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Research Communication

Stability of Dimeric Interface in Banana Lectin: Insight from Molecular Dynamics Simulations

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Summary

Banana lectin (Banlec) is a homodimeric non-glycosylated protein. It exhibits the β-prism I structure. High-temperature molecular dynamics simulations have been utilized to monitor and understand early stages of thermally induced unfolding of Banlec. The present study elucidates the behavior of the dimeric protein at four different temperatures and compares the structural and conformational changes to that of the minimized crystal structure. The process of unfolding was monitored by following the radius of gyration, the rms deviation of each residue, change in relative solvent accessibility and the pattern of inter- and intra-subunit interactions. The overall study demonstrates that the Banlec dimer is a highly stable structure, and the stability is mostly contributed by interfacial interactions. It maintains its overall conformation during high-temperature (400–500 K) simulations, with only the unstructured loop regions acquiring greater momentum under such condition. Nevertheless, at still higher temperatures (600 K) the tertiary structure is gradually lost which later extends to loss of secondary structural elements. The pattern of hydrogen bonding within the subunit and at the interface across different stages has been analyzed and has provided rationale for its intrinsic high stability.

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Keywords banana lectin (Banlec); molecular dynamics simulations; hydrogen bond analysis; cluster analysis; root mean square deviation; solvent accessible surface area.

Abbreviations Banlec, banana lectin; MD, molecular dynamics; MPA, maclura pomifera agglutinin; Mc, main chain; Sc, side chain; RMSD, root mean square deviation; RGYR, radius of gyration; ASA, accessible surface area; RRMS, residue wise RMSD; PNA, peanut agglutinin.

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INTRODUCTION

Protein-protein interactions and oligomerization are important determinants for the formation of many functional proteins. The unique three-dimensional structure of both monomeric and oligomeric proteins is encoded in their sequence (*1, 2*). It is believed that that oligomerization and inter-protein interactions endow additional structural and conformational stability to oligomeric proteins (*3*). Thermal or denaturant induced unfolding of proteins has been studied extensively by spectroscopy or calorimetry to determine their thermodynamic stability (*4–8*). The details of this process at atomic level are, however, diffused and remain elusive by the use of traditional experimental/theoretical procedures. Over the years, the advent of molecular dynamics (MD) simulations has proved to be a powerful tool in shedding some light on these processes (*9, 10*). These simulations often complement the experimental information available and provide further insights on the process of unfolding/folding, along with information on folding intermediates and factors affecting the stability at various time points during the process.

In order to unfold a protein, high-temperature simulations (300–600 K) are carried out. Such perturbations are necessary because there exists a large difference in the experimental time scale for unfolding and compared to what is accessible computationally. High-temperature simulations accelerate the unfolding process as it would for a traditionally activated process without affecting the pathway itself (*11*). Previously, such simulations have been carried out for proteins like Chymotrypsin inhibitor 2, Barnase, Ubiquitin, Hen egg lysozyme, T4 lysozyme and more recently for peanut agglutinin (*11–16*). High-temperature MD simulations have been done to elucidate the process of deoligomerization and to study the changes that occur at interfaces during the process which shed some light on the necessity of formation of oligomeric structures by proteins. MD simulations have also been utilized to investigate binding mechanisms of some oligomeric proteins which involve different degrees of flexibility and plasticity during their assembly, as well as to characterize the interactions involved during ligand binding

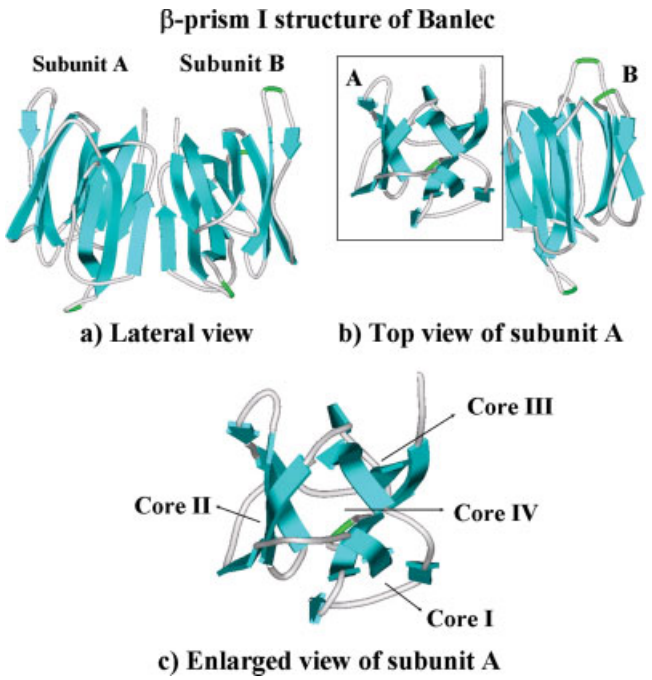

Figure 1. Banana lectin is a homodimer exhibiting the β-prism I structure. The β-prism topology is shown (a) as a lateral view and (b) view from top of subunit A. (c) The three greek-keys making the prism enclose three core regions (I, II and III), whereas together they form another core in the center of the prism (core IV). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

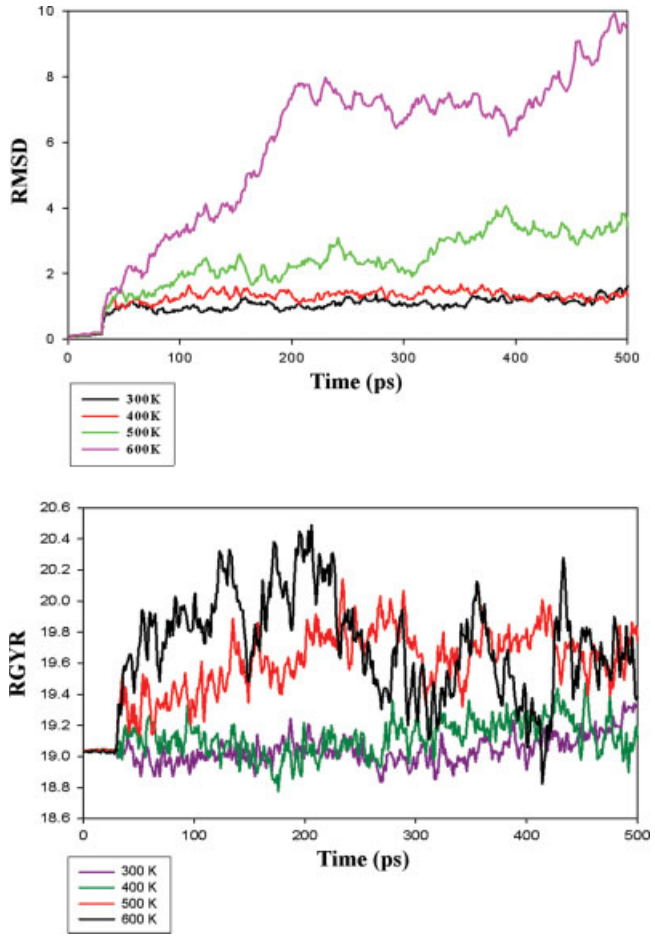

Figure 2. The upper panel depicts the comparative RMSD of Banlec at different simulation temperatures for the 500 ps simulation time. The lower panel depicts the change in RGYR of the molecule for the same simulations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(*17*). The present study investigates early stages of unfolding in Banlectin (Banlec).

Banlec is a homodimeric protein consisting of 282 residues. It belongs to the β-prism I class of structural proteins (*18*). In β-prism I fold, three greek keys are arranged like the triangular face of a prism (Fig. 1). Other members of this structural family are Jacalin (first to be identified), Artocarpin, Maclura Pomifera Agglutinin (MPA), Heltuba, Calsepa, MornigaM and Parkia lectins. Banlec is unique among them in retaining more than one primary ligand binding site per subunit. Banlec among the β-prism I family is the only one, which belongs to a monocot family of plants, whereas all other members are dicots. It also exhibits interesting sugar binding properties which are different from other members of the structural family (*19–22*).

In a separate study, we have investigated the structural stability of Banlec in the presence of chaotropes. It was observed that despite a small interfacial area, Banlec displays substantial conformational stability in the presence of chaotropic agents (*23*). It is, however, not a good system for studying thermal denaturation experimentally as it aggregates immediately at its melting temperature which is quite high; therefore, high-temperature MD simulations were performed. A detailed investigation of the behavior of individual subunits at the atomic level during simulation at various temperatures has been carried out. Interestingly, a graph spectral approach, together with the analyses of hydrogen bonds, reveals that the relative abundance of Main chain—Main chain hydrogen bonds at the inter-subunit interface imparts the protein considerable stability than what can be expected if it were populated by Side chain—Side chain hydrogen bonds even when hydrophobic interactions at the interface are sparse.

METHODOLOGY

All simulations have been carried out on the crystal structure of Banlec (PDB code 1X1V) (*22*) using AMBER8 package (*24*) with parm98 parameters (*25*). Explicit water molecules (TIP3P) (*26*) were used in the simulations and the solvation box was 12 Å from the farthest atom along any axis. Particle Mesh Ewald summation (*27*) was used for the long-range electrostatics and van der Waals cut-off was 10 Å. Pressure and temperature relaxation was 0.5 ps^{-1}. All simulations have been

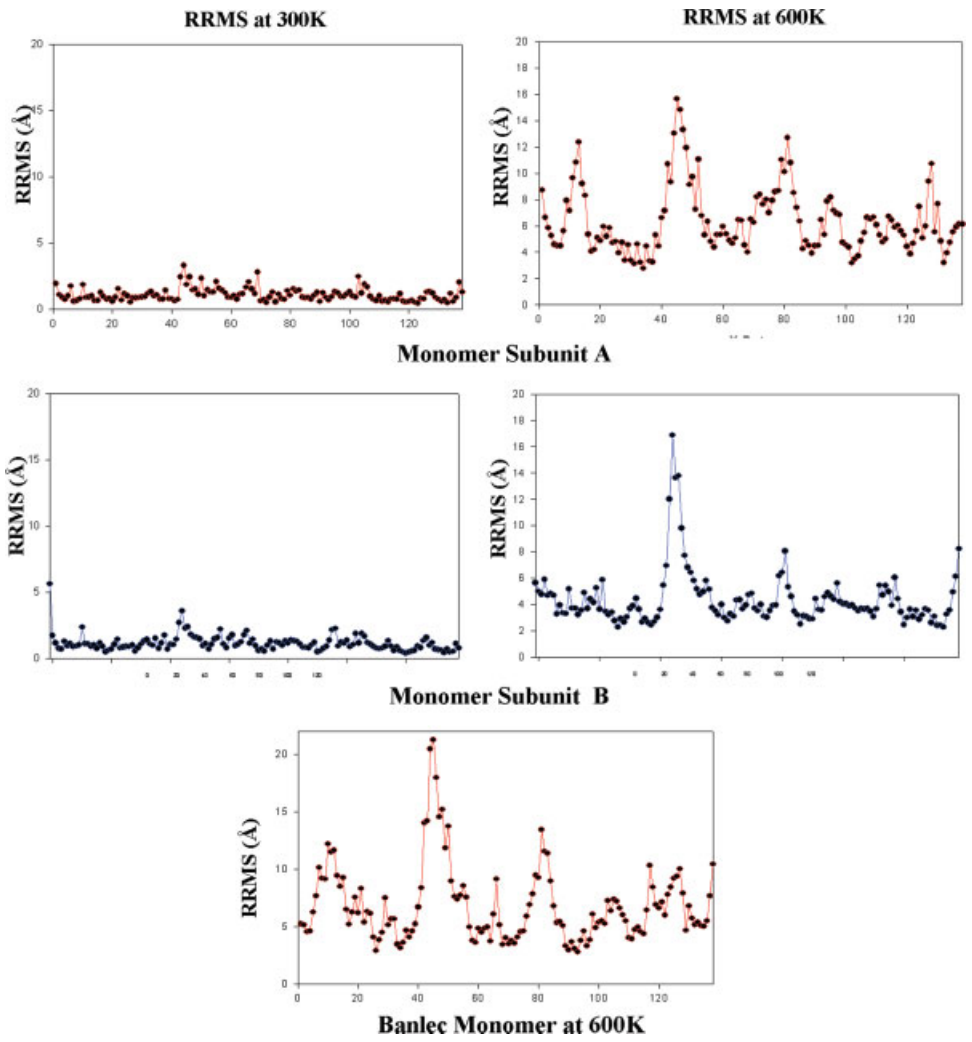

Figure 3. The upper panel in red compares the change in residue-wise RMSD of Banlec monomer subunit A from 300 to 600 K. The second panel in blue does the same for monomer subunit B. The last panel shows the residue-wise RMSD of the monomer at 600 K when simulated alone. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

performed at constant pressure and temperature conditions. The simulations were carried out at temperatures 300, 400, 500 and 600 K for 500 ps and were repeated twice to validate the consistency of results. The simulations were performed with constraint on the protein initially for 30 ps during which the desired temperature was reached. This was followed by unrestrained simulation at the particular temperature.

The hydrogen bond analysis has been carried out by the CARNAL module of AMBER. The cut off values set for Donor–Acceptor were $\leq$3.5 Å for D....A bond distance and $\geq$ 120° for the D—H...A bond angle. The hydrogen bonds which exist for ≥50% in any simulation have been considered as dynamically stable and used for interpretation of stability. For comparison of protein stability hydrogen bonds involving only main chain atoms (Mc–Mc) as well as those involving at least one side chain (Sc) atom (Mc–Sc, Sc–Mc and Sc–Sc) have been considered.

Solvent accessible surface area (ASA) of various core regions of Banlec has been calculated by NACCESS (*28*). A probe of 1.5 Å has been used to determine ASA. A clustering algorithm has been used to identify residues forming the interface and cores (*29, 30*). In this method, the residues of a protein are considered as nodal points. On the basis of the strength of non-covalent interaction, an edge is constructed, which is denoted as I_{min}. The protein graphs thus constructed are analyzed for interface and core clusters. Generally, it is recognized that a transition in the size of the largest cluster occurs around I_{min} value of 3–4% (*30*). The clusters obtained at I_{min} values >4% are considered to represent strong interactions. The range of non-covalent interactions with their side-chains is considered

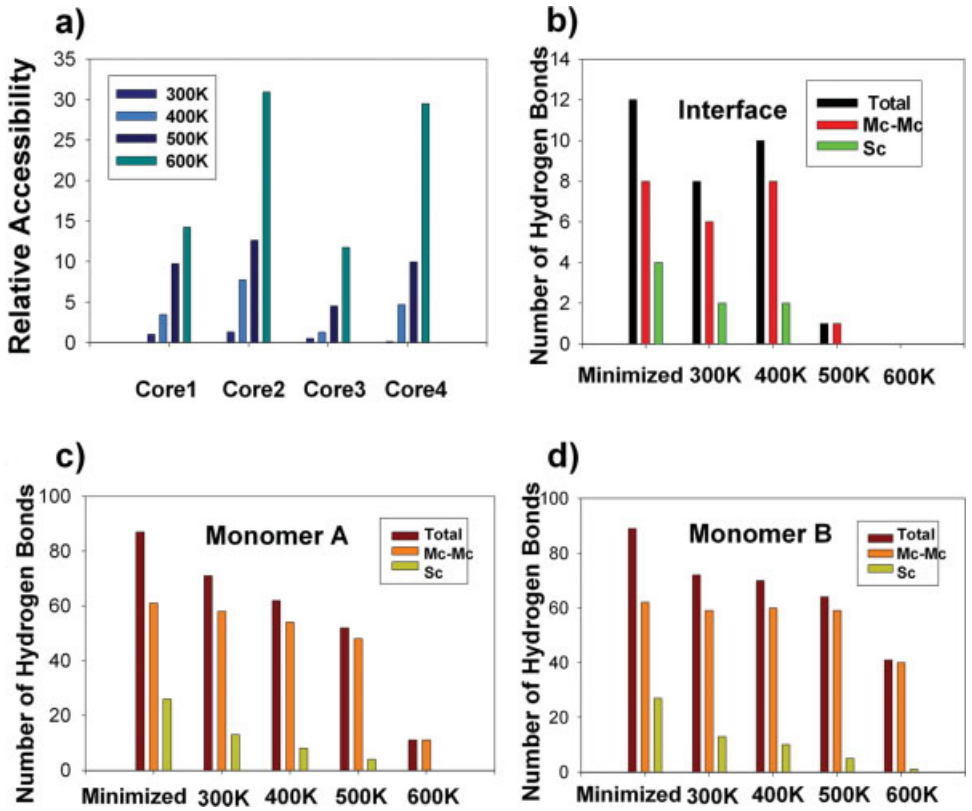

Figure 4. (a) The bar-chart represents the relative accessibility of the four core regions of Banlec at different simulation temperatures. Differences in each core region across the temperatures have been depicted here. (b) The bar-diagram represents change in hydrogen bonding pattern at the interface of the dimer across the different temperatures of simulation. (c) The panel shows the progressive loss of hydrogen bonds in monomer A of Banlec and compares the total loss with loss of Mc-Mc and Sc involved hydrogen bonds. (d) The panel shows the progressive loss of hydrogen bonds in monomer B of Banlec and compares the total loss with loss of Mc-Mc and Sc involved hydrogen bonds. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

as the edge. I_{min} is the strength of the non-covalent interactions. A protein graph is built on the basis of obtained I_{min} values. The clustering algorithm identifies the residues forming clusters with strength greater than the specified I_{min}.

RESULTS AND DISCUSSION

In this study, we have looked into the effect of temperature on quaternary structure of Banlec by carrying out simulations between 300 and 600 K. The overall stability of the dimer was measured by estimating the root mean square deviation (RMSD) of the molecule, its radius of gyration (RGYR) and solvent accessible surface area (ASA). Monitoring changes at the residue level was done by assessing the residue wise RMSD (RRMS), change in hydrogen bonding pattern and cluster analysis. All comparisons are with respect to the minimized Banlec crystal structure. The analyses carried out shed light on the behavior of interacting residues, either via hydrogen bonds or by non-covalent interactions, both inter- and intra-subunit. These results put together bring forth the picture of changes occurring collectively in the dimer and at individual residue level. Comparison of monomer stability with that of monomer in the dimer has also been done to emphasize the role of oligomerization in the overall stability of the molecule. Results of individual analyses are presented below.

RMSD and RGYR

The comparative RMSD and RGYR profiles of Banlec simulation are shown in Fig. 2. It is observed that at both 300 and 400 K simulations the average RMSD value is 1 Å for Banlec. On increasing the temperature to 500 K the value rises gradually, but the average value remains around 2 Å. It is only at extreme temperature of 600 K that the RMSD increases steadily. It attains an average value of 8 Å between 200 and 400 ps simulation time, after which it rises to a value of 10 Å. Simulation at 500 K for 500 ps makes the protein lose its compactness to an extent, but the unfolding process occurs only at 600 K. From the RGYR plot, we observe a small change of only 1 Å from 19 Å at 300 K to 20 Å at 600 K. It indicates that the

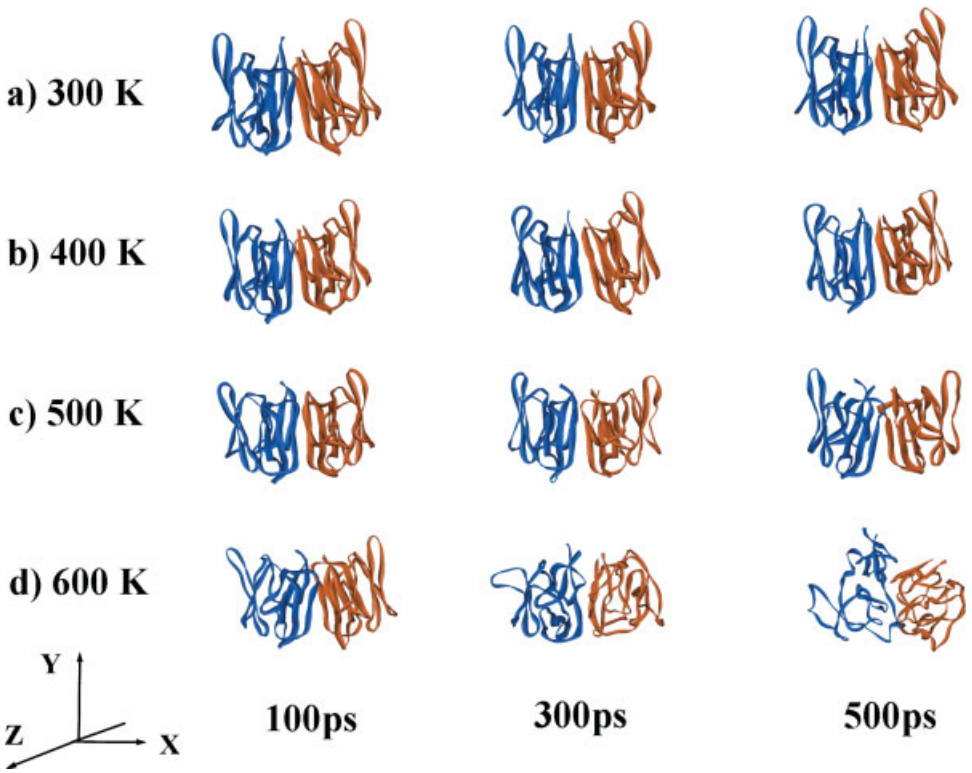

Figure 5. The change in conformation of Banlec during the high temperature molecular dynamics simulations is represented by ribbon diagrams. Three snapshots at each temperature corresponding to 100, 300 and 500 ps have been chosen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

dimer is reasonably compact even at 600 K, consistent with experimental observation (*23*) that strong perturbations are required to destabilize the Banlec dimer. However, it is to be noted that fluctuations in RGYR has considerably increased at 600 K, indicating perturbations from its native structure.

Residue-wise RMSD (RRMS) and Accessible Surface Area (ASA)

The RRMS of each monomer of Banlec (Subunit A and B) has been plotted as shown in Fig. 3. It is evident from the plots that the residues undergoing the most fluctuation are those that make up the loop regions of the greek-key motifs, especially that of greek-key II (residues 25–65) which are farthest from the interface. At 300 K, the average RRMS of each monomer is approximately 1 Å, with that of loop region in greek-key II going up to 4 Å. However, at 600 K, it is observed that the average value hovers at 4 Å with that of greek-key II loop going as high as 17 Å. At this temperature, loop regions of other greek-keys also show higher fluctuation, however, the maximum perturbation is at the second greek-key. It is interesting to note that the perturbations at 600 K is asymmetric *i.e.* fluctuations are higher in one of the subunits (subunit A) than in the other one (subunit B).

As can be observed from the structure of Banlec, there are primarily three hydrophobic core regions present at the centre of each greek-key. Core I, II and III for greek-keys I, II and III, respectively. Additionally, there is a Core IV formed by the arrangement of the three greek-keys in β-prism topology as shown in the Fig. 1. The changes in the ASA of the residues forming the four core regions in comparison to the minimized structure have been calculated and presented in Fig. 4a. The relative accessibility of each core region is very low at 300 K implying that the hydrophobic residues are well buried from the solvent. Simulations at higher temperatures gradually expose the buried surface area to the solvent thereby increasing the relative accessibility. The maximum exposure occurs at 600 K as expected. Comparing the three primary cores, it is observed that the Core II has the highest change in its relative accessibility, the change being more than twice as much as the other two cores (this result is also consistent with RRMS profile). Core II is present in the greek-key farthest from the interface region with no involvement in formation of interface whereas greek-keys I and III are involved in the formation of interface of the Banlec dimer. The hydrogen bonds across the interface hold these two greek-keys in position and resist perturbation, which is reflected in the lower change in ASA of Cores I and III. Core IV is a result of positioning of the three greek-keys in the β-prism. It reflects the total perturbation of the greek-keys. Therefore, increased change in ASA of Core II is reflected in the cumulative change in ASA of the β-prism in Core IV.

Sugar-binding does not alter the stability significantly, offering only mild reduction in fluctuation of the loops at primary binding sites (results not shown). It was also observed that the monomer in the dimer tends to retain its native conformation for a longer period of time, compared with the separated monomeric subunit (Fig. 3).

Hydrogen Bond Analysis

The hydrogen bond analysis was carried out over the entire simulation time. The hydrogen bonds (Mc–Mc, Mc–Sc, Sc–Mc, and Sc–Sc) present in the minimized structure were determined for each monomer subunit. The comparison of hydrogen bonding pattern in Banlec at the temperatures 300–600 K was then carried out with that of the minimized structure. The details of the analysis are given in Figs. 4c and 4d. As the temperature is increased from 300 to 600 K, there is a decrease in the total number of hydrogen bonds, the effect being more profound in bonds involving side-chains. Upto the simulation at 500 K, both monomers in the dimer show similar behavior and approximately 33% loss in total hydrogen bonds. Only 15% of side-chains involved in H bonds are retained at 500 K, whereas the Mc–Mc bonds are retained up to 78%. Retention of Mc–Mc bonds resist much disintegration of the secondary structural fold, yet towards the last stages of the simulation disruption of a few of the β-strands can be observed (Fig. 5).

MD simulation of Banlec at the temperature of 600 K demonstrates the accelerated unfolding process. Under this highly perturbing condition; however, the monomers differ in bond-disruption pattern. Although monomer A shows a drastic loss of 87% of total H bonds, monomer B shows only 54% loss. Therefore, as also seen in the snapshots in Fig. 5, monomer A loses its secondary structure elements earlier than monomer B. However, on increasing the simulation time from 500 ps, similar loss in monomer B was observed. Thus, the unfolding process is initiated in the subunit A of the dimer and then propagates to its B subunit.

From 300 to 600 K during the 500 ps simulation, a total loss of H bonds involving side chains (Mc–Sc, Sc–Mc and Sc–Sc) was observed. Only the stronger Mc–Mc H bonds remained. Retention of most Mc–Mc H bonds up to 500 K imparts the molecule sufficient secondary structural integrity which is lost gradually in the simulation at 600 K. Comparing the results with that of monomer simulated alone, it was observed that the lone subunit in the absence of any intermolecular bonding is highly unstable (Table 1). The monomer loses its hydrogen bonds even at lower temperatures (*e.g.* 500 K) compared with that of the dimer, along with the simultaneous loss of its secondary structure, suggesting that oligomerization endows more stability to the protein structure. The numbers of dynamically stable H bonds at the interface have been listed separately in Table 1 and discussed below.

Table 1
Hydrogen bond analysis

	Total	Mc-Mc	Sc involved (Mc-Sc/Sc-Mc, Sc-Sc)
Monomer subunit A			
Minimized (K)	87	61	26
300	71	58	13
400	62	54	8
500	52	48	4
600	11	11	0
Monomer subunit B			
Minimized (K)	89	62	27
300	72	59	13
400	70	60	10
500	64	59	5
600	41	40	1
Interface			
Minimized (K)	12	8	4
300	8	6	2
400	10	8	2
500	1	1	0
600	0	0	0
Lone Monomer			
Minimized (K)	87	62	25
300	61	49	12
400	48	43	5
500	37	35	2
600	8	8	0

Interface Hydrogen Bond Analysis and Cluster Analysis

Graph theory-based cluster analysis on crystal structure of lectins has provided valuable insights into stability of various interface types in some of the previous studies (*29, 30*). Cluster analysis on Banlec was carried out to identify presence of interface clusters. Snapshots of every 100th ps from each temperature simulation were identified and analyzed. $I_{min} \geq 5\%$ was used to identify the clusters held by non-covalent interactions. The number of clusters and the residues involved in the formation of clusters were found to be influenced by the temperature of simulation. As the temperature is increased, the native clusters (present in minimized structure) are lost. New clusters form as the protein undergoes constant dynamic conformational changes at higher temperatures, especially at 600 K (Fig. 6). Overall, Banlec does not display high density of clusters at the interface compared with other proteins previously studied by this method (*16*), implying that not many non-covalent interactions between interface residues exist other than hydrogen bonding.

As can be observed from the values in Table 1, the interface is stabilized mainly by Mc–Mc hydrogen bond interactions. Side chains of interface residues have very little role to play. Till the end of 400 K simulation it can be seen that there is very little change in the interface. It is, however, perturbed during the 500 K simulation, with all H bonds (in comparison to

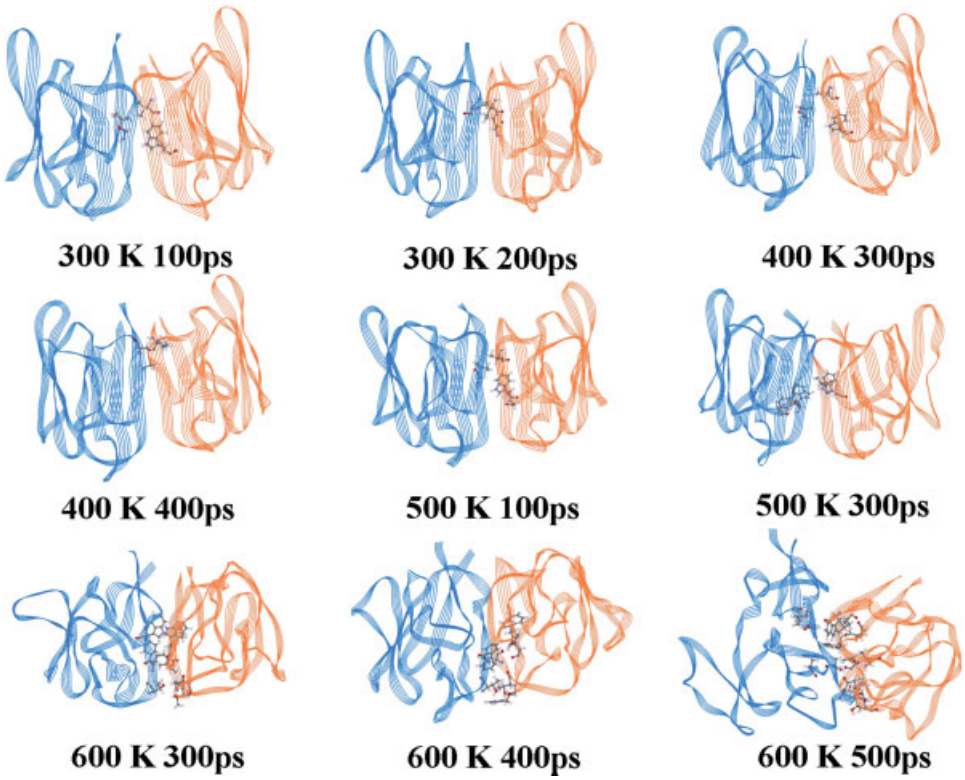

Figure 6. Occurrence of clusters at the interface of Banlec at different simulation periods are elucidated here. The residues involved are highlighted by ball and stick representation with the subunit backbone in ribbon form. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the minimized structure) being lost by the end of 600 K simulation (Fig. 4b). There are other interactions that do persist during the 600 K simulation as described subsequently. The minimized structure of Banlec displays presence of 12 hydrogen bonds across the interface, of which eight are Mc–Mc, two Mc–Sc and two Sc–Sc. Other than these 12 H bonds, there exist three water bridges across the interface. The interface seems to be stabilized by the presence of only these H bonds, which seem to form a continuous β-sheet like structure and water bridges. There are no hydrophobic interactions or cation-pi interactions, which may play a role in stabilizing the structure unlike most other lectins like ConA, SBA or PNA. The simulations at 300 and 400 K result in minor loss of native H bonds at the interface with the occurrence of one cluster at I_{min} of 5%. This cluster is not a very extensive one and consists of only three residues (Val, Trp and Leu). Minor movements changing the orientation of the interface β-strands are the probable cause of such an observation. At 500 K, there is >90% loss of native interface H bonds and 100% loss at 600 K, however, there are some non-native interactions that result due to a mutual reorientation of the β-strands with respect to each other. These non-native interactions hold the subunits together. At these temperatures, the conformational movements are rapid. Consequently, the clusters at interface are constantly changing, as different regions are brought into closer proximity frequently. At certain snapshots, it can be observed that clusters formed consist of >3 residues. In spite of this, not all snapshots analyzed show presence of clusters.

In most lectins non-covalent interactions, such as hydrophobic interactions, play a major role in subunit association in addition to hydrogen bonding. This is clearly not true in the case of Banlec. The only other lectin that has been analyzed very recently in a similar manner is Peanut Agglutinin (PNA) (*16*). PNA is a large tetramer with a completely different fold (jelly-roll fold) from Banlec (β-prism I) and also displays three different interface types (*31, 32*). Nonetheless, keeping aside these differences, some comparisons among the two can be made. The interfaces of PNA largely involve residue sidechain-sidechain (Sc–Sc) interactions. This provides it with presence of significant number of clusters consisting of many residues at an I_{min} of 6% also. They are a consequence of non-covalent interactions that are absent in the case of Banlec. The Sc–Sc interactions among residues endow greater flexibility in relative movement across subunits. The subunits can move freely at higher temperatures without much change in interacting partners at the interface. This is not the case if the interface is held together by H bonds between main chain atoms. There is definitely more restriction of relative subunit movement. This is perhaps the reason why Banlec shows great resistance to change in conformation during simulation and only under the high-temperature

condition of 600 K, there is the collapse of interface and the whole structure when simulated beyond 500 ps. Therefore, in spite of the fact that the interfacial area of Banlec is small and despite the absence of significant non-polar interactions as well as Sc–Sc H bonds, the strong Mc–Mc H bonds across the interface are sufficient to provide it substantial stability.

Despite increasing interest in plant lectins and their multiple uses, little is known about their native functions within the plant itself. They may have many different and important roles to play such as defense against pathogens and predators, storage and transport of carbohydrates (*33*). High-stability of lectins and their resistance to denaturation and degradation are properties which enhance the functionality conferred to them within the plant. Recently, in another light, it has also been shown that more stable proteins are more evolvable because they are able to tolerate functionally beneficial but destabilizing mutations with ease (*34*). Banlec dimer, as a highly stable structure, is in all probability optimized for carrying out the above functions.

CONCLUSIONS

In summary, the high-temperature MD simulations carried out demonstrate that Banlec is a stable dimer. The early thermally induced unfolding stages are manifested only at temperatures as high as 500 K and become clearer at 600 K. Unlike most other proteins, Banlec is stabilized by the presence of only strong hydrogen bonds across the interface and no other noncovalent interactions among the residues appear to play a significant role in the stabilization of this dimeric protein. Thus, dimerization imparts stability to the whole protein structure and monomeric Banlec is unable to maintain its structure and conformation unlike that exhibited by its dimeric counterpart.

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