HOMO-OLIGOMERIZATION OF TRANSMEMBRANE α-DOMAIN OF INTEGRIN

Amir Shamloo, Ehsan Nikbin, Nastaran Mehboudi and Behzad Damirchi

Abstract— Integrins contribute to form focal adhesions complex. Therefore, simulation of integrin interactions can be helpful in clarifying the mechanism of focal adhesion formation. Interactions of integrins can also initiate signal transduction in the focal adhesions. Since integrins contain α and β subunits that are separated in an active state, studying both subunits separately is crucial, since, in the active state of integrins, the distance between these subunits is long enough that they do not influence one another significantly. Thus, this study aims to investigate the tendency of α subunits of integrins to form homodimers. All simulations were carried out via MARTINI coarse grain (CG) molecular dynamics technique. α subunits were placed in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid bilayer at a distance of 5 nm, and they were allowed to diffuse in the lipid bilayer. All simulations showed that α subunits have a tendency to form stable dimers.

I. INTRODUCTION

Cells need to communicate with their environment and with the other cells in order to survive, migrate and differentiate. In order to regulate cells' functions, signals are both transferred from one cell to the other cells and received from extracellular matrix (ECM). Focal adhesion formation is one of the cells' critical functions that occurs when cells require to transfer mechanical signals to the other cells or attach to the extracellular matrix. First and foremost, the initiators of this process are integrins which commence the signaling process by attaching to ligands or Arg-Gly-Asp (RGD) sequence of ECM proteins in order to transfer signals.

Integrin-ligand rigidity of binding can influence focal adhesion formation since the strength of the focal adhesion directly depends on the rigidity of the binding between integrin and the ligand. The integrins often influence one another and interact together to regulate cell's function. Integrins consist of α and β subunits that make them capable of performing multiple functions. αIIbβ3 Integrin is one of the important receptors that initiates focal adhesion formation [1]. In the inactive state, α and β subunits are bound, but in the active state they are distant from each other and can cluster in the form of homodimers or heterodimers [2]. As a result, both α and β subunits are important for studying the mechanism of focal adhesion formation. In addition, studying of α and β subunits together and separately are important, because clustering of these subunits in different manners can cause variations in the strength of the focal adhesions and consequently can affect cell signal transduction.

In many integrins, α subunits have several binding sites for divalent cations such as Ca^{2+} [3], and some researches have indicated that Ca^{2+} increases focal adhesion formation, thus it is essential to study the clustering of α subunits to partly understand the mechanism of focal adhesion formation [4]. In this study, the clustering of α subunits is simulated using molecular dynamics technique to investigate their potential role in focal adhesion formation.

There exist many research studies that have been carried out on α subunit homodimers. For instance, Li et al.[5] found out that α and β subunits have a tendency to form dimers and trimmers respectively, although Wang et al.[6] observed that α and β subunits don't have any tendency to form homo-oligomers. Furthermore, Mehrbod et al. modeled transmembrane and cytoplasmic domains (TMC) of αIIbβ3 integrin using molecular dynamics simulation. They showed that the hydrophobic residues in α subunits are more available than in β subunits; therefore lipid packings around β subunits are less than the ones around α subunits. Moreover, they showed that β subunits can get closer to each other via talin [7]. In addition, Kalli et al. investigated α and β subunits heterodimeration by CG MARTINI and compared their simulation results with NMR structure of these subunits. His simulation and experimental results were in a good agreement [8]. Chng et al. investigated αLβ2 integrins heterodimer by CG MARTINI method and then compared it with αIIbβ3 integrins heterodimer[9]. Thus, CG MARTINI method is used extensively for investigation of heterodimer conformation of α and β subunits. Accordingly, CG MARTINI method may an appropriate method for investigation of α subunits cluster formation in the active state and we have employed this technique for our studies.

II. MATERIAL AND METHOD

A. METHOD

The current investigation utilized MARTINI coarse grain mapping to construct the model [10]. The MARTINI force field includes parameters for a wide range of biomolecules, such as amino acids and lipids. Also, it is a protocol to model proteins. The results of the MARTINI

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model simulation was compared with experiment and the result showed that the model usually performs efficiently for a variety of systems [11]. In this technique, first, the atomic structure is provided completely and then it maps onto the CG structure by a standard protocol [10]. In this mapping, four heavy atoms (non-hydrogen) are assumed as single "bead". Four water molecules are mapped onto one bead. Amino acids are embodied by 2-5 beads. The mass of CG bead is the same as the mass of the atoms exist in each group. Mass of each bead is assumed to be 72 amu in order to provide computational efficiency for lipids, ions, and water [12]. In comparison to all-atom simulation, however, molecular friction is missing, this kind of mapping have smoother potentials. In the following, the interaction among the beads in bonded and none-bonded forms are explained. Bonded interactions are defined as series of potential energy functions below:

$$V_b = \frac{1}{2} K_b \left(d_{ij} - d_b \right)^2 \quad (1)$$

$$V_a = \frac{1}{2} K_a \left[\cos\left(\phi_{ijk} \right) - \cos\left(\phi_a \right) \right]^2 \quad (2)$$

$$V_d = K_d \left[1 + \cos\left(\theta_{ijkl} - \theta_d \right) \right] \quad (3)$$

$$V_{id} = K_{id} \left(\theta_{ijkl} - \theta_{id} \right)^2 \quad (4)$$

In all above equations, the acting bonded sites are presented as indexes i,j,k,l with equilibrium distance d_b, angle ϕ_a, and dihedral angles θ_d and θ_{id}. The force constants K are generally weak, implying flexibility of CG mapping. V_b represents chemical bond potential and V_a is used for chain stiffness. V_d is proper dihedral presenting the secondary structure of the peptide backbone, and the improper dihedral angle potential. V_{id} is used to avoid out-of-plane distortions of planar groups.

None-bonded interactions are comprised in two groups. Each pairs of particles i, j at distance r_{ij} interact via Lenard-Jones (LJ) potential which can be written as:

$$V_{LJ} = 4\varepsilon \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^{6} \right] \quad (5)$$

Well-depth ε_{ij} represents the strength of interaction due to particle types. The effective size of the particles is governed by the LJ parameter $\sigma = 0.47nm$ for almost normal particle types. For the special class of particles such as ring-like molecules, slightly reduced parameters are set to model ring–ring interactions; $\sigma = 0.43nm$, and ε_{ij} is scaled to 75% of the standard value [11]. The full interaction matrix can be found in Ref. [13].

Charged groups interact via a Coulombic energy function as below:

$$V_{el} = \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon_{rel} r_{ij}} \quad (6)$$

Where, relative dielectric constant ε_{rel} is considered 15. In order to gain smoother potential for none-bonded potential, interactions are cut off at r_{cut} and begin to shift at r_{shift}

B. MODEL AND SIMULATION DETAILS

Models for the transmembrane-cytoplasmic α domain of integrin αIIbβ3 were taken from the published αIIbβ3 structure (Protein Data Bank ID 2KNC). Two identical α subdomains were implanted 50 Å apart along the line joining their geometrical centers in the 100×50 Å patch of POPC lipid bilayer and overlapping lipid chains were eliminated using software VMD [14]. The atomistic models were then converted to their coarse-grained counterparts. The membrane is solvated and the water box was extended ± 15 Å from the protein. The system was ionized with NaCl with concentration of 8 mM [15]. 15% of water beads are anti-freeze CG beads. A cutoff of 12 Å was considered to calculate none-bonded interactions with a shifting begins at 9 Å to apply a smooth cutoff. The time step of simulations is 30 fs. Initial system was minimized for 2000 steps. Nose–Hoover Langevin piston, with period of 2000 fs and a decay time of 1000 fs, was used to sustain a constant pressure of 1 atm and also, Langevin dynamics, with a damping coefficient of 1 ps^{-1}, was used to maintain a constant temperature of 323 K. It is important mentioning that the simulations were implemented in NPT ensemble and building CG model from all-atom model was implemented using VMD software v1.9.1 [14] and all simulations was done using Nightly Build of NAMD molecular dynamics program[16]. In order to study the homomeric interactions between two α sub-units, they were permitted to diffuse freely in the lipid bilayer membrane. 10 equilibrium simulations were performed for 100-500 ns.

III. RESULTS AND DISCUSSION

Molecular dynamics simulations were targeted to investigate the homomeric interactions between two α subunits when they are in an active state so they are distant enough from β subunits that they do not interact significantly. In all simulations, at first the two α subunits were located at a distance of approximately 50 Å away from each other. This separation between the α subunits is larger than the cutoff radius for interactions that occur in electrostatic and van der Waals linkage, and so there is no interaction between monomers at the beginning and the initial spot does not favor dimer formation. During the simulations, two monomers diffuse randomly within the lipid bilayer towards each other, until they form a stable dimer during a few hundred nanoseconds.

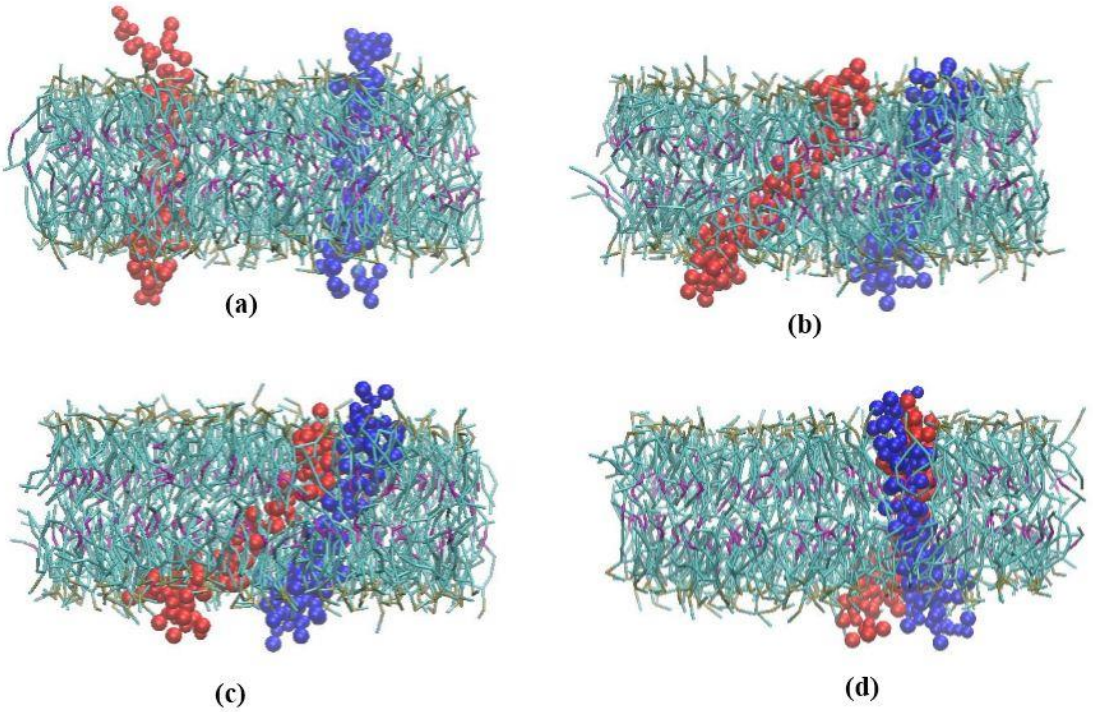

Figure 1. Sequences of snapshots of dimer formation. (a) Initial position of two α subunits within lipid bilayer was considered so that they are 50 Å apart from each other. (b) After 50 ns (c) After 90 ns and (d) Formation of α homodimer after 150 ns. (Water molecules have not been shown)

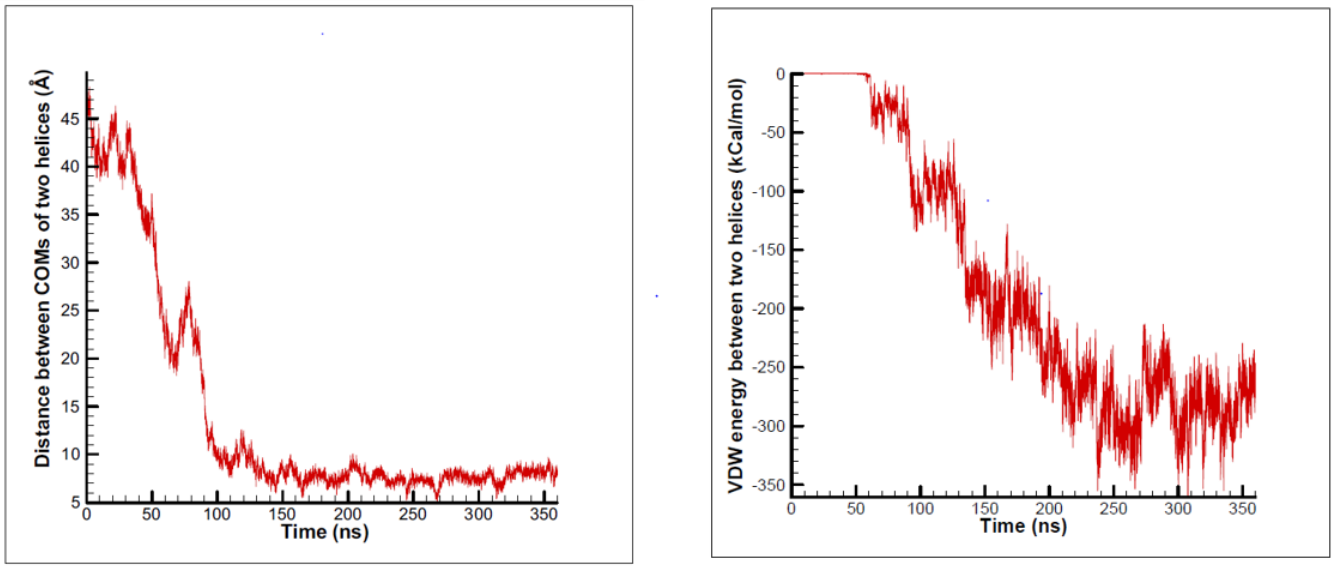

Figure 2. (a) Distance between centers of masses of two α subunits decreases during simulation time until it forms a dimer and the distance reaches a stable amount. (b) The Van Der Waals interaction energy of the two α subunits decreases during the simulation time.

Transmembrane α homodimer formation was observed in all 10 independent αIIbβ3 simulations within 100-300 ns (Figure 1). All simulations have shown that α subunits have a tendency to form a dimer. Diagram of the distance between the centers of masses of two monomers for one of the simulations is shown in figure 2(a). At first, the distance between two monomers was 50 Å; however, as the time passed, this distance decreased. Then, the two monomers stopped moving towards each other at the distance of 8 Å after 150 ns, which is the total radius of the two monomers. It was shown that the two monomers of α subunits form a stable dimer.

Diagram of van der waals interaction energy between two monomers is shown in figure 2(b). As it was expected, at the first stage of simulation, these two monomers don't have any van der waals interaction and the van der waals energy of the two is zero, however as the time goes by, two monomers get closer and the van der waals interaction energy decreases. Consequently, after several fluctuations, van der waals interaction energy reaches to the significantly negative amount which again confirms that the α subunits form a stable dimer.

IV. Conclusion

In this study, homodimer formation of TMC α domain of αIIbβ3 integrin was examined with coarse grained molecular dynamics approach. Simulation results showed that α subunits can form a stable dimer in the membrane bilayer. In fact, interactions of α subunits in molecular dynamic simulations can help partly explain the mechanism for the formation of focal adhesions. However, researchers have not studied comprehensively the possibility of the formation of bindings between α subunit of αIIbβ3 integrin. In this study, we observed that these subunits can significantly interact with one another and form stable dimers. It has been reported that the diffusion of α subunits is not affected by blocking cytoplasmic tails of these subunits with cytoskeleton [7]. Therefore, it is reasonable to allow α subunits diffuse randomly in the lipid bilayer to investigate their homomeric interaction. Although, the distances between α and β subunits in active state are far enough not to influence each other, the existence of β subunit in the membrane and simulating them concurrently can lead to a more comprehensive understanding of the mechanism of focal adhesion formation that we are currently working on this combined model. In summary, the results of this study can be helpful in understanding the mechanism of focal adhesion formation and may be useful in the development of drugs for stimulating or blocking focal adhesion formation.

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