Molecular Insights into the Antiviral Mechanism of Ebselen Inhibitor Against the Main Protease (Mpro) of SARS-CoV-2

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Abstract: The coronarivus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus depend on various essential enzymes for replication, including the main protease (M^{pro}). In this paper, we carried out a semi-empirical computational study to gain molecular insights into the antiviral mechanism of the organoselenide inhibitor, ebselen. The reaction energy profile of the reaction mechanism from the unreacted ebselen up to the formation of the covalent S-Se product was determined. The energy profile showed several key transition states showing that the antiviral mechanism is a multistep process. The reaction mechanism could be summarized as follows: (1) deprotonation of Cys145 by His41, (2) attack of the deprotonated Cys145 to Se, (3) breaking of the Se-N bond in ebselen, and (4) transfer of the proton from His41 to the N atom of ebselen. The results gave insights and better understanding of the antiviral mechanism of ebselen and could provide hints for the design of new organoselenide inhibitors for SARS-CoV-2.

Key Words: COVID-19; SARS-CoV-2 main protease; ebselen; semi-empirical quantum method; reaction mechanism

1. INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the SARS-CoV-2 virus.(Krammer, 2020; Wu et al., 2020) During the virus replication process, several essential enzymes are needed, and one of which is the main protease. The main protease, also called, M^{pro}, is the enzyme responsible for cleaving the translated proteins, and its inhibition can effectively block the replication of the virus.(Jackson et al., 2022; Ullrich & Nitsche, 2020)

The Mpro structure forms a homodimer with each protomer consisting of three domains I, II, and III. The catalytic dyad of M^{pro} consists of a histamine residue (His41) and a cysteine residue (Cys145). Due to the presence of the reactive sulfur atom in the active site, this residue is a very good target for many covalent and non-covalent inhibitors.

Recently, an organoselenide type of inhibitor, [(2-phenyl-1,2-benzoisoselenazol-3(2H)-one], or most commonly known as ebselen was identified and observed to show a very strong antiviral inhibition activity against SARS-CoV-2 Mpro.(Amporndanai et al., 2021; Menéndez et al., 2020) Ebselen is a repurposed drug with reported biological activities including as an antioxidant, anti-inflammatory, and therapeutic potential in neurological disorders, cancer, and others.(Schewe, 1995; Sies, 1993) Ebselen was also found to inhibit other cysteine proteases from other viruses such as 2Apro or 3Cpro and SARS-CoV-2 papain-like protease, which could indicate that ebselen is a good multi-target drug candidate.(Sies & Parnham, 2020)

Determination of the actual reaction mechanism of the observed antiviral activity typically involves computational methods. By studying the reaction at the atomic level, new insights can be found that is typically hard to observe using experimental

methods.(Fernandes et al., 2021; Ramos-Guzmán et al., 2020) Previously, various computational methods, e.g., molecular docking, molecular dynamics, DFT, QM/MM, or combinations of these methods were employed to determine the antiviral reaction mechanism of various drugs against Mpro. (Arafet et al., 2021; Madabeni et al., 2021; Parise et al., 2021) In these studies, they showed that the first step is the deprotonation of Cys145 by His41, and followed by the formation of the bond between the S atom of the protein and the ligand/drug.(Świderek & Moliner, 2020; Zanetti-Polzi et al., 2021) The crystal structure of Mpro treated ebselen as the inhibitor, showed that the S atom of Cys145 is indeed bonded to the Se atom of ebselen, confirming the proposed mechanisms.

This study focuses on the determination of transition state structure/s in the reaction of ebselen and an ebselen derivative, EB2-1, with the catalytic dyad of SARS-CoV-2 M^{pro}, Cys145 and His41. The computations were done at a much lower cost as compared to previous study by using a semi-empirical method, PM6-D3H4, in a truncated model of the protein containing only the reactive residues and carried out in gas-phase. Although other residues in the catalytic site may affect the protein-receptor interaction, the mechanism is assumed to proceed similarly. The reaction energy profile was mapped from the unreacted ebselen up to the covalently bonded configuration of Cys145 and ebselen.

2. METHODOLOGY

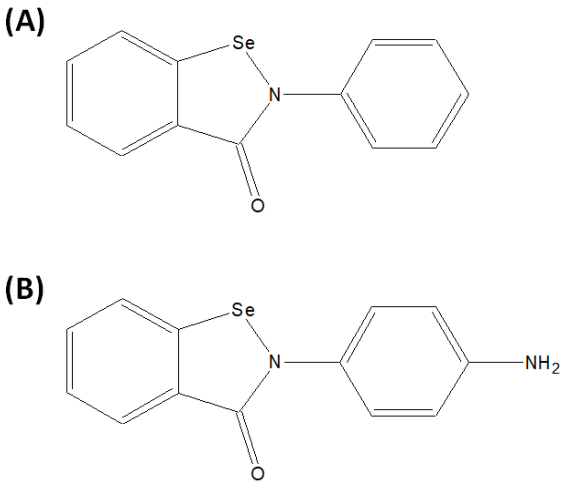

Fig. 1. Chemical structures of (A) ebselen and (B) the ebselen derivative, EB2-1, used in the study.

Fig. 1 shows the chemical structures ebselen and the ebselen derivative, EB2-1, used in the study. Semi-empirical quantum chemical calculations were carried out with the AMPAC10 package.(*AMPAC 10*, n.d.) The PM6-D3H4 method was used to model the system in gas phase. The PM6-D3H4 method has been shown to model biochemical systems with very high accuracy and whose results are even comparable to much more expensive quantum chemical methods.(Christensen et al., 2016; Kříž & Řezáč, 2020). The structures of the reactant and product were first optimized and used as the starting and final structures for the CHN calculation to determine transition states between the two input geometries. FORCE calculations were carried out to verify that the structures are at a minimum or a maximum. The GaussView6 program(Dennington et al., n.d.) was used to generate starting structures, input, and view the output.

3. RESULTS AND DISCUSSION

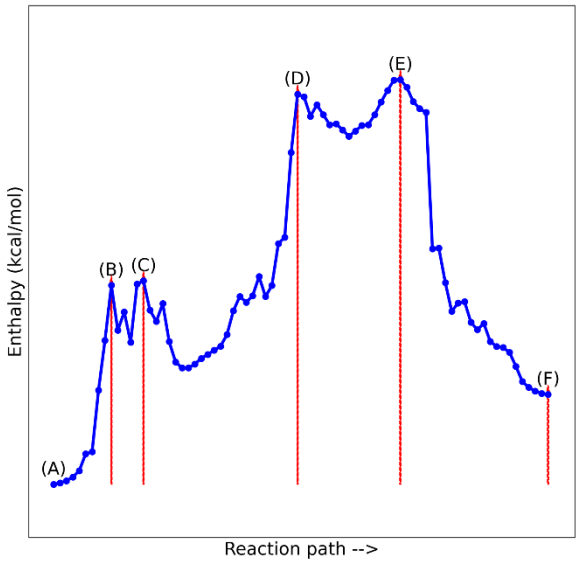

Fig. 2. Energy profile of the reaction mechanism of ebselen with the active site residues of M^{pro}. The determined transition states are labelled from B to E, while the reactant and product were labelled A and F, respectively.

Fig. 2 shows the energy profile of the reaction mechanism between ebselen and the active site of Mpro. From node A (Fig. 3A) to node B, it was observed that the S-H bond in Cys145 was broken and

the proton was transferred to His41 forming the CysS-/HisH+ pair which was also observed in the previous studies with an activation energy of 18.7 kcal/mol. The lengthening of the S-H and shortening of the H-N(H) distances are shown in Table 1. The transition state configuration is shown in Fig. 3B. From B to C, the S- atom leaves the proton and attacks the Se atom as shown by the shortening of the distance between S and Se in Table 1. The activation energy for this step is 19.1 kcal/mol and the configuration is shown in Fig. 3C. From node C to node D, we observed that the distance between the Se and N(E) are increasing resulting in the breaking of the Se-N bond, while the Se-S distance decreased resulting in bond formation. This step has an activation energy of 36.7 kcal/mol, with the configuration shown in Fig. 3D. In nodes D to E, the Se-N bond is fully broken, and the Se-S bond is formed. The H-N(H) starts to increase and the H-N(E) decreases. This step has an activation energy of 38.03 kcal/mol and shown in Fig. 3E. The final step is the formation of the product where the N(H) atom is deprotonated by the N(E) atom, the Se-N(E) broken, and the Se-S bond is formed. The final configuration is shown in Fig. 3F.

Table 1. Distances and bond lengths of several important atom/bonding pairs along the reaction path in the reaction of ebselen with the active site residues of M^{pro}. N(H) refers to the N atom involved in His41, while N(E) corresponds to the N atom involved in ebselen.

Node	S-H	N(H)-H	S-Se	Se-N	N(E)-H
A	1.36	2.06	5.6	1.86	5.97
B	2.32	1.07	3.73	1.85	4.43
C	2.73	1.05	2.91	1.88	4.69
D	4.9	1.04	2.26	3.17	3.83
E	4.86	1.04	2.29	3.55	2.55
F	4.58	2.88	2.28	3.65	1.02

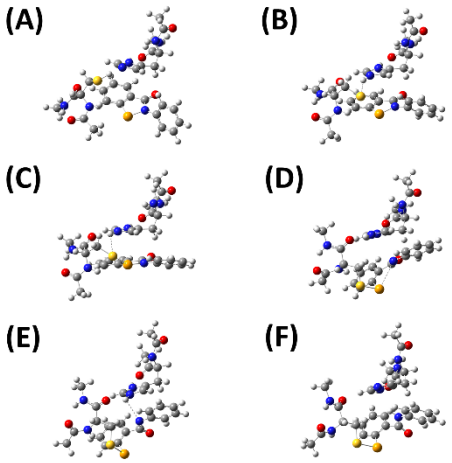

Fig. 3. Structural configurations of the (A) reactant, (B to E) transition states, and (F) product in the reaction of ebselen with the active site residues of M^{pro}.

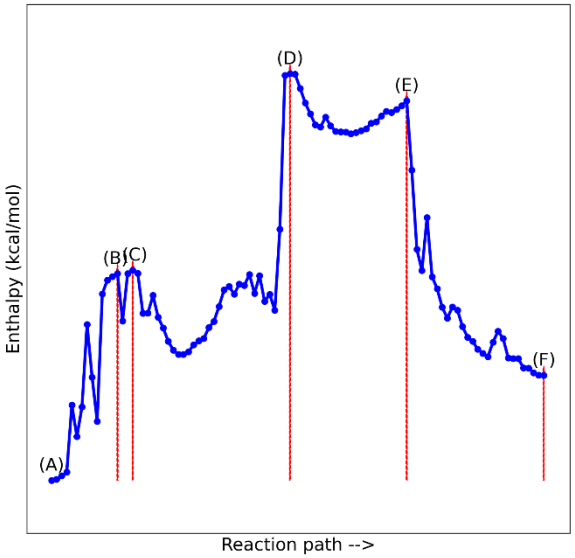

Fig. 4. Energy profile of the reaction mechanism of the ebselen derivative, EB2-1, with the active site residues of M^{pro}. The determined transition states are labelled from B to E, while the reactant and product were labelled A and F, respectively.

To see how the changes in the molecular

structure of ebselen may affect its antiviral reaction mechanism, we also carried out the same calculations and analysis using an ebselen derivative, EB2-1. EB2-1 has a 4-aminophenyl substitution in the phenyl ring doesn't cause a great increase in the molecular size and should possibly have a similar energy profile with the parent ebselen compound. In fact, the %inhibition at 0.3 µM of the two compounds are not very far from each other.(Qiao et al., 2021)

Fig. 4 shows the energy profile of the antiviral reaction mechanism for EB2-1 and it can be observed that it has the same overall shape with ebselen. Similar to the energy profile for ebselen shown in Fig. 2, there are four transition states as shown in nodes B to E. The first transition state has an activation energy of 19.5 kcal/mol and corresponds to the deprotonation of Cys145, resulting in the formation of CysS-/HisH+ ion pair and the configuration and bond distance changes are shown in Fig. 5B and Table 2. The second step involves the transition state at node C where the S atom attacks the Se atom and has an activation energy of 19.9 kcal/mol. The configuration is shown in Fig. 5C. The third step, which involves the breaking of the Se-N(E) bond has an activation energy of 38.4 kcal/mol and demonstrated by the increase in the distance between Se-N(E) and decrease in the distance between S and Se. The configuration is shown in Fig. 5D. The fourth step is the deprotonation of N(H) by N(E) forming the final product and has an activation energy of 35.9 kcal/mol.

Table 2. Distances and bond lengths of several important atom/bonding pairs along the reaction path in the reaction of the ebselen derivative, EB2-1, with the active site residues of M^{pro}. N(H) refers to the N atom involved in His41, while N(E) corresponds to the N atom involved in EB2-1.

Node	S-H	N(H)-H	S-Se	Se-N	N(E)-H
A	1.37	2.06	5.53	1.86	5.97
B	2.87	1.06	3.49	1.84	4.48
C	2.76	1.05	2.91	1.88	4.66
D	4.91	1.04	2.26	2.86	4.1
E	5.04	1.06	2.28	3.63	2.15
F	4.63	2.91	2.28	3.66	1.02

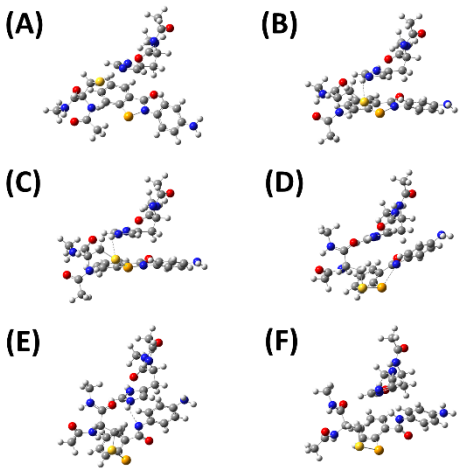

Fig. 5. Structural configurations of the (A) reactant, (B to E) transition states, and (F) product in the reaction of the ebselen derivative, EB2-1, with the active site residues of M^{pro}.

4. CONCLUSIONS

This study described the antiviral mechanism of ebselen in the M^{pro} of SARS-CoV-2. The study showed that Cys145 and ebselen form a covalent bond and the reaction is a multistep process and involves at least four reactions/transition states: (1) deprotonation of Cys145, (2) attack of S in Cys145 to the Se of ebselen, (3) the breaking of the Se-N bond, and (4) protonation of the N atom of the ebselen moiety. Since the antiviral activity of drugs depend on the reaction mechanism, understanding of the reaction mechanism, activation energies, and structural considerations would greatly help in the design of future drugs. Also, the procedure described in this study could be used as a low-cost quantum chemical method to evaluate potential drug candidates.

5. ACKNOWLEDGMENTS

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