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Research Article

Investigation of Binding Free Energy of Different Peptides by Jarzynski's Equation

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Abstract: Selection and binding of peptides to MHC-I molecules play an important role in the adaptive immune system. Some molecular dynamic (MD) simulations methods have been developed to understand the MHC class I-peptide binding mechanism. Behçet's disease (BD) is a chronic multisystem inflammatory disorder of unknown etiology. In this study, we use steered molecular dynamics (SMD) simulations to unravel the binding mechanism of different peptides to human leukocyte antigens (HLA-B*51:01), an antigen associated with Behcet's disease (BD). We approached this problem by employing 195 ns simulations. The maximum force as a function of time was determined to 2.16 ns for NPYD peptide. The Jarzynski's equation was used to obtain the binding free energy. This result showed that the experimental IC_{50} values with calculated IC50 values are not compatible. Then, the present study showed that SMD simulation is an important approach in the MHC I-peptide binding mechanism for autoimmune diseases and methodology to drug design and peptide immunogenicity.

Keywords: Jarzynski's equation, binding free energy, Peptide, Steered Molecular Dynamic Simulation.

1. INTRODUCTION

Behçet's disease (BD) is one of the vasculitides with devastating multi-systemic symptoms that possibly will involve most tissues of the body, such as the mucocutaneous, ocular, musculoskeletal, vascular and central nervous system (CNS) [1, 2]. BD is known to be strongly associated HLA genotype B5 and its subclass (split antigen) 51 allele on chromosome 6 (HLA-B*51:01) [3] and this B51 association has been confirmed in many different ethnic groups in Turkey, Greece, Japan and Iran [3, 5, 6, 7, 8].

Human Leukocyte Antigens (HLA) is the human version of the major histocompatibility complex (MHC), which initiates a specific immune response [9]. The MHC is divided into three lokus: classes I, II, and III. MHC class I encoded HLA-A, B and C antigens, which is responsible for the antigen presentation. The MHC class I molecules are found on all nucleated cell bodies and these molecules present antigens to CD8+ cytotoxic T cells (CTLs) [10, 11, 12]. MHC class I molecules (Figure-1) are composed of heavy chain α, non-covalenty associated β_2-microglobulin (β_2m) and 8–10 residue peptide [13, 14]. The peptide-binding groove of MHC class I is made up of the α1 and α2 domains, which are polygenis and polymophic. A small number of high affinity peptides derived from signal sequences of ER translocated proteins can also be loaded by class I molecules that affect the stability of HLA proteins [15, 16]. This peptide loading takes place in the endoplasmic reticulum (ER) [17], after which peptide-loading complex (PLC) is formed.

There are huge numbers of possible bindings between HLAs and peptides. In the last decades, molecular dynamics (MD) simulations have been developed for measuring the binding affinity between HLAs and peptides [18, 19, 20, 21]. The steered molecular dynamics (SMD) is a non-equilibrium computational method [22], which has been primarily useful in a variety of dynamic systems to study drug binding [23, 24] and in the characterization of the mechanical response of biological complexes, such as protein (un)folding, ligand binding, etc. [25, 26, 27, 28]. In

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SMD, a constant velocity or force is applied to the groups of atoms (e.g. ligand bound to a protein surface) to move the system from one state to another.

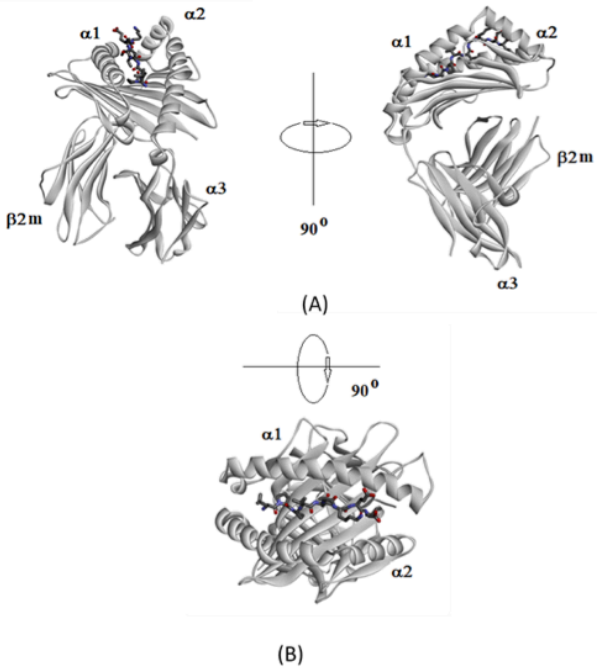

Figure 1: (A) Structural basis of HLA-B*51:01. HLA contains the α_1 and α_2 subdomains (grey), the α_1 and α_2 domains form the antigen peptide (Ag) binding groove. (B) View from the top into the class I peptide-binding groove.

In the literature, investigation of different peptides bound by the MHC class I for HLA-A and-B alleles associated with BD has been reported in molecular dynamic simulations studies. For example, Sirilak et al. [29] have investigated the link between BD and two specific HLA-A*26:01 and HLA-B*51:01 in terms of their binding affinity to the MICA-TM peptide, using MD simulations. They showed that all the structural, dynamics and energetics information explain to some extent the recognition and selective binding of the MICA-TM peptide.

The hydrogen bond plays a key role in the peptide binding. The two ends of the MHC class I binding cleft are defined N- and C-termini of peptide ligands, which are held by hydrogen bond networks in the cleft and they make the highest contribution to the binding energy of the peptide [30, 31, 32].

How peptides could become bound within the peptide-binding groove of HLA-B*51:01 is still largely unresolved in the atomic level for Behcet's disease (BD). In the present work, we aimed to investigate the analysis of effects of peptide binding free energy on the one HLA allele associated with BD (HLA-B*51:01) in terms of their binding affinity to the different peptide using SMD simulations. One of the objectives of this study was to investigate the disruption of hydrogen bonds between HLA-B*51:01 and the peptide.

2. METHODS

2.1 Simulation Details

The X-ray structure of the HLA-B51:01 bound to a 9-mer, LPPVVAKEI (PDB accession code 1e27), peptide from HIV-1 were retrieved from the Protein Data Bank (PDB) [33].

As seen in Table 1, HLA peptides downloaded from the SYFPEITHI database [34] were studied with SMD. The last column of Table 1 shows theinhibiting potencies (IC50 values) obtained by experimental methods using NetMHCPan v2.4 [35]. For protein-peptide docking, to obtain unbound form of HLA-B51:01 the peptide was removed from 1e27 by using Discovery Studio (Accelrys Inc., San Diego, CA, USA). The GalaxyPepDock method was used for protein-peptide docking.

All Molecular Dynamics (MD) simulations were performed by using NAMD 2.8 [36] package with CHARMM27 [37] force field. MD simulations were performed in the isobaric-isothermal (NPT) ensemble at 300 K temperature and 1 bar pressure. To control the temperature and the pressure of the system Langevin Dynamics and Langevin

piston Nose-Hoover method were used respectively. The equations of motion were integrated with a 2 fs timestep. Long range electrostatic interactions were calculated using the particle-mesh Ewald (PME) [38] summation scheme. The cutoff distance for both Coulomb and van der Waals potentials was set to 1 nm and 1.4 nm, respectively. SHAKE algorithm was applied to all bond lengths involving hydrogen [39]. The protein-peptide complex was soaked with TIP3P water molecules [40] with no less than 20 A˙ from the edge of the water box to the nearest protein atom and simulated using cubic periodic boundary conditions in all three directions. To represent a more typical biological environment, the electroneutrality of the system was achieved by adding Na+/Cl- ions to a final concentration of 0.15 mol/L. Each protein-peptide complex was energetically minimised with 5000 steps and after minimisation, the whole complex were subject to a further 2 ns equilibration in isobaric-isothermal (NPT) ensemble. Each system was subjected to the five stages of the restrained MD simulations at 300 K with force constants of 40, 20, 10, 5 and 0 (kcal/mol/(A))˙ on the heavy atoms (Cα) of the entire protein for 100 ps at each stage accordingly.

Table 1: 8 and 9-mer epitopes used in SMD analysis.

NO	Peptide	Epitope Source Molecule	IC_{50} (nM)
1	DPYEVSYRI	B cell transloc. gene 1 protein 107-115	146.78
2	FPFPEDYPN	Flavin containing monooxygenase 71-79	3143.24
3	LPNAVITRI	DNA-polymerase epsilon p17 10-18	85.27
4	NPYDSVKKI	Diubiquitin 25-33	612.54
5	NPLPSKETI	Thymosin beta-4 27-35	1583.53

The SMD simulations of the whole protein-peptide complex were performed by applying some force on the Cα atom (SMD atom) of the peptide in the (x-y-z) direction with the NAMD programme [36] using the CHARMM27 potential energy function for all the atomic models for proteins [37]. Here, the direction of pull is determined by the direction of the vector that links the fixed and the SMD atoms, which is the x, y, and z- components of the normalized direction between the fixed and the SMD atom [41]. The SMD simulations were applied with a spring constant 5 pN/A and pulling speed 5 A/ns on the SMD atom. The snapshots of SMD simulations were recorded with 100 ps intervals. Each simulation took 6.5 ns and the mean force profile and PMF for each peptide were calculated by averaging the outcomes of 6 independent runs.

The topology file and subsequent analysis of each protein-peptide complex was used by Visual Molecular Dynamics (VMD) tool [42]. Force and work were calculated at every SMD step.

2.2 Free Energy Calculations:

The force applied is usually in the form of a harmonic restraint on the chosen SMD atom:

$$F(t) = \frac{1}{2} k\,[r\ -\ r_0(t)]^2 \tag{1}$$

Where r and r_0 are the instantaneous position and the initial position of the SMD atom respectively and k is the spring constant. The external work from nonequilibrium simulations was calculated by:

$$W(t) = \int_0^t F(t) v dt \tag{2}$$

In nonequilibrium MD simulations, the Jarzynski's equality derived an expression that allows for the calculation of the extracted equilibrium free energy difference from the nonequilibrium work distribution [43]:

$$e^{-\beta \Delta F} = < e^{-\beta W} > \tag{3}$$

where $< >$ denotes an ensemble average of the work W from nonequilibrium simulations, β is the inverse temperature $(1/kT)$ and ΔF is the free energy difference between these two states. The goal is to obtain the potential of mean force (PMF) of the whole complex which is the free energy profile as a function of that chosen coordinate and its form is a good indicator of the stability of a system as you do work on it along a coordinate path.

3. RESULTS AND DISCUSSION

We analyzed the binding mechanism of different peptides to HLA-B*51 an antigen associated with Behcet's disease using steered molecular dynamics simulation (SMD) technique.

The average force profiles as a function of the simulation time can be seen in Figure 2. The force profile in Figure 2 showed one peak, which is defined as the maximum force (before breaking) exerted during a given SMD simulation. The maximum peak heights for all peptides ranged from 700 pN to 800 pN. The force maximum was reached at 2.16 ns in the high binding peptide (Figure 2). As seen from Fig. 2, the low binding affinity peptide is completely out of the binding cleft after 3.03 ns.

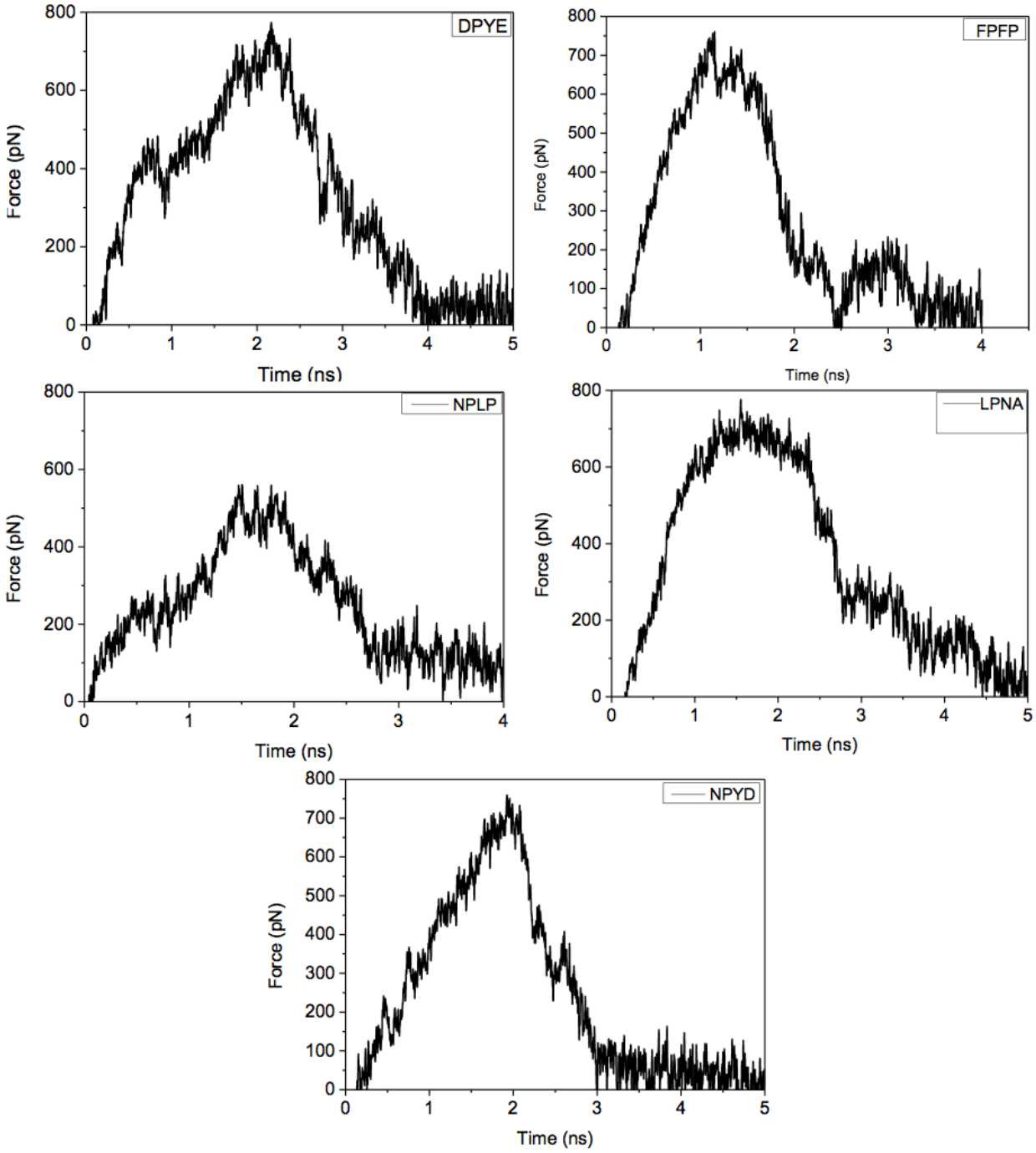

Figure 2: The average force profiles as a function of time of the protein-peptide complex for six replicates by SMD.

For all the peptides, we calculated potentials of mean force (PMF), which is the free-energy difference between the bound and the free states, from the Jarzynski's equality, shown in Figure 3 and was assessed. For all the peptides, the average binding free energy was 98.94, 65.26, 40.27 and 27.73 kcal/mol, respectively. The binding free energy can be approximated to the Gibbs free energy [44].

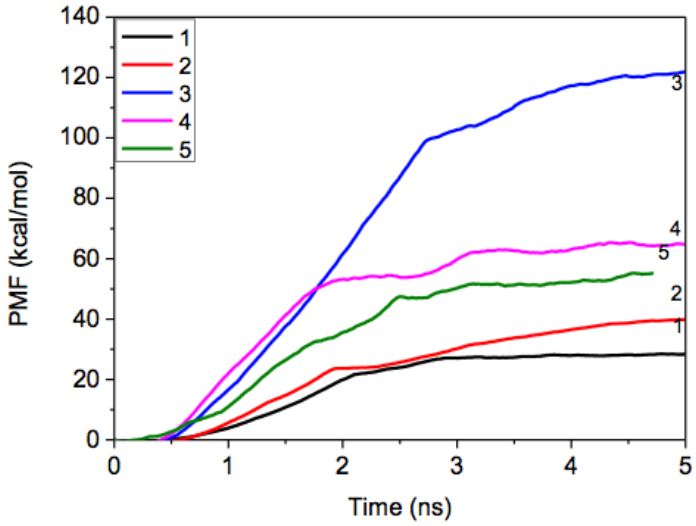

Figure 3: The potentials of mean force (PMF) profiles calculated from SMD trajectories for all the peptides.

Then, the theoretical Gibbs free energy for all the peptides are compared with the experimental IC50 values (see Table 1). The calculated IC50 values according to equation-4 and the IC50 values given in Table 1 are not compatible. These results show the correlation between PMFs and experimental IC50 values due to the unfavourable conformational changes occurring during SMD simulations in protein-peptide complex. Only the force profile of FPFPEDYPN peptide showed three peaks after 1.77 ns. It is clear from the PMF profiles given in Fig. 3 that the binding affinity of NPYDSVKKI peptide is much weaker than the "binding" affinity of DPYEVSYRI peptide.

$$\Delta G = -RT \log (IC_{50}) \quad (4)$$

The position of the DPYEVSYRI peptide within peptide binding cleft the conclusion of the production MD simulations are shown in Fig. 4. Detailed simulation trajectory analysis suggests that the hydrogen bonds start to break (Fig.4) and peptides begin to break up from peptide binding cleft after maximum peak values (Fig. 2).

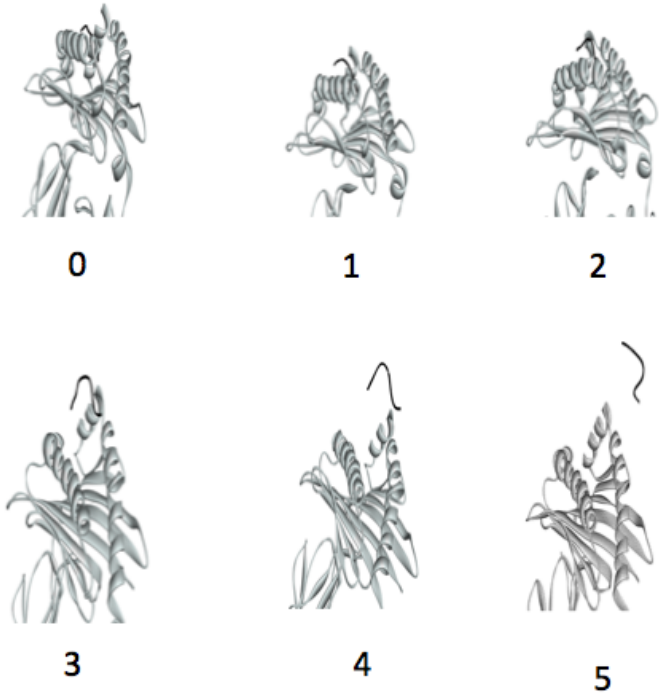

Figure 4: Snapshots from the SMD trajectory as a function of time for DPYEVSYRI peptide (black) configurations.

4. CONCLUSION

The binding free energy of peptide to protein-peptide complex is an important thermodynamic property. The goal of this study was to investigate the binding free energy of protein-peptide complex by means of steered molecular dynamics (SMD) simulations. Here, we have shown that SMD can provide insight into the force-time profiles of protein–peptide complex. From PMF results, DPYEVSYRI peptide was found to be more effective than the other peptides. This study highlights SMD simulation is emerging as a promising tool in the binding affinity of a complex. We think that there are many problems that we need to solve about this issue and that our results will encourage further research.

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