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Research Article

Investigation of Molecular Dynamics of Peptides Bound with HLA * B51: 01 Protein

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Abstract: In the human body, there is the human leukocyte antigen (hereafter HLA) gene system, which recognizes whether the gene belongs to that body or not. This system encodes HLA antigens proteins to function. HLA antigens are classified as Class I, Class II and Class III according to their structural and functional properties. Class I and Class II HLA proteins form stable complex structures by binding with various peptides. However, some mutations on these proteins affect the binding process by causing lack of binding or poor binding of peptides and causes the development of many important diseases such as ankylosing spondalitis, systemic lupus, type 1 diabetes, Behçet's disease and autoimmune chronic hepatitis-B. The statistical thermodynamic properties of peptides (experimentally shown to be associated with HLA-B * 51: 01 Class I protein) that causes Behçet's disease (a very common disease in Turkey), has been investigated. Within the scope of the research, molecular dynamics (MD) method was used to determine the common aspects of peptides' behavior. In the MD method, peptides were carried out in solvent medium using the TIP3 water model.

Keywords: Molecular Dynamics Simulation (MD), protein, peptide, statistical thermodynamic properties

1. INTRODUCTION

Human leukocyte antigens (HLA) are cell surface molecules that present and activate T cells and determine the direction of the T cell-mediated immune response. HLA Class I proteins are composed of α and β2-microglobulin polypeptide chains. The α chain of HLA Class I proteins has 3 'folds' (α1, α2 and α3) located outside the cell and a short part extending into the cell. Of these, the top of the α1 and α2 folds are the most polymorphic parts of the chain and form the binding site of the antigen in the peptide structure of the protein (Figure 1). Only small peptides of 8-10 residues can be attached to this site [1].

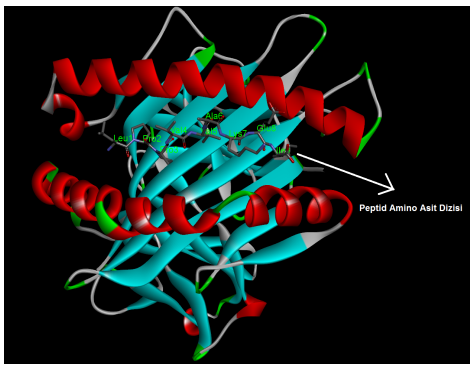

Figure 1: Schematic representation of the HLA Class I molecule [2]

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It is known from studies that these molecules constitute the basis for the factors that occur during the presentation of the peptide antigens to this region (poor binding or binding) and disease relationships (providing susceptibility / susceptibility or protection to the disease) [3]. Knowledge of disease-causing relationships gained by this study is essential for folding mechanisms, molecular function, signal transduction, drug design, and protein engineering. [4-6]. Protein dynamics is based on time-dependent changes in the position of atoms that create both equilibrium and out-of-balance fluctuations. There are many theoretical and experimental methods used to study the dynamics of proteins: X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy, electron paramagnetic resonance (EPR), small-angle X-ray scattering (SAXS), molecular dynamics (MD), normal mode. analysis (NMA) [7-9]. In the proposed study, molecular dynamics (MD) simulation, which is one of these methods and applied in the field of biology in recent years, was used.

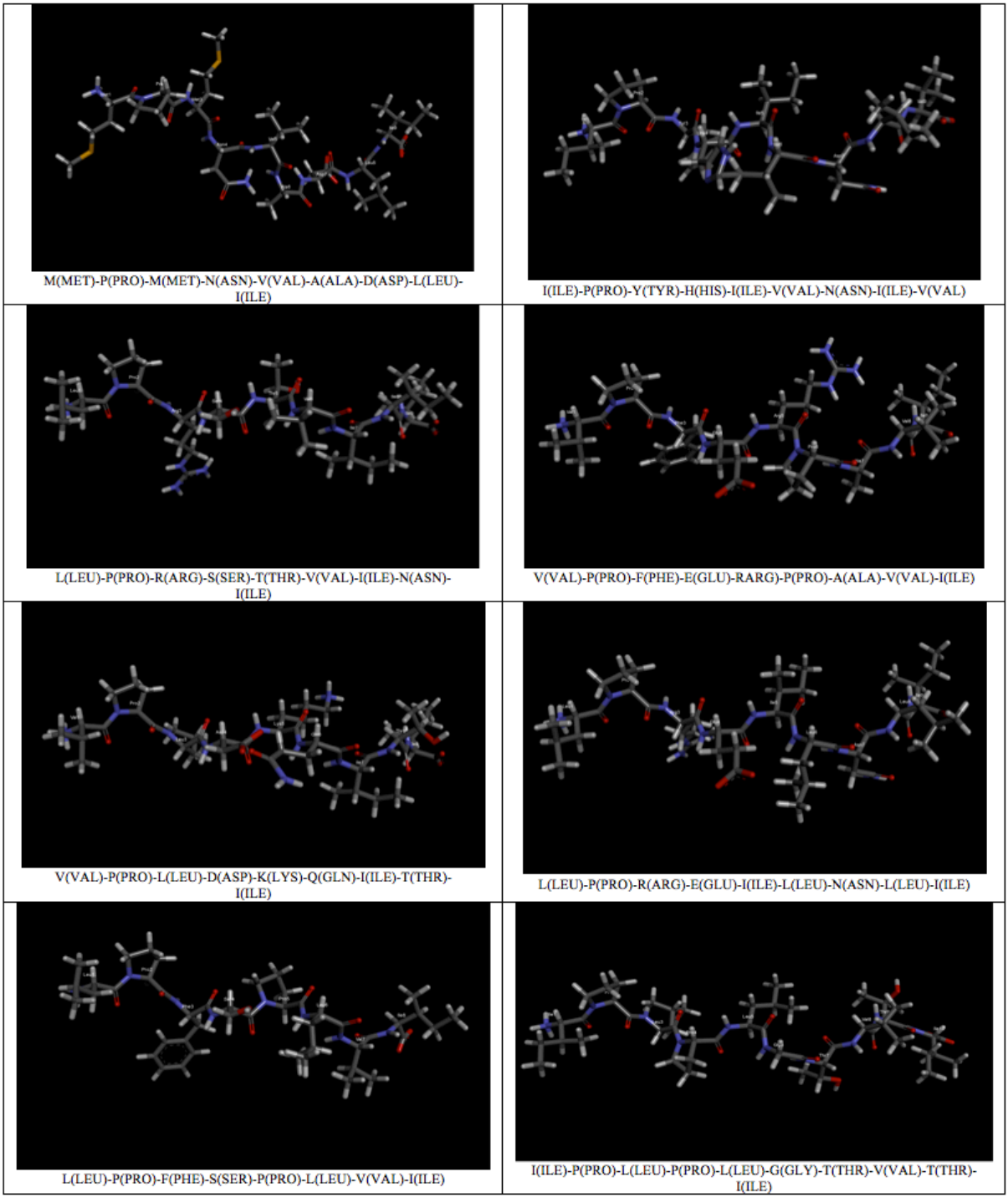


Figure 2: Amino acid sequences of the peptides used within the scope of the project.

Previously HLA Class I proteins have been investigated using MD simulation methods [10, 11]. In 2006, F. Sieker et al. Investigated the situation with and without peptide bound to HLA-B * 4402 Protein Data Bank (pdb file name: 1M6O and 1SYV for HLA-B * 4405) by MD simulation studies [12]. Similarly, U. Omasits et al. Investigated the behavior of the system formed by the binding of two different peptides (amino acid sequences respectively; ARAAAAAAA and GRFAAAIAK) forming a complex with the HLAB * 2705 protein by using the GROMACS method [13]. In 2004, M. Zacharias and S. Springer analyzed the cases where a tumor antigen peptide derived from melanoma antigen (amino acid sequence: GVYDGREHTV) is bound and not bound to the HLA-A * 0201 Class I protein using the MD simulation method [14]. The difference of the proposed study from these studies is that the pure properties of peptides associated with a specific disease, regardless of proteins, were examined.

2. MATERIALS AND METHODS

Eight peptides to be examined within the scope of the research were determined from the web page of SYFPEITHI [15] and the crystal houses belonging to these peptides were recorded in separate files. The amino acid sequences of the peptides to be used are given below (Figure-2).

The binding affinities of the selected peptides are: **M P M N V A D L I** (IC_{50}: 28.69 nM), I P Y H I V N I V (IC_{50} = 59.09 Nm), V P F E R P A V I (IC_{50} = 85.78), **L P R S T V I N I** (IC_{50}: 86.80 nM), V P L D K Q I T I (IC_{50} = 91.88 Nm), **L P R E I L N L I** (IC_{50} = 95.72 Nm), L P F S P L V I (IC_{50} = 115.93 Nm), **I P L P L G T V T I** (IC_{50} = 118.90 nM), V P Y E P P E V (IC_{50}: 205.08 nM), **I P Y Q D L P H L** (IC_{50}: 344.89 nM), I P M G K S M L V (IC_{50}: 401.43 nM), **Y P F K P P K I** (IC_{50} = 431.55 nM), Y P L L I S R I (IC_{50} = 481.58 nM), **Y P F K P P V I** (IC_{50}: 950.40 nM).

The research carried out consists of two different stages: (I) Making the necessary preparations by using the VMD program for Molecular Dynamics (MD) simulation, and (II) MD simulations.

2.1 Preparation Stage

(a1) Protein structure files (PSF) were created for each peptide, (a2) the appropriate biological environment was created, (a3) A water box of 20 Å liquid medium was created using the TIP3 water model to create a solvent medium. (a4) The periodic boundary conditions of the created box are determined by the code, (a5) to neutralize the structure, an appropriate number of ions were added according to the number of negative or positive charges of the peptides.

2.2. MD Simulations

A 5000-step minimization process was performed to bring the system into equilibrium at the beginning. 2.500.000 step with 2fs time step MD simulation was performed in three different temperatures (308, 310, 312 and 314 K) and in separate NPT (constant pressure) conditions. Langevin thermostat was used for temperature and pressure control of the system in simulation studies, As a result of the simulation process, the instantaneous atomic coordinates of the atoms of each peptide R, their velocities, pressure and energy properties were recorded. The necessary programs were written for the calculation of statistical thermodynamic properties from the atomic positions and velocities, and the calculations of the research results were made, and Using the obtained calculation results, the temperature-time, total energy-time (Total Energy-Timestep), root mean square deviation (Root Mean Square Deviation, RMSD) graphics for each peptide were plotted. Root mean square fluctuations (RMSF) and Entropy graphics were also plotted.

3. EXPERIMENTAL STUDIES AND EVALUATION

3.1. Temperature and Energy Calculations

Within the scope of the project, eight peptides with the closest binding affinities were studied at 308, 310, 312 and 314 K temperatures. In order to understand whether the simulation studies were in good progress, temperature and total energy changes were examined during and at the end of the experiment depending on time. The temperature and total energy graphs of the MPMNVADLI peptide are given in Figure 3.

When Figure-3 is examined, it is seen that there are not very serious fluctuations in both temperature and total energy graphs depending on time. This result is an indication that the experimental work is going smoothly and unproblematic.

3.2. Root mean square fluctuations (RMSF) calculations

As the structure of the peptide changes throughout the simulation, it is not equal. Generally, the flexibility of some regions differs from others due to differences in amino acid sequence. Root mean square fluctuations (RMSF) correspond to fluctuations in mean position for each atom and often to crystallographic temperature (or b) factors. The higher the temperature factor, the more mobile the atom is. An interesting proof of this analysis is that it supports the calculation of an average structure that can be used for future analysis. In this project, the RMSF values of the main chain C_α atoms of each peptide were calculated depending on the temperature and are shown in Figure-4.

$$RMSF = \sqrt{\frac{1}{T}\sum_{t_j=1}^{T}(x_i(t_j) - \hat{x}_i)^2}$$

Where T is the duration (time steps) of the simulation and x_i (t_ (j)) is the coordinates of x_i atoms with respect to time t_j.

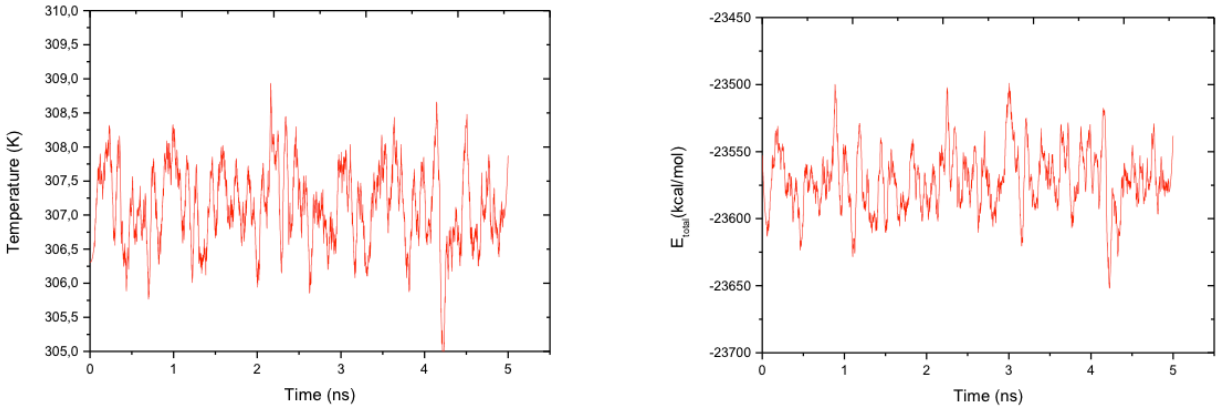


Figure 3: (a) temperature (308 K) and (b) total energy (ethotal (kcal / mol)) change of MPMNVADLI peptide depending on time

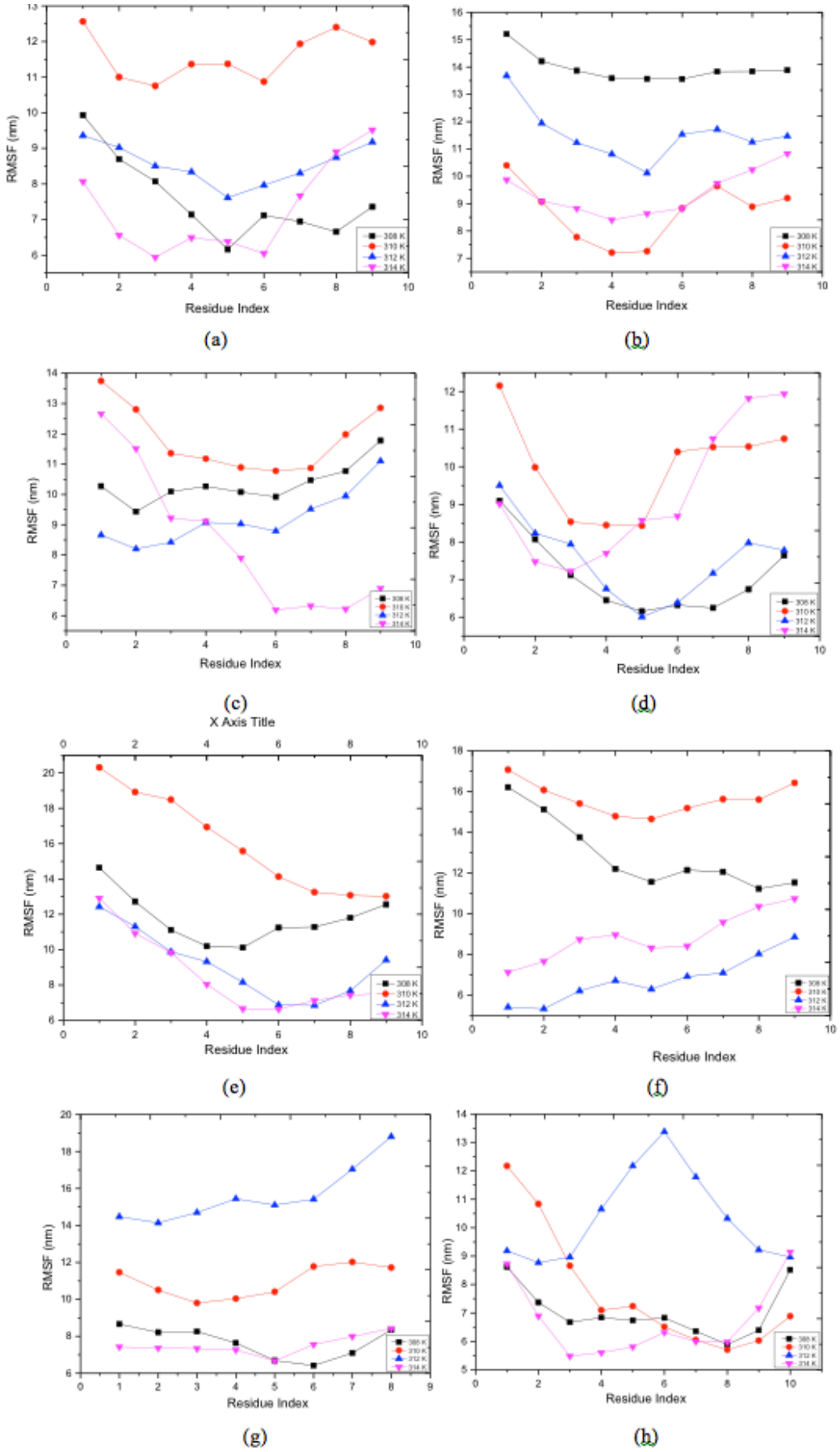


Figure 4: RMSF values of 8 different peptides: (a) MPMNVADLI, (b) IPYHIVNIV, (c) LPRSTVINI, (d) VPFERPAVI, (e) VPLDKQITI, (f) LPREILNLI, (g) LPFSPLVI and (h) IPLPGTVTI

As seen in Figure-4, the maximum RMSF value of the MPMNVADLI peptide belongs to the MET1 amino acid of the N-terminal and its value is 12 nm. Similarly, the minimum RMSF value belongs to the ALA6 amino acid located in the middle of the peptide and its value is 6 nm. The maximum RMSF value of the IPYHIVNIV peptide belongs to the ILE1 amino acid of the N-terminal and its value is 15 nm. Similarly, the minimum RMSF value belongs to the HSE4 amino acid located in the middle of the peptide and its value is 7 nm. The maximum RMSF value of the LPRSTVINI peptide belongs to the LEU1 amino acid of the N-terminal and has a value of 13 nm. Similarly, the minimum RMSF value belongs to the VAL6 amino acid in the middle of the peptide and its value is 6 nm. The maximum RMSF value of the VPFERPAVI peptide belongs to the amino acid VAL1 of the N-terminal and its value is 12 nm. Similarly, the minimum RMSF value belongs to the amino acid ARG5 located in the middle of the peptide and its value is 6 nm. The maximum RMSF value of the VPLDKQITI peptide belongs to the VAL1 amino acid of the N-terminal and its value is 20 nm. Similarly, the minimum RMSF value belongs to the GLN6 amino acid located in the middle of the peptide and its value is 6 nm. The maximum RMSF value of the LPREILNLI peptide belongs to the LEU1 amino acid of the N-terminal and its value is 17 nm. Similarly, the minimum RMSF value belongs to the PRO2 amino acid located in the middle of the peptide and its value is 5 nm. The maximum RMSF value of the LPFSPLVI peptide belongs to the ILE8 amino acid of the C-terminus and its value is 18 nm. Similarly, the minimum RMSF value belongs to the LEU6 amino acid located in the middle of the peptide and its value is 6 nm. The maximum RMSF value of IPLPGTVTI peptide belongs to GLY6 amino acid and its value is 13 nm. Similarly, the minimum RMSF value belongs to the LEU3 amino acid located in the middle of the peptide and its value is 5 nm. RMSF behavior of each peptide at 308, 310, 312 and 314 K values is given in Figure-5.

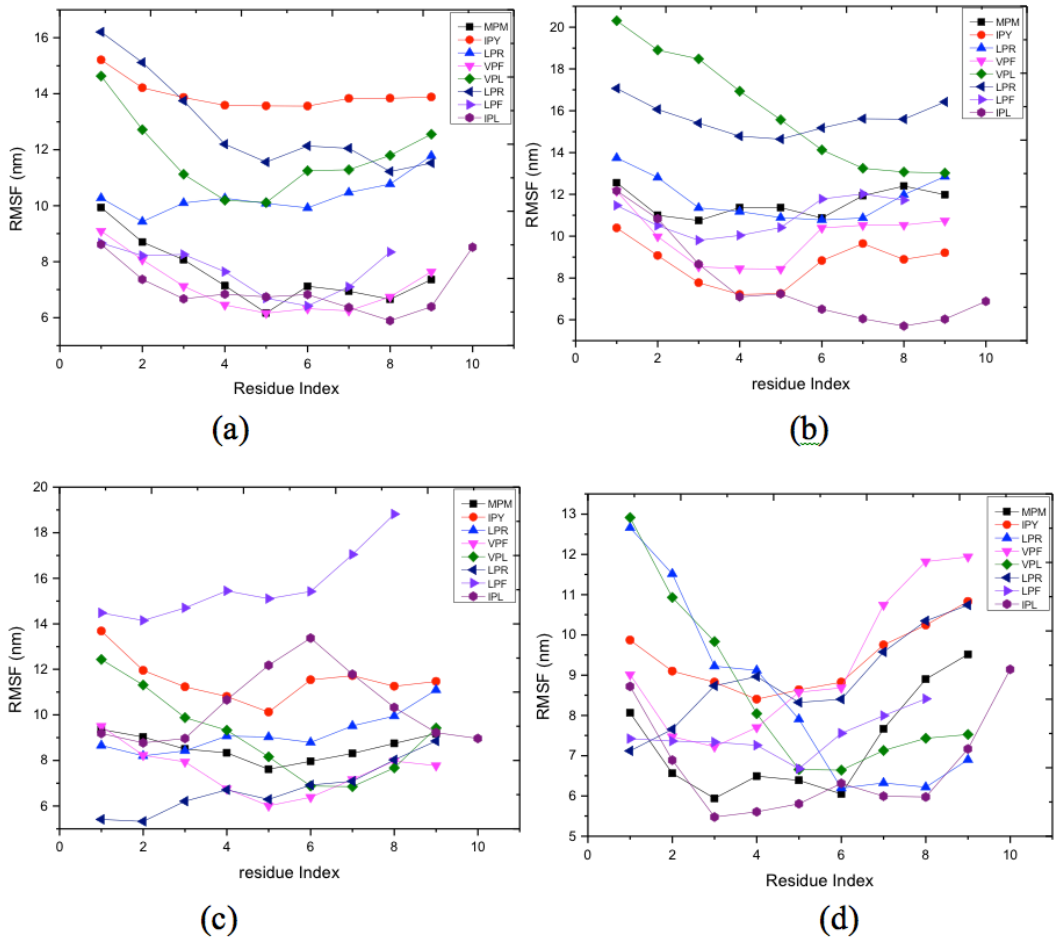


Figure 5: Behavior of each peptide at different temperatures: (a) 308 K, (b) 310 K, (c) 312 K and (d) 314 K.

It can be seen from Figure-5 that as the temperature increases, there is a large increase in the mobility (fluctuations) of the peptides. According to the temperature increase, the most stable peptide is MPMNVADLI, while the most mobile peptide is VPLDKQITI. In addition, the results of the graphical analysis for each temperature value are

given separately below. At 308 K; The greatest motility was in the LPREILNLI peptide (RMSF = 11.78 nm), while the most stable peptide was identified as IPYHIVNIV. At 310 K; The greatest motility was in the VPLDKQITI peptide (RMSF = 20.30 nm), while the most stable peptide was found to be MPMNVADLI. At 312 K; the greatest mobility was in the LPFSPLVI peptide (RMSF = 18.81 nm), while the most stable peptide was found to be MPMNVADLI. At 314 K; VPLDKQITI peptide (RMSF = 12.91 nm) has the greatest mobility at this temperature while there is an increase in the stability of all peptides.

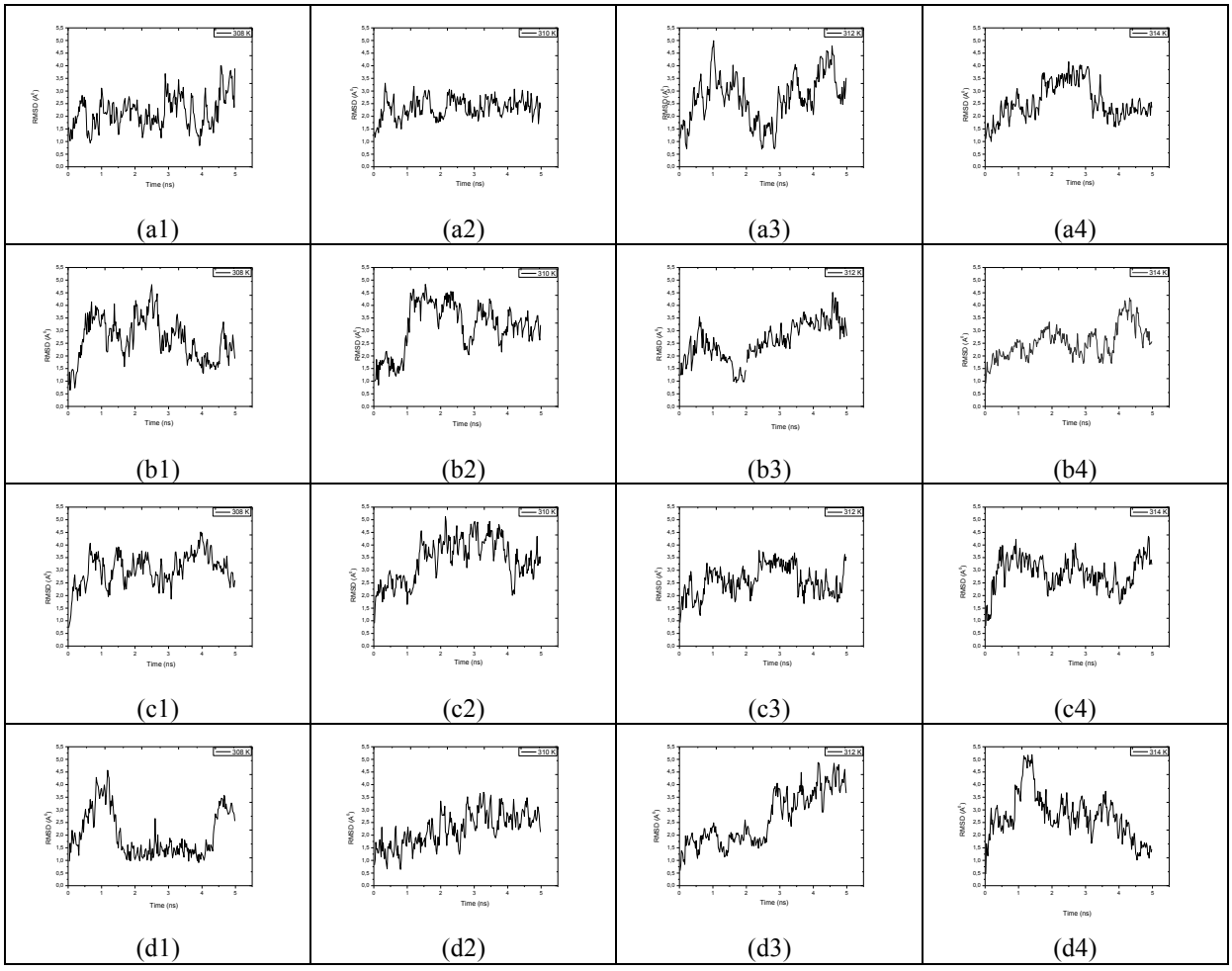
When we compare peptides according to their experimental binding affinity, the most stable peptide according to temperature increase is MPMNVADLI.

3.3. RMSD Calculations

RMSD graphs of each peptide at each temperature are also given in Figure-6. The RMSD value of each peptide for different temperature was calculated by the formula below;

$$RMSD = \frac{1}{N}\sqrt{\sum_{i=1}^{N}(x_i^m - x_i^f)^2 + (y_i^m - y_i^f)^2 + (z_i^m - z_i^f)^2}$$

Here, x, y and z represent the time coordinates of the peptide atoms.



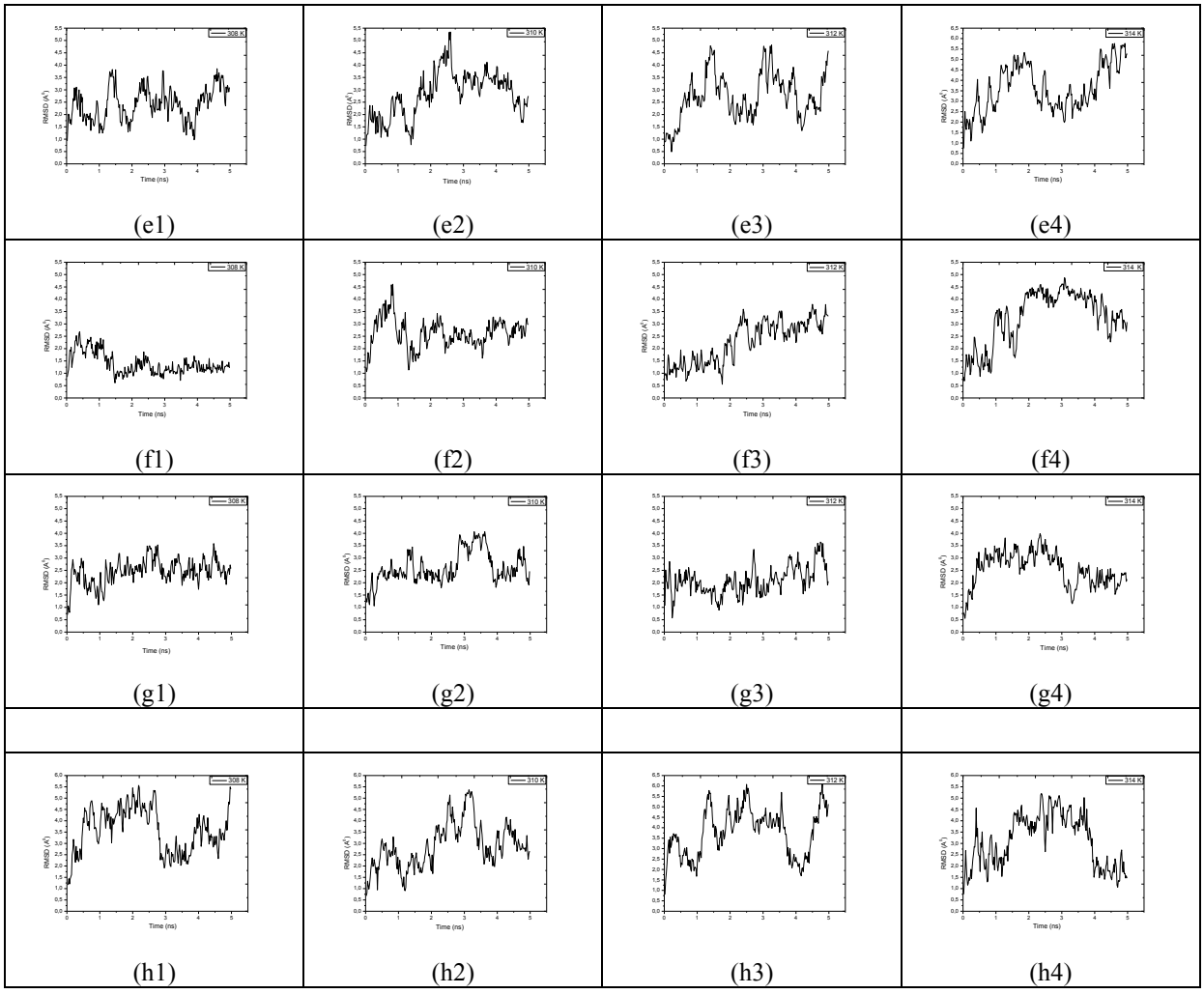


Figure 6: RMSD graphics of the main chain atoms (N, Cα and C) during the simulation of peptides: (a) MPMNVADLI, (b) IPYHIVNIV, (c) LPRSTVINI, (d) VPFERPAVI, (e) VPLDKQITI, (f) LPREILNLI, (g) LPFSPLVI and (h) IPLPGTVTI.

The graph results in Figure-6 are the analysis of the peptides with the best and the worst mobility. MPMNVADLI: It is the most stable peptide during simulation. Especially at 310 K, it is seen that the RMSD value varies between 1.73 - 3.04 A0. Increases in mobility were detected after 310 K. IPLPGTVTI: It is the most unstable peptide with high mobility during simulation. Especially at 312 K it is seen that the average RMSD value is 6.05 A0.

When each peptide was examined within itself depending on the temperature, it was found that there was no regular change in RMSD values. Also, considering the peptides showing the best and worst mobility behavior, it can be said that binding affinity has a significant effect on RMSD results

3.4. Conformational Entropy Calculations

Conformational entropies of peptides calculated from simulation results at different temperatures were calculated according to Schliter's equation and the results are shown in Figure-7.

$$S = \frac{1}{2}\, k_B ln det\, [1 + \frac{k_B T_e^2}{\hbar^2}\, M\sigma]$$

In the formula here, where S is the entropy, k_B is the Boltzman constant, T is the simulation temperature (or temperature absorbed by the system), e is the Euler number, h is the Plank constant, M is the mass matrix (holding masses of atomic Cartesian degrees of freedom diagonally) and σ represents the covariance matrix of 3N Cartesian coordinates. N is the number of atoms in the molecule or molecules.

Although, the choice of atoms set determines which movements are translational and rotational excluded, it has little effect on relative comparisons as it can affect the computed entropy.

$$\sigma_{ij} = \langle (x_i \langle x_i \rangle)(x_j \langle x_j \rangle) \rangle$$

While calculating the entropy, the positional fluctuations of the peptide's main chain atoms (N, Cα and C) for each case were taken into account.

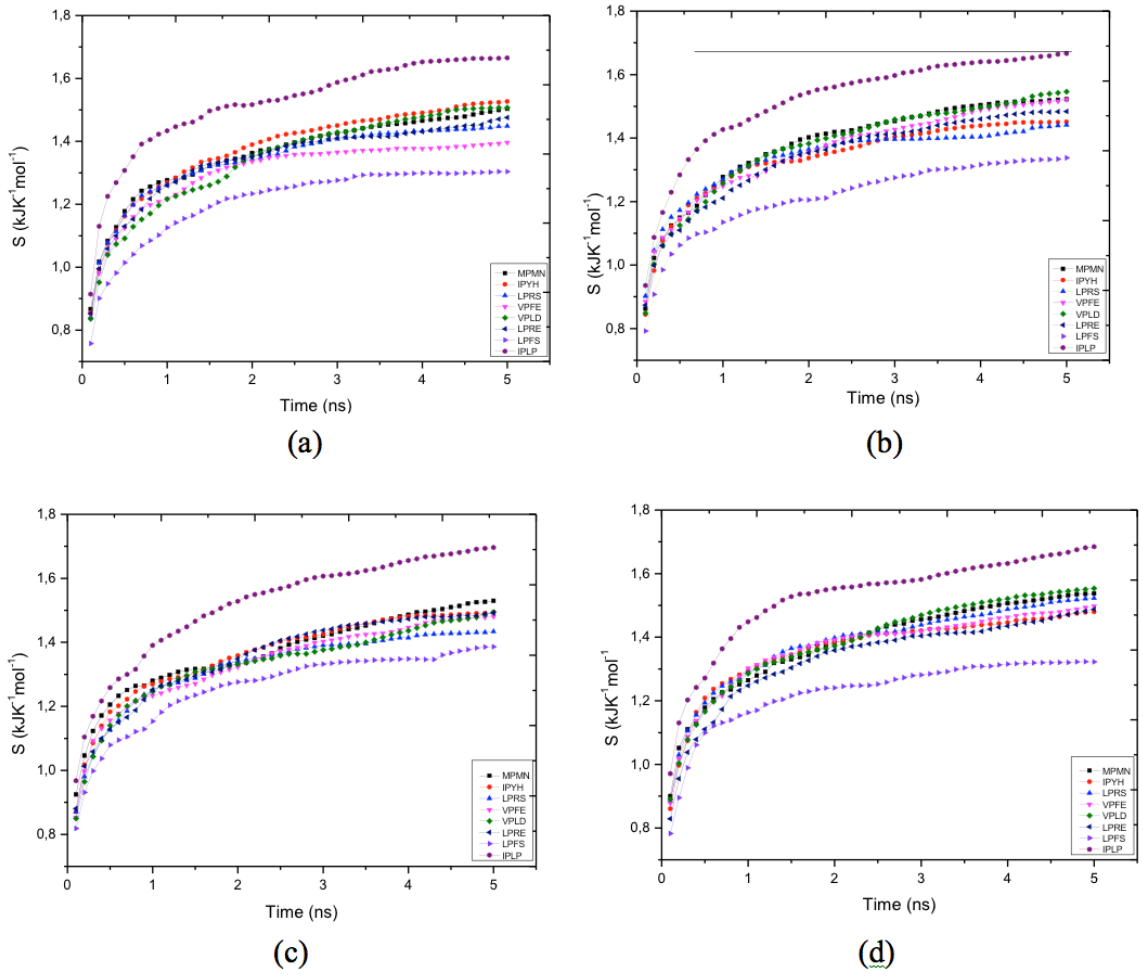


Figure 7: Plots of the configuration entropies of the main chain atoms of all peptides depending on time and temperature: (a) 308 K, (b) 310 K, (c) 312 K and (d) 314 K.

When the graphs in Figure 7 are examined, among the eight peptides, IPLPLGTVTI peptide was found to be the highest binding affinity with the largest configurational entropy (1.665 kJK-1mol-1 at 308 K; 1,666 kJK-1mol-1 at 310 K, 1.696 at 312 K and 1,684 kJK-1mol-1 at 314 K). When the entropy results of this peptide are examined for 4 different temperature values, it is seen that the results are very close to each other. This result is very interesting and requires further investigation.

4. CONCLUSION

In this study, eight different peptides were examined under 4 different temperatures. The results of the investigation are presented below:

1) Total energy-time and temperature-time graphs of each peptide were drawn to show whether the simulations were running smoothly, and it was understood from the graphs that the experimental studies were running smoothly. This result has shown us that the method chosen is suitable for these peptides.

2) When we compare the peptides according to the experimental binding affinity of all peptides from the RMSF graphs at different temperatures and for each temperature, it was found that the most stable peptide according to the temperature increase was MPMNVADLI.

3) When each peptide was examined within itself depending on the temperature, it was found that there was no regular change in RMSD values. Also, considering the peptides showing the best and worst mobility behavior, it can be said that binding affinity has a significant effect on RMSD results.

4) Conformational entropies of peptides calculated from simulation results at different temperatures were calculated according to Schliter's equation. The graphs drawn with the results obtained from the calculation showed that the peptide with the highest binding affinity has the highest conformational entropy value. In addition, when the entropy results of this peptide were examined for four different temperatures, it was seen that the results were very close to each other. This result is very interesting and requires further investigation.

The molecular dynamics method here was shown to be used to investigate and determine the mobility and conformational entropy of peptides. Working with more peptides requires fast and powerful computers and sufficient time.

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