conformation of some binding site residues was modified. These residues are Tyr 12, Tyr 100, Asp 208, Thr 226 and Arg 228.

In this model the α-PNM H2′, H3′ distances from Mn^{2+} are: $D(H2' - Mn^{2+}) = 1.316$ nm, $D(H3' - Mn^{2+}) = 1.424$ nm also in good agreement with the distances measured by n.m.r.

However, an important observation in this case, is that the aglycone group is not in contact with the outer surface of the protein. This minimizes the number of its energetically favourable interactions with the protein.

Table 3b shows the coordinates of the atoms of α-PNM in this case, in the coordinate system of the protein.

Another attractive model (called thereafter model 2′) would result by an approximate rotation of 180° of the second model about an axis joining the atoms C2, C5 of the pyranose ring. Nevertheless, the resulting structure suffers from the same disadvantage as model 2: the aglycone part of the molecule does not interact favourably with the protein. In this case, however, in contrast to the second model, the hydrophobic 'face' of the pyranose ring, containing hydrogens H3 and H5, interacts favourably with Leu 99 in an analogous way as in model 1.

Every other docking attempt led to stereochemically disallowed structures.

Discussion

It should be emphasized that our models of binding of α-PNM to Con A satisfy best contact criteria and constraints imposed from n.m.r. measurements, but they are intended as a first approximation only to the docking of the molecule on the protein surface.

In our modelling studies, it was apparent to us (and also to other workers, Refs 15–17, 25–27) that in order to 'dock' satisfactorily the saccharide part of the molecule into the binding site, the conformation of some of its binding-site amino acid residue side chains has to be modified.

One of the basic postulates of our models (and also of other workers, Refs 15–17, 25–27) is that the salt bridge between Arg 228 and Asp 16 must be broken to accommodate saccharides in the site, in agreement with experimental observations (Ref. 15 and references therein). Another prediction of the models is that the side chains of Tyr 12 and Tyr 100 must reorient. This probably causes the perturbations in the u.v. and c.d. spectra of Con A observed on the binding of sugars[28–30].

The interactions of the saccharide part of α-PNM with the protein via hydrogen bonds is in fair agreement with the 'Goldstein rules'[3]. In our models, the hydroxyls at C2, C3, C4 and C6 are all involved in hydrogen bonds.

From our modelling work and energetic considerations we conclude that the first model (*Figure 2*) is clearly more satisfactory than the second (*Figure 3*) and its variant (model 2′) described above. Perhaps, it is interesting to note that, a structure closely resembling model 2 has been proposed as a possible model of binding of D-mannose derivatives to Con A[17].

The theoretical principles of our search have also been employed by other workers in studies of carbohydrate binding to Con A[15–17,25–27]. However, our attempts were directed primarily to maximize favourable hydrophobic interactions of α-PNM with the residues comprising the binding site of Con A and then to look for possible hydrogen bonding schemes and favourable van der Waals contacts: it is obvious that the binding site of Con A has a profound hydrophobic character towards Leu 99 and Gly 98, whereas it is clearly hydrophilic towards Asn 14, Arg 228, Tyr 12, Tyr 100 and Asp 208.

Our conclusion was that the 'face' of the pyranose ring containing the hydrophobic hydrogens H3 and H5 has greater probability of being directed towards the hydrophobic 'surface' of the binding site, than its 'face' containing the hydrophilic hydroxyls 2, 3 and 4. The reason for considering model 1 as more attractive than model 2′ is that in the latter case the aglycone part of the molecule is directed away from the protein surface, which, consequently, results to fewer favourable interactions of more than half of the molecule with the protein.

It is hoped that current X-ray crystallographic studies of saccharide – Con A complexes[32] will help to resolve the ambiguities concerning the details of carbohydrate binding to this protein.

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