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Changes in soybean trypsin inhibitor by varying pressure and temperature of processing: A molecular modeling study

Sai Kranthi Vanga[a,*], Ashutosh Singh[b], Vijaya Raghavan[a]

[a] Department of Bioresource Engineering, Faculty of Agricultural and Environmental Sciences, McGill University, Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada
[b] Department of Biological Engineering, School of Engineering, University of Guelph, Guelph, ON N1G 2W1, Canada

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ABSTRACT

In recent years, molecular dynamic (MD) simulations have been used to understand the effects of various processing methods on the structural properties and stability of proteins. In this study, the conformational changes in soybean trypsin inhibitor molecule were evaluated with the use of high temperature and pressure. The MD simulations have been performed at various temperatures (300 K, 345 K and 373 K) and pressure (1 bar, 3 kbar, 6 kbar) combinations. The results showed that the soybean trypsin inhibitor (STI) molecule is relatively stable at high temperatures, primarily due to the presence of disulphide bonds. However, at higher pressures, significant compaction was observed. Root mean square deviations, Radius of gyration and volume of the STI molecule were evaluated. Furthermore, Ramachandran plots were used to evaluate the stability of the various simulated molecules. It was found that the compaction resulted in high steric interferences among the core residues.

1. Introduction

Proteins are one of the major nutrients present in the food and play a primary role in immune response, muscle formation and maintenance. They can also act as a vital source of energy to the human body in the absence of carbohydrates, providing 4 kcal per gram of protein (Phillips & Williams, 2011; Vanga, Singh, & Raghavan, 2017). They are also responsible for a diverse range of biological functions (Vanga et al., 2017; Whitford, 2013). Furthermore, proteins also play a major role in stability of food products due to their wide array of functional and sensory properties which are highly dependent on their structural integrity. They affect hydration, surface and rheological properties of foods which are dictated by the intrinsic structure of the proteins due to the constituent amino acid residues and protein interaction with surrounding molecules like water and salts which result in the formation of hydrogen bonds. These indirectly influence the stability of proteins (Singh, Vanga, Orsat, & Raghavan, 2017; Yada, 2017).

The functional properties exhibited by proteins are highly dependent on their secondary and tertiary structures which can change due to external stress which is of research interest for engineering proteins (Nakai, 1983). In the case of food proteins, various thermal and non-thermal processing methods act as a source of stress that can trigger structural changes and in turn functional property changes (Singh et al., 2015; Vanga et al., 2016; Vanga et al., 2017). These processing methods play a primary role in improving the shelf life and organoleptic properties of various food products by changing the reactivity and functional properties of enzymes and proteins. Furthermore, they are responsible in increasing the digestibility by reducing the content of antinutritional factors like protein inhibitors, phytic acids and oxalates (Vagadia et al., 2017; Vagadia, Vanga, Singh, Gariepy, & Raghavan, 2018).

Molecular dynamics (MD) simulation technique can be used as an alternative method to traditional experimental techniques for evaluating the secondary structural changes in various proteins. Experiments have already shown that the unfolding and denaturation of proteins is a 'slow process' that can vary widely from few seconds to hours depending on the level of physical and chemical stresses on the protein. And the direct application of MD simulations on protein denaturation and unfolding is not computationally feasible even with the current access to supercomputing infrastructure which could improve in probable future (Sarupria, Ghosh, García, & Garde, 2010; Woenckhaus et al., 2001). However, numerous studies have been performed to investigate the pressure and temperature induced structural transformations and corresponding changes in the functional properties. A study evaluated the structural changes in ubiquitin molecule due to increasing pressure. They observed that pressure changes can influence the water penetration capacity of the protein which is the primary driving force in protein denaturation (Imai & Sugita, 2010). Grigera and

* Corresponding author.
E-mail address: sai.vanga@mail.mcgill.ca (S.K. Vanga).

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McCarthy (2010) investigated the influence of pressure on lysozyme and apomyoglobin biomolecules. They found that hydrophobic interactions and the surface properties of the protein play a primary role in denaturation. At 3 kbar, they found that apomyoglobin molecule started to denaturize, whereas lysozyme molecule was still stable and showed native-like conformation due to the presence of four disulphide bonds (Grigera & McCarthy, 2010). In another study, the protein denaturation dynamics were evaluated using temperature and pressure deviations and used parameters like root mean square deviation (RMSD), radius of gyration (Rg) and hydrophobic surface interactions to evaluate the folding-unfolding kinetics of a protein fragment (Paschek & García, 2004).

In our study, we are evaluating the conformational changes in the STI molecule due to temperature and pressure deviations. The objective of this study is to provide insights into the stability of the STI protein during the application of non-thermal processing techniques like ultrasonication and high-pressure processing. On an experimental scale, high-pressure processing is a technique where pressures in the range of 3 kbar–9 kbar (~300 MPa–900 MPa) are applied to process foods for improving their physicochemical properties. It is reported that moderate pressures in the order of 1 kbar–2 kbar can result in unfolding of protein depending on the temperature. However, higher pressures (4 kbar–8 kbar) can result in tertiary and secondary structure changes which can be permanent and irreversible (Balny & Masson, 1993). These changes can result in enzyme inactivation as observed in various pressure treatments (Basak & Ramaswamy, 1996; Serment-Moreno, Barbosa-Cánovas, Torres, & Welti-Chanes, 2014).

However, understanding ultrasonication in terms of pressure is more indirect. High-energy ultrasonication is used in food and pharmaceutical industry which is the interaction of ultrasonic sound waves (16 kHz–100 kHz) with the biological material. The process-related effects, for example enzyme inactivation is observed due to a combination of stresses, primarily physical and chemical which is the result of cavitation (Islam, Zhang, & Adhikari, 2014). The phenomenon of 'cavitation' refers to the initiation, growth and collapse of tiny bubbles due to the ultrasound progression in the solution. The whole process is known to occur within few microseconds along with the collapse of the cavitation bubbles which are known to last less than a nanosecond. This collapse of the bubbles is generally violent and results in extremely high temperatures (1000 K–5000 K) and high pressure (50–1000 MPa) along with shear forces at the molecular level (Manickam & Rana, 2011; Mawson, Gamage, Terefe, & Knoerzer, 2011; Raviyan, Zhang, & Feng, 2005). Ultrasonication is widely considered as non-thermal processing technique despite the high temperature on the molecular scale due to enormous dissipation rates (10^{10} K/s) (Manickam & Rana, 2011; Suslick, McNamara III, & Didenko, 1996). To increase the inactivation efficiency and further reducing the processing time, researchers have used ultrasonic processing in combination with temperature called thermosonication, in combination with high-pressure (up to 200 MPa) called manosonication and combination of temperature and high pressure which is called manothermosonication. These methods are reported to be effective against various enzymes and proteins from dairy, nuts, fruits and vegetables like peroxidase, lipoxygenase (O'Donnell, Tiwari, Bourke, & Cullen, 2010). The MD simulations presented here are performed in known boundary conditions of temperature and pressure based on these theoretical understanding of ultrasonic and high-pressure processing. This can provide valuable insights in understanding the mechanism involved in denaturation of Soybean trypsin inhibitor due to temperature and pressure.

Conformational changes in soybean trypsin inhibitor (STI) molecule have been previously evaluated using the thermal stresses and oscillating electric fields. This study showed that the aromatic core of the STI molecule plays a primary role in the structural and functional stability of the molecule. A rise in surface area concerning the cysteine residues could result in disulphide bond stability (Vagadia, Vanga, Singh, & Raghavan, 2016). The experimental data revealed that the STI molecule is relatively stable due to the presence of a large number of 'disordered' β-sheets (Roychaudhuri, Sarath, Zeece, & Markwell, 2003, 2004). The changes in the conformation are evaluated in terms of RMSD, Rg, surface area and STRIDE analysis. Furthermore, Ramachandran plots are used to evaluate the final stability of the molecule after simulations.

2. Materials and methods

2.1. Molecular modeling

All the MD simulations presented in the study have been performed using the classical MD algorithm as implemented in Groningen machine for chemical simulations (GROMACS) software package, version 5.0.7 from the Stockholm Center for Biomembrane Research, Stockholm, Sweden (Van Der Spoel et al., 2005). The Kunitz-type trypsin inhibitor molecule (PDB Code: 1AVU) was downloaded from the Protein Data Bank (Berman et al., 2000; Song & Suh, 1998). The method to run the simulation was similar to the one described in Vanga et al. (2016) and (Vanga, Singh, & Raghavan, 2015) with few modifications mentioned below.

A total of nine MD simulations have been run using all combinations of temperatures 300 K, 345 K and 373 K and pressures 1 bar, 3 kbar and 6 kbar. Berendsen thermostat (Berendsen, Postma, Van Gunsteren, Dinola, & Haak, 1984) and Parrinello–Rahman barostat (Parrinello and Rahman, 1980, 1981) were used to maintain temperatures and pressures to the set values during each simulation. The data obtained was analysed using the GROMACS in-built analyzing tools. Visual molecular dynamics (VMD) software was used to evaluate the secondary structure deviations using the STRIDE algorithm (Humphrey, Dalke, & Schulten, 1996).

2.2. Ramachandran plot

The three dihedral angles in any protein chain are called the φ (phi), ψ (psi) and ω (omega) angles. Of these, the phi (between N-Cα) and psi (between Cα-C) angles play a primary role in determining the structural stability and rotation of backbone of the proteins. These angles are called the Ramachandran angles and are used to plot the Ramachandran plot (Ramachandran, Ramakrishnan, & Sasisekharan, 1963; Ramakrishnan & Ramachandran, 1965). The procedure used is similar to Vagadia et al. (2016) and MolProbity software is used to verify the stability of the structures generated (Chen et al., 2009; Davis, Murray, Richardson, & Richardson, 2004).

3. Results and discussions

3.1. Root mean square deviations (RMSD)

RMSD is one of the most common measures to evaluate the structural composition and integrity of simulated folding and unfolding of proteins. Specifically, in the case of MD simulations, RMSD is used in predicting the atomic deviations caused by external stresses (Budi, Legge, Treutlein, & Yarovsky, 2004; Kuzmanic & Zagrovic, 2010).

Table 1 summarizes the average RMSD values of the STI molecule under different combinations of temperature and pressure. The RMSD values decreased significantly from 0.269 ± 0.026 nm to 0.225 ± 0.019 nm when the pressure was raised from 1 bar to 6 kbar at 300 K. Similar reduction in the RMSD values was observed at different temperatures with increasing pressure. When simulations were performed at a constant pressure of 1 bar with only the temperature as a varying factor, the RMSD raised dramatically which was reported as 0.269 ± 0.026 nm, 0.292 ± 0.029 nm and 0.342 ± 0.031 nm at 300 K, 345 K and 373 K respectively. Similar unfolding in the molecule was observed at 3 kbar with RMSD increasing from 0.236 ± 0.022 nm to 0.266 ± 0.03 nm as temperature increased from 300 K to 345 K. On