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A Study on interactions of the SARS-CoV-2 spike proteins with the hTLR4 using molecular dynamic simulations

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Abstract

The several studies have showed that interaction between S1 subunit of SARS-CoV-2 spike proteins and human toll-like receptor 4 (hTLR4) activates the innate immune responses. It leads to the cytokines over secretions and may facilitate the 'cytokines storm' that contributes to the severity the COVID-19 patients. However, the atomic level explanation of the interaction between spike proteins and hTLR4 has not yet elucidated. In this study, we performed the molecular dynamics simulations of spike protein and hTLR4 to clarify their interaction. We show the possible interactions of spike protein and hTLR4.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has speared around the world since December, 2019. SARS-CoV-2 induce the human innate and adaptive immune systems, and the activation pathways are not still fully understood. However, the several studies have showed that interaction between S1 subunit of SARS-CoV-2 spike proteins and human toll-like receptor 4 (hTLR4) activates the innate immune responses [1-2]. It leads to the cytokines over secretions and may facilitate the 'cytokines storm' that contributes to the severity the COVID-19 patients [1-3]. However, the atomic level explanation of the interaction between spike proteins and hTLR4 has not yet elucidated. Spike protein of SARS-CoV-2 has an important role in an infection as it regulates viral entry into host cells [4]. Only the trimer spike protein could induce IL1B and IL6 by TLR4 pathway [2]. For dimerization, TLR4 and MD2 forms hydrophobic and hydrophilic interactions directly with LPS [5, 6]. These interactions may be interacted with SARS-CoV-2 trimer spike proteins. In this study, we performed the molecular dynamics simulations of spike protein and hTLR4 to clarify their interaction. We show the possible interactions of spike protein and hTLR4.

Methods and Materials

The crustal structures of spike protein and TLR4 were retrieved from the Protein Data Bank [7] (PDB; 3FXI and 6XLU ,respectively). Spike protein structure has the missing residues filled with MODELLER. We prepared a two independent systems: hTLR4 dimer complex with MD2 and closed spike trimer for each of wild-type. Residues 27-627 were considered in hTLR4 dimer structure and 11-555 in the MD2 structure. Residues 1-1148 were considered in closed trimer spike structure. Two systems were set up using GROMACS additional tools with standard parameters, including a physiological salt concentration of 0.15 M NA-CL. All-atom, molecular dynamics (MD) simulations were performed using the GROMACS [8] 2022.1 with the CHARMM36 force field. Minimization and equilibration were performed with NVT (volume and temperature) conditions, and the production runs were performed under NPT (pressure and temperature) conditions at 1 bar and 300 K using 2 fs time steps. The two systems were run for 150 ns each. The RMSD, RMSF and cluster analysis were calculated using the g_rms, g_cluster and g_rmsf tools of GROMACS, respectively, provided with GROMACS. Visualization of all structures were done with VMD.

Results and Discussion

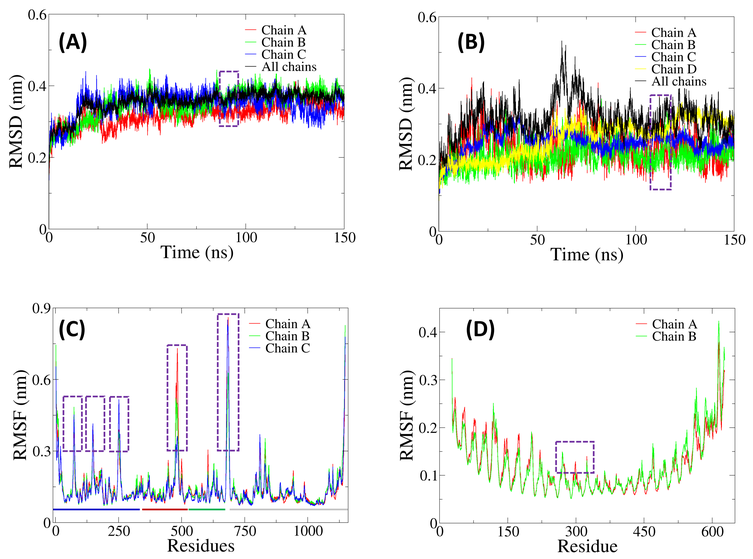

Fig 1: Root-mean-square deviations and fluctuation of SARS-CoV-2 spike and the hTLR4 protein. (**A**) RMSD of each chains of SARS-CoV-2 spike protein, (**B**) RMSF of each chains of SARS-CoV-2 spike protein, (**C**) RMSD of each chains of hTLR4 and MD2, (**D**) RMSF of each chains of hTLR4. The highlighted box shows the important residues.

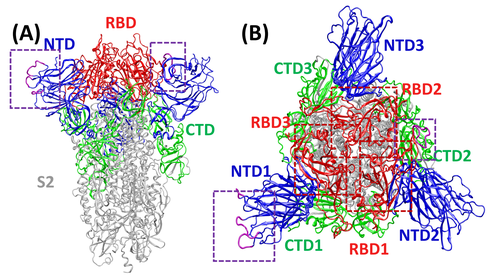

Fig 2: The stabled structures of the closed trimer spike proteins. (**A**) Top view of the trimer. (**B**) Side view of the trimer. The highlighted box shows the important residues.

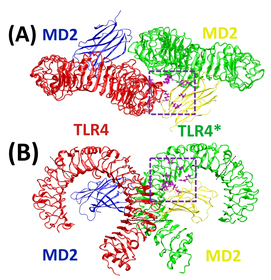

Fig 3: The stabled structures of the dimer TLR4 and MD2. (**A**) Top view of the dimer. (**B**) Side view of the dimer. The highlighted box shows the important residues.

In this study, we performed the molecular dynamics simulations of spike protein and hTLR4 to clarify their interaction. For larger structures, each chains of trimer spike (Fig 1A) and dimer hTLR4 (Fig 1B) and show the root mean square deviation (RMSD) levels off to ~0.4 nm (4 Å) during the simulations, indicating that the structure is stable. To find the most stable structure for each of dimer hTLR4 and trimer spike, each of the conformations in the last 100 nanoseconds of the simulations was clustered by RMSD the atoms of the main chain. For trimer spike, conformation in 92 nanoseconds of the simulation is most stable (Fig 1A, 2). For dimer hTLR4, conformation in 113 nanoseconds of the simulation is most stable (Fig 1B, 3). To understand the flexibility of the spike protein and hTLR4, we calculated the average root-mean-square fluctuations (RMSF) of the all residues (Fig 1C, 1D). In simulation of trimer spike, A five loops consisting of 71-73, 147-150, 248-254 residues of the amino-terminal (N-terminal) domain (NTD), 475-487 residues of receptor binding domain (RBD) and 678-688 residues of carboxy-terminal (C-terminal) domains of the trimer spike is showing the larger fluctuation than the other region during simulations (Fig 1C, 2) without the residues of N- and C-terminal. The four loops of these may be interacted with hTLR4 (Fig 1C, 2). The last one loop is involve that the spike proteins is cleaved into S1 and S2 subunits by furin protease (Fig 1C, 2) [4]. The three RBDs of the trimer spike form the apex of the S protein (Fig 2) [4]. The extracellular domains of TLR4 consist of 22 leucine-rich repeats (LRRs) with a horseshoelike shape [5]. In simulation of hTLR4, residues of beta strands of concave surface of each LRRs is showing the larger fluctuation than residues of convex surface (Fig 1D) [6]. It is show that residues of concave surface has an important role to stability of the horseshoelike structures [6]. The central region consisting 200-500 residues of TLR4 is showing the more fluctuation than N- and C- terminal regions (Fig 3). It is show that central region is important role to dimerization of hTLR4 and MD2 proteins. For dimer structures, R264, D294, D296, K341, K362, K388 residues of hTLR4 and K58, S118, K122 residues of MD2 forms hydrophilic bonds directly with LPS [4]. The these residues of central region of TLR4 is showing the fluctuation than other residues of central region, which may be interacted with S1 subunit of trimer spike protein [Fig 1D, 3]. We show the possible interactions of spike protein and hTLR4. These results will be important information for understanding the mechanism of interaction between the human TLR4 protein and the SARS-CoV-2 spike protein, and will help predict drug molecules to prevent complications of COVID-19 disease.

Conclusions

The several studies have showed that interaction between S1 subunit of SARS-CoV-2 spike proteins and human toll-like receptor 4 (hTLR4) activates the innate immune responses. However, the atomic level explanation of the interaction between spike proteins and hTLR4 has not yet elucidated. In this study, we performed the molecular dynamics simulations of spike protein and hTLR4 to clarify their interaction. In simulation of trimer spike, A four loops consisting of 71-73, 147-150, 248-254 residues of the amino-terminal (N-terminal) domain (NTD), and 475-487 residues of receptor binding domain (RBD) of the trimer spike shows the larger flexibility. The four loops of these may be interacted with hTLR4. For dimer structures, R264, D294, D296, K341, K362, K388 residues of hTLR4 and K58, S118, K122 residues of MD2 forms hydrophilic bonds directly with LPS. These residues may be interacted with S1 subunit of trimer spike protein. We show the possible interactions of spike protein and hTLR4. These results will be important information for understanding the mechanism of interaction between the human TLR4 protein and the SARS-CoV-2 spike protein, and will help predict drug molecules to prevent complications of COVID-19 disease.

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