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## The Influence of Sequence Variability and Dimerization on Mannose Binding in Monocot Mannose Binding Lectins<sup>#</sup>

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### Abstract

**Motivation.** A model of the lectin from *Aloe arborescens* was built by homology modeling. Docking studies with mannose were performed on this model and the known crystal structures of monocot mannose binding lectins from snowdrop and garlic. On the basis of these results association of monomers to form dimers is found to be necessary for successful binding of mannose by site III of these lectins, by providing the fourth strand of the  $\beta$ -sheet that is a supporting edge for the site. From an analysis of the carbohydrate binding sites (I, II and III) of the above lectins and the docking studies, the mannose binding site I of aloe lectin is predicted to retain the ability to bind mannose with all of the key residues involved in binding unchanged. Site II and III lose residues specific for hydrogen bonding and are predicted to be unable to bind mannose. Aloe lectin monomers are shown to be able to associate as dimers but docking is still unsuccessful in site III.

**Method.** Protein homology modeling and AutoDock docking studies were used in this study.

**Results.** A homology model of aloe lectin was created by both manual and automatic methods and its ability to bind the natural substrate mannose was assessed by docking studies using the genetic algorithm approach in the AutoDock program. The results of the docking studies were correlated with those on lectins for which X-ray crystal data is known and rationalized in terms of specific mutations in the aloe lectin binding sites.

**Conclusions.** Aloe lectin is predicted to be able to bind mannose in its site I binding site, unable to bind in site II because of key residue mutations and also unable to bind in site III.

**Keywords.** Homology modeling; lectin; docking; mannose; binding sites.

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## 1 INTRODUCTION

Lectins are proteins that selectively and reversibly bind carbohydrates [1]. Lectins are found in most organisms as a facilitator of host biological recognition processes such as host pathogen interactions, fertilization, lymphocyte homing and for the purposes of combating parasites and herbivores. They are proteins that are capable of recognizing complex arrays of carbohydrates and

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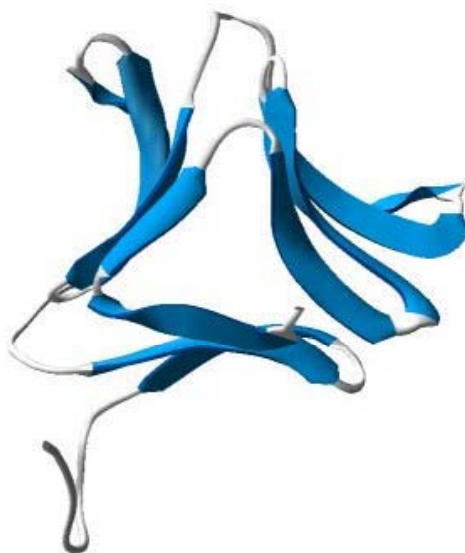
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are currently being used extensively as biological research tools utilizing this ability to bind to cell surface carbohydrates [1,2]. Specifically, monocot mannose binding (MMB) lectins have been shown to bind to carbohydrates on the surfaces of retroviruses such as HIV [3,4].

There are two categories of carbohydrate-binding proteins. Group I have deep binding pockets, which move to close off the bound molecule completely from the environment outside the protein. Group II proteins have shallow binding sites and, as a consequence, lower binding affinities. All lectins fall into the category of group II carbohydrate binding proteins [5,6]. At no time does the binding site move to enclose the substrate, nor does the site change much more than 1 Å upon binding. Moreover, there is no evidence to suggest distortion of the geometry of the sugar ring during binding as is observed in other carbohydrate binding systems [7]. As a result of these characteristics, extended binding sites can exist, utilizing van der Waals forces and aromatic stacking to improve selectivity for oligosaccharides [2,7]. Despite their highly polar nature, carbohydrates have non-polar patches which can be utilized by the protein to provide extra binding/selectivity by packing a non polar area against a hydrophobic residue.

Affinity for various carbohydrates has been noted to increase with the formation of oligomers, which gather together many binding sites [9]. A MMB lectin monomer consists of three bundles of four anti parallel  $\beta$ -sheets joined by loops to form a twelve-stranded  $\beta$ -barrel in the shape of a triangular prism (Figure 1).



**Figure 1.** Schematic of an MMB lectin structure showing sites I, II and III.

These lectin monomers associate as dimers or tetramers with varying degrees of homology between the subunits [9], gathering together as many as 12 binding sites. This clustering of subunits enables these proteins to bind to cell surfaces for their role within the plant, which is to selectively

bind large, relatively planar, groups of carbohydrates. The binding sites of MMB lectins consist of an Asn residue which forms a hydrogen bond with the axial 2–OH of the mannose acting as a hydrogen bond acceptor. Asp and Gln are polar planar residues, which form contacts with the 2–OH and 3–OH respectively as hydrogen bond donors. A Tyr residue is an OH containing residue hydrogen bonding with the 4–OH of the mannose. A hydrophobic interaction between a Val and the C3 and C4 of the mannose is also thought to be important [2,9,10]. The primary structures of lectins show significant sequence identity and homology within each lectin group. It is thus possible within these groups of proteins to predict the secondary structure of a sequence for which there is no available three–dimensional model and to build a model by homology modeling [2]. Here we look at the Monocot Mannose Binding (MMB) family of lectins and build a homology model of aloe lectin in order to assess its potential ability to bind mannose. All of the crystal structures available in the Brookhaven Protein Data Bank (PDB) for these proteins have three highly conserved mannose binding sites. There are no such structures that have the residues instrumental in binding altered, however models of such lectins have been studied [9,11] and binding is not considered to be possible. Alterations in two of the three binding sites are apparent from the primary sequence of aloe lectin. A modeling study of this lectin and its family was undertaken to understand the modes of binding of these proteins to polysaccharides on the surfaces of cells and viruses in plants. These results may also eventually be useful to identify the potential of such lectins for various therapeutic purposes, such as the molecular recognition of viruses in other systems.

## 2 MATERIALS AND METHODS

Homology modeling was performed manually using InsightII (Biosym Technologies, San Diego), run on a Silicon Graphics Iris Indigo XZ4000 workstation. Protein sequences were aligned using ClustalW, the Internet service at the European Bioinformatics Institute <http://www.ebi.ac.uk/clustalw> [12]. Protein structures were viewed, manipulated and superimposed using the Builder and Biopolymer module of InsightII and also with Deep View, also known as Swiss PDB Viewer from Glaxo–Wellcome in Switzerland [13]. SWISSMODEL, the artificial intelligence based server at (<http://www.expasy.ch>) [13,14,15] was also used to create homology models. The Discover module of InsightII was used for potential energy minimizations. The force field used for all of the calculations undertaken using InsightII was AMBER [16]. Molecular Dynamics simulations were carried out using AMBER5 [17] on the supercomputer facilities (Columbus) at the Rutherford Appleton Laboratory, Oxford, UK. Docking was carried out using the AutoDock suite of programs [18] using the Lamarckian genetic algorithm with default settings. AutoDock uses a linear free energy approach to calculate the free energies of binding, including a solvation model. The final docked energies quoted = Final Intermolecular Energy + Final Internal Energy of the ligand. The program Autodock Tools [19] was used to prepare the ligand for docking.

## 2.1 Homology Model

The lectin from *Galanthus Nivalis* (1jpc.pdb [5]), with a resolution of 2.0Å, was used as the template to model the aloe lectin monomer (sequence retrieved from the SwissProt database, lec\_aloar [20]). In total, 43 residues were replaced and one inserted using the library of allowed conformations available with InsightII. The aligned sequences have 109 residues each, including a gap in 1jpc, giving a sequence identity of 60% (Table 1).

**Table 1.** Sequence alignment of aloe lectin with snowdrop, daffodil and garlic lectin. Name of plant followed by PDB code and chain identifier where necessary or sequence identifier. ■ = residues to be replaced, ■ = residue to be inserted, Pink type = residues involved in binding. Green type = sequence additionally required for constructing binding site.

	1	10	20	30	40	50
Snowdrop (1jpc)	DNILYSGETLSTGEFLNYGSFV	FIMQEDCNLVLVYD	VDKPIWATNTGGLSR			
Aloe (lec_aloar)	DNILYSSEVLHENQYISYGPYEFIMQEDCNLVLVYESGNPTWASNTGGLAL					
Daffodil (1npl)	DNILYSGETLSPGEFLNNGRYV	FIMQEDCNLVLVYD	VDKPIWATNTGGLDR			
Garlic (1bwu_a)	RNILRNDEGLYGGQSLDVN	PHYHFIMQEDCNLVLVYD	HSTSVWASNTGILDK			
Garlic (1bwu_b)	RNILTNDDEGLYGGQSLDVN	PHYHLMQEDCNLVLVYD	HSTAVWSNSTDIPDK			
	60	70	80	90	100	
S.	SCFLS-MQTDGNLNVVYNPSNKP	IWASNTG-GQNGNYVCILQKDR	NVVIYGTDRWATGTHT-			
A.	HCRAT-LQTDGNLNVVONSANRTI	IWQSNTGTGTNGDYLLVLQKNG	NVVIYGPPIWATGTGRG			
D.	RCHLS-MQSDGNLNVVYSPRNNPI	IWASNTG-GENGNYVCVLQKDR	NVVIYGTARWATGTNIH			
G (a)	KGCRAVLQSDGNFVVYDAEGRSL	WASHSVRG-NGNYVLVLQEDGNVVI	YRSDIWSTN----			
G (b)	KGCKAVLQSDGNFVVYDAEGASL	WASHSVRG-NGNYVLVLQEDGNVVI	YRSDIWSTNTYR-			

Studies of lectins have been published with models built from sequence identities between 40% to 50% [11,21,22]. Owing to the ease of automated homology modeling the aloe sequence was also submitted to the SWISSMODEL server (www.expasy.ch) to produce a model of both the monomer and the dimer. The X-ray structure of the lectin from *Galanthus Nivalis* (1msa) was used as a template for the automatic homology model [23]. We have called the resulting monomer models, aloe and swiss-aloe respectively. In summary, the monomer was modeled by both manual and automatic methods to provide a comparison of the models produced and the dimer by the automatic method only.

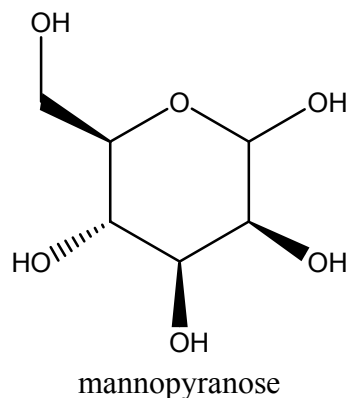
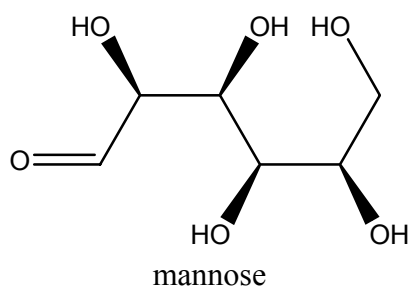
## 2.2 Minimization

In order to assess the binding sites of the monomers the entire lectin structures were first compared before continuing to look in more detail at the comparison of the binding sites. Four MMB lectin structures were chosen from the Protein Data Bank for comparison with the two aloe models. These four were chosen because their sequence homology with aloe lectin is greater than 50% and their resolution is below 3 Å. All six lectin structures were energy minimized: *in vacuo* using the AMBER force field in DISCOVER. No solvent or constraints were used in these minimizations. The models that were energy minimized were the two aloe models, 1bwua.pdb [24] (from Garlic) and 1msa.pdb (chain A only), 1jpc.pdb and 1npl.pdb (Daffodil, *Narcissus*

*Pseudonarcissus*) [25]. All six were treated the same in order that artifacts of crystal packing forces be removed from the crystal structures and thus meaningful comparisons could be made with the two aloe models. Initially the steepest descents method was used. After 100 iterations the energy was below 100kcal/mol and further minimization using the conjugate gradients method was carried out. The convergence criterion was set at 0.001kcal/mol for the root mean square deviation (rmsd) of the energy. The final energies of the models were around –1000kcal/mol. The models and all four crystal structures were analyzed using Procheck [26]. The resulting Ramachandran plots indicated that the minimized structures and the models both contain few residues in disallowed regions. The X-ray structures and models were superimposed using the DALI server at the European Bioinformatics Institute (www.ebi.ac.uk). The RMSD values are given for all aligned residues (Table 2).

**Table 2.** Root mean squared deviation (rmsd) and % identity of all lectin models and all four minimized x-ray structures together with the resolution of the crystal structures used.

Resolution Å	Model	%ID						
		Aloe	Swiss aloe	jpc	msa	bwu	daff	
–	R	aloemin		100%	60%	60%	52%	57%
–	M	swissaloemin	0.2		60%	60%	52%	57%
2.00	S	jpcmin	0.3	0.3		100%	51%	86%
2.80	D	msamin	0.2	0.2	0.3		51%	86%
2.29	(Å)	bwumin	0.5	0.5	0.2	0.3		50%
2.00		daffmin	0.3	0.3	1.0	0.3	0.3	



**Figure 2.** Structure of the mannose ligand used in the docking simulations.

## 2.3 Molecular Dynamics

The aloe dimer was equilibrated for a period of 25ps after initial heating (in 10 ps steps/50K up to the production temperature of 300K) and then subject to a 250ps constant pressure molecular dynamics run at 300K, in a solvated water box at 1bar. The resulting structure has an rmsd from the original (using the above criteria for superimposition) of 0.38Å. From these simulations it is likely that aloe lectin will retain the overall fold of a MMB lectin and associate to form dimers.

## 2.4 Chemical Data

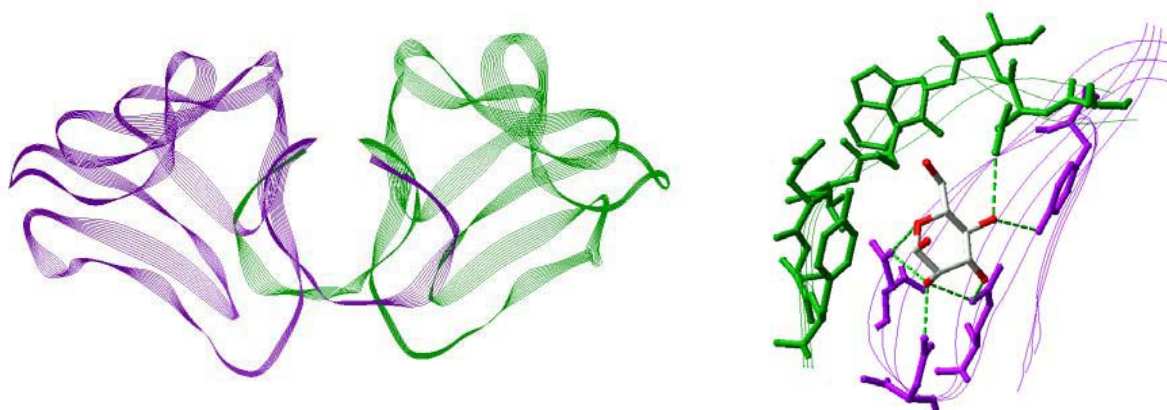
Structures of the mannose ligand used in this study in both open chain and pyranose form. The pyranose form was used exclusively for the docking studies (Figure 2).

# 3 RESULTS AND DISCUSSION

## 3.1 The Binding sites

In each of the three binding sites of the crystal structures of 1jpc, 1bwu, 1msa and 1npl monomers, the binding site residues Asn, Asp, Gln and Tyr which form hydrogen bonds to the ligand, and are considered to be prerequisite for binding, are identical. There is also a conserved Val in all three binding sites which forms a hydrophobic interaction. Mannose ligands are seen in the binding sites of the x-ray structures.

Some of these ligands are oligosaccharides but hydrogen bonds occur to only the first mannose, the rings of the other mannoses becoming involved in binding only when dimers associate to form tetramers. The appearance of 1npl and 1jpc in the PDB as monomers with ligands present in all three binding sites may be misleading as the crystals from which they are derived contained tetramers [2,25].



**Figure 3.** AutoDock docking validation to the X-ray structure of garlic lectin. The structure of the garlic dimer is shown on the left hand side and the best docked position of mannose in binding site III is shown on the right hand side. Monomer 1 is shown in purple with the four key residues for binding displayed on the right. Monomer 2 is shown in green.

### 3.2 Docking

Firstly AutoDock was validated for this type of system by performing dockings to each of the binding sites of the minimized garlic lectin monomer with methyl- $\alpha$ -D-mannose that is found in the crystal structure 1bwu (Figure 3).

Gasteiger charges [28] were assigned to the ligand using the AutoDock Tools package and the mannose was placed in the binding site in the same position as in the crystal structure. AutoDock uses a solvation potential which requires the addition of solvation parameters to the starting pdb file. This file termed a pdbqs file was produced with AutoDock Tools. AutoDock was used to produce a final docked conformation within a fixed protein structure. This proved successful for sites I and II after only 10 runs of conformational space search. A docking was considered to be successful when the ligand was seen to be in a position to form hydrogen bonds with all four of the residues directly involved with mannose binding in MMB lectins (Table 3); essentially the same position as seen in the crystal structure.

**Table 3.** The above are the lowest Final Docked Energy positions from 10 docked positions calculated by Autodock. The Final Docked Energy = Final Intermolecular Energy + Final Internal Energy of Ligand; these energies are of a similar magnitude for each docking performed here. The four key residues are not always observed to be involved in binding.

Mannose group	Bwu-min (residue:Å)	Aloe-min (residue:Å)	jpc-min (residue:Å)
O2	H Asn30: 2.01	H Asn30: 2.02	H Asn30: 1.86
O2H	O Gln26: 2.05	O Gln26: 2.18	O Gln26: 1.95
	O Asn28: 2.66	–	O Asn28: 2.73
O3	H Gln26: 2.23	H Gln26: 2.04	H Gln26: 2.33
O3H	O Gln26: 1.85	O Gln26: 2.16	H Tyr30: 1.72
O4	–	–	–
O4H	O Tyr34: 1.83	O Tyr34: 2.04	–
O6	H Ser39: 2.48	H Ser39: 2.08	–
O6H	–	O Asn44: 1.76	O Asn44: 2.04
O7	–	–	–
Final Docked Energy kcal/mol	–4.03	–4.79	–3.68

The lowest energy structure was used for analysis. The distance between Asp28 and 2-OH of mannose is observed to be slightly greater than might be considered usual for a hydrogen bond at around 2.7 Å, however, in the crystal structures this interaction is also seen to be longer than those with other residues. From an analysis of the models, the crystal structures and the docking studies examined here, the hydrophobic interaction with the conserved Val seems to be from C3–C6 of the mannose. This hydrophobic patch on the mannose is crucial for the recognition of mannose in MMB lectins, as it is in other mannose binding lectins [6]. Docking of mannose was unsuccessful to Site III of 1bwu, consisting of Gln90, Asp92, Asn94 and Tyr98, even after several hundred runs of the simulations. This appears to be due to the importance of a sequence of the protein required to provide structure to the shallow binding pocket, similar to the role of loop D in leguminous lectins [7,28,29]. In site I and II this sequence occurs directly after the sequence containing the site and folds back to form the fourth strand of the  $\beta$ -sheet. This sequence (Table 1) is not as highly

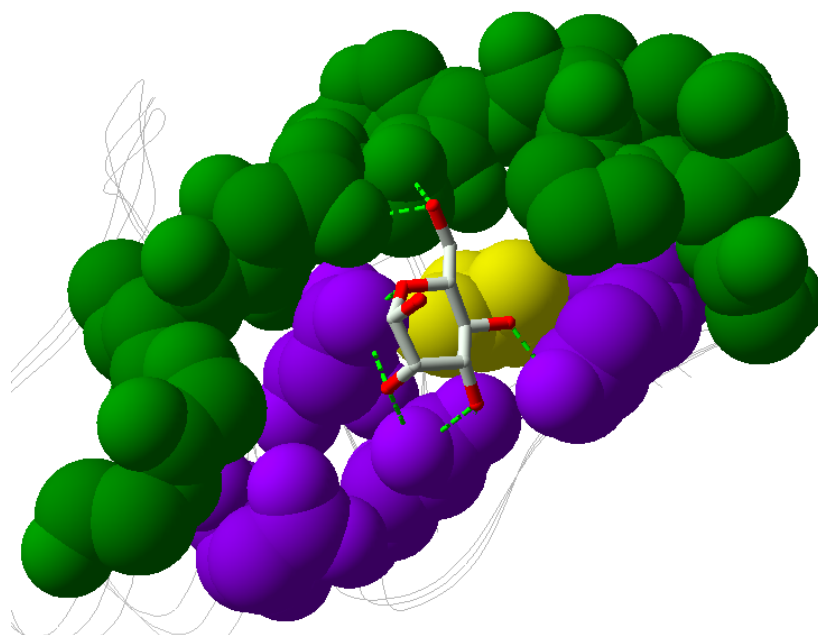
conserved throughout this family as the previously mentioned binding sites and does not have specific residues that are a prerequisite for binding, although a hydrogen bond with Asn44 is observed. There is an invariant Trp residue that is oriented towards the center of the monomer (away from the binding site) in all three complete sites. Site III is completed by the C-terminal tail of a second monomer when dimerization occurs, connected by hydrogen bonds and van der Waals contacts, which provides the fourth strand of the  $\beta$ -sheet and the third Tyr residue oriented into the center of the  $\beta$ -barrel.

The absence of binding capability in site III of MMB lectin monomers was further substantiated by attempting to dock mannose to *jpc*-min and *daff*-min, whose crystal structures appear in the PDB as monomers with ligands in all three sites. Both of these monomers failed to dock mannose to site III. The same ligand was successfully docked to site III of minimized garlic and snowdrop dimers from the *lbwu* and *lmsa* crystal structures from the PDB. From this it seems evident that this site is only active when stabilized in the dimeric form.

The mechanism of the association of monomers to form dimers has been examined previously [9], the increased affinity of oligomers for carbohydrates [19] and the effect of oligomerization on saccharide specificity [2] has also been noted. However, the necessity for the association of two monomers for binding to occur in site III has not previously been observed. It is hoped that experimental work would confirm this observation.

### 3.3 Aloe Docking

Docking was successful to site I of *aloe*-min (Figure 4). This site has no alterations to the main residues involved in binding and hydrogen bonds are observed in a similar pattern to that for the garlic docking above (Table 3), though there is not a bond between 2-OH and Asp28. In this model, Asp28 is orientated in such a way that it is not involved in binding at all and yet docking is still successful. Either three out of the four residues are sufficient for binding (indeed many of the docked conformations have hydrogen bonds to only three residues) or specifically Asp28 is not a requirement for mannose binding to occur. It is possible that an alteration in this residue that did not introduce steric, or other, interference would not necessarily render site I unable to bind mannose. Asp28 in Site I of *swiss*-*aloe*-min is closer to the snowdrop template and as such has hydrogen-bonding characteristics similar to *msa*-min. The orientation of Gln26 in *swiss*-*aloe*-min and *msa*-min is such that there is a rotation in the end of the side chain resulting in oxygen and nitrogen being transposed. This is not so for site I of *aloe*-min or the other structures looked at here, but this variation is observed in many of the sites and does not seem to affect the docking capability. The protein and the mannose will be in constant motion and it is likely that hydrogen bond contacts are continuously being broken and reformed. These two models offer an interesting look at two different possible conformations of the same protein, *i.e.* different local minima on the potential energy surface.



**Figure 4.** AutoDock result of mannose docked into aloe lectin binding site I. Key residues involved in binding are shown in purple. The key hydrophobic valine residue is shown in yellow and additional residues forming the edge of binding pocket are shown in green.

Site II was not found to dock mannose in either model during these simulations. The replacement of Tyr66 with Gln65, although retaining the possibility of a hydrogen bond, does not reach far enough into the binding area. The replacement of Ala75 with Gln74 within the supporting region of aloe–min sterically hinders the positioning of mannose in the binding area making docking impossible. Swiss–aloe has no such clash but docking is also unsuccessful; it would seem that the Tyr is a vital residue for binding. Site III also does not dock mannose in either model. The important residues for binding are altered quite considerably with a conservative replacement of Asp with Asn, similar in shape and retaining the ability to form a hydrogen bond and Tyr replaced with Val which is a large alteration in shape and size and removes a vital hydrogen bond donor. More importantly, docking cannot occur to the monomer as a consequence of the lack of a second monomer to provide the fourth strand of the  $\beta$ -sheet and the support for the back edge of the site, as was found to be the case with the garlic, snowdrop and daffodil monomers.

Docking to site III was unsuccessful in the swiss–aloe dimer also, the alterations in the key residues are predicted to be too severe for binding to occur.

## 4 CONCLUSIONS

Lectins are carbohydrate binding proteins that exhibit a high degree of sequence identity and homology within a family. Characteristic folds are observed for each family group and binding sites are often highly conserved; any alterations in key residues directly involved in binding often

resulting in loss of ability to interact with carbohydrates. All lectins show a propensity to form  $\beta$ -sheets as the predominant secondary structure motif and all associate to form dimers and tetramers. The necessity of this oligomerization for the mannose binding capability of binding site III of MMB lectins has been suggested here: we have seen that this site will not dock mannose in the monomeric form. There are few similarities in sequence between lectin families, but they have separately evolved means of achieving similar physiological roles. An example of this is association to form dimers and tetramers to facilitate the binding of large groups of cell surface carbohydrates. This is supported by the observation of higher mannose binding affinities of dimers and tetramers [19].

The aloe models constructed show that this lectin is likely to retain the overall fold of a MMB lectin and suggests that at least one of the three binding sites will remain active as it is identical to all others observed in this study. Docking of mannose to this site provides further evidence that this will be the case. The highly conserved site I binds mannose successfully but site II, with one alteration, although retaining the possibility of a hydrogen bond with the replaced residue does not bind mannose in the docking simulations. Site III of the aloe monomer has further changes that remove the possibility of a hydrogen bond from the fourth residue involved in binding and as such is less likely to retain any affinity for mannose. In addition the supporting edge of the binding site supplied by dimerization is missing and indeed mannose does not dock to this site of the monomer. Further modeling suggests that aloe lectin monomers are able to associate as dimers, as is the norm for the rest of this family, and that site III in the dimer is also incapable of binding mannose in the dimeric form. Literature also supports this as changes in key residues of the carbohydrate binding sites, especially non conservative ones, will render these sites inactive [9].

The affinity of this particular lectin for mannose will probably remain low, even as a dimer, as binding sites II and III are inactive. However, it is possible that aloe as a tetramer could bind branched mannopentoses utilizing site I, which has retained its ability to bind mannose. Site I is an integral part of the areas of association of dimers which contribute to extended binding sites that recognize  $\beta$ -1,3 sugar linkages, which is considered to be a biologically relevant mode of binding [30]. Aloe lectin has the potential to form tetramers and thus this area would be fruitful for further study. The structural comparisons of lectins combined with homology modeling and ligand docking studies is a powerful tool for understanding the mode of ligand binding in lectins and providing information about the behavior of as yet unelucidated structures.

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