Investigation on the Effect of Temperature on the Structure of β-Lactoglobulin Based on Molecular Dynamics Simulation

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Abstract: In this study, the structural changes of β-lactoglobulin, the core component of whey protein, in different temperatures (303.15 K, 348.15 K, 373.15 K and 423.15 K) were investigated from a molecular perspective employing the molecular dynamics (MD) simulation method. By analyzing structural parameters such as root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), and solvent-accessible surface area (SASA), the influence of temperature on the structure of β-lactoglobulin was explored. The results show that within the temperature range of 303.15 K to 373.15 K, the molecular conformation of β-lactoglobulin is relatively stable. However, the relative high temperature (e.g., 423.15 K) might significantly affect the structure of β-lactoglobulin, causing significant aggregation and denaturation, which may subsequently affect the gel properties and nutritional functions of the β-lactoglobulin. Furthermore, based on the visualization results, high temperatures might lead to the protein denaturation.

Keywords: β-lactoglobulin; molecular dynamics; temperatures

1. Introduction

β-Lactoglobulin, as the primary protein component in bovine milk whey, plays a critical role in determining dairy product quality and exerts significant effects on human health due to its structural and functional properties (1–4). To eliminate pathogenic microorganisms and extend product shelf life, dairy products are typically subjected to various sterilization treatments, such as pasteurization, ultra-high temperature (UHT) processing, and boiling. Previous studies have indicated that the molecular structure of β-lactoglobulin may undergo alterations under different temperature conditions, which directly influences the quality of dairy products. For instance, various experimental techniques, including circular dichroism spectroscopy, gel electrophoresis, and spectral analysis, have been employed to investigate the effects of temperature on the structure and function of β-lactoglobulin (5–9). However, these investigations have largely relied on macroscopic experimental characterizations and have not elucidated the effects of temperature on β-lactoglobulin at the molecular level, thereby limiting a comprehensive understanding of the underlying mechanisms governing temperature-induced structural changes. Molecular dynamics (MD) simulation enables the dynamic modeling of structural changes in biomolecules at the atomic scale, offering direct visualization of microscopic conformational transitions and providing molecular-level insights that are often inaccessible through conventional experimental approaches (10–18).

In this study, MD simulations were employed to investigate the effects of temperature conditions associated with common dairy sterilization methods, namely pasteurization, boiling, and UHT processing, on the molecular structure of β-lactoglobulin. Structural parameters, including radius of gyration (Rg), root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and solvent-accessible surface area (SASA), were analyzed at temperatures of 348.15 K, 373.15 K, and 423.15 K, corresponding to the respective sterilization processes. Molecular structure at 303.15 K was used as a control to assess temperature-induced conformational changes. By characterizing the structural features of β-lactoglobulin at different temperatures,

this study aims to elucidate the molecular mechanisms by which temperature influences its conformation. The findings in this study will provide a theoretical basis for understanding the thermal stability of β-lactoglobulin from a molecular perspective.

2. Methods

MD simulations were performed using the GROMACS software package. The initial structure of β-lactoglobulin was obtained from the RCSB Protein Data Bank (PDB ID: 3NPO) (19). Molecules of water and extraneous ions were removed using the PACKMOL software. The CHARMM force field, which is widely applied in simulations of proteins, lipids, carbohydrates, and small molecules (20–26), was selected for this study. The simulation temperatures were set to 303.15 K, 348.15 K, 373.15 K, and 423.15 K, with 303.15 K serving as the ambient temperature reference.

Prior to simulation, the β-lactoglobulin molecule was placed in a cubic water box with dimensions of 40 × 40 × 40 Å to mimic its native solvation environment and ensure adequate interaction with solvent molecules. Based on the electrostatic properties of the protein, K^+ ions were added to neutralize the net charge of the system. Energy minimization was carried out using the conjugate gradient method. Subsequently, the system was equilibrated under NVT and NPT ensembles to achieve stable temperature and pressure conditions. The molecular dynamics simulation was conducted for 50 ns with a time step of 2 fs to ensure adequate conformational sampling. Structural differences of the protein at various temperatures were analyzed and visualized using the VMD software, a molecular visualization program.

3. Results and Discussions

3.1 Radius of Gyration

Figure 1 illustrates the temporal evolution of the Rg of β-lactoglobulin at different temperatures (303.15 K, 348.15 K, 373.15 K, and 423.15 K). The Rg value primarily reflects the overall compactness of a protein structure, representing the distribution of all atoms relative to the center of mass, and can indicate the degree of protein folding or unfolding (29). A decrease in Rg suggests a more compact structure (e.g., protein folding), whereas an increase in Rg indicates a looser structure or the onset of denaturation.

The results demonstrate that at 303.15 K (orange curve), the Rg remained relatively low and stable throughout the simulation, with minimal fluctuations. This indicates that at this temperature, β-lactoglobulin maintains a compact conformation with strong intramolecular interactions and high structural stability. As the temperature increased to 348.15 K (yellow curve) and 373.15 K (blue curve), the overall Rg values exhibited a gradual increase, accompanied by a slight broadening of fluctuation ranges. This suggests that elevated temperatures begin to influence the protein structure, inducing partial unfolding, reducing structural compactness, and initiating a trend toward expansion. However, within the temperature range of 303.15 K to 373.15 K, despite a modest average increase in Rg, three curves displayed non-fluctuated changes. This indicates that β-lactoglobulin does not undergo significant structural alterations within this termperature range, and that intramolecular hydrophobic interactions remain sufficient to maintain its fundamental architecture. This finding is generally consistent with previous results obtained using circular dichroism spectroscopy (8).

Upon reaching 423.15 K (purple curve), the Rg values were not only markedly higher than those observed in the other temperature groups but also exhibited pronounced fluctuations, particularly a significant increase during the later stages of the simulation. This behavior suggests that at 423.15 K, the structure of β-lactoglobulin undergoes substantial disruption, characterized by an expanded atomic distribution around the center of mass and a sharp decline in structural stability.

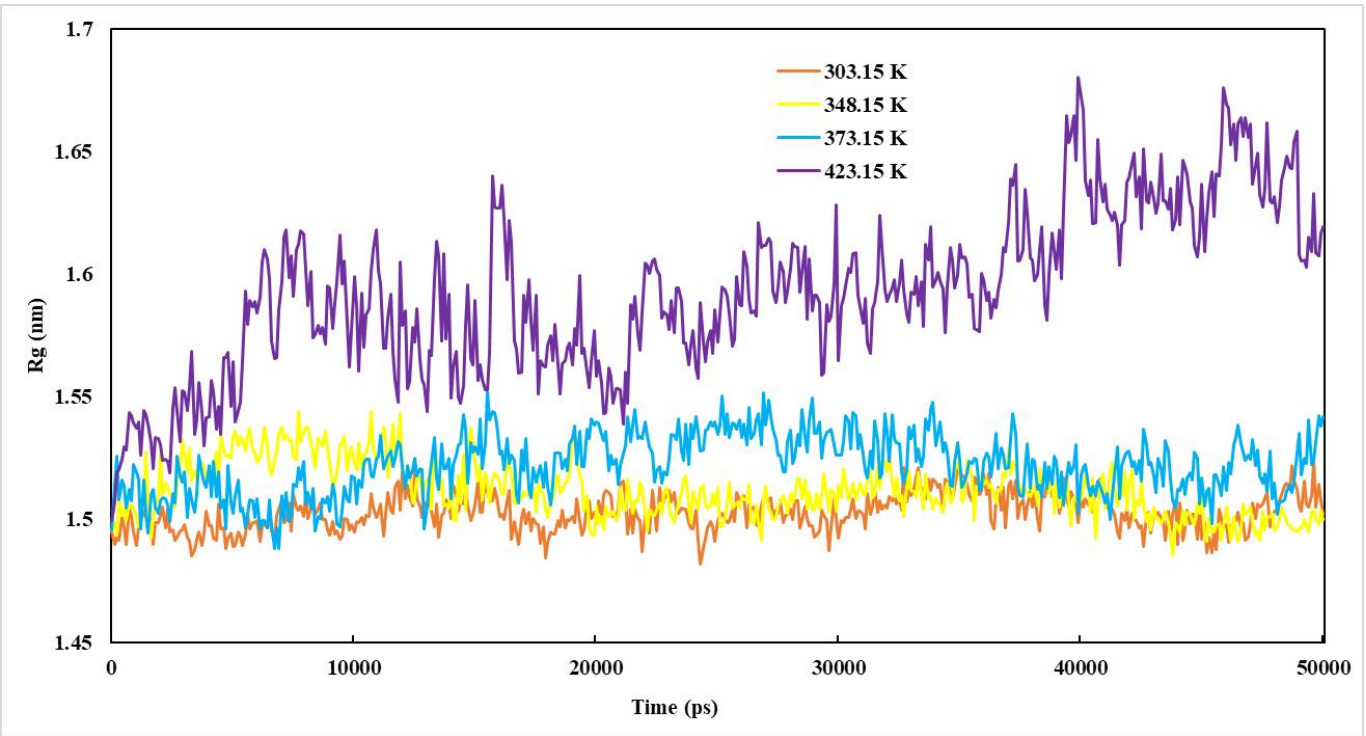

Figure 1. The Rg of β-lactoglobulin under different temperature conditions

3.2 Root-Mean-Square Deviation

Figure 2 illustrates the evolution of the RMSD of β-lactoglobulin at temperatures of 303.15 K, 348.15 K, 373.15 K, and 423.15 K. RMSD reflects the overall structural stability of a protein by measuring the average deviation of the simulated structure from a reference structure, typically the initial conformation (27). A lower RMSD value and an earlier attainment of a stable plateau indicate a more stable system.

At 303.15 K (orange curve), the RMSD remained consistently low throughout the simulation, exhibiting minimal fluctuations. This indicates that at this temperature, the structure of β-lactoglobulin is stable and deviates little from its original conformation. Within the temperature range of 348.15 K to 373.15 K, the RMSD values were notably higher than those observed at 303.15 K. However, within this specific interval, the RMSD remained relatively stable without dramatic fluctuations, and the increase in RMSD with rising temperature was not pronounced. This suggests that within a certain temperature range, elevated temperatures have a limited impact on the structure of β-lactoglobulin. This finding is largely consistent with previous results obtained using techniques such as circular dichroism spectroscopy (8).

When the temperature was increased to 423.15 K (purple curve), the RMSD was significantly higher than that in the other temperature groups. It exhibited a rapid increase during the initial phase, followed by a sustained period at a relatively high value with substantial fluctuations. This behavior clearly indicates a marked deviation of the β-lactoglobulin structure from its original conformation, demonstrating that high temperature induces significant structural distortion and a sharp decline in conformational stability.

In summary, the influence of temperature on the RMSD of β-lactoglobulin exhibits a stage-dependent pattern. Within a certain temperature range (303.15 K to 373.15 K), the molecular conformation of β-lactoglobulin remains relatively stable despite the temperature increase. However, upon reaching 423.15 K, the high temperature leads to a significant alteration in its molecular conformation.

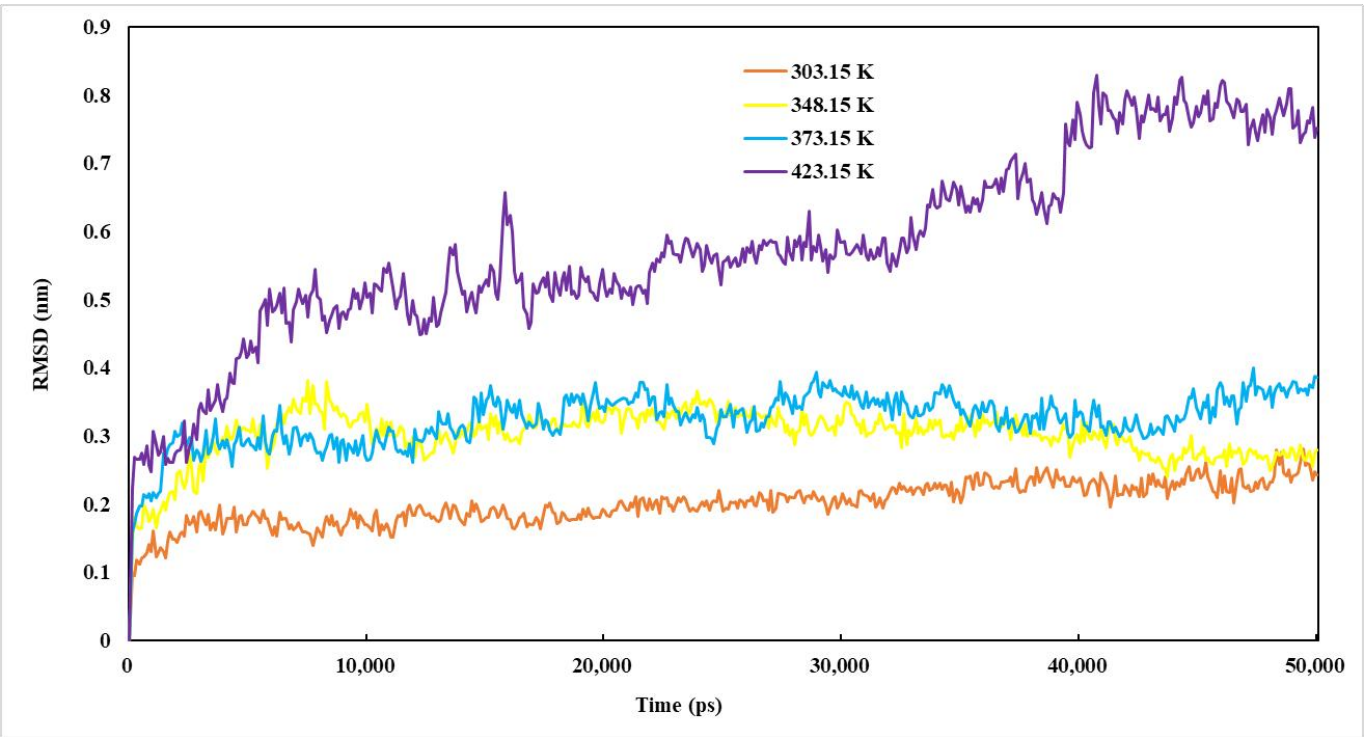

Figure 2. The RMSD of β-lactoglobulin under different temperature conditions

3.3 Root-Mean-Square Fluctuation

The RMSF reflects the local structural flexibility of a protein molecule by measuring the average fluctuation amplitude of each residue or atom relative to its mean position during the simulation (28). High RMSF values indicate greater regional flexibility, whereas low RMSF values suggest higher regional rigidity.

As shown in Figure 3, at relatively low temperatures (e.g., 303.15 K), the RMSF values of β-lactoglobulin remained low and stable, indicating that intramolecular interactions, such as hydrogen bonds and hydrophobic effects, effectively constrained atomic motions, resulting in limited movement of amino acid residues. At this temperature, the structure of β-lactoglobulin exhibited high rigidity and stability.

With increasing temperature (348.15 K and 373.15 K), some residues displayed markedly elevated RMSF values, indicating enhanced thermal motion and increased flexibility of specific amino acid residues, reflecting greater conformational dynamics. Overall, within the temperature range of 348.15 K to 373.15 K, the RMSF profiles showed no substantial differences, and neither curve exhibited dramatic fluctuations. This suggests that temperature elevation within this range does not significantly affect the thermal motion of β-lactoglobulin residues.

When the temperature was further increased to 423.15 K, the RMSF values increased sharply, indicating a substantial enhancement in the thermal motion of amino acid residues within β-lactoglobulin. This pronounced increase suggests a loss of structural rigidity and heightened local flexibility under extreme thermal conditions.

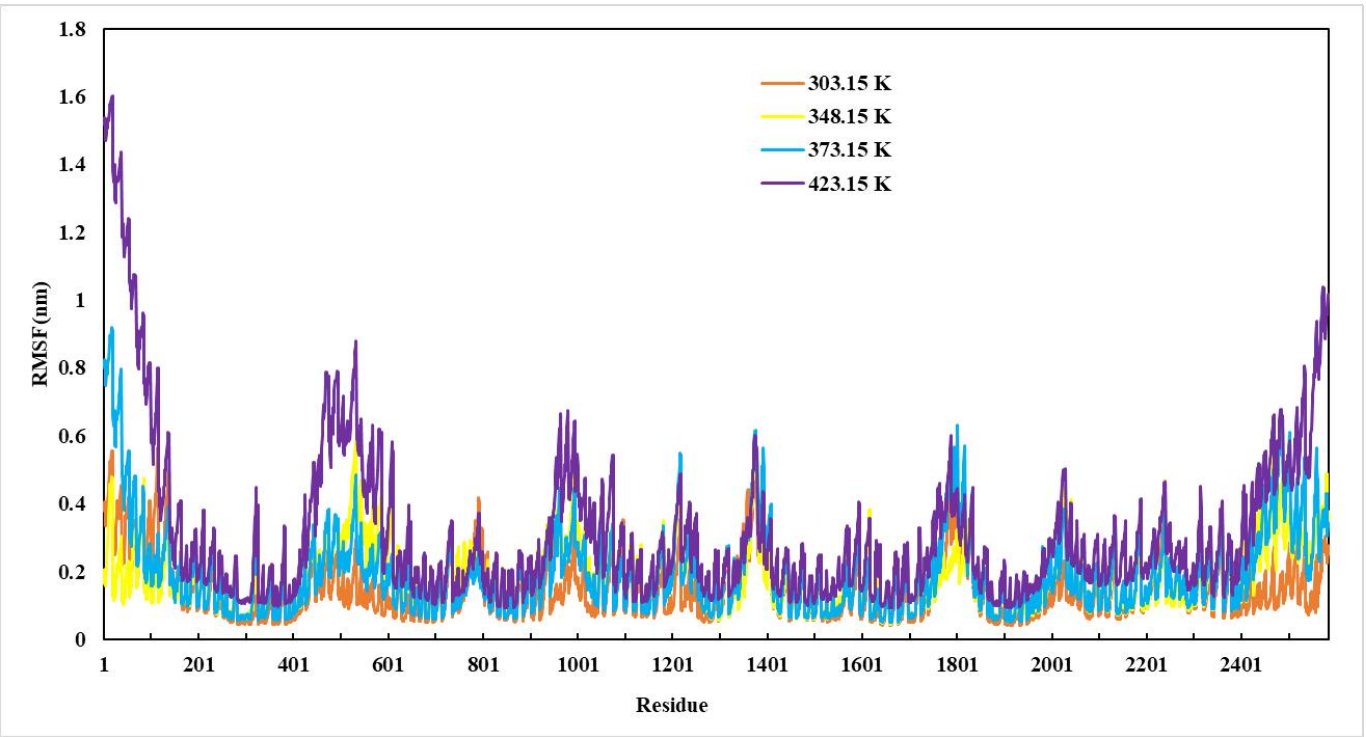

Figure 3. The RMSF of β-lactoglobulin under different temperature conditions

3.4 Solvent-Accessible Surface Area

Figure 4 illustrates the dynamic changes in the SASA of β-lactoglobulin at temperatures of 303.15 K, 348.15 K, 373.15 K, and 423.15 K. SASA reflects protein surface accessibility and hydrophobic effects, serving as a metric for the surface area of a molecule that is accessible to solvent molecules. An increase in SASA typically results from structural unfolding, dissociation, or conformational opening, which exposes more of the hydrophobic core to the solvent. Conversely, a decrease in SASA is often associated with structural compaction, folding, or binding events (30).

The results demonstrate that at 303.15 K (orange curve), the SASA remained at a relatively low level throughout the simulation, indicating limited contact area between the protein and the aqueous solution. At this temperature, β-lactoglobulin maintains a compact structure with hydrophobic groups predominantly buried within the interior of the molecule.

As the temperature increased to 348.15 K (yellow curve) and 373.15 K (blue curve), the SASA exhibited a gradual upward trend, suggesting that elevated temperatures progressively increase the contact area between the protein and solvent, leading to a gradual loosening of the β-lactoglobulin structure.

Upon reaching 423.15 K (purple curve), the SASA increased markedly, indicating a substantially larger contact area between the protein and the aqueous solution. This pronounced increase suggests that high temperature induces structural expansion of β-lactoglobulin, exposing hydrophobic groups originally buried in the interior and consequently enhancing solvent accessibility.

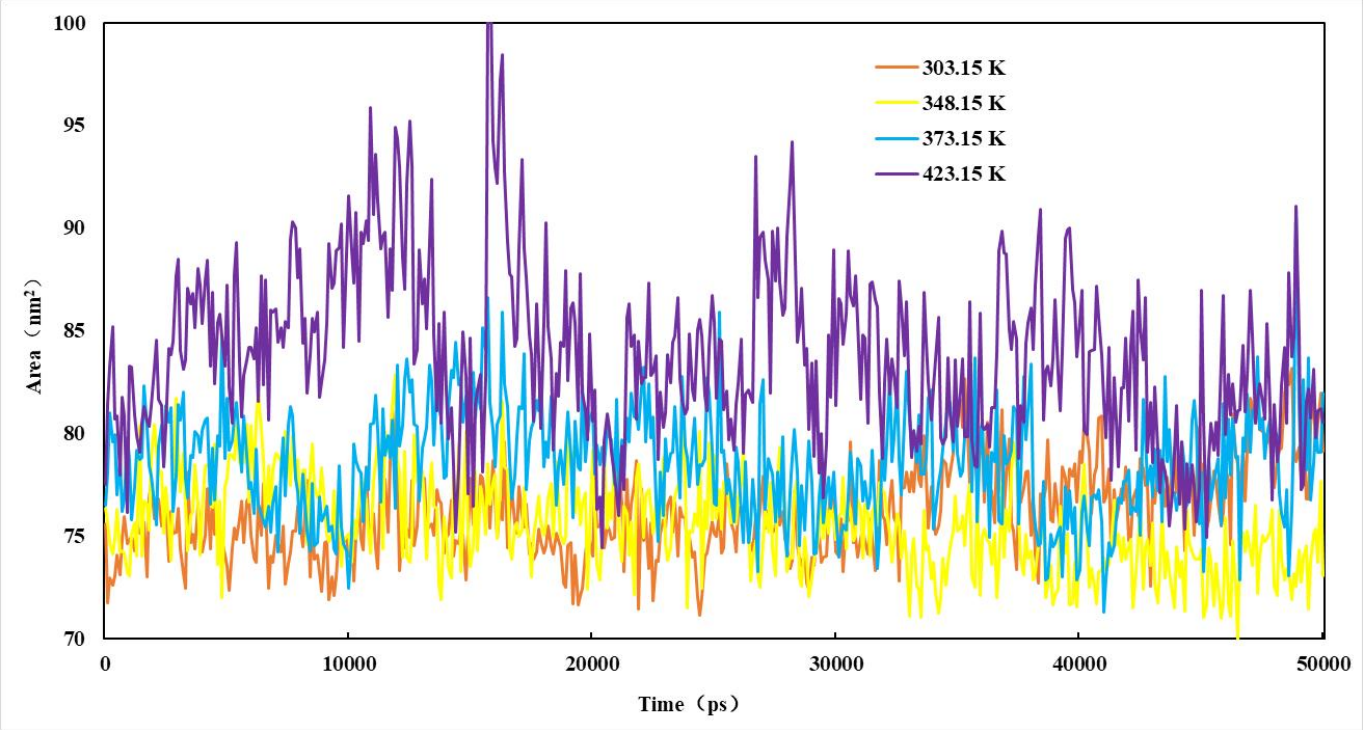

Figure 4. The SASA of β-lactoglobulin under different temperature conditions

3.5 Structural Evolution of β-Lactoglobulin

Figure 5 presents VMD visualizations depicting the thermal denaturation process of β-lactoglobulin across temperatures ranging from 303.15 K to 423.15 K. At low temperatures, the protein remains thermodynamically stable, maintaining its native conformation through balanced intramolecular interactions. As temperature increases, thermal energy progressively dominates, disrupting structural order and leading to conformational changes in the β-lactoglobulin molecule. Within the temperature range of 303.15 K to 373.15 K, the β-lactoglobulin structure exhibited loosening, but substantial overall conformational changes were not observed. However, upon reaching 423.15 K, the structure became notably compact, with reduced spatial occupancy and decreased atomic distribution dispersion. Certain regions displayed contraction tendencies, indicating that high temperature disrupted the original structural organization.

The findings reveal that at low temperatures, inter-domain interactions maintain spatial positioning and ensure functional integrity. At high temperatures (e.g., 423.15 K), visualization results unexpectedly showed structural compaction. Combined with Rg and SASA results indicating maximal protein loosening, this suggests a state of "compact loosening", wherein the protein adopts an overall contracted yet internally destabilized conformation. It is hypothesized that high temperature induces domain rearrangement, disrupts original spatial coordination, and potentially alters inter-domain positioning. RMSF analysis revealed intense amino acid residue motion at 423.15 K, which likely disrupts the secondary and tertiary structures of β-lactoglobulin, resulting in significant conformational changes. At this stage, β-lactoglobulin has entered a denaturation phase, accounting for the observed structural compaction. Such conformational alterations may impair molecular function, with biological activity potentially diminishing due to structural rearrangement, potentially leading to loss of β-lactoglobulin bioactivity and functionality.

Figure 5 illustrates the transition of β-lactoglobulin from a stable to an unstable state, clearly demonstrating the dynamic structural progression from relatively compact to loose and ultimately to compact conformation. These observations provide visual structural evidence for understanding the thermal stability and denaturation mechanism of β-lactoglobulin.

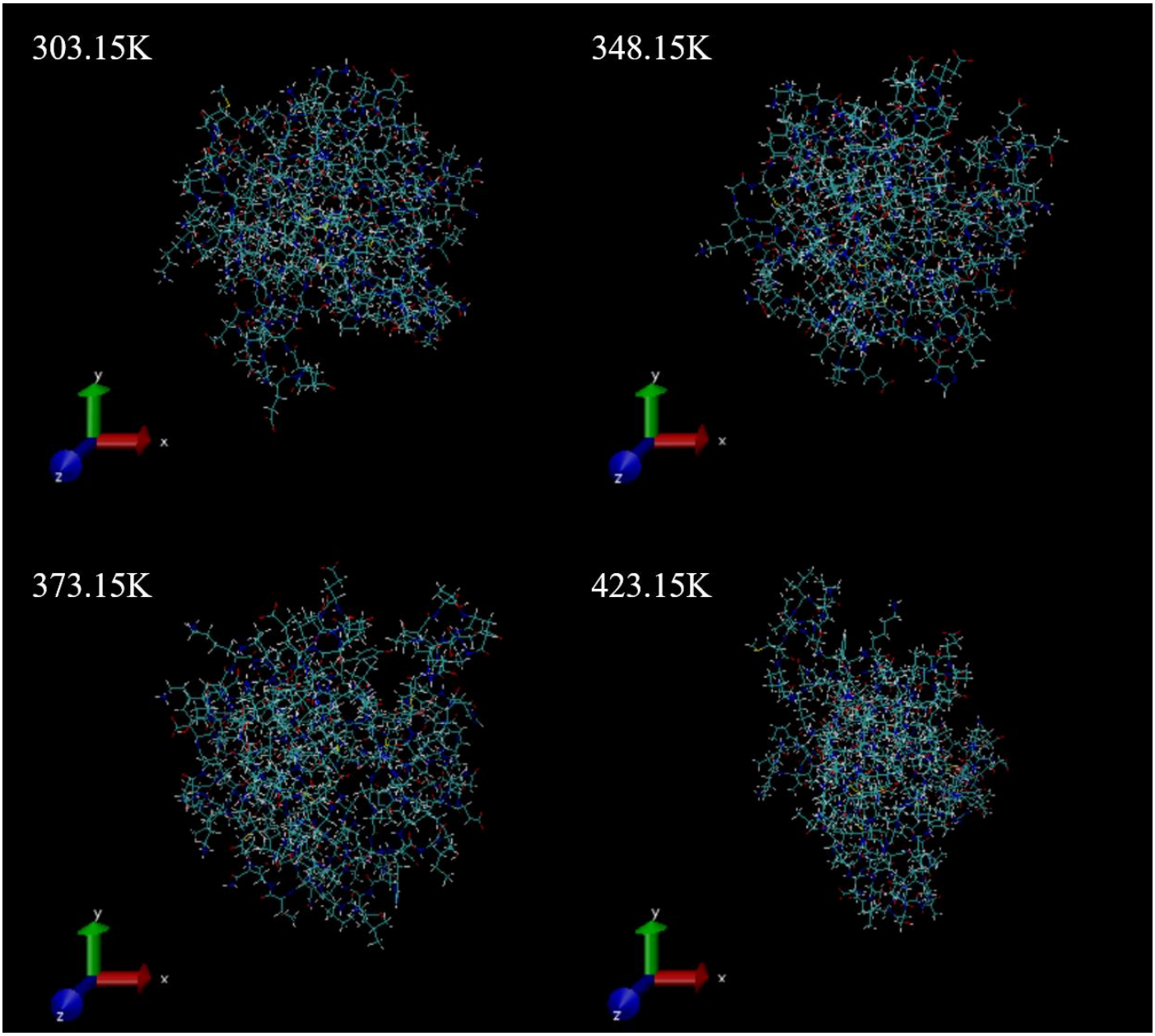

Figure 5. VMD visualization of β-lactoglobulin structural evolution at different temperatures

Overall, within the temperature range of 303.15 K to 373.15 K, the structural parameters of β-lactoglobulin exhibited certain variations with increasing temperature. However, the overall changes were not substantial, indicating that the protein structure remained essentially stable under these conditions. In contrast, upon reaching 423.15 K, significant alterations in the structural parameters were observed. The values of Rg, RMSF, RMSD, and SASA increased markedly with pronounced fluctuations, reflecting a sharp decline in the structural stability of β-lactoglobulin. Combined with the visualized structural models, these findings suggest that substantial thermal denaturation likely occurs at this temperature.

The results of this study elucidate the mechanism by which elevated temperature influences the conformation of β-lactoglobulin: increasing temperature intensifies the thermal motion of amino acid residues, leading to the progressive exposure of hydrophobic groups that were originally buried within the protein core.

4. Conclusion

This study has analyzed the impact of temperature, as employed in different sterilization methods, on the structure of β-lactoglobulin from a molecular perspective. Using MD simulations, the structural evolution of β-lactoglobulin was investigated

at four temperatures: 303.15 K, 348.15 K, 373.15 K, and 423.15 K. The structural parameters, Rg, RMSD, RMSF, and SASA, remained relatively low and exhibited minimal fluctuation within the lower temperature range (303.15 K–373.15 K). Corroborated by visualizations, this indicates that the β-lactoglobulin structure is relatively loose yet stable in this interval. In contrast, at the highest temperature (423.15 K), these parameters increased significantly and fluctuated markedly. Combined with visualizations showing a more compact protein structure, this suggests a deviation from the native stable state, characterized by intense amino acid residue motion, exposure of hydrophobic groups, and conformational changes indicative of potential protein denaturation. These findings demonstrate that within a certain temperature range, the effect on β-lactoglobulin is relatively minor, whereas excessively high temperatures can induce profound structural alterations.

The results imply that sterilization methods such as pasteurization (348.15 K) and boiling (373.15 K) may preserve the nutritional integrity of dairy products to a certain extent. In contrast, ultra-high temperature sterilization may compromise nutritional quality by inducing protein denaturation and aggregation. This research provides a crucial molecular-level foundation for understanding the mechanisms of protein structural changes during dairy processing. It offers theoretical support for optimizing dairy sterilization techniques, assessing the impact on food proteins, and guiding the application of proteins in food products. Given the complexity and diversity of actual dairy processing environments, which involve the interplay of factors such as temperature, pressure, and pH, future research should focus on developing multi-field coupling models. Such models would enable a comprehensive investigation of the synergistic effects of these factors on the structure and function of β-lactoglobulin, facilitating a deeper understanding of protein dynamics under various conditions. This would, in turn, provide more targeted theoretical guidance for industrial applications.

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