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## 論文の要旨
## Abstract of thesis

Influenza A virus causes epidemics almost every year. Several anti-viral drugs are already approved by the authorities, MHLW, FDA and EMEA, but drug-resistant viruses have been emerged due to the error-prone genome replication. There are several drug targets in the influenza A virus such as viral polymerase, neuraminidase (NA), matrix protein 2 (M2), etc. Among them, the influenza viral polymerase is a replicative enzyme highly conserved among different strains, and is expected to be a prominent candidate of anti-viral drug target. But the structural information of viral polymerase is very limited because of the difficulty to express the recombinant viral polymerase. NA inhibitors such as oseltamivir are used as a first-line drug, but patients should receive NA inhibitors within 48 h after illness onset. On the other hand, M2 is essential for influenza virus replication, and is a proven drug target. Amantadine is a representative M2 blocker, but is no longer recommended for the treatment of influenza A because the majority of recent circulating influenza strains show the resistance.　Recently, adamantyl bromothiophene was reported to be a dual blocker targeting both amantadine-sensitive M2 (S31 M2) and amantadine-resistant M2 (N31 M2). Therefore, the applicant selected M2 as a target, and evaluated the binding kinetics of M2 blockers, amantadine and adamantyl bromothiophene, with M2 using metadynamics and conventional molecular dynamics studies.

First of all, the applicant elucidated the binding processes of M2 channel blockers into their binding sites and the nature of binding transition states, using metadynamics simulation, which is one of biased simulation

techniques, in order to make M2 blockers bind to M2 effectively and smoothly. The binding trajectories of the M2 channel blockers in the channel pore of S31 M2 showed that amantadine first binds to 2 of the 4 Asp24 residues via salt bridges, to yield a metastable conformation separated by a high free energy barrier. In contrast, adamantyl bromothiophene could only form one salt bridge with Asp24 of S31 M2 due to the steric hindrance, thus, the metastable binding state of adamantyl bromothiophene at Asp24 was more unstable than that of amantadine. This may explain why the antiviral activity of adamantyl bromothiophene is weaker than that of amantadine. In N31 M2, the binding of adamantyl bromothiophene to Asp24 was not metastable possibly due to the halogen bond between the bromine atom of adamantyl bromothiophene and Asn31 since halogen bonds tend to be more directional than hydrogen bonds. Drugs, which bind to the target protein, shuttle between metastable binding sites and the binding pocket. Thus, to separate metastable binding sites from the binding pocket by transition states with high free energy barriers is important to increase residence time of the drug in the binding pocket.

Next, the applicant examined the possibility of the emergence of the resistant viruses against adamantyl bromothiophene. M gene mutant virus libraries prepared from either S31 or N31 virus strain were passaged 3 times in the presence of M2 channel blockers. The M gene mutant libraries were generated by error-prone PCR, and were subjected to reverse genetics to produce the mutant virus libraries. The population of the resistant virus against amantadine was reached to 98%, in contrast, any resistant viruses against adamantyl bromothiophene did not appear from either S31- nor N31-based mutant virus libraries, indicating that adamantyl bromothiophene has the higher genetic barrier to drug resistance than amantadine. Adamantyl bromothiophene inhibits both S31 M2 and N31 M2 by binding in a different orientation. Thus, adamantyl bromothiophene does not allow the appearance of revertant of N31 M2 to S31 M2, thereby drug resistant mutants against adamantyl bromothiophene are thought to be hardly produced under its selection pressure.

The binding profiles of the M2 channel blockers with M2 protein examined by an unbiased simulation technique, conventional molecular dynamics simulation, showed that amantadine has only one pharmacophore in the M2 channel pore, on the other hand, adamantyl bromothiophene has multiple pharmacophores. It was also observed that adamantyl bromothiophene interacts with irreplaceable His37 residues of M2. It is likely that this binding specificity is also responsible for the lower incidence of drug resistant mutants against adamantyl bromothiophene. The drug design strategy targeting the amino acid residues, which is not replaceable to other amino acids, such as His37, may provide new M2 blockers which do not allow the emergence of resistant mutants.

The amantadine derivatives are thought to be high-profile drug candidates since M2 is a proven drug target and essential for influenza A virus replication. Many efforts have been made to discover novel M2 blocker, however, in spite of recent progress in structure-based anti-influenza drug design, the drug discovery targeting M2 has been impeded, and no M2 blockers have entered clinical trials since the approval of rimantadine in1993. Molecular dynamics simulation has provided insights into the structure and dynamics of compound-protein complexes at the atomistic level for drug design by focusing on the affinity and selectivity of a compound to its target. However, it is also known that the binding of compounds to proteins is often restricted through several metastable states separated by high free energy barriers from stable states, leading to kinetic bottlenecks. Taking these situations into consideration, the findings about the binding kinetics and the pharmacophoric features of M2 channel blockers, which provided throughout this project will contribute to open new concept to design further dual M2 blockers.

# 審査の要旨
# Abstract of assessment result

【批評 Review】

The applicant revealed the possibility to discover a novel drug for influenza A virus with no or little emergence of the resistant viruses using metadynamics and conventional molecular dynamics simulations. The target is not novel, but the applicant opened novel frontiers by a state-of-the-art technique. Influenza A virus causes epidemic almost every year. Therefore, the applicant' insight has a deepest impact, which is expected economically and socially. In addition, the thesis is well written and understandable.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 12, 01, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.