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Research Article

IN SILICO STUDY OF CRYO-EM STRUCTURES OF ANTIGEN-ANTIBODY COMPLEX OF CHIKUNGUNYA FOR THE DEVELOPMENT OF DIAGNOSTIC AGENT

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ABSTRACT

Objective: Despite the availability of the commercial rapid tests of chikungunya, the difference of pathogen's genotypes amongst different countries has created some causes for concern. It is found that the sensitivity of the current chikungunya rapid tests on Asian strain was only 20.5%, as compared to 90.3% when tested on the African phylogroup. Therefore, the development of diagnostics that is specific for the current strain circulating in the country is important to be done. The cryo-electron microscopy (cryo-EM) structures of antigen-antibody complex can be used as an insightful structural basis to the development of the tailored antibody for diagnostics purposes. However, cryo-EM structures usually were resolved in low resolution, thus some sterical clashes between residues are expected. This work aims to refine the cryo-EM structures of E1-E2 of chikungunya virus in complex with antibody using molecular mechanics method, to calculate the binding energy of antigen-antibody complex, and to compare it with the experimental results.

Methods: The cryo-EM structures were refined *in vacuo* by short minimization scheme using AMBER 14. The binding energies were calculated using FireDock and Molecular Mechanics Generalized Boltzmann Surface Area methods.

Results: The results showed that the direct calculation of binding energies of cryo-EM structures reflected high repulsive forces. While the calculation on the refined structure showed lower binding energies. Visual inspections on the complex structures also indicated that the refined structures showed better interactions.

Conclusion: The refinement of cryo-EM structures should be useful to gain more insight into the binding mode of interactions between antigenic protein and antibody, at the atomic level.

Keywords: Antibody, Chikungunya, *In silico.*

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INTRODUCTION

Chikungunya, an arthralgic disease, was first documented in East Africa as an epidemic in 1952-1953 [1,2]. This disease is transmitted to human by *Aedes* mosquitoes and caused by chikungunya virus (CHIKV) which belongs to *Togaviridae* family, genus *Alphavirus* [3]. CHIKV infection has caused a number of outbreaks in East and South Africa and also in Southeast Asia during the past 50 years. Chikungunya has similar symptoms with dengue fever and is characterized by painful and acute syndrome with fever, headache, skin rash, and joint pain (arthralgia) [4]. The latter symptom distinguishes CHIKV from dengue virus [5].

The most recent epidemic of CHIKV infections was recorded in the year of 2000 in Kinshasa (50,000 estimated cases) and 2001-2003 in Indonesia. It has also spread to the Indian Ocean Islands of Mayotte, Mauritius, Réunion, and the Seychelles with 270,000 cases in 2005-2006 in La Réunion Island), and India with 1.4-6.5 million estimated cases in 2006-2007 [3]. Chikungunya was recently reported to be endemic in Bandung, Indonesia [6].

Although the commercial rapid tests of chikungunya have been available, the difference of pathogen's genotypes among different countries has created some causes for concern. The sensitivity of the current chikungunya rapid tests on Asian strain was only 20.5% of that of African strain [7]. Therefore, the development of diagnostics that is specific for the current strain circulating in the country is important to be done. The cryo-electron microscopy (cryo-EM) structures of antigen-antibody complex can be used as an insightful structural basis to the development of the tailored antibody for diagnostics purposes. However, cryo-EM structures usually were resolved in low resolution, thus, some sterical clashes between residues are expected. The refinement of cryo-EM structures should be useful to gain better understanding of the intermolecular interactions, at the atomic level.

In 2013, four Fab fragments of monoclonal antibodies, namely, CHIK 9, CHIK-152, m10, and m242, were complexed with pseudo-atomic CHIK virus-like particles (VLPs). These antibodies bound to the E1-E2 antigenic protein of CHIKV. The cryo-EM structures of the four Fabs have been deposited in Protein Data Bank (PDB) [8]. Different neutralization mechanism between the Fabs was observed. Based on the analysis of these cryo-EM structures, differences on the Fab's footprint Fabs on VLP were shown. The footprints of CHK-9 and m242 Fabs were dominantly localized on the domain A while m10's on the domain B. CHK-152's footprint was spanned at both domains A and B. It was noted that CHK-152 prevents the viral fusion. Three other Fabs neutralized CHIKV infectivity by blocking the cellular binding site on the A domain of E2 protein [8]. Nevertheless, a detailed picture of the atomic interactions between antigen and antibody in these cryo-EM structures are remain unclear.

The objectives of this study were to refine the cryo-EM structures of E1-E2 of CHIKV in complex with antibody using molecular mechanics method, to calculate the binding energy of antigen-antibody complex, and to compare it with the experimental results.

METHODS

Cryo-EM structures

Cryo-EM structures of antigen-antibody complex of chikungunya which used in this study were downloaded from PDB. The PDB IDs for CHK-9, CHK-152, m10, m242, and E1-E2 CHIKV are 3J2Y, 3J30, 3J2Z, 3J2X, and 3J2W, respectively [8].

Molecular mechanics refinement of Cryo-EM structures

The cryo-EM structures of antigen-antibody complex of E1-E2 CHIKV were minimized *in vacuo* by 250 steps of steepest descent and followed by 250 steps conjugate gradient methods, using sander module in AMBER 14 [9]. The cutoff for non-bonded distance was 12 Å.

Binding energy calculations

Two methods were used to calculate the binding energy of antigen-antibody, i.e., FireDock [10] and Molecular Mechanics Generalized Boltzmann Surface Area (MM/GBSA) method implemented in MMPBSA.py [11]. In FireDock, options of antibody-antigen complex type, 100 cycles, and full refinement level were selected. In MM/GBSA calculation, salt concentration of 150 mM was used.

RESULTS

First, the antigen-antibody complex structures were prepared by combining the coordinates of E1-E2 CHIKV (PDB ID 3J2W) with that of the antibody. Then, the complex structures were submitted to FireDock server (http://bioinfo3d.cs.tau.ac.il/FireDock/index.html) to get the calculated binding energy. The result showed that the repulsive van der Waals force dominated the binding energy of m10 (Table 1). Hydrogen bond energies, one of the most important non-covalent bonds, were also generally weak in all of the complexes. This indicated that the atomic conformations in cryo-EM structures were not in a favorable position.

Furthermore, all of the antigen-antibody complexes were refined by molecular mechanics method. A short minimization (500 steps) was conducted *in vacuo*, to remove any bad contacts or sterical clashes that may lead to the repulsive forces. It is showed that when the refined complexes were submitted to FireDock server, the calculated binding energies improved (Table 2). It is worth noting that a high repulsive force in m10 system was removed. In general, the global binding energy, attractive van der Waals force, and hydrogen bond energy of all the refined systems were stronger than that of the initial conformations of cryo-EM structures.

The changes of energies reflected that the atomic conformations after minimization were in more favorable positions than before. Visual inspection on the refined structure showed no sterical clashes among the side chains of antigen and antibody. Fig. 1 shows that the primary amino group of Lys733 was in a very near distance with the side chain of Asp600 (0.938 Å). Furthermore, it is noted that the distance has increased to 3.895 Å after being refined by geometry minimization using molecular mechanics method.

However, despite the stronger binding energy (Table 2), the correlation between calculated binding energy and experimental effective concentration 50 (EC50) was not satisfying (R^2=0.1224). A possible reason behind this result was due to the simplified solvation model that is used in FireDock, i.e., atomic contact energy approach [10]. Therefore, the binding energy of the refined complexes were further calculated by MM/GBSA method, which uses a continuum solvent model (Table 3).

While FireDock shows m10 as the most active Fab, MM/GBSA was successfully identified CHK-152 as the best Fab with the strongest binding to E1-E2 CHIKV, although the third and fourth ranks of Fab were not really in agreement with the experimental values. However, the correlation coefficient of MM/GBSA was better than FireDock in this case.

DISCUSSION

Based on the binding energy, CHIK-152 was identified as the best antibody bound to E1-E2 CHIKV. This is in agreement with the fact that CHIK-152 has a better neutralization effect than m10 [8]. Pal and colleagues also reported that CHIK-152 prevented CHIKV infection up to 90% in all phylogroups, i.e., African and Asian strains [12]. The binding profile of CHIK-152 on E1-E2 CHIKV, at the atomic level, should be useful to provide structural basis in designing a specific antibody that can be used for a

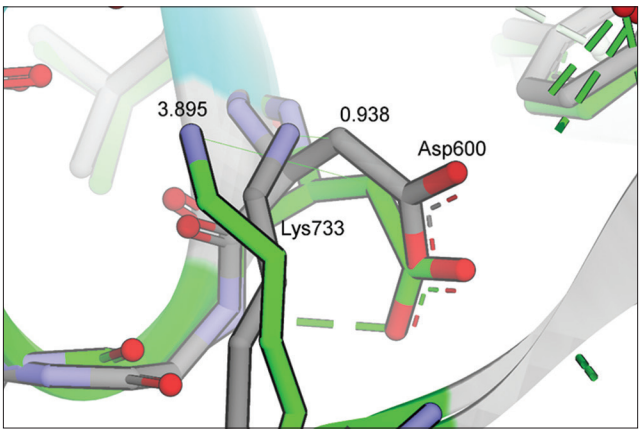

Fig. 1: Superimposition of the refined cryo-electron microscopy structure (green-colored carbon stick) on the initial structure (gray-colored carbon stick)

Table 1: Direct calculation of binding energy of cryo-EM structures using FireDock [8]

Fab	PDB ID	Experimental EC50 (µg/ml)*	Binding energy (kcal/mol)	Attractive VdW (kcal/mol)	Repulsive VdW (kcal/mol)	H-bond energy (kcal/mol)
CHIK-152	3J30	0.013	−9.7	−13.94	11.70	−0.81
m10	3J2Z	2.9	189.46	−10.75	335.50	−2.26
CHIK-9	3J2Y	18	22.85	−25.27	11.85	−2.07
m242	3J2X	20	−6.98	−7.40	0.75	−1.11

EC: Effective concentration, cryo-EM: Cryo-electron microscopy, CHIK: Chikungunya virus, PDB: Protein Data Bank

Table 2: Calculation of binding energy of the refined cryo-EM structures using FireDock [8]

Fab	PDB ID	Experimental EC50 (µg/ml)*	Binding energy (kcal/mol)	Attractive VdW (kcal/mol)	Repulsive VdW (kcal/mol)	H-bond energy (kcal/mol)
CHIK-152	3J30	0.013	−76.54	−55.89	17.19	−9.57
m10	3J2Z	2.9	−100.64	−74.59	18.44	−12.35
CHIK-9	3J2Y	18	−56.19	−69.94	17.32	−7.03
m242	3J2X	20	−89.78	−57.95	16.98	−6.69

EC: Effective concentration, cryo-EM: Cryo-electron microscopy, CHIK: Chikungunya virus, PDB: Protein Data Bank

Table 3: Binding energies calculated by FireDock and MM/GBSA as compared to the experimental EC50 [8]

Fab	Experimental EC50 (μg/ml)*	FireDock binding energy (kcal/mol)	FireDock R^2	MM/GBSA binding energy (kcal/mol)	MM/GBSA R^2
CHIK-152	0.013	−76.54	0.1224	−3.45	0.7306
m10	2.9	−100.64		17.39	
CHIK-9	18	−56.19		35.26	
m242	20	−89.78		25.62	

EC: Effective concentration, cryo-EM: Cryo-electron microscopy, CHIK: Chikungunya virus, PDB: Protein Data Bank, MM/GBSA: Molecular Mechanics Generalized Boltzmann Surface Area

sensitive diagnostic agent, or even therapeutics. A tailored antibody can be further developed into a paper-based diagnostics or biosensors [13]. Recently, four new methods to immobilize antibody on the nitrocellulose were introduced to support the rapid test developer [14].

In this study, binding energy calculated by MM/GBSA has better correlation coefficient with the experimental values, as compared to FireDock. This result is in agreement with the previous studies which showed that MM/GBSA method is often useful to refine the binding scores resulted from docking-based virtual screening [15]. Nevertheless, a better conformational sampling for MM/GBSA method should be able to improve the accuracy of binding energy calculation. This can be achieved by molecular dynamics simulations.

CONCLUSION

This study showed that the cryo-EM structures should be refined first before any structural analysis, especially for the purpose of designing antibody. Sterical clashes in the initial cryo-EM structure were observed by visual inspection. Structural refinement by short minimization using molecular mechanics method was able to improve the binding energy between antigen and antibody. However, the correlation between the calculated binding energy resulted from FireDock, and the experimental EC50 was not satisfying. Recalculation of binding energy using MM/GBSA has improved the correlation coefficient between the calculated and experimental values from 0.1224 to 0.7306. This study is an important initiative to the further development of antibody for a diagnostic agent.

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