
Quaternary association in β -prism I fold plant lectins: Insights from X-ray crystallography, modelling and molecular dynamics

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Dimeric banana lectin and calsepa, tetrameric artocarpin and octameric heltuba are mannose-specific β -prism I fold lectins of nearly the same tertiary structure. MD simulations on individual subunits and the oligomers provide insights into the changes in the structure brought about in the protomers on oligomerization, including swapping of the N-terminal stretch in one instance. The regions that undergo changes also tend to exhibit dynamic flexibility during MD simulations. The internal symmetries of individual oligomers are substantially retained during the calculations. Energy minimization and simulations were also carried out on models using all possible oligomers by employing the four different protomers. The unique dimerization pattern observed in calsepa could be traced to unique substitutions in a peptide stretch involved in dimerization. The impossibility of a specific mode of oligomerization involving a particular protomer is often expressed in terms of unacceptable steric contacts or dissociation of the oligomer during simulations. The calculations also led to a rationale for the observation of a heltuba tetramer in solution although the lectin exists as an octamer in the crystal, in addition to providing insights into relations among evolution, oligomerization and ligand binding.

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1. Introduction

The importance of quaternary association in the structure and function of proteins began to be recognized from the early days of protein structural studies, particularly because one of the first two proteins to be X-ray analysed was haemoglobin (Perutz 1970), a hetero-tetramer involved in oxygen transport. The basic principles of quaternary structure were first enunciated in the context of its role in allostery (Monod *et al.* 1965; Cornish-Bowden and Koshland 1971). It was subsequently realized that quaternary association is widespread in nature, even when allostery is not involved (Schulz and Schirmer 1979; Goodsell and Olson 2000; Levy *et al.* 2008). In fact, a vast majority of proteins with known structure are oligomers. Among oligomers, those with identical subunits (homo-oligomers)

form the majority. Almost all of them, with a few notable exceptions, possess point group symmetry (Steitz *et al.* 1976; Banerjee *et al.* 1994). The requirement of symmetry and indeed the possible biological rationale for the widespread occurrence of oligomers have been examined (Klotz *et al.* 1970; Goodsell and Olson 2000). The nature of protein–protein interfaces, including those involved in quaternary association, has also been extensively explored (Janin *et al.* 1988; Bahadur *et al.* 2003; Nooren and Thornton 2003; Ali and Imperiali 2005; Brinda and Vishveshwara 2005; Guharoy and Chakrabarti 2005).

The variability in the quaternary association of proteins with essentially the same tertiary structure is an aspect that has received some attention in recent years. Lectins, which are multivalent carbohydrate-binding proteins of non-immune origin that bind specifically to different carbohydrates, have

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received particular attention in this regard (Sharon and Lis 1989; Vijayan and Chandra 1999; Sharon 2007). The variability in the quaternary association of proteins with similar subunit structure was first seriously explored in legume lectins, particularly after the structural studies of peanut lectin (Banerjee *et al.* 1994; 1996). Subsequently, it was established that legume lectins are a family of proteins in which small alternations in essentially the same tertiary structure lead to large variations in the mode of oligomerization (Drickamer 1995; Loris *et al.* 1998; Prabu *et al.* 1998; 1999; Vijayan and Chandra 1999; Manoj and Suguna 2001; Kulkarni *et al.* 2004; Sinha *et al.* 2007). Such variations have since been observed in β -prism I fold and β -prism II fold lectins also (Hester *et al.* 1995; Chandra *et al.* 1999; Singh *et al.* 2005; Meagher *et al.* 2005). In fact, almost all known plant lectins have one or the other of five subunit folds (<http://www.cermav.cnrs.fr/lectines/>). Variability among them arises from differences in oligomerization and carbohydrate specificity (Bouckaert *et al.* 1999; Vijayan and Chandra 1999). A wealth of information on both these aspects has been provided by X-ray crystallographic studies (<http://www.cermav.cnrs.fr/lectines/>). Sugar specificity has been explored using modelling and molecular dynamics (MD) simulations as well (Bradbrook *et al.* 1998; 2000; Bryce *et al.* 2001; Pratap *et al.* 2001; Colombo *et al.* 2004; Sujatha *et al.* 2004; Di Lella *et al.* 2007; Konidala and Niemeyer 2007; Fujimoto *et al.* 2008; Mishra *et al.* 2008; Veluraja and Seethalakshmi 2008; Kaushik *et al.* 2009; Sharma *et al.* 2009; Sharma and Vijayan, 2011). The work on β -prism I fold lectins, presented here, probably represents the first attempt to explore the variability in the quaternary association in a lectin family using modelling as well as MD simulations based on crystallographic results.

Galactose-specific jacalin, one of the two lectins in jackfruit seeds, was the first lectin shown to have the β -prism I fold (Sankaranarayanan *et al.* 1996). The structure and carbohydrate specificity of the lectin was further elaborated through X-ray and MD simulations studies on its many ligand complexes (Jeyaprakash *et al.* 2002; 2003; Goel *et al.* 2004; Jeyaprakash *et al.* 2005; Sharma *et al.* 2009). Another tetrameric β -prism I fold galactose-specific lectin from *Maclura pomifera*, which, like jacalin, has a long (α) chain and a short (β) chain produced through post-translational proteolysis in each subunit, was subsequently studied (Lee *et al.* 1998). The second lectin from jackfruit seeds, artocarpin, also has a β -prism I fold and is tetrameric (Pratap *et al.* 2002). In the absence of post-translational modification, it has a single-chain subunit and the lectin is mannose specific. Subsequently another single-chain, mannose-specific, tetrameric lectin, namely MornigaM, was also X-ray-analysed (Rabijns *et al.* 2005). The other mannose-specific β -prism I fold plant lectins of known

structure also have a single-chain subunit. However, among them, heltuba (Bourne *et al.* 1999) is octameric and banana lectin (Singh *et al.* 2005; Meagher *et al.* 2005) and calsepa (Bourne *et al.* 2004) are dimeric. The modes of the dimerization in the last two are, however, entirely different. Thus, mannose-specific, single-chain β -prism I fold lectins exhibit a variety of quaternary structures although all of them have essentially the same tertiary structure. The present study, which combines computational approaches and results of X-ray analyses, provides valuable insights into the structural basis for the variability in their quaternary association and the changes in subunit structure arising from oligomerization.

2. Materials and methods

Coordinates of the crystal structures were downloaded from a locally maintained PDB-FTP anonymous server at the Bioinformatics Centre, Indian Institute of Science, Bangalore. Coordinates with PDB codes 1X1V, 1OUW 1J4T and 1C3M were used for banana lectin, calsepa, artocarpin and heltuba, respectively.

2.1 Energy minimizations and molecular dynamics simulations

Energy minimizations and MD simulations were performed using the GROMACS v3.3.1 package (Van Der Spoel *et al.* 2005) running on parallel processors with the OPLS-AA/L force field (Jorgense *et al.* 1996). Modelled oligomers were generated by superposing the lectin subunits of one protein on the subunits of other protein. The inter-subunit interfaces of modelled oligomers were carefully examined, and small adjustments, such as rotamer variation, were performed manually as and when required. For energy minimization of native oligomers, the crystallographic water molecules were removed from the protein models. The simulation box was generated using the editconf module of the GROMACS package with the criterion that the minimum distance between the solute and edge of the box was at least 7.5 Å. Subsequently, the protein models were solvated with the TIP4P water model using the program genbox available in the GROMACS suite. Sodium or chloride ions were added to neutralize the overall charge of the system wherever necessary. Energy minimizations were carried out using the conjugate gradient and steepest descent methods with a frequency of latter at 1 in 1000. A maximum force of 1 kJ mol⁻¹ nm⁻¹ was chosen as convergence criterion for minimization. On account of severe steric clashes in the inter-subunit regions, some of the modelled oligomers could not be energy minimized with these methods. Thus, they were first energy minimized in vacuum with the low-memory Broyden-Fletcher-Goldfarb-Shanno approach and

then were further treated using the same protocol as employed in the case of native oligomers. Energy minimizations were followed by solvent equilibration by position-restrained dynamics of 10 ps where positions of protein atoms were restrained and solvent was allowed to move.

MD simulations utilized the NPT ensembles with Parrinello-Rahman isotropic pressure coupling ($\tau_p=0.5$ ps) to 1 bar and Nosé-Hoover temperature coupling ($\tau_t=0.1$ ps) to 300 K. Long-range electrostatic interactions were computed using the Particle Mesh Ewald (Darden *et al.* 1993) method with a cut-off of 12 Å. A cut-off of 15 Å was used to compute long-range van der Waals interactions. Simulations were performed with full periodic boundary conditions. Bonds were constrained with the LINCS algorithm (Hess *et al.* 1997). The dielectric constant was taken as unity.

2.2 Analysis of structures

Analysis was performed with various tools available in the GROMACS suite and self-written programs and scripts. Figures were prepared using PyMOL (<http://www.pymol.org>). Graphs were prepared using Xmgrace (Paul J Turner, Center for Coastal and Land-Margin Research Oregon Graduate Institute of Science and Technology Beaverton, Oregon). Accessible surface areas were computed using NACCESS (<http://www.wolf.bms.umist.ac.uk/naccess/>). Programs Sc (Lawrence and Colman 1993) available in the CCP4 suite of programs (Collaborative Computational Project 1994) and ALIGN (Cohen 1997) were used to calculate shape complementarity values and superposition of structures, respectively. HBPLUS (McDonald and Thornton 1994) was used to calculate hydrogen bonds in the X-ray structures. A donor-acceptor distance less than or equal to 3.2 Å and donor-hydrogen-acceptor angle greater than or equal to 120° were used as criteria for delineating hydrogen bonds. Burial of 1 Å² or more surface area with a probe radius of 1.4 Å was used as a criterion to select interfacial residues. Structure alignment and structure-based sequence alignments were performed using MUSTANG (Konagurthu *et al.* 2006).

3. Results and discussion

3.1 Observed variability in quaternary association

The subunits of all the four lectins considered in the present study have essentially the same tertiary structure with a fold schematically illustrated in figure 1a. The fold is made up of three Greek keys, two of which are contiguous while the N-terminus and the C-terminus come

together in the third. The strands in the three keys form the three sides of a triangular prism with loops at both the ends. One end carries the sugar binding site(s). The amino acid sequences of the four lectins align well with sequence identities between pairs varying between 33% and 38% (figure 1b). The three-fold symmetry of the tertiary structure is weakly reflected in the sequence of the lectin from banana, a monocot. It is not in the sequence of the other three lectins, all from dicots. Also, banana lectin has two mannose-binding sites on each subunit, whereas the other lectins have only one. These differences have been suggested to have arisen from the different evolutionary paths monocot and dicot lectins have followed from a common ancestor (Sharma *et al.* 2007).

Despite the striking similarities in their sequences and tertiary structures, the four lectins exhibit very different quaternary structures, as illustrated in figure 2. Artocarpin can be considered as a dimer of two dimers AB and CD, with 222 symmetry. Heltuba, an octamer with 422 symmetry, is made up of four AB dimers. The geometry of the AB dimer in both these structures is essentially the same as that of dimeric banana lectin (figure 2a). The dimer of calsepa, designated as A'B', is entirely different from that of the AB dimer (figure 2b). Among other things, the AB dimer has a parallel arrangement of protomers whereas the A'B' dimer in calsepa has an anti-parallel arrangement. The N-terminal and the C-terminal stretches are involved in the interface of the AB dimer as well the A'B' dimer. Part of Greek Key I and an edge of Greek key III are involved in the inter-subunit interface of the banana lectin dimer. These Greek keys are involved in the interfaces in calsepa as well, but in a different way. Greek key II occurs only in the calsepa interface. Tetramerization in artocarpin involves the bringing together of an AB dimer and the equivalent CD dimer (figure 2c), which are related by a two-fold symmetry. Subunit A interacts with subunit C (and B with D) through an N-terminal stretch (residues 1–3), and the loop that connects Greek keys I and II (residues 20–25). In octameric heltuba, subunit A of the AB dimer interacts with subunit B of a neighbouring AB dimer (figure 2d). This interface has some similarity with the inter-subunit interface in the A'B' dimer in calsepa. However, the mutual orientations of the two subunits at the interface are entirely different.

3.2 Modelling, energy minimization and MD simulations

The present study involved, in part, construction of models involving subunits of one protein with the quaternary structure of another. For example, calsepa on banana lectin was constructed by superposing the calsepa subunit on each of the subunits in the banana lectin. Banana lectin on calsepa, on artocarpin, and on heltuba, calsepa on banana

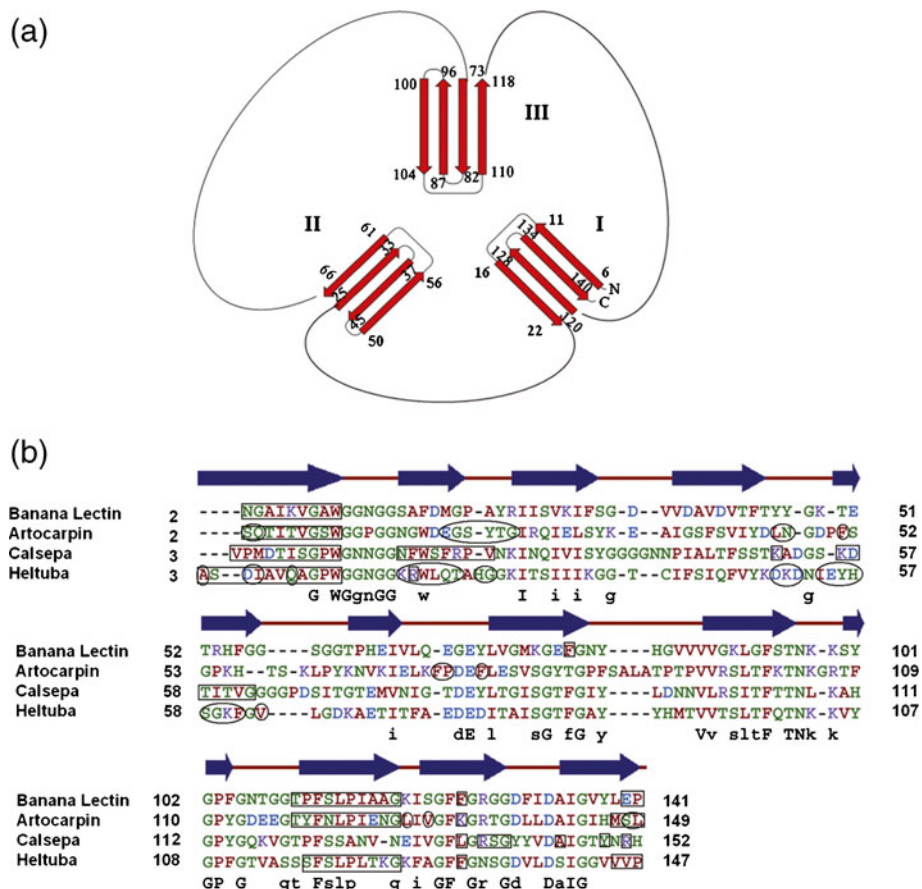


Figure 1. (a) Schematic representation of a subunit of a single-chain mannose-binding β -prism I fold lectin. Numbers correspond to the banana lectin sequence. (b) Structure based sequence alignment of banana lectin, artocarpin, calsepa and heltuba. Secondary structure elements shown on top correspond to the crystal structure of banana lectin. Residues involved in dimerization are boxed and those involved in dimer-dimer interactions are circled.

lectin, on artocarpin and on heltuba, artocarpin on heltuba and heltuba on artocarpin were similarly constructed. The models thus constructed and the native structures were then energy minimized. The root mean square deviations (rmsd) of α -carbon positions of the minimized structures from the native models were in the range 0.50 Å to 1.0 Å in the case of experimentally determined native structures. Those in the case of the constructed models were understandably higher (0.60 Å to 1.5 Å). For reasons discussed later, banana lectin on artocarpin and calsepa on artocarpin could not at all be energy minimized in a sensible manner.

50 ns MD simulations were carried out on minimized native structures and all the models except the two that could not be constructed and two others which dissociated by the time the simulation reached 20 ns (supplementary figures 1–4). Simulations for 50 ns were also carried out on individual subunits of banana lectin, calsepa, artocarpin and heltuba. Understandably the constructed models took a longer time to stabilize. Therefore, further analyses were carried out using frames in the 15 to 50 ns period.

3.2.1 Changes in subunit structure consequent to oligomerization: The population distributions of individual monomers in terms of rms deviations from a subunit in the appropriate crystal structure were reasonably sharp with peaks at rmsd in C^α positions varying between 1.25 Å and 2.50 Å. The population distributions of individual subunits in oligomeric simulations have in general peaks with lower rmsd (0.75 Å to 1.75 Å) from subunits in the respective crystal structure. This is understandable as the restraints imposed on subunit conformation on account of oligomerization are the same in the crystal structures, where the lectins exist as oligomers, and in the simulations involving oligomeric molecules. These restraints do not obviously exist in simulations involving monomers. Indeed, the results of the simulations of monomers and the oligomers together form an appropriate resource for exploring the intrinsic conformational features of monomers and the changes in them resulting from oligomerization.

The subunits from the four lectins have essentially the same structure with the chain length varying between 141



similar superpositions in octameric heltuba in which two interfaces of each subunit is involved in oligomerization.

When the most probable structures of the four subunits, corresponding to the peaks in the appropriate population distributions, are superposed the rms deviations for 127 to 136 C α positions range from 1.70 Å to 1.90 Å. The residues, which deviate by 3 Å or more in any of the pair-wise superpositions, were identified to obtain a rough and ready estimate of the regions that exhibit differences among the subunits. These residues, in banana lectin numbering, are 2–5, 13–15, 21–25, 44–46, 48–54, 56–60, 63–64, 71, 74–75, 77, 83, 98–100, 107, 111, 113–122, 126, 129 and 140–141. Not surprisingly, a majority of these residues belong to the N-terminal and C-terminal stretches and loops. The maximum deviation is exhibited by the N-terminal stretch. The root mean square fluctuations (rmsf) along the polypeptide chain for four monomers are illustrated in the figure 4a. By and large, the regions that exhibit large fluctuations are the same as those that show large deviations among the four monomers (figure 4b). Thus, the variability among the structures appears to have correlation with the mobility in each of them. The

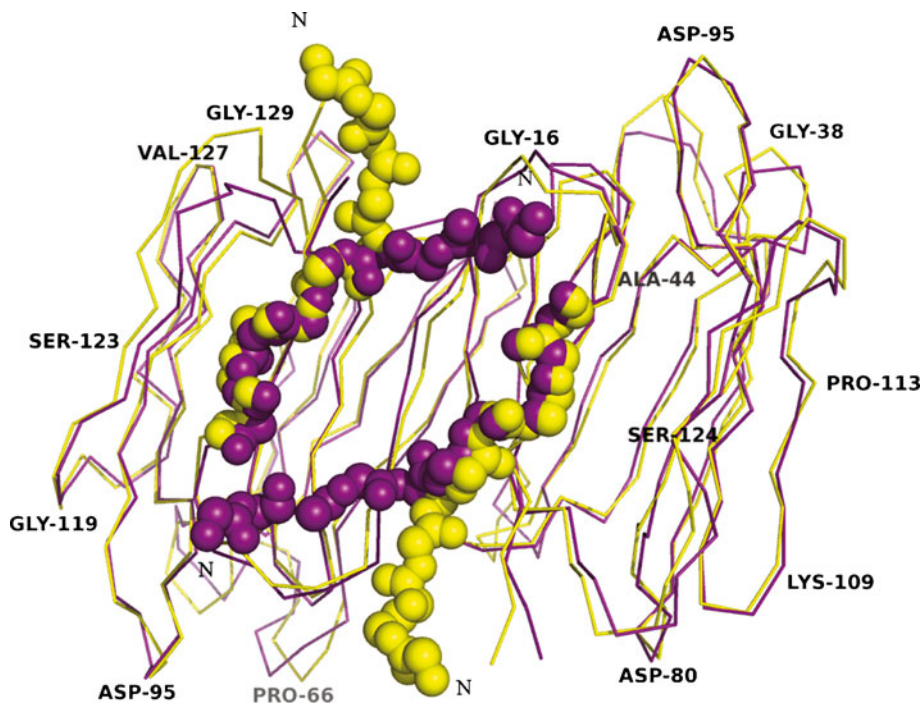


Figure 3. Superposition of structures corresponding to peaks in the population distributions of monomer (yellow) and dimer (magenta) simulations of calsepa. First 10 N-terminus residues are shown in spheres.

fact that many of these residues belong to loops might be a factor responsible for this correlation. But that does not appear to be the only factor. All loop residues do not belong to the variable or mobile regions. Furthermore, these regions also contain residues belonging to strands. Many residues involved in oligomerization, particularly those belonging to the AB interface, also belong to the variable/mobile regions. Interestingly a similar observation was made in separate studies involving legume lectins and jacalin as well (Natchiar *et al.* 2004; Sharma *et al.* 2009).

A detailed comparison of the most probable structure of the free subunits and those of the corresponding oligomers indicate the structural changes that occur during oligomerization. The changes in all cases involve movement of the N- and the C- terminal regions to different extents. The interactions between subunits in the oligomer often involve strands as well. Thus, the movements during oligomerization necessitate some readjustment in the whole subunit. The core structure of the subunit involving strands in the three Greek keys is stabilized by the hydrophobic core and inter-strand hydrogen bonds and is therefore relatively rigid. Thus, the effect of readjustment on oligomerization is felt mainly in the loops. It also turns out that a vast majority of the residues that move during oligomerization belong to regions that exhibit structural differences among the subunits of the four lectins. Thus, there appears to be correlation among dynamic variability, differences among

the four lectins and movements during oligomerization. The picture of a subunit of β -prism I fold lectin that emerges is one with a comparatively rigid core surrounded by flexible regions that vary among the members of the family and also undergo changes during oligomerization.

It is well known that oligomeric proteins are expected to obey, and indeed do with only very few exceptions, an appropriate point group symmetry. Banana lectin and calsepa are two-fold symmetric while artocarpin has 222 symmetry and heltuba has 422 symmetry. No non-crystallographic symmetry restraints were employed during simulations. Yet the symmetries are substantially preserved. By and large, departures from the expected rotation angles were within $\pm 10^\circ$, indicating the robustness of the observed quaternary arrangements.

3.2.2 The dimeric models: Banana lectin and calsepa represent two distinctly different and mutually exclusive modes of dimerization in β -prism I fold lectins. The population distributions of the two native structures and the two models in terms of rms deviations from the respective initial models are shown in figure 5a. The two native structures stabilize with rms deviations of around 1 Å. The calsepa on banana lectin model stabilizes with an rms deviation of less than 2 Å, whereas in the stable population of the banana lectin on calsepa model, the rms deviation from the initial model is close to 3 Å. Thus, much larger readjustments are necessary to accommodate the banana lectin

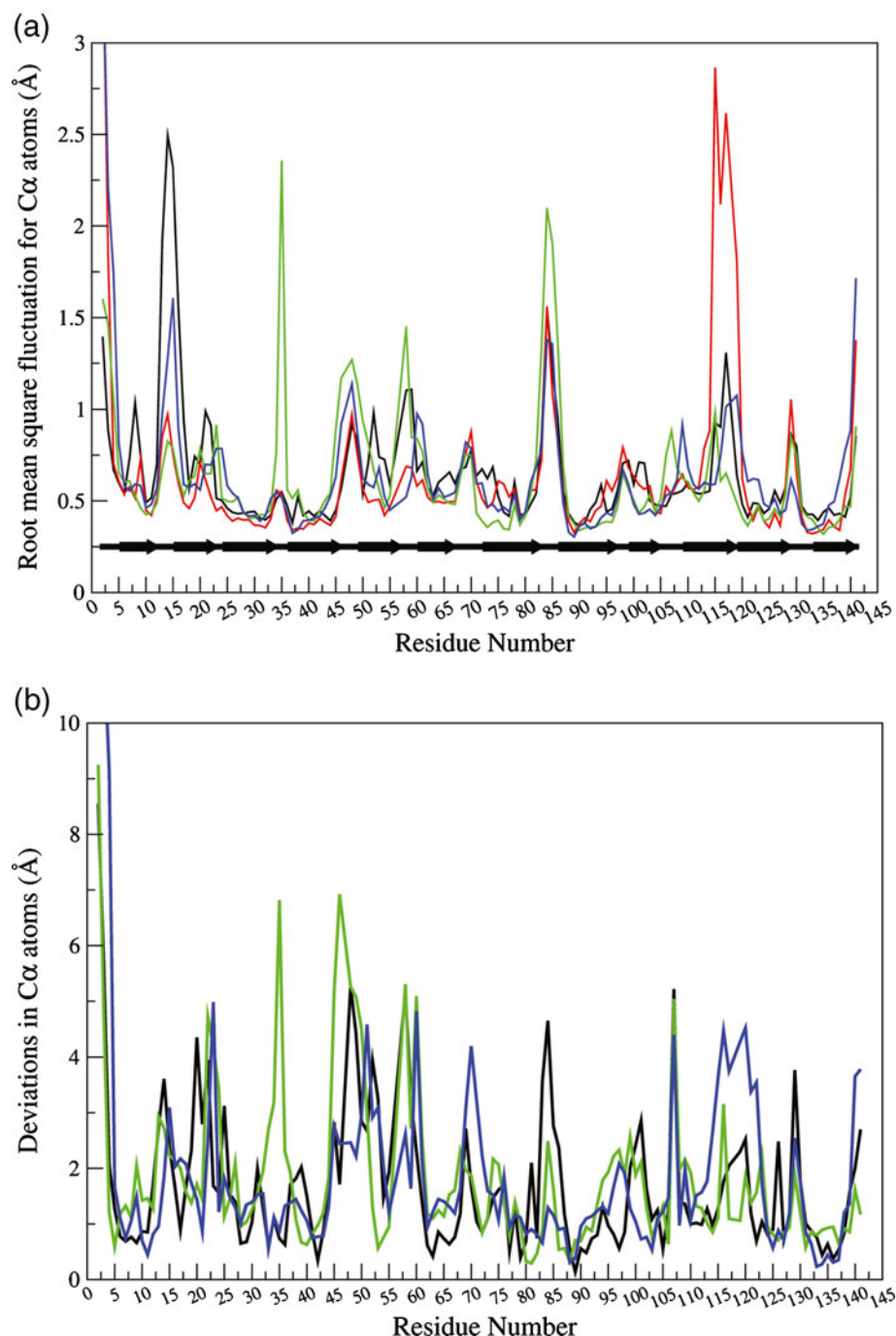


Figure 4. (a) Average root mean square fluctuations (Å) of C α atoms in the simulations of banana lectin (red), artocarpin (black), calsepa (green) and heltuba (blue) monomers. Residue numbers and secondary structure elements correspond to banana lectin. (b) Deviations in C α atoms of most probable structures of calsepa (green), artocarpin (black) and heltuba (blue) from that of banana lectin in simulations involving individual subunits. Numbers correspond to banana lectin.

subunit in the calsepa dimer than what are required to accommodate the calsepa subunit in the banana lectin dimer. This conclusion is reinforced by the average values of the parameters that define the interactions between the two

subunits in the dimers, listed in table 1. The difference between calsepa and banana lectin on calsepa in each parameter is by and large greater than that between banana lectin and calsepa on banana.

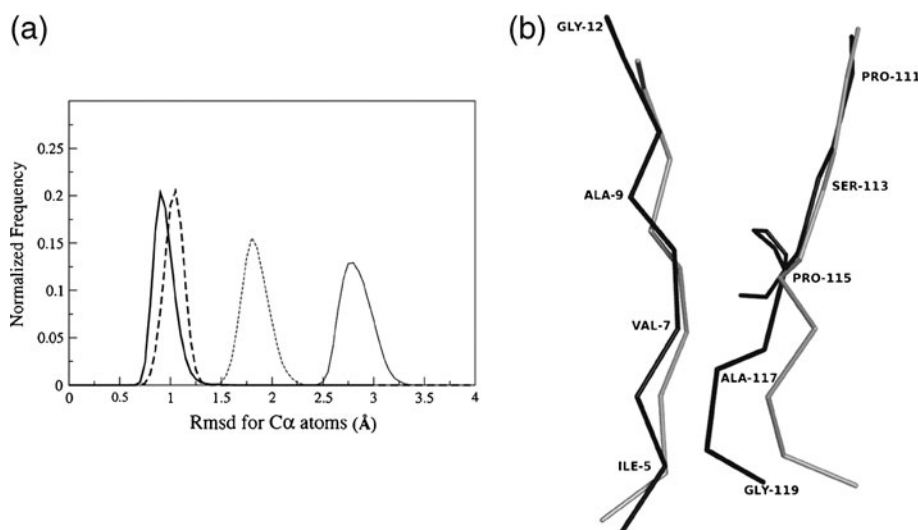


Figure 5. (a) Population distributions in terms of rmsd (Å) of C α atoms from the relevant crystal structure in the case of banana lectin (thick solid line), calsepa (thick dashed line), banana lectin on calsepa (thin solid line) and calsepa on banana lectin (thin dashed line) simulations. (b) The 110–120 segment and the segment in the other molecule with which it interacts in the superposed structures of banana lectin (black) and calsepa on banana lectin (grey). Numbers correspond to banana lectin. The structures were taken from the last frames in the two simulations.

In spite of the comparatively large re-adjustments necessary for the banana lectin on calsepa model, the final model has interaction parameters comparable to those in the banana lectin structure (table 1). The interaction energy is in fact slightly better in the model. However, the non-polar surface area buried on dimerization, which is a measure of the entropy component, is larger in the banana lectin structure. There are only marginal differences between the two in the average number of hydrogen bonds and shape complementarity. However, most of the hydrogen bonds in banana lectin are of the main chain – main chain type, while those in the model are more variable on account of the involvement of side chains. Perhaps most significantly, the two-fold symmetry, a necessary prerequisite for dimeric proteins, is substantially violated in the model, whereas it is reasonably well preserved in the banana lectin structure. Thus, overall it would appear that for the banana lectin

subunit, the observed pattern of dimerization is only slightly better than that employed in the calsepa structure.

An entirely different picture emerges when the native calsepa structure is compared with the calsepa on banana lectin model. The native structure is emphatically superior in terms of all the parameters that define inter-subunit interactions. Among the four structures/models considered, the calsepa dimer is the most stable, a distant second is the banana lectin dimer closely followed by banana lectin on calsepa and calsepa on banana lectin. Thus, in addition to indicating the relative stabilities of the different possible dimers, the broad parameters obtained from simulations provide a rationale for the observed choice of dimeric arrangements in the banana lectin and calsepa. These parameters do not provide indications on the specific role of individual residues. It is the overall surface properties of the subunits that determine them. However, a close

Table 1. Average inter-subunit interaction parameters for native and modelled dimers

	Banana lectin	Calsepa on Banana lectin	Calsepa	Banana lectin on Calsepa
Total buried area (Å ²)	1506 (35)	1546 (61)	2449 (52)	1617 (178)
Non-polar buried area (Å ²)	1112 (30)	978 (40)	1613 (50)	814 (117)
Total interaction energy (–kJ/mol)	569.4 (27.1)	534.4 (50.6)	1093.6 (37.9)	759.0 (58.9)
Number of hydrogen bonds	7.0 (1.3)	5.8 (1.5)	11.3 (1.4)	8.3 (1.5)
Inter-subunit rotation angle (°)	177.8 (1.7)	175.3 (2.6)	178.9 (0.9)	166.7 (2.4)
Shape complementarity	0.69 (0.03)	0.69 (0.04)	0.72 (0.02)	0.68 (0.03)

Averages were taken from 15–50 ns simulation trajectories. Standard deviations are given in parentheses.

examination brings to light the specific role of three consecutive residues in the case of the banana lectin dimer. In addition to the N- and C-terminal stretches, the 110–119 stretch is involved in the dimerization of banana lectin. This stretch is highly conserved not only in banana lectin, artocarpin and heltuba, but also in the related two-chain galactose specific lectins jacalin and maclura, with a consensus sequence of –T/S-X-F-N/S-L-P-I/L-X-X-G. All the five lectins involve banana lectin type dimerization. The corresponding stretch in calsepa departs substantially from the consensus sequence. In particular, the hydrophobic triplet –L-P-I/L– is replaced by –S-A-N– in calsepa. This change appears to be such as to introduce a shift in the course of the polypeptide chain as illustrated in figure 5b, which compares the relevant region of the banana lectin and calsepa on banana lectin. This shift is such as to diminish, if not abolish, the possibility of a few hydrogen bonds. It could also contribute to the reduction in the non-polar surface area buried on dimerization.

3.2.3 Tetrameric and octameric models: As mentioned earlier, banana lectin on artocarpin and calsepa on artocarpin could not be properly energy minimized. Severe steric clashes involving loop 45–50 in the banana lectin subunit that occur when it is superposed on subunits in artocarpin could not be removed during minimization. As pointed out in the discussion on the structure of banana lectin (Singh *et al.* 2005), the disposition of this loop and the stretch that follows it (51–62) are different in banana lectin and artocarpin. In fact, all the tetrameric lectins (jacalin, maclura, morniga and artocarpin), irrespective of their sugar specificity and of whether they have been post-translationally modified or not, have the same conformation for this region, which is different from that observed in banana lectin (figure 6). The observed conformation in the tetrameric lectins is such as to permit the dimerization of two AB dimers, whereas that in banana lectin prevents this dimerization of dimers. Thus, the difference in the oligomerization of the tetrameric lectins and banana lectin can be readily understood in terms of specific differences in the tertiary structures. A situation involving steric clashes is encountered also when a calsepa monomer is superposed on artocarpin. The severe short contacts now involve the N-terminal stretch. Thus, the deviations in the common tertiary structure are such that the subunit of the two dimeric lectins of known structure cannot be accommodated in quaternary association observed in tetrameric artocarpin.

An acceptable model of heltuba on artocarpin results on energy minimization. (figure 7a). The interaction parameters for the model derived from MD simulations (table 2) are not as good as those for octameric heltuba, but are comparable to those for the well-established tetrameric structure of

artocarpin. The expected 222 symmetry of the model is substantially retained during the rather long simulation. Thus, MD simulations appear to indicate that a tetrameric model of heltuba is possible.

Subunits in octameric heltuba could be successfully replaced by those of banana lectin, calsepa and artocarpin and the resulting models energy minimized. However, the energy minimized models behave differently from one another when MD simulations were performed on them. The structural integrity of the octamer is lost entirely during simulation in banana lectin on heltuba and artocarpin on heltuba (figure 7). The integrity of the octamer is retained in calsepa on heltuba. However, as can be seen from table 2, the indices that define inter-subunit interactions are much weaker in the model than in native heltuba and calsepa. This is particularly evident in the AA' type interface. Thus, although the model is feasible, it is much inferior to the native heltuba structure. The average interaction parameters are much poorer than those in the native calsepa structure and even those in calsepa on banana lectin.

3.3 Evolution, oligomerization and ligand binding

Interestingly, the difference in the disposition of the 45–62 stretch in banana lectin referred to earlier in the context of quaternary association appears to be related to the retention of a second carbohydrate-binding site in the lectin. All β -prism I fold lectins of known structure bind to carbohydrates with a consensus motif G...GXXXD anchored on the top loop region of Greek key I (Sharma *et al.* 2007). Banana lectin is the only known case that has this motif on Greek key II also and thus has two carbohydrate-binding sites per subunit. Carbohydrate-binding residues in the banana lectin on site P1 (that on Greek key I) are Gly15 and Gly129-Asp133 and those on site P2 (that on Greek key II) are Gly60 and Gly34-Asp38 (figure 8) (Singh *et al.* 2005). The distal glycine in both the sites is believed to have a structural role as the main chain torsion angles for these residues are appropriate only for glycine among proteinaceous amino acids (Sharma *et al.* 2007). G at P2 is replaced by an L-amino acid in the other lectins. This presumably contributes to the altered conformation of the 45–62 stretch in them. This alteration constricts P2, which can no longer accommodate a carbohydrate molecule, thus presenting an interesting example of the relation among evolution, oligomerization and ligand binding.

Yet another insight on a possible change in ligand binding caused by oligomerization can be obtained by comparing the results of the MD simulations on banana lectin and banana lectin on calsepa. As indicated in figure 8a, the population distributions of the atoms in the carbohydrate-binding P2, in terms of rms deviations from those in the crystal structure, show a major peak and a

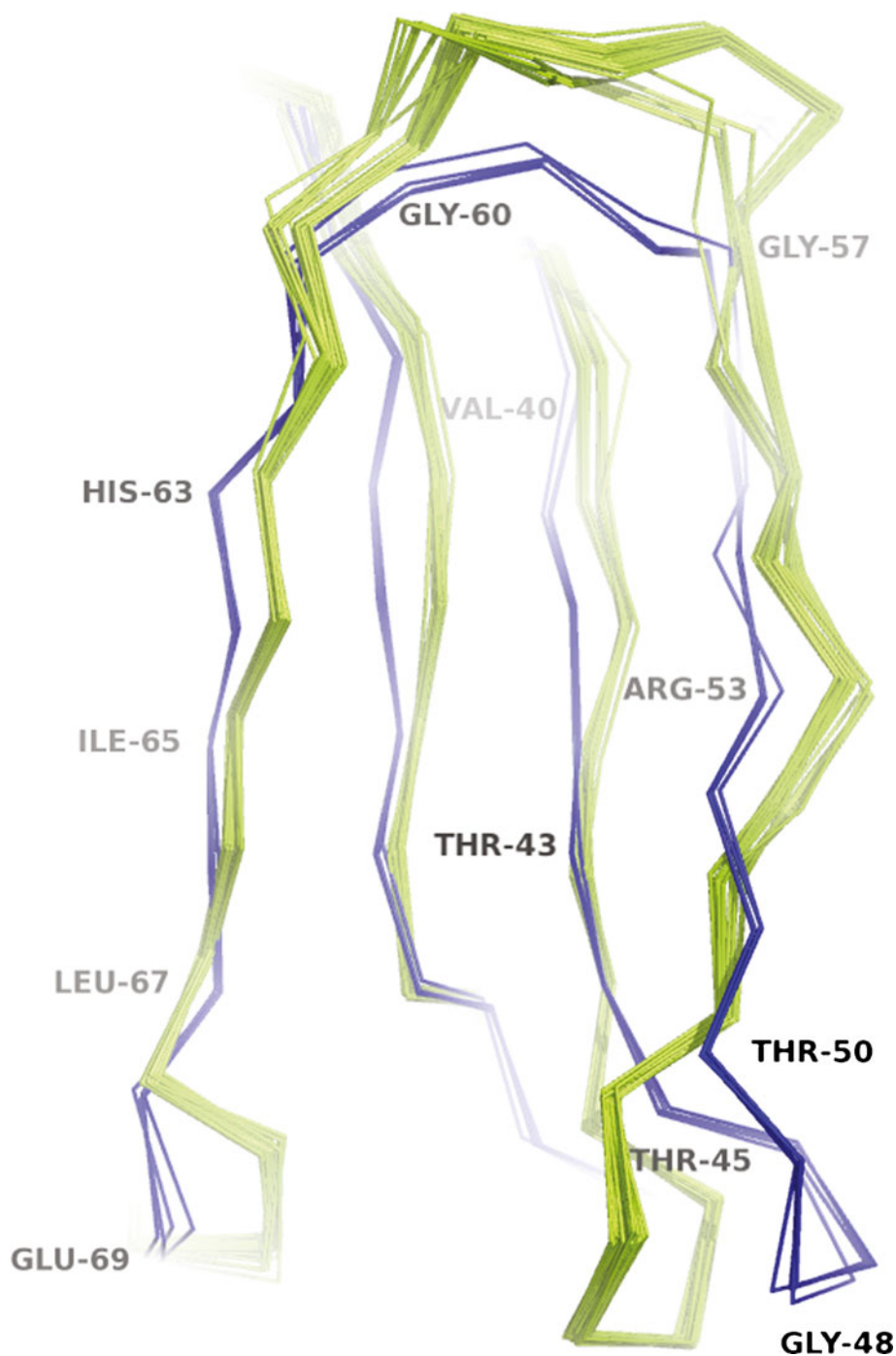


Figure 6. The 40–69 residue stretch (banana lectin numbering) of the superposed subunits of banana lectin (blue) and those of all β -prism I fold tetramer lectin subunits (green). (PDB CODES, 1X1V, 1JAC, 1KU8, 1KUJ, 1M2B, 1PXD, 1UGX, 1UGY, 1UH0, 1UH1, 1WS4, 1WS5, 1J4S, 1JOT, 2BMY, 2BMZ, 2BN0, 1XXR and 1XXQ).

minor peak, indicating that although the combining site is substantially preformed, deviations from the preformed geometry is possible. Such deviations have earlier been noticed in the MD studies on jacalin as well (Sharma *et al.* 2009). The major peak corresponds to the disposition of

atoms found in the crystal structure of the banana lectin–carbohydrate complex. The disposition corresponding to the minor peak involves an expansion of the carbohydrate-binding site (figure 8b), which, as seen from docking, leads to weaker binding on account of the lengthening of some

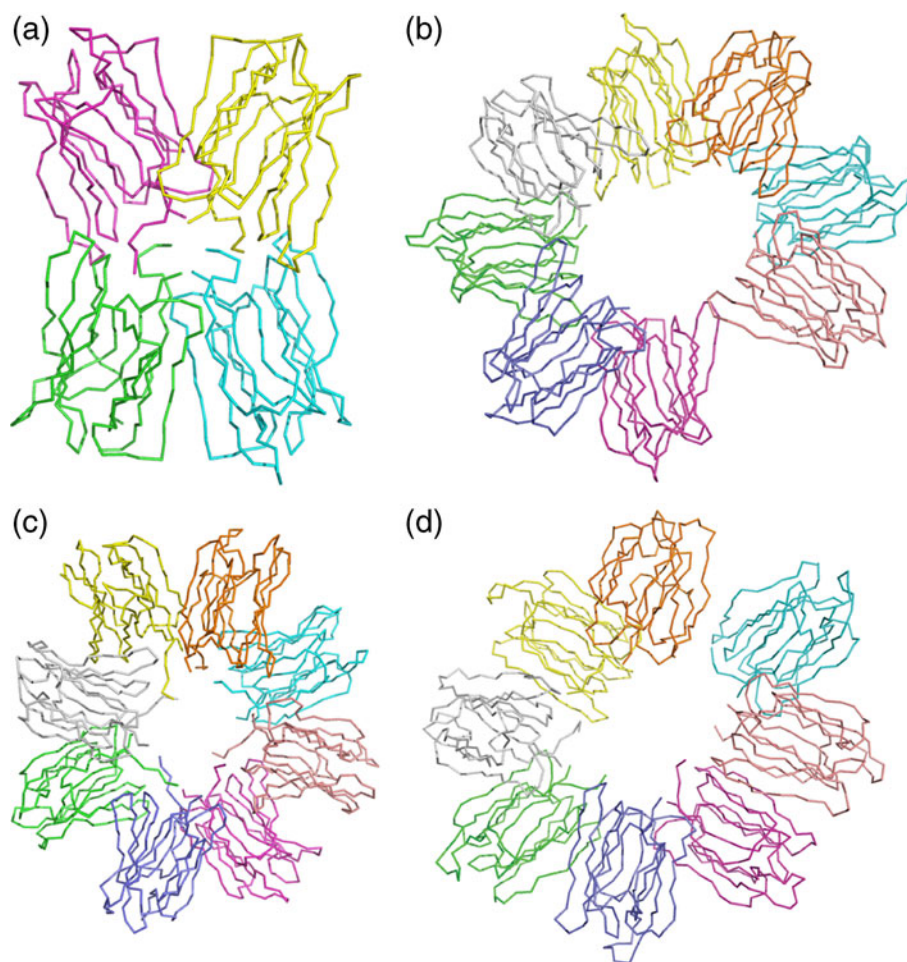


Figure 7. The assembly of subunits as in the final frames of simulations of (a) heltuba on artocarpin, (b) banana lectin on heltuba, (c) calsepa on heltuba and (d) artocarpin on heltuba.

protein–carbohydrate hydrogen bonds and the disappearance of some of them. Interestingly, the peak in the population distribution of the carbohydrate binding atoms in P2 in banana lectin on calsepa is located close to the minor peak in the banana lectin simulation (figure 8b). The structure of the binding site corresponding to the two peaks is also very similar. Thus, normal binding of a carbohydrate molecule at site P2 in banana lectin is not compatible with a dimeric association of the type found in calsepa. This appears to provide a good example for oligomerization dependent conformational selection (Kumar *et al.* 2000). Conformational selection involving binding sites has been described in the case of jacalin as well (Sharma *et al.* 2009).

4. Conclusions

Structure determination using X-ray crystallography cannot obviously reveal the favoured conformations of individual subunits in an oligomeric protein and the changes introduced

in it on quaternary association. The work reported here, in part, demonstrates how this problem can be approached using MD simulations. The simulations on the four monomers reveal their preferred conformations, while the simulations on the corresponding oligomers throw light on the changes brought about on account of oligomerization. The changes are most striking in calsepa, where dimerization involves a swapping of the N-terminal stretch. By and large, the magnitude of overall changes in the monomers on oligomerization is related to the number of interfaces involved in the quaternary structure. The regions of the molecules affected by subunit association interestingly exhibit commonalities despite the differences in the mode of oligomerization in the four lectins. It also turns out that the regions in the monomer that show differences among the protomers in the four lectins correlate well with those that exhibit larger dynamic variability. The fact that the oligomers substantially maintain the expected symmetry during

Table 2. Average inter-subunit interaction parameters for tetramers and octamers

Interface type	Artocarpin		Heltuba		Heltuba on artocarpin		Calsepa on heltuba	
	AB	AC	AB	AA'	AB	AC	AB	AA'
Total buried area (\AA^2)	1766 (64)	746 (71)	1523 (25)	1927 (73)	1626 (39)	881 (29)	1312 (49)	1300 (80)
Non-polar buried area (\AA^2)	1132 (48)	365 (35)	952 (16)	1120 (48)	1013 (21)	499 (20)	834 (28)	841 (39)
Total interaction energy (-kJ/mol)	900.2 (75.3)	441.6 (85.1)	659.3 (18.8)	981.5 (59.5)	690.5 (34.8)	645.6 (45.8)	454.9 (33.9)	420.1 (82.4)
Number of hydrogen bonds	10.4 (1.4)	3.6 (1.0)	8.4 (0.7)	8.3 (0.8)	9.0 (1.1)	5.05 (0.7)	4.5 (1.3)	2.0 (0.9)
Inter-subunit rotation angle ($^\circ$)	173.8 (2.4)	170.4 (2.7)	176.2 (1.1)	178.5 (0.6)	175.9 (14.7)	174.3 (14.4)	173.4 (2.6)	173.9 (2.1)
Shape complementarity	0.70 (0.02)	0.73 (0.04)	0.72 (0.01)	0.64 (0.02)	0.72 (0.03)	0.64 (0.06)	0.61 (0.02)	0.53 (0.054)

Averages were taken from 15–50 ns simulation trajectories. Standard deviations are given in parentheses.

simulations even in the absence of symmetry restraints, leads to added confidence in the results of the simulations.

Mannose-binding proteins with β -prism I fold present an interesting case in which subunits with nearly the same tertiary structure assume widely different quaternary structures, with the number of protomers ranging between 2 and 8. The inability of a certain protomers to assume a specific quaternary arrangement can be explained in a straightforward manner on the basis of steric considerations only in two instances. They involve banana lectin and calsepa protomers in the quaternary arrangement found in artocarpin. Even energy minimization does not permit the unambiguous exclusion of any other possibility. The relative merits of different arrangements become obvious only through MD simulations.

The results involving banana lectin and calsepa, which exhibit distinctly different dimerization patterns, are particularly interesting. A banana-lectin-type dimer is found not only in artocarpin and heltuba but also in tetrameric galactose-binding two-chain lectins such as jacalin and maclura. Thus, the observed arrangement in calsepa is unique. At least one reason for this uniqueness appears to be the change from –L-P-I/L– in all the other lectins to –S-A-N– in calsepa, referred to earlier. The change in the disposition of the polypeptide stretch resulting from this substitution appears to contribute to the destabilization of calsepa on banana lectin. A critical substitution of three consecutive residues thus appears to contribute to tilting the balance between two possible modes of dimerization. Amino acid substitutions arising during evolution also appear to contribute to the inability to accommodate the banana lectin dimer in an artocarpin-type tetramer. The changes resulting from the substitutions also cause the disappearance of a carbohydrate-binding site, thus providing correlation involving evolution, quaternary association and ligand

binding. MD simulations further indicate that a banana lectin subunit involved in a calsepa-type dimer cannot retain an efficient second carbohydrate-binding site.

The octameric assembly of the type exhibited by heltuba is favoured only when heltuba subunits are the protomers. The octamer either dissociates or has very poor oligomerization parameters when other subunits are used to construct the oligomer. However, interestingly, the results of MD simulations indicate that an artocarpin type tetramer can be formed with artocarpin subunits as well as heltuba subunits. Thus, it would appear that heltuba can exist as an octamer or a tetramer, although only octamer has been observed in the crystal structure. This is in consonance with the observation of a tetrameric species of heltuba in solution (Van Damme *et al.* 1999).

Admittedly, the ultimate objective of computational studies on protein folding is to be able to predict the three-dimensional structures of proteins from sequences alone. Knowledge-based prediction of tertiary structures has achieved a measure of success, but *ab initio* prediction is still at its infancy. Quaternary association involves the next level of organization. The work presented here does not attempt to address the general issue of this level of association in terms of the primary structure. However, the results obtained have reasonable predictive value in relation to the quaternary structure of β -prism fold lectins. For instance, the two modes of dimerization have been strongly suggested to have resulted from specific differences in sequence. Likewise, the inability of banana lectin to tetramerize as artocarpin has been related to the length and possibly the composition of a loop region. These observations should be useful in anticipating the quaternary association of β -prism fold lectins of known sequence, but unknown structure. All the same, as in the case of many computational studies on large biological systems, the main

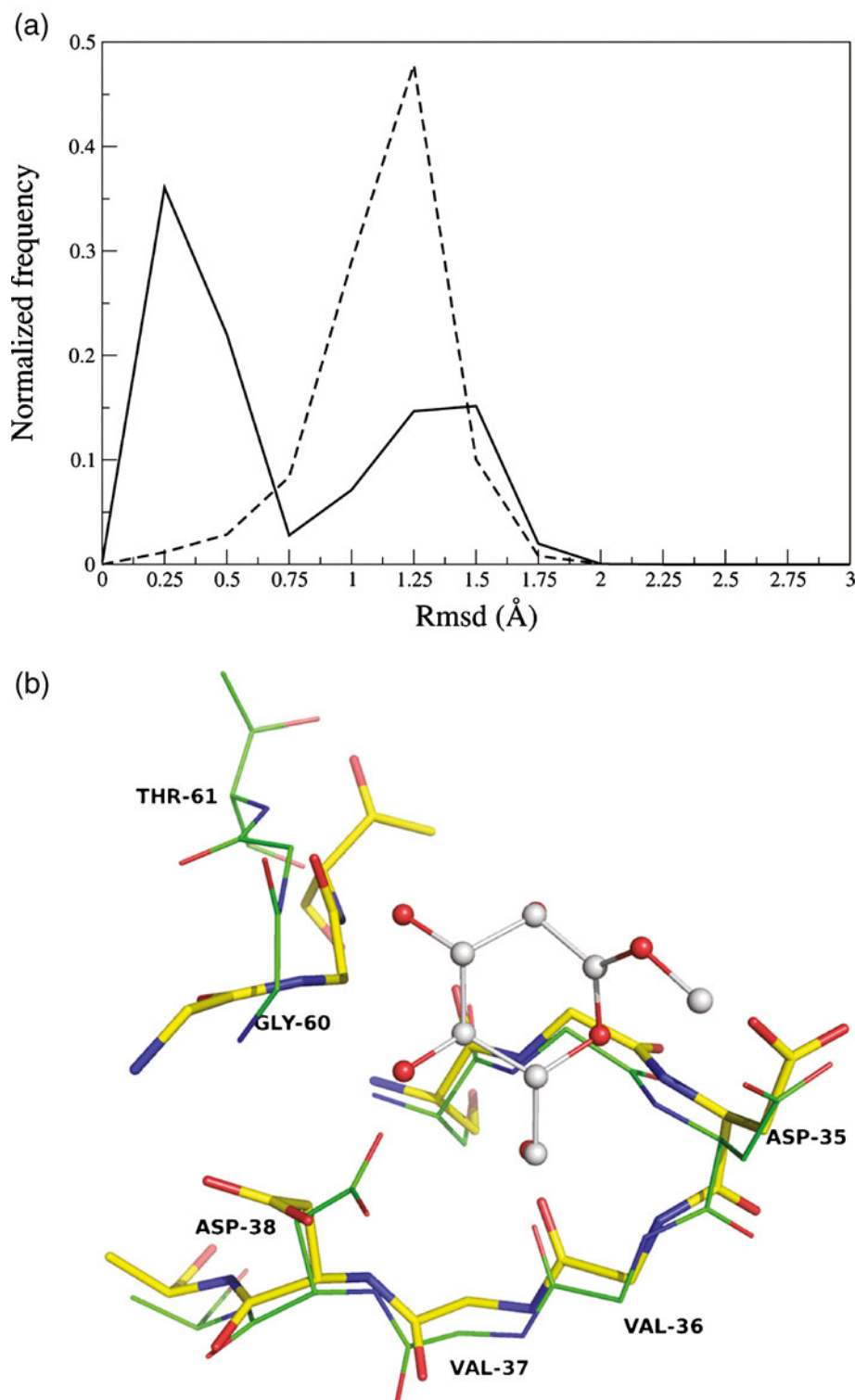


Figure 8. (a) Population distributions in terms of rmsd (Å) of all atoms, from the relevant crystal structure, of carbohydrate-binding residues of site P2 in banana lectin (solid line) and banana lectin on calsepa (dashed line) simulations. (b) Superposition of residues in carbohydrate-binding site P2 corresponding to major peak in the population distribution of banana lectin (thick lines) and those corresponding to peak in the population distribution of banana lectin on calsepa (thin lines) simulations. The carbohydrate ligand, α -methyl mannose, is shown in the ball and stick representation.

thrust of the current effort has been to rationalize experimental observations and to gain useful insights with some predictive value.

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