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journal homepage: www.elsevier.com/locate/ifset

Effect of thermal and electric field treatment on the conformation of Ara h 6 peanut protein allergen

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ARTICLE INFO

Article history:

Received 13 August 2014

Received in revised form 31 December 2014

Accepted 2 March 2015

Available online xxxx

Keywords:

Peanut allergen

Root mean square deviation

Ara h 6

Radius of gyration

Molecular dynamic simulation

ABSTRACT

The study uses molecular dynamic simulation to evaluate the effect of static and oscillating electric field (2450 MHz) of intensity 0.05 V/nm at different temperatures 300 K, 380 K and 425 K on structural conformation of Ara h 6 peanut protein allergen. The conformational changes in the protein were studied with respect to root mean square deviation, radius of gyration, dipole moment and solvent accessible surface area. The increase in temperature and application of external electric fields, both static and oscillating fields had significant effect on the conformation of Ara h 6, specifically the helical secondary structures. It was observed that the root mean square deviation increased with a rise in temperature and application of external electric fields had no significant effect on it at any given temperatures. This study also demonstrated that exposure to external stresses including thermal and electric fields induces conformational changes in the protein structure, which may impact its physico-chemical properties.

Industrial relevance:

- The work was performed to understand the influence of food processing on protein and the changes in their structure using molecular modeling concept.
- Molecular Dynamics Simulations have been applied to visualize the folding and unfolding of the protein structure depending on the amount of stress applied on the system.
- This work can help in modification and optimization of process parameters (like temperature and time) to enhance the protein functional properties and digestibility in the end product.
- Increasing digestibility would automatically result in higher nutritional absorption in the body, decreased immunoreactivity and overall better nutritional quality.

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1. Introduction

Food allergy is defined as an immune response towards a particular protein or a group of proteins present in a food. Although, any food may cause an immune response, peanuts, tree nuts, milk, soybean, wheat, egg, fish and shellfish are responsible for most of the food allergies around the world (Scott H. Sicherer & Sampson, 2006). It has been

estimated that approximately 3% of the adult population and 3–8% of the infants are allergic to one or more foods and this number is growing (Rona et al., 2007; Venter et al., 2008; Zuidmeer et al., 2008). Several researchers have suggested that genetic predisposition (Arshad, Stevens, & Hide, 1993; Sampson, 2004) could also be another major issue. The inter-exchange between various cultures confers dissipation of various forms of cuisines increasing the diversity in food available for consumption. This exposure to numerous forms of food has been indicated as one of the reasons for an increase in population that suffer from food allergies (Helm & Burks, 2000b). Moreover, hygiene hypothesis, which states that excessive cleanliness around an individual interrupts with the normal development of immune system because of lack of external environmental triggers may also lead to an increase in susceptibility to allergens related to environment and food (Guarner et al., 2006; Helm & Burks, 2000a; Yazdanbakhsh, Kremsner, & van Ree, 2002).

In North America, peanuts are one of the major sources of food allergy. Sicherer, et al. (Scott H. Sicherer, Muñoz-Furlong, Godbold, &

Abbreviations: IgE, Immunoglobulin E; SASA, Solvent Accessible Surface Area; RMSD, Root Mean Square Deviation; SR, Systemic Allergic Reaction; OAS, Oral Allergy Syndrome; NMR, Nuclear Magnetic Resonance Imaging; MD, 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; PPI, Peanut Protein Isolate.

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<http://dx.doi.org/10.1016/j.ifset.2015.03.003>

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Please cite this article as: Vanga, S.K., et al., Effect of thermal and electric field treatment on the conformation of Ara h 6 peanut protein allergen, *Innovative Food Science and Emerging Technologies* (2015), <http://dx.doi.org/10.1016/j.ifset.2015.03.003>

Sampson, 2010) estimated that about 1% of the population in United States of America is sensitive to peanuts. They also suggested that over the years there has been an overwhelming increase from 0.6% in 1999 to 2.1% in 2008 in the younger population (<18 years) who are affected by sensitivity towards peanuts (Scott H. Sicherer et al., 2010). Within Peanuts, there are about 13 protein allergens recognized by The International Union of Immunological Societies Nomenclature subcommittee, (*Arachis hypogaea* – Ara h) Ara h 1 to Ara h 13, of which Ara h 1, 2 and 3 are major peanut allergens (Breiteneder & Radauer, 2004; Burks et al., 1992; Sampson, 2004; Schmitt, Cheng, Maleki, & Burks, 2004; Scott H. Sicherer & Sampson, 2007). These allergens of peanuts cause immunoglobulin E (IgE) - mediated immune responses. The portion of the allergen, which is recognized by IgE is called an epitope. These epitopes consist of linear contiguous stretch of amino acids or three dimensional structural motif that crosslink with IgE triggering an allergenic response in a sensitized individual (Sathe & Sharma, 2009).

The allergenicity of a specific compound can be defined as its ability to introduce allergic response and it may vary with varying environmental conditions. In food, proteins are found within a complex matrix where its interaction with other food components such as carbohydrates may alter its functionality and make it allergic (S. H. Sicherer & Sampson, 2010; Clare Mills, Sancho, Rigby, Jenkins, & Mackie, 2009). Processing of food also impacts proteins and alters their digestibility and conformation, which alters their ability to induce allergenic responses (S. H. Sicherer & Sampson, 2010). In recent years several studies have revealed that food processing can also play an alternative role in moderating the allergic response of food proteins (Clare Mills et al., 2009; Cucu, De Meulenaer, Bridts, Devreese, & Ebo, 2012; Thomas et al., 2007). In 2012, Cucu et al. (Cucu et al., 2012) studied the impact of thermal processing and glycation on the basophil activation by hazelnut proteins. They reported that thermal processing of hazelnut protein in the presence or absence of wheat protein had no significant effect on the stimulatory activity of basophil for patients with systemic allergic reaction (SR) or oral allergy syndrome (OAS). They also observed that incubation of hazelnut protein with glucose completely suppressed the stimulatory activity of basophil in OAS patients. They concluded that SR patients were more susceptible to allergic reaction for both processed and unprocessed hazelnut protein as compared to OAS patients. In another study Yang et al. (Yang, Mwakatage, Goodrich-Schneider, Krishnamurthy, & Rababah, 2012) applied pulsed ultraviolet light on raw and roasted peanuts and peanut buttery slurry to reduce peanut allergenicity. Their SDS-PAGE and ELISA analysis revealed that with increase in treatment time the allergenicity of peanut protein reduced compared to control. Hence it can be assumed that by introducing novel food processing techniques allergenicity of food proteins can be altered and reduced to a significant level.

It is well known that during processing food components undergo several chemical and physical changes; hence protein subjected to external stresses such as thermal, chemical and electrical stresses may lead to conformational changes to the molecular structure (Singh, Orsat, & Raghavan, 2013b). The functional properties of proteins depend on their structure and any change in it may alter the functionality (Singh, Munshi, & Raghavan, 2013a). In recent years several studies have been conducted to understand the impact of processing on protein conformation and its relation to the functional properties (Clare Mills et al., 2009; Davis, Smales, & James, 2001; Mondoulet et al., 2005; Singh, Orsat, & Raghavan, 2012; Singh et al., 2013a, 2013b). Several techniques including Fourier Transformation Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance Imaging (NMR), X-ray diffraction can be used to study the conformational changes in protein during processing, but these techniques are expensive and can only relate the structure of proteins before and after processing. To overcome these shortcomings Singh et al. (Singh et al., 2013b) applied molecular dynamic (MD) simulation

technique to gain insight into protein dynamics under the influence of external static electric field. MD simulation technique is widely used to study the structural and dynamic properties of biomolecules and has been applied in the field of pharmacology and molecular biology for development of accurate and novel drug systems.

In this study, the conformational changes in Ara h 6 peanut protein allergen under the influence of external stresses including thermal and static/oscillating electric field have been evaluated. Ara h 6 is selected as the model protein for this study because it is an important allergen in peanuts and is approximately 59% homologues with the Ara h 2, which is regarded as a major peanut allergen. Several researchers have reported that Ara h 2 and Ara h 6 allergens are harder to digest in humans compared to the Ara h 1 or Ara h 3 (Koppelman, Hefle, Taylor, & De Jong, 2010; Koppelman et al., 2005). Moreover, recent studies have also suggested that allergic reactivity to various isoforms of Ara h 6 can be observed in the growing allergic population (Bernard et al., 2007; Flinterman et al., 2007; Peeters et al., 2007). The isoforms are the same protein but with a slight disparity in the sequence i.e., a very similar duplicate. In 2007, Bernard et al., (Bernard et al., 2007) found an isoform of Ara h 6 allergen and its derivative compound from peanut protein that were as reactive or more reactive than that of Ara h 2. All the aforementioned findings suggested that studying the behavior of Ara h 6 under the influence of external stresses might provide an insight on how various food processing techniques might affect their allergenic properties of peanut proteins.

Hence, in this study emphasis has been given to the quantification of conformational change using root mean square deviation, radius of gyration, dipole moment and its effect on surface hydrophobicity and hydrophilicity of Ara h 6.

2. Materials and methods

MD simulation on Ara h 6 was performed using a classical MD algorithm as implemented in Groningen machine for chemical simulations (GROMACS) software package, version 4.5.5 from the Stockholm Center for Biomembrane Research, Stockholm, Sweden (Hess, Kutzner, Van Der Spoel, & Lindahl, 2008). Peanut allergen Ara h 6 consists of 127 amino acid residues, where more than 40% of the secondary structure consists mainly of helices (6 helices) and one 3/10 helix. Ara h 6 starting configuration with the Protein Data Bank (PDB) (Berman et al., 2000) accession code 1W2Q (Lehmann et al., 2006) was used for this study. All atom CHARMM27 force field was used to describe the potential energy and provide functions and parameters for every type of atom in the system (Astrakas, Gousias, & Tzaphlidou, 2012). The protein configuration was enclosed in a periodic cubic water box of dimensions 10.215 X 10.215 X 10.215 (nm) containing 34838 water molecules to satisfy the minimum image convention. The water model selected for this study was TIP3P and three sodium ions were added to neutralize the system. The neutral solvated protein system was first energy minimized with converging criterion of maximum force value of 10 kJ/nm/mol using steepest descent for 20000 steps and equilibrated to constant volume (NVT) and temperature (NPT) for 200 ps.

During the MD simulation the temperature was maintained using Berendsen thermostat and pressure was set at 1 bar. The constant temperature and pressure coupling was 0.1 ps and 2 ps respectively and to limit the short-range non-bonded interactions, van der Waals interaction and long-range electrostatic interactions, a cut-off of 1 nm was used. PME algorithm was used with grid spacing of 0.16 nm and the time step during the simulation was 2 fs. A total of nine MD simulations were run to evaluate the effect of temperature 300 K (27 °C), 380 K (107 °C) and 425 K (151 °C) static electric field of intensity 0.05 V/nm and oscillating electric field of intensity 0.05 V/nm and frequency of 2450 MHz. All the electric fields were applied at the x axis of the equilibrated solvated protein system.

The thermal treatment conditions were chosen to repeat the conditions of study performed by Beyer et al. (Beyer et al., 2001). The electric field conditions were selected to simulate microwave assisted processing conditions. All simulations were run for 1 ns and the results obtained were sufficiently distinct to conclude the observation.

The effect of temperature and external electric field on secondary structure of the Ara h 6 protein was characterized using STRIDE algorithm implemented in visual molecular dynamics (VMD) (Humphrey, Dalke, & Schulten, 1996). Root mean square deviation (RMSD) of backbone atoms was calculated with starting structure as a reference. Also radius of gyration (Rg), which is defined as the root mean square distance from each atom of the protein to their centroid was calculated as indication of conformational changes in the protein. Surface hydrophobicity and hydrophilicity of the system were also estimated to evaluate the effect of temperature and external electric field on the surface properties of the protein. Changes in these parameters were studied using GROMACS analyzing tools. The snapshots of protein conformational change were taken using VMD.

3. Results and discussions

The main purpose of this study was to examine the effect of thermal treatments at 300 K, 380 K and 425 K with or without the application of static or oscillating electrical fields on conformation of Ara h 6 protein (Fig. 1). The aforementioned temperatures were selected to compare the results with those obtained by Beyer et al. (Beyer et al., 2001). They reported that boiling peanuts in water at 100 °C for 20 min or frying them in vegetable oil for about 5–10 min moderated the IgE binding intensity of peanut protein allergens as compared to roasting them at 170 °C for about 20 min. The modifications to the IgE binding properties of the peanut caused during the processing may be due to the conformational deviations in the structure.

3.1. Secondary structure analysis

VMD STRIDE algorithm was used to study the effect of temperature and external static and oscillating electric field on secondary structure of Ara h 6 (Fig. 2). Ara h 6 allergen is a fraction of a large protein present in peanuts called the 2S Albumin. Apart from the Ara h 6, Ara h 2 and Ara h 7 are also present in the seed storage proteins of the peanuts and are members of the 2S Albumin family (Lehmann et al., 2006). All these allergens belong to prolamine protein superfamily (Kreis, Forde, Rahman, Miflin, & Shewry, 1985) which also includes α -amylase/trypsin inhibitors, nsLTP's (non-specific Lipid Transfer Proteins) (Shewry, Beaudoin, Jenkins, Griffiths-Jones, & Mills, 2002). 2S albumin is a protein with its

molecular mass ranging from 12 to 15 kDa (Lehmann et al., 2006). The Ara h 6 has a molecular weight calculated to be about 14.5 kDa (Koppelman et al., 2003, 2005) and comprises of 127 amino acids, where more than 44% of the secondary structure consists of helices (five α -helices and one 3/10 helix) (Fig. 2 (a)). The five α -helices are formed between the residues 16–20 (CYS-VAL), 26–33 (LYS-MET), 53–66 (SER-GLU), 74–84 (MET-ASN) and 95–109 (VAL-ASN) residues of the allergen molecule and the 3/10 helix is between 35 and 37 (ARG-MET) residues.

The STRIDE analysis is done to understand the changes that take place within the protein structure during the period of simulation. This analysis provides the secondary structural variations in the protein subjected to stress corresponding to the time period for which the simulation is run in the form of a chart (X axis: the residues and Y axis: Frames/Time). When these charts are observed as in Fig. 2, it can be seen that as the simulation time progresses representing the increasing stress on the protein secondary structures, changes in the color are observed which represent the variations in the specific secondary structure. The STRIDE analysis aids in observing the change in secondary structure, the residues involved in this secondary structure variations and the exact time when the change was initiated can also be pointed out. Moreover, the comparison between the initial structural representations and the final will provide us with the secondary structures that deviated the most during the course of simulation with application of known external stresses.

It can be observed from Fig. 2(a) and (b) that temperature of 300 K had no significant effect on the secondary structure of the protein with or without the application of static electric field (0.05 V/nm), but application of oscillating electric field of 2.45 GHz, 0.05 V/nm (Fig. 2(c)), resulted in conversion of α -helix (CYS (residue: 16)-VAL (residue: 20)) into 3/10 helix and turns. As the temperature was increased to 425 K from 380 K in the absence of external electric field, significant changes in the secondary structure of the protein were observed (Fig. 2. (d)). The most significant change was the transformation of the α -helix (SER (residue: 53)-GLU (residue: 66)) into a π -helix apart from the turns and 3/10 helices for a brief period of time during the simulation. Similar results were obtained when external static and oscillating electric field were applied at 425 K, the α -helix (SER (residue: 53)-GLU (residue: 66)) transformed into 3/10 helices and turns. Thus, it is clear that the stresses caused by the increasing temperature and electric field (static and oscillating) have an effect on the secondary structure of the Ara h 6 allergen molecule.

3.2. Root mean square deviation (RMSD)

The root mean square deviation provides the arithmetical value of the deviations in the structure of the molecule. The RMSD is calculated by evaluating the changes in the structure with a reference molecule that is normally the control (not under any external stress) (Budi, Legge, Treutlein, & Yarovsky, 2004). RMSD of the protein can be represented using the following equation (Singh et al., 2013b):

$$RMSD = \sqrt{\frac{1}{N} \sum_{i=1}^N |r_{final}(i) - r_{initial}(i)|^2}$$

Where $r_{final}(i)$ is the final coordinates of an atom i , and $r_{initial}(i)$ is the initial coordinate of the atom i , and N is the number of atoms.

It was observed that the application of temperature had a significant effect on the RMSD value of the Ara h 6 allergen. There was a significant difference in values of RMSD obtained as the temperature increased from 300 K to 380 K (Fig. 3 (a)). When the temperature was further raised to 425 K, it was observed that the RMSD values obtained were similar to that of 380 K.

From Table 1 it can be concluded that application of static and oscillating electric field had no significant effect on the RMSD values at 300 K

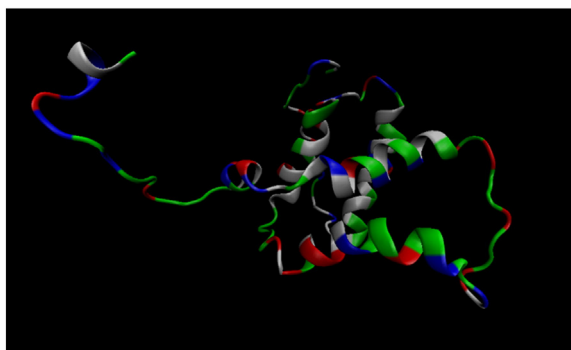


Fig. 1. The three dimensional structure of the Ara h 6 allergen protein.

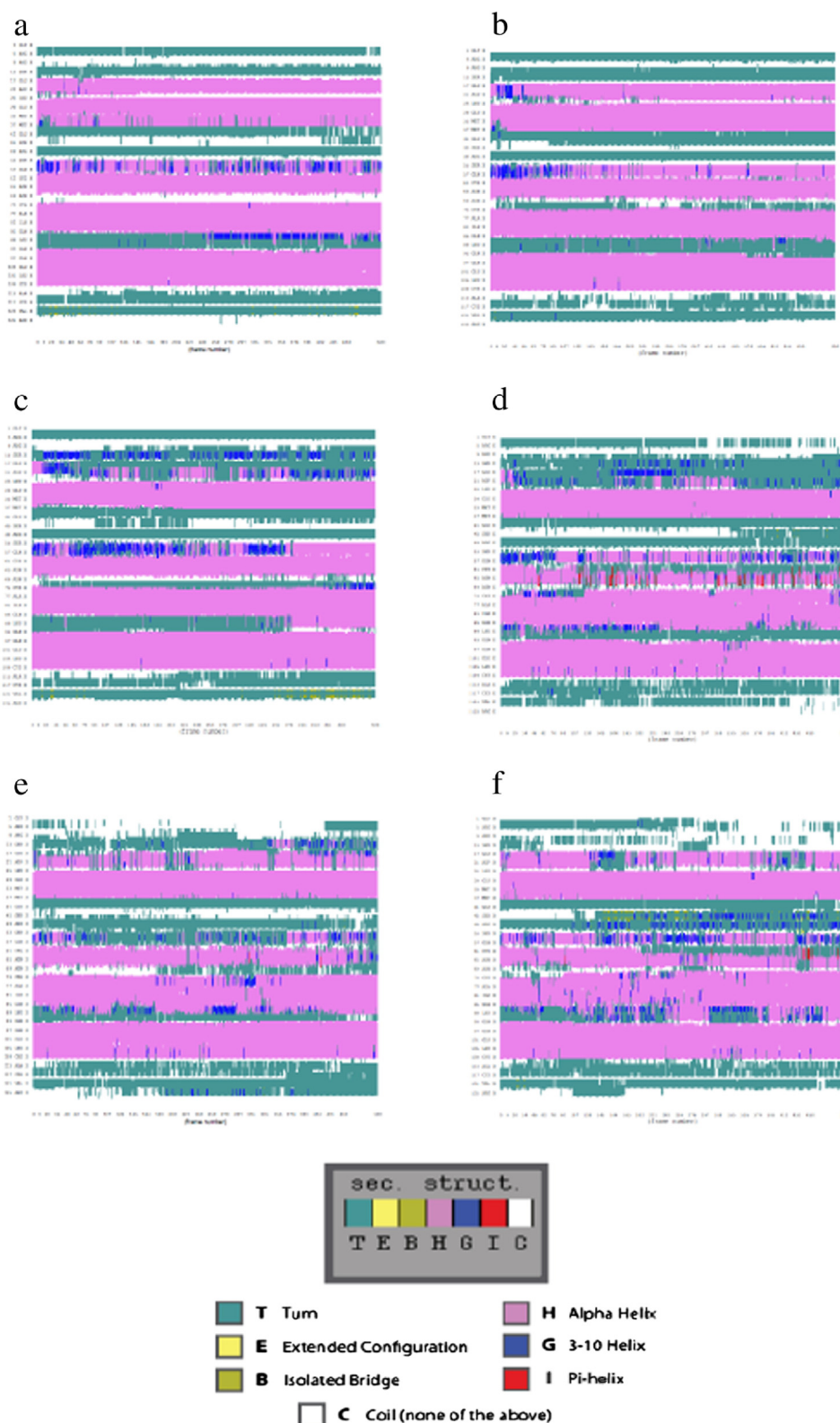


Fig. 2. STRIDE analysis showing the evolution of molecule (a) 300 K, (b) 300 K, static electric field of 0.05 V/nm, (c) 380 K oscillating electric field of 0.05 V/nm at 2450 MHz, (d) 425 K, (e) 425 K, static electric field of 0.05 V/nm (f) 425 K, oscillating electric field of 0.05 V/nm at 2450 MHz. Note: Color code: Magneta denotes α (alpha)-helix, red denotes π (pi)-helix, cyan denotes turn, blue denotes 3–10 helix and white denotes coil. (The X-axis shows the residue numbers and names (from 1 to 127) and the Y-axis shoes the time frames (1 frame = 2 ps) of the simulation). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and 380 K when compared to control. At 425 K static and oscillating electric field had higher RMSD values compared to the control (Fig. 3 (b–c)). These results confirm the STRIDE analysis of secondary structure

in which it was observed that secondary structures changed as the temperature rose from 300 K to 425 K under both the static and oscillating electric fields.

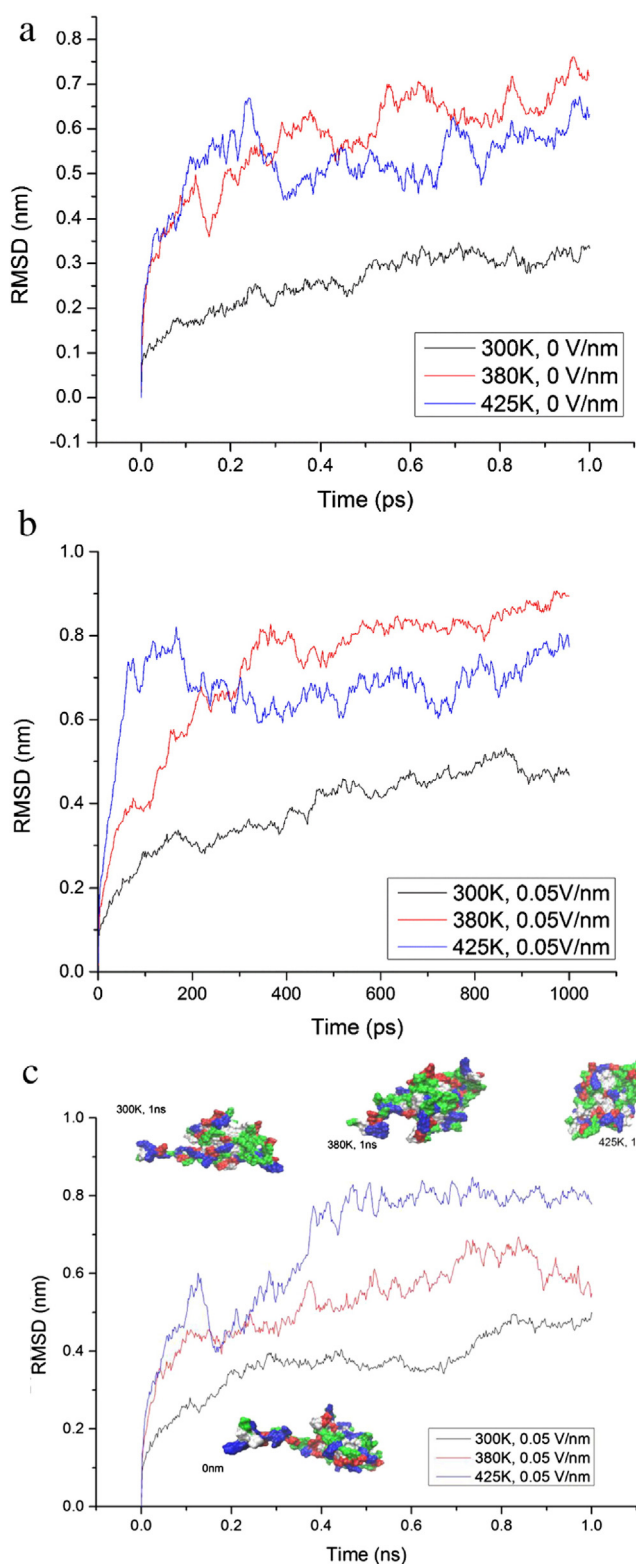


Fig. 3. RMSD variations observed in Ara h 6 Allergen (a) heat treatment, (b) heat treatment + static electric field, (c) heat treatment + oscillating electric field of 2450 MHz.

3.3. Radius of gyration (R_g)

Radius of gyration is defined as the measurement of the overall spread of the atoms in a given molecule with respect to its center of mass (Budi et al., 2004; Singh et al., 2013b). It was observed that there was a significant reduction in radius of gyration of the Ara h 6 molecule

Table 1

RMSD values obtained for Ara h 6.

Temperatures (K)	No electric field (nm)	Static electric field (0.05 V/nm) (nm)	Oscillating electric field (0.05 V/nm, 2450 MHz) (nm)
300	7.281 ± 2.420	7.895 ± 3.750	6.808 ± 2.428
380	10.557 ± 4.300	10.771 ± 4.755	10.976 ± 4.644
425	10.492 ± 4.113	11.391 ± 3.393	11.309 ± 3.849

at temperatures 380 K and 425 K compared to the control temperature of 300 K. Application of external electric field provided similar observations (Fig. 4 and Table 2). Radius of gyration for the protein can be calculated by using the following equation (Singh et al., 2013b):

$$R_g = \sqrt{\frac{1}{N} \sum_{i=1}^N |r(i) - r_{center}|^2}$$

where $r(i)$ is the coordinates of an atom i and r_{center} is the coordinates of the protein's center of mass, N is the number of atoms.

Lobanov, Bogatyreva, and Galzitskaya (2008) suggested that radius of gyration for a protein can act as an indicator for the compactness of atomic packing (Lobanov et al., 2008). They reported that lower the radius of gyration of the molecule the more packed is the structure. In 2012, Liu et al., (Liu, Zhao, Zhao, Ren, & Yang, 2012) indicated that the peanut protein isolate (PPI) had compacted on dry-heating. In their work, they suggested that the mixture and conjugates of PPI and Dextran when subjected to dry-heat had more compaction and that they had exhibited better functional properties. In the current case of Ara h 6 allergen, with the application of temperature and external electric fields, a compaction in the molecule was observed. The effects of this compaction on the functional properties and their clinical usability have to be studied further.

3.4. Dipole moment

The Dipole moment of a protein molecule occurs because of its secondary structure conformations. With an application of external electric field, the protein tries to align itself in the direction of the applied field (Singh et al., 2013b). Following is the equation used for representing the dipole moment of protein (Singh et al., 2013b):

$$\vec{d} = \sum_{i=1}^n q_i(i) \vec{r}_i$$

where $\vec{d}$ is the dipole, q_i is the charge of the atom i , $\vec{r}_i$ is the directional vector of each atom and N is the number of atoms.

From Fig. 5(a) and Table 3 it can be observed that the dipole moment of the Ara h 6 protein has reduced significantly with the electric field. Previous research has shown that the helical structures in the protein have their own dipole moment, which contribute to the overall dipole moment of the protein molecule (Budi et al., 2004). In the case of Ara h 6 allergen, the STRIDE analysis performed has clearly shown that the α -helices present were converted to 3/10 helices and turns with the application of temperature (425 K) in the simulation as shown in Fig. 1. The deviations in helical structures would result in a change in their individual dipole moments in return causing an alteration in the total dipole moment of the molecule.

Fig. 5(b) shows the relation between the RMSD and the dipole moment of the Ara h 6 molecule at a temperature of 425 K in an oscillating field of 0.05 V/nm. It can be observed that the molecule underwent a significant change in terms of the RMSD and total dipole moment in the time period of 0.13 ns to 0.34 ns (Fig. 5(b)). The snapshots of molecule of Ara h 6 has shown a considerable amount of change and thus the variation in both the RMSD and Dipole moments are to be observed in the figure. The RMSD values increased from about 0.43 nm to 0.75 nm

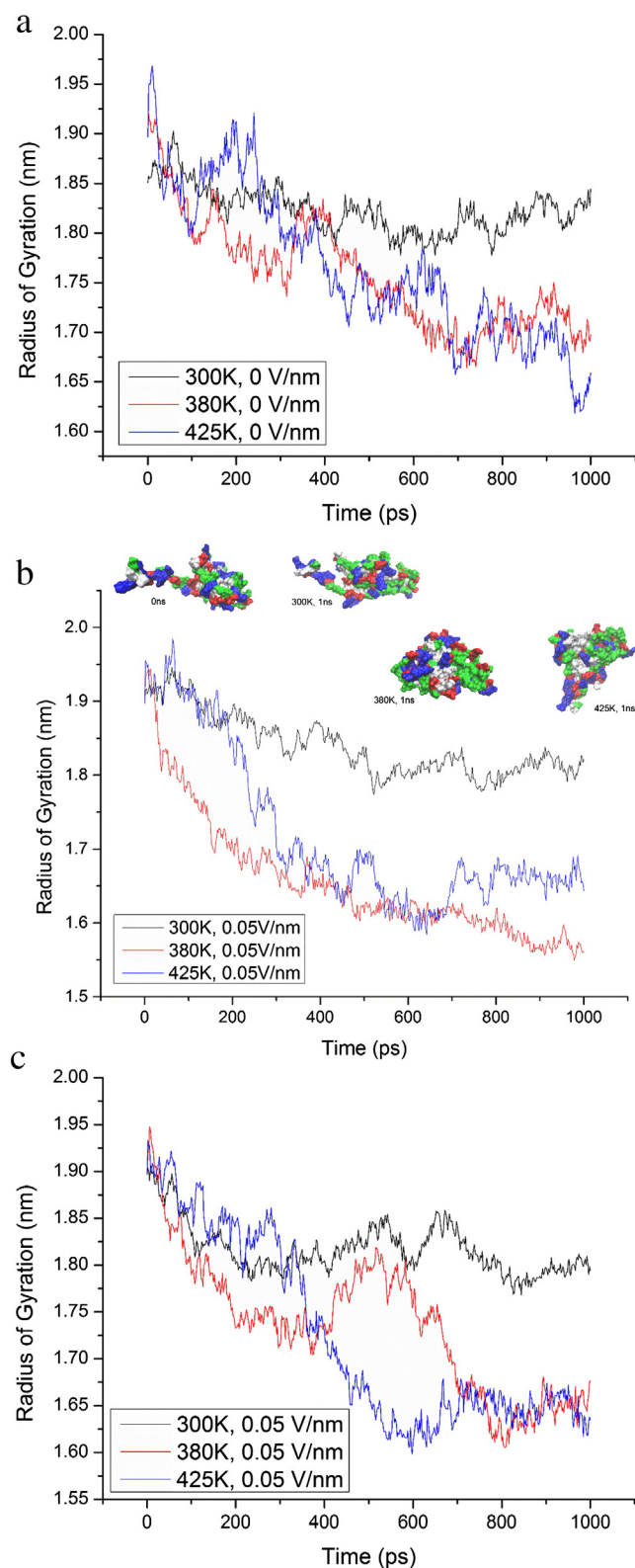


Fig. 4. Radius of gyration variations observed in Ara h 6 allergen (a) heat treatment, (b) heat treatment + static electric field, (c) heat treatment + oscillating electric field of 2450 MHz.

and remained mostly constant after that for the rest of simulation, whereas the dipole moment of the molecule decreased from 762 to 394 Debye's. When the same time interval is observed in Fig. 2(f) (between frames 100 and 200), it can clearly observe a lot of deviation in the residues 43–51 and α -helix between 74 and 84 residues.

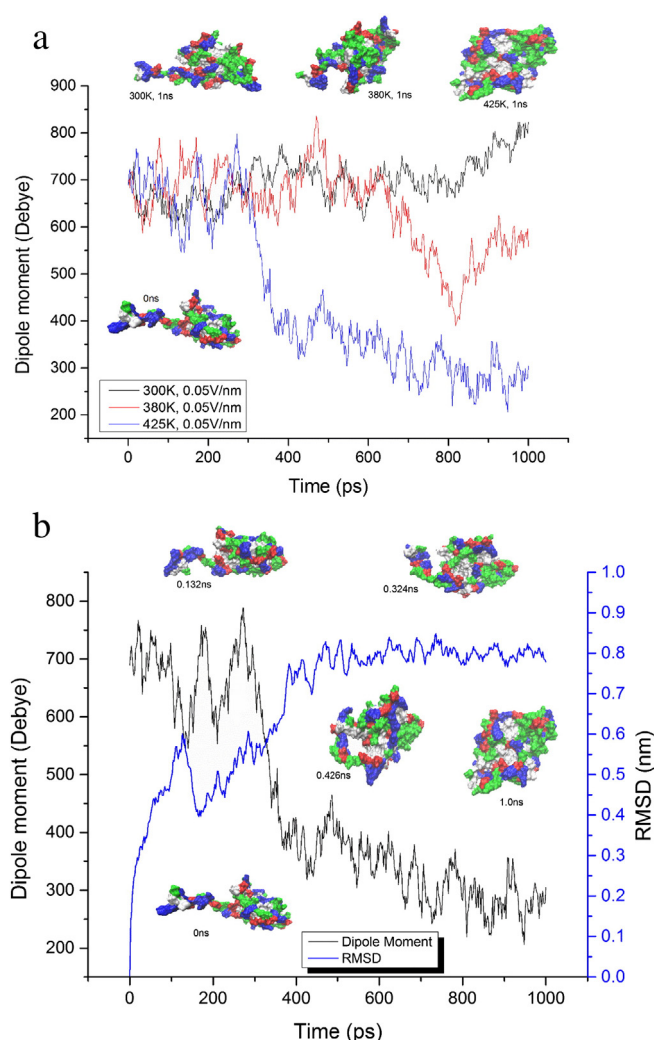


Fig. 5. Dipole moment variations in Ara h 6 allergen (a) change in dipole moments with temperature in oscillating electric field (0.05 V/nm), (b) dipole moment and RMSD variations at 425 K and oscillating electric field (0.05 V/nm).

From Fig. 4(c) it can be clearly observed that the radius of gyration also decreased significantly in this time interval.

3.5. Solvent accessible surface area (SASA)

The surface properties play a vital role in determining various functional characteristics of the protein (Fig. 6). But, these surface properties are highly dependent on the secondary structures and thus change in these structures can result in alteration of the surface properties, which in turn cause functional property variation. The surface properties of the protein also play a critical role in determining the molecular interactions. Thus, a change in the secondary structure can result in modifications of the molecular interaction (interactions with various solvents and enzymes) property of the protein (Singh et al., 2013a, 2013b; Wellner et al., 2005). The SASA basically indicates the area available for a direct contact between protein and solvents or molecules of similar size. In this study SASA was estimated using inbuilt functions available in the computational software GROMACS and an assumption that the solvent molecule is a sphere of radius 1.4 Å (Fig. 7). In 1971, Lee and Richards using the aforementioned assumption provided the means for estimation of SASA, they stated that the accessible surface area A , of an atom of radius R is the area on the surface, on each point of which the center of a solvent molecules can be placed without

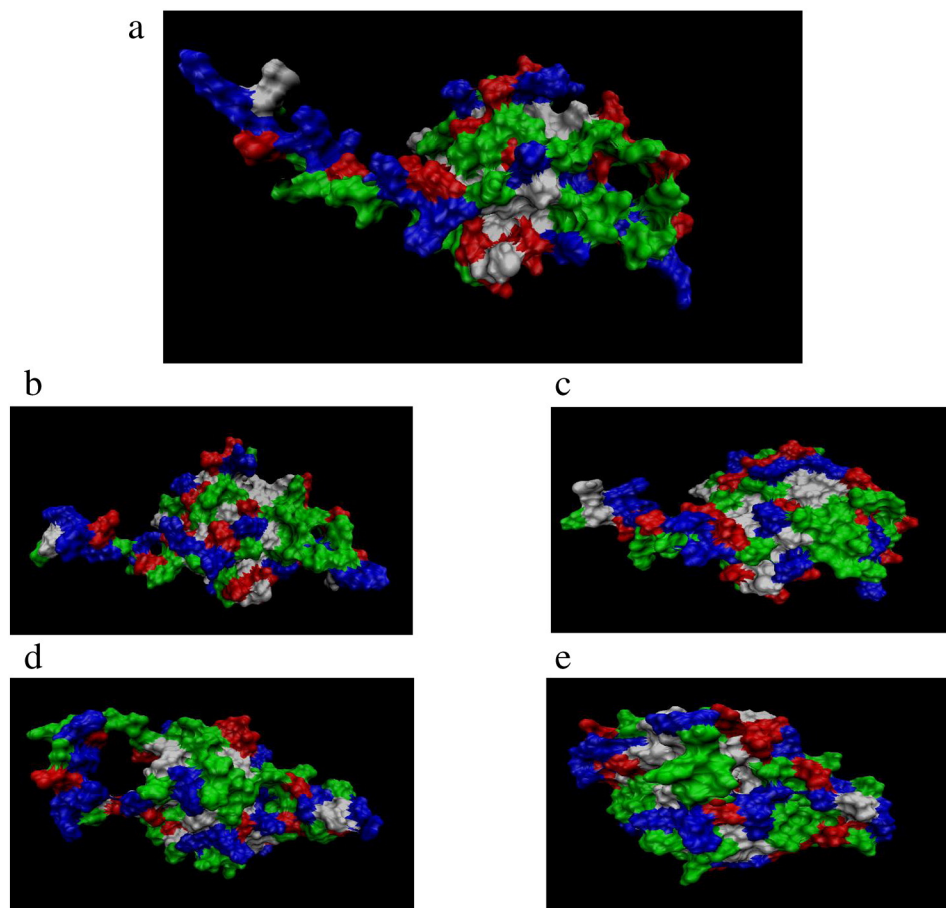


Fig. 6. Snapshot of surface properties of Ara h 6. (a) 300 K, (b) molecule at the end of 300 K and oscillating electric field (2450 Mhz), (c) molecule at the end of 380 K and oscillating electric field (2450 MHz), (d) molecule at 425 K and oscillating electric field (2450 MHz) at 400 ps, (e) molecule at end of 425 K and oscillating electric field (2450 MHz). Note: non-polar residues (white), basic (blue), acidic (red) and polar residues (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

altering other atoms of the molecules. They summarized their estimation in an equation (Lee & Richards, 1971):

$$SASA = A = \sum \left(R / \sqrt{R^2 - Z_i^2} \right) \times D \times L_i$$

Where, L_i is the length of the arc drawn on a given section i ; Z_i is the perpendicular distance of section i from the center of the sphere. It is important to note that the SASA estimated by Lee and Richards refers to static accessibility and it does not take into account potential flexibility of the structure.

In this study it was observed that the application of the static and oscillating electric field to the protein molecule resulted in surface area reduction similar to that with the application of only heat. In comparison, the oscillating fields had a little more pronounced effect on the molecule to the static fields [Fig. 8(b), (c), (e) and (f)]. Thus, effect of the overall reduction in the SASA must be further analyzed to determine the changes in the molecular interactive properties. In this study, it has been observed that the heat treatment resulted in a change the hydrophobic and hydrophilic surface areas as in Table 4. There is a considerable amount of reduction in the surface areas, especially the hydrophilic compared to the hydrophobic with an increase in temperature from control of 300 K to 380 K and then to 425 K as shown in Fig. 8(a) and (d). The simulations in this study were run for 1 ns due to computational limitations. But, the results observed clearly showed that there is an effect of the applied external stresses on the surface area of the molecule. Further research is required to extensively analyze the aforementioned effect using simulations ran for longer time periods such as 10–100 ns.

From Fig. 6(a) it can be observed that there is a protrusion that lies out of the core structure of the Ara h 6 molecule to which α -helix (CYS (residue: 16)-VAL (residue: 20)) almost acts as the connecting point. In the case of application of temperatures 300 K and 380 K, the

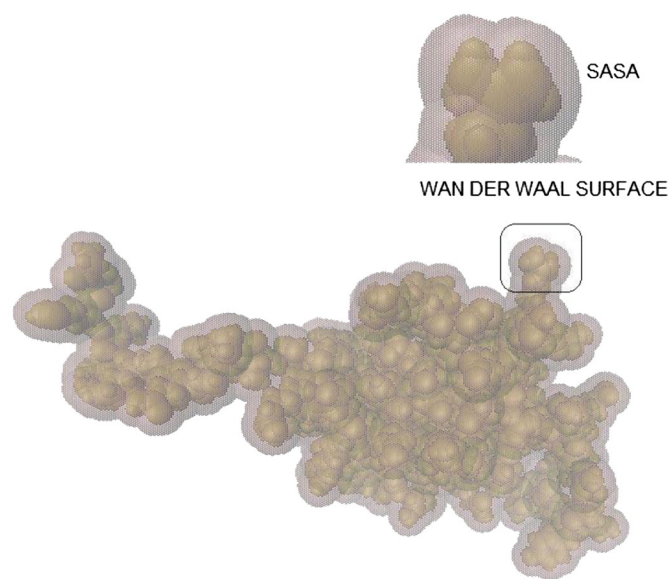


Fig. 7. Solvent accessible surface area of the Ara h 6 molecule. The protein Van der Waals surface is shown in solid and a radius of 0.14 nm was used to generate the solvent accessible surface area shown in mesh.

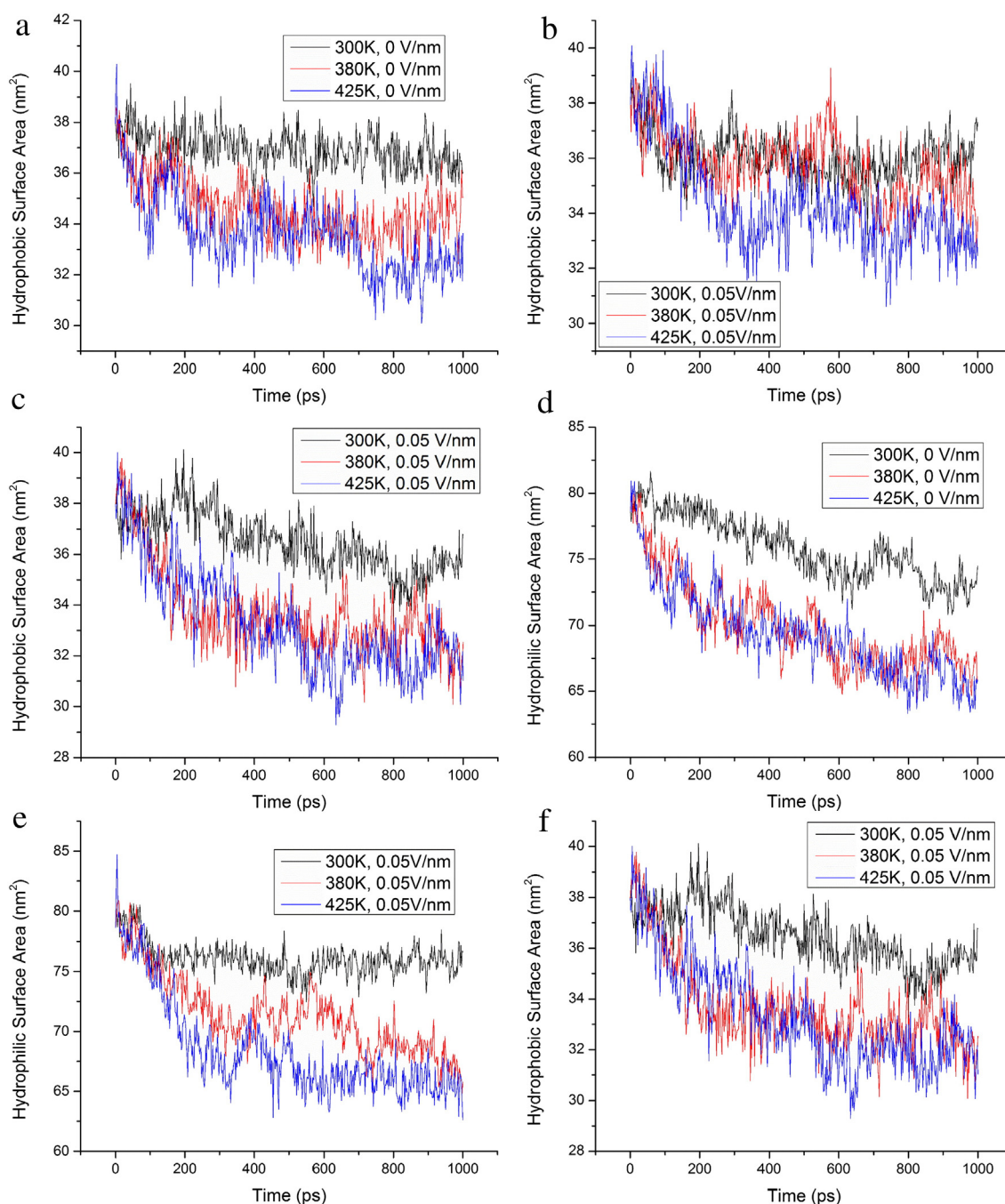


Fig. 8. Solvent accessible surface area (SASA) variation with heat treatments and electric fields (static and oscillating) of Ara h 6. (a) Hydrophobic surface area with heat treatment, (b) hydrophobic surface area with heat treatment + static electric field, (c) hydrophobic surface area with heat treatment + oscillating electric field, (d) hydrophilic surface area with heat treatment, (e) hydrophilic surface area with heat treatment + static electric field, (f) hydrophilic surface area with heat treatment + oscillating electric field.

rapid vibrations of the protruded part must have caused a strain on the secondary structure as suggested in Fig. 2(a), (b) and (c) causing deviations resulting in formation of 3/10 helices and turns in α -helix (CYS (residue: 16)-VAL (residue: 20)). But when the 425 K temperature is

applied along with an electric field, both static and oscillating, the vibrations were so high that the protruded part folded back into the core molecule and hence putting lower amount of strain on the α -helix (CYS (residue: 16)-VAL (residue: 20)) of the allergen Fig. 2(e) and (f).

Table 2
Radius of gyration.

Temperatures (K)	No electric field (nm)	Static electric field (0.05 V/nm) (nm)	Oscillating electric field (0.05 V/nm, 2450 MHz) (nm)
300	1.823 $\pm$ 0.023	1.840 $\pm$ 0.041	1.817 $\pm$ 0.027
380	1.757 $\pm$ 0.056	1.655 $\pm$ 0.081	1.735 $\pm$ 0.071
425	1.765 $\pm$ 0.076	1.719 $\pm$ 0.105	1.724 $\pm$ 0.098

Table 3
Dipole moments.

Temperatures (K)	Oscillating electric field (0.05 V/nm, 2450 MHz) (nm)
300	698.03 $\pm$ 46.06
380	639.97 $\pm$ 86.42
425	445.94 $\pm$ 167.33

Table 4

(a) Hydrophobic surface areas and (b) hydrophilic surface area.

Temperatures (K)	No electric field (nm)	Static electric field (0.05 V/nm) (nm)	Oscillating electric field (0.05 V/nm, 2450 MHz) (nm)
(a)			
300	36.934 ± 0.754	36.028 ± 0.848	36.693 ± 1.157
380	34.659 ± 1.232	35.725 ± 1.242	33.694 ± 1.751
425	33.644 ± 1.751	34.340 ± 1.803	33.331 ± 2.042
(b)			
300	75.836 ± 2.409	76.095 ± 1.335	75.164 ± 2.689
380	69.956 ± 3.356	71.367 ± 3.215	70.584 ± 3.311
425	69.373 ± 3.317	68.423 ± 4.026	68.561 ± 3.254

But, this folding had visually aided in showing the compaction of the molecule with the application of external thermal and electric field stress. Thus structural deviations can be achieved with the application of external stresses on a molecule, but the effect that these changes in the Ara h 6 molecule on the functional properties have to be further studied.

Fig. 6(a) shows the structure of the Ara h 6 molecule with no external stress application. With the increase in the temperature from 300 K to 380 K shown in Fig. 6 (b) & (c), the overall structure of the molecule has a very slight change in the structure as the molecule compacted. But, when the temperature increased to 425 K and oscillating electric field is applied, after 400 ps (Fig. 6 (d)) into the simulation, it was observed that the residue 1 which is actually protruded out of the molecule reverted back and joined in with the core of the molecule. By the end of simulation, the protrusion of the molecule almost joined into the core structure resulting in change of secondary structure of the protein.

Moreover, the hydrophobic dipole moment also contributes to the overall dipole moment of the molecule. The hydrophobic dipole moment is the measure of the irregularities in the surface between the hydrophobic and hydrophilic ends of the protein. When any protein has a high value of hydrophobic dipole moment it suggests that the structure of the protein is asymmetrical, i.e. one end of the protein has a high amount of hydrophobic surface while the other end of the protein has a higher amount of hydrophilic surface contributing to the dipole moment of the whole molecule. In the case of globular proteins, this is a little different because the dipole moments are directed towards the center of the molecule. Rose, Geselowitz, Lesser, Lee, and Zehfus (1985) have showed that the surfaces cluster around a center and this also contributes to the folding of the molecule.

The Ara h 6 molecule initially has an extended structure as shown in Fig. 1 at 300 K. But, with an increase in temperature to 425 K with an oscillating field of 0.05 V/nm, the structure of the molecule compacted leading to rearrangement of the surface resulting in change of the hydrophobic and hydrophilic surfaces. This overall reduction in the surface area of the Ara h 6 molecule leading to this compaction resulted in a structure similar to globular protein at the end of 1 ns. This change in the overall structure should have resulted in the reduction of the hydrophobic dipole moment also resulting in the overall dipole moment reduction.

When an external stress is applied on a protein it is bound to change its conformation to obtain a more stable state and that the change in the secondary structure can directly affect the functional properties. Previously, the exact relation between the protein structure and the protein functions was quite unclear. Fischer (Fischer, 1894; Lemieux & Spohr, 1994) first proposed the “lock and key” mechanism in 1894. In 1931, Wang (Wu, 1931) gave the first reference relating the protein functions to its structure when he proposed the first theory of protein denaturation which was later also documented by Edsall (Edsall, 1995). As mentioned earlier, Liu et al., (Liu et al., 2012) reported a compaction in the peanut protein isolate (PPI) and dextran mixture on dry heating and they also mentioned that this had a considerable effect on the functional properties of the protein. They observed that the solubility of the PPI dextran mixture was higher compared to that of the PPI alone.

Emulsifying and foaming properties were also tested and it was found out that the PPI dextran mixture were better emulsifying and foaming agents than the PPI alone. They have attributed this change to the predicted compaction in the molecule on dry heating the mixture of PPI and Dextran. This shows that the change in the secondary structure of the protein can result in a change in the functional properties of the molecule.

4. Conclusion

It is clear from the above observations that the secondary structure of Ara h 6 allergen can be altered under applied external stresses including thermal and electric fields. The results observed suggest that the effect of heat on the protein molecule is much more than compared to that of the electric field, both static and oscillating. But, the effect of these changes in the secondary structure of the molecule leading to further changes in the functional properties has to be studied. In the case of Ara h 6, further tests have to be conducted to verify that the variations caused in the secondary structure and in turn in the SASA would have any effect on the allergenicity of the protein in peanuts.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgment

The authors are grateful to NSERC (Natural Sciences and Engineering Research Council of Canada) for its financial support for this study.

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