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Effects of thermal and electric fields on soybean trypsin inhibitor protein: A molecular modelling study

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ABSTRACT

This study uses molecular dynamics (MD) simulations in investigating the unusual stability of Soybean Trypsin Inhibitor (STI) protein. The effects of temperature and oscillating electric fields (0.5 V/nm and 2.45 GHz) have been used to perform simulations using GROMACS software. The conformational changes in the protein were studies using root mean square deviations and secondary structure analysis (STRIDE). It was found that significant rearrangements took place within the protein especially in 'turns' and 'coils', but the core structure was stable under external stresses because of the antiparallel β-sheet structure. This study also provides evidence that the aromatic residues play a major role in stabilizing the STI protein using Solvent Accessible Surface Area (SASA) analysis. Ramachandran plots were also used to analyze the stability of the molecules obtained on treatment with temperatures (300 K to 393 K) and oscillating electric fields.

Industrial relevance: Molecular dynamics (MD) simulation techniques have been applied to visualize and predict the behaviour of proteins during food processing, and changes in their structure under the influence of external field stress.

This study can be used to understand the changes at molecular level that could help industrialists to design processing methods with increased nutritive and sensory appeal of food products.

This work can also contribute to the optimization of process parameters to enhance the functional properties of protein and increase protein digestibility.

1. Introduction

Proteins are one the most important nutrients present in food apart from carbohydrates and fats, which together are called the major nutrients. These proteins exhibit various functional properties which can be defined as the characteristic physicochemical properties that dictate the protein behaviour in foods during numerous stages of processing and storage. These properties are governed by the changes in the protein structure. Thus, the structure directly influences the functional properties that in turn influence various organoleptic and nutritional properties of the final food product (Messens, Van Camp, & Huyghebaert, 1997; Vanga, Singh, & Raghavan, 2015b). Various researchers have shown that processing affects the protein structures within the food matrix. From late 70s, both researchers and the food industry have been interested in technology to predict the structural changes of these food proteins that would help engineer high quality food products with superior nutritional output and digestibility, but only with limited success (Nakai, 1983).

During processing, external stresses are applied using thermal (boiling, roasting, infrared heating and dielectric heating) and non-thermal (fermentation, high pressure processing, pulsed electric field and high electric fields) processes. These processes help in increasing the shelf life of food products and also improve their organoleptic properties by producing conformational changes in the protein structure (Vangaet al., 2015b). They can also result in modified protein–protein interactions and protein–carbohydrate reactions through Maillard-type reaction contributing to the variation in functional properties of proteins in the final product (Messens et al., 1997). Therefore, researchers have put more emphasis in understanding the effects of processing on protein structures in recent years (Gomaa & Boye, 2015; Gomaa & Boye, 2013; Mozhaev, Heremans, Frank, Masson, & Balny, 1996; Schulz & Schirmer, 2013; Singh, Vanga, Nair, Gariepy, Orsat & Raghavan, 2015). Recently, several techniques including Fourier Transformation Infrared Spectroscopy (FTIR) (Singh, Lahlali, Vanga, Karunakaran, Orsat & Raghavan, 2016; Vanga, Singh, Kalkan, Gariepy, Orsat, & Raghavan, 2016), Nuclear

Abbreviations: MD, molecular dynamics; SASA, Solvent Accessible Surface Area; RMSD, Root Mean Square Deviation; STI, Soybean Trypsin Inhibitor; KTI, Kunitz-type Trypsin Inhibitor; NMR, Nuclear Magnetic Resonance Imaging; VMD, Visual Molecular Dynamics; FTIR, Fourier Transformation Infrared Spectroscopy; PDB, Protein Data Base; PME, Particle Mesh Ewald; NVT, ensemble in which number of atoms, volume and temperature are constant; NPT, ensemble in which number of atoms, pressure and temperature are constant; DSSP, Define Secondary Structure of Protein.

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Magnetic Resonance Imaging (NMR) (Kainosho et al., 2006), X-ray diffraction (Engh & Huber, 1991; Frauenfelder, Petsko, & Tsernoglou, 1979) and Circular Dichroism (CD) (Provencher & Gloeckner, 1981; Sreerama & Woody, 2000) have been used to study and understand the protein structures. However, they have various limitations depending on the technique used and are very expensive. Moreover, these techniques can only assess the protein structure before and after processing which further complicates the means of evaluating the mechanism involved in protein structural changes in various complex biological structures (Astrakas, Gousias, & Tzaphlidou, 2012; Vanga, Singh, & Raghavan, 2015a).

Molecular dynamics (MD) simulation techniques can act as a viable alternative. It can be applied to overcome the abovementioned issues and can be used to further understand the conformational changes in the protein. The effects of electric fields have been evaluated on chignolin which is the smallest protein stable in solution form using MD simulations. This study concluded that by applying enough external stress the ten residue chignolin protein rotated and aligned itself in the direction of external electric field. With a continuous application of the stress the protein unfolded with changes in the total dipole moment (Astrakas, Gousias, & Tzaphlidou, 2011a, 2011b; Astrakas et al., 2012). Studies by Wang, Li, He, Chen, and Zhang (2014) showed that the secondary structure of protein (insulin) was intact under the electrical field strength below 0.15 V/nm. Disruptions in the structure were observed on application of electric field strengths of 0.25 V/nm or higher. The results also showed that intensity of the external electric field could either speed up protein folding or destroy the secondary structure of proteins (Wang et al., 2014). Marracino, Apollonio, Liberti, d'Inzeo, and Amadei (2013) have also used MD simulations in understanding the effects of pulsed and static electric fields on protein folding and unfolding in myoglobin. They used electric fields in the range of 1–10 V/nm and found that fields in the range of 10 V/nm have produced

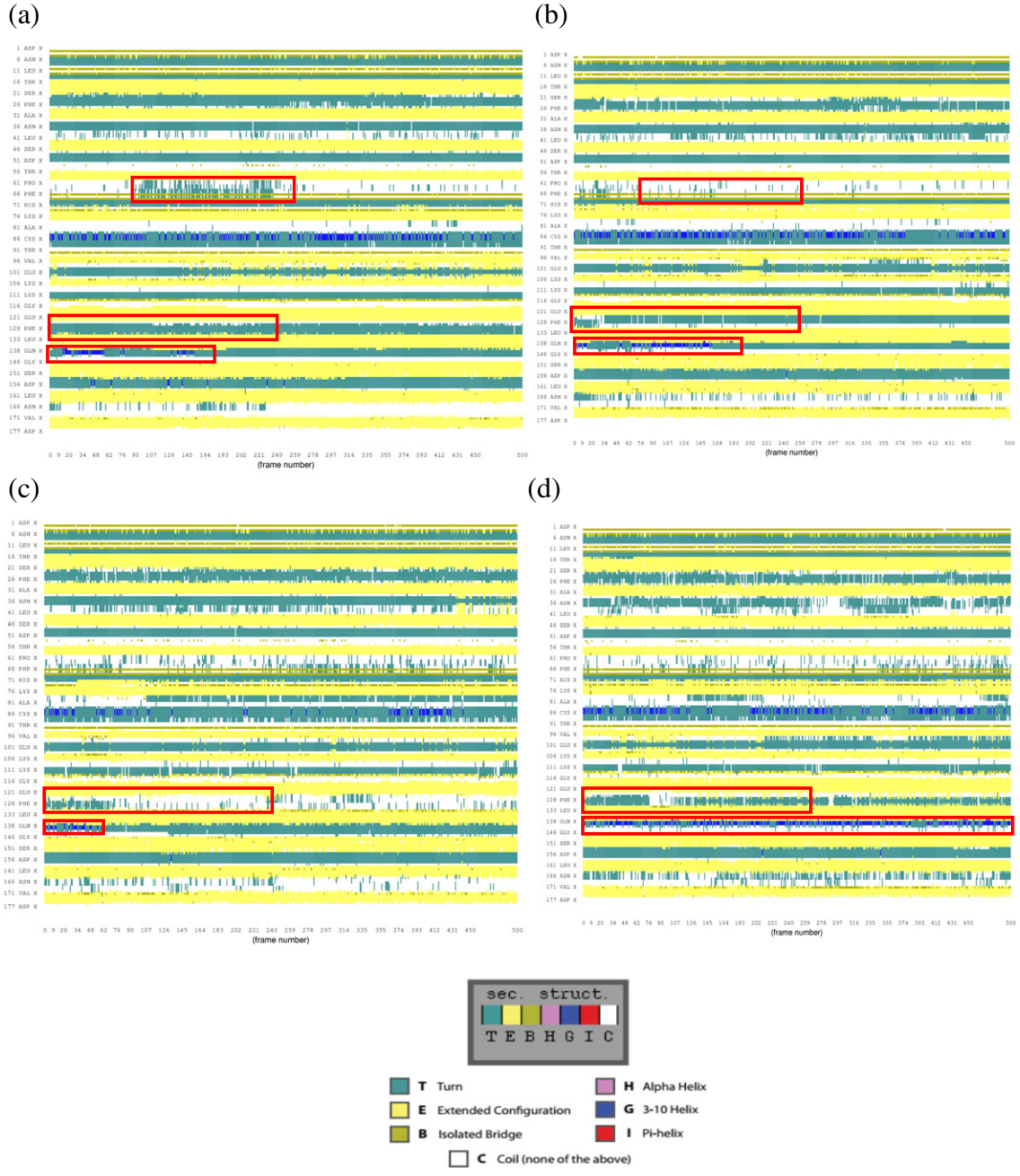

Fig. 1. STRIDE analysis showing the deviations in 1AVU with no electric field. (a) 300 K, (b) 343 K, (c) 373 K, (d) 394 K.