Molecular Modeling Evaluation of the Binding Abilities of Ritonavir and Lopinavir to Wuhan Pneumonia Coronavirus Proteases

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Keywords
Coronavirus; Homology Modeling; Molecular Docking; Lopinavir; Ritonavir; Wuhan Pneumonia

Abstract
An anti-HIV drug named Kaletra, composed of two protease inhibitors, ritonavir and lopinavir, might have therapeutic effect on coronavirus diseases like Wuhan pneumonia. In this study, we built the structure models of two Wuhan pneumonia coronavirus proteases, coronavirus endopeptidase C30 and papain like viral protease, by homology modeling, followed by docking ritonavir and lopinavir to the protease models, respectively. In all the simulations, the binding between ritonavir and coronavirus endopeptidase C30 was most suitable. In addition, both ritonavir and lopinavir seemed more suitable to bind to coronavirus endopeptidase C30 than papain like viral protease. According to these results, we suggest that the therapeutic effect of Kaletra on Wuhan pneumonia, might be mainly due to the inhibitory effect of ritonavir on coronavirus endopeptidase C30.

1. Introduction

Coronavirus is a group of positive single-stranded RNA virus with coronary appearance [1]. Besides the recently discovered coronavirus which causes Wuhan pneumonia, six other coronaviruses have been found infectious to human [1-3]. Like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), Wuhan pneumonia can also cause severe respiratory disease [4] . Finding drugs that can fight against new coronavirus is a top priority.

According to the previous studies, an anti-HIV drug named Kaletra, which is composed of two protease inhibitors, ritonavir (CAS#: 155213-67-5) and lopinavir (CAS#: 192725-17-0), might have therapeutic effect on SARS and MERS [5-9]. Recently, the diagnosis and treatment guideline of pneumonia caused by new coronavirus infection (trial fourth edition) issued by the National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/), also recommends Kaletra to treat Wuhan pneumonia. At the same time, many randomize clinical control trials are carried out on studying the efficacy of Kaletra on new Coronavirus in China.

However, the mechanism of that Kaletra treat coronavirus diseases is still unclear. As coronaviruses, including Wuhan pneumonia coronavirus, synthesize polyproteins followed by hydrolyzed to produce their structure and function proteins [1, 10-12], it is suggested that Kaletra may block their multiplication cycle by inhibiting their proteases. To preliminarily understand the inhibitory effect of Kaletra on Wuhan pneumonia coronavirus proteases, in this study, we built molecular models of the proteases by homology modeling, followed by docking the two components of Kaletra, ritonavir and lopinavir, to the proteases in order to evaluate their inhibitory effects.

2. Methods and Results

2.1 Selection of Proteases Sequences

Since there was no gene of protease identified in the ten Wuhan pneumonia coronavirus genes directly, we chose to find the protease-like conserved domains in the polyprotein of the virus first. We analyzed the orf1ab polyprotein (GenBank: QHO60603.1) by NCBI Conserved Domain Search Service (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [13-16], and found two protease-like conserved domains. One domain is coronavirus endopeptidase C30 (CEP_C30), located from 3292 to 3569 of the polyprotein sequence; the other domain is papain like viral protease (PLVP), located from 1564 to 1880 of the sequence. We used these two sequences for further modeling and analysis.

2.2 Homology Modeling of Proteases

We modeled CEP_C30 and PLVP by Homology Modeling through SWISS-MODEL (https://swissmodel.expasy.org/) [17-21]. Before modeling CEP_C30, we deleted the 5 residues at the C-terminal of the sequence to minimize disordered structures. While modeling CEP_C30, 8 models were built according to 8 automatically found templates. The top-rated model in the 8 models is a homo-dimer with no ligand, and was selected to represent the structure of CEP_C30. While modeling PLVP, 29 models were built according to 16 automatically found templates. The top-rated model in the 29 models

is a monomer with a zinc ion ligand, and was selected to represent the structure of PLVP. The structures of the two selected models are available in Supplemental Files S1 and S2. The detail information, evaluation scores and Ramachandran plots of the two models are shown in Figure 1.

2.3 Molecular Docking of Ritonavir and Lopinavir

We used Discovery Studio software (version 2.5) to dock ritonavir and lopinavir to the proteases, respectively. First we set CEP_C30 as receptor, and found all its possible docking sites. A sphere whose radius was 17 units was set in the optimal docking site, followed by rigid docking of ritonavir and lopinavir to CEP_C30, respectively. While docking, the dockligands (libdock) module of Discovery Studio software was used, and the ritonavir or lopinavir were limited in the defined sphere. The same procedure was executed to dock ritonavir and lopinavir to PLVP, respectively.

Through docking, 100 poses were found when docking ritonavir to CEP_C30, with the libdock score of the optimal pose 192.346; 88 poses were found when docking lopinavir to CEP_C30, with the libdock score of the optimal pose 147.123; only 4 poses were found when docking ritonavir to PLVP, with the libdock score of the optimal pose 164.153; and only 3 poses were found when docking lopinavir to PLVP, with the libdock score of the optimal pose 107.137. The interactions between the proteases and drugs at the optimal pose are shown in Figure 2, with the interactions between PLVP and the drugs obviously less than between CEP_C30 and the drugs. The structures of the docking proteases and drugs at the optimal pose are available in Supplemental Files S3, S4, S5, and S6.

3. Discussion

In this study, we built two models of two protease-like domains, CEP_C30 and PLVP, of orf1ab polyprotein of Wuhan pneumonia coronavirus. Since these two models were built by taking highly homologous crystal structures, their qualities are quite considerable. As it is difficult to obtain the crystal structure of CEP_C30 and PLVP in a short time, there is great significance to use these reliable structure models to predict their dynamic interactions with other molecules.

As the catalytic mechanisms of CEP_C30 and PLVP domains are both unknown, it is unable to determine whether ritonavir and lopinavir are competitive or non-competitive inhibitors, although these two drugs are both peptide analogues and seem to be competitive. However, as both competitive and non-competitive inhibitors need to bind tightly to the enzyme, we evaluated the binding abilities of ritonavir and lopinavir to the proteases in order to preliminarily estimate their inhibitor effects on these proteases. It is suggested that the binding between CEP_C30 and ritonavir is most suitable in all our simulations, considering both libdock scores and intermolecular interactions. In addition, compared with CEP_C30, both ritonavir and lopinavir seem not suitable to bind to PLVP. Therefore, we speculate that the therapeutic effect of Kaletra on Wuhan pneumonia and other coronavirus disease may be mainly due to the inhibitory effect of ritonavir on CEP_C30, which suggests that further studies should focus on finding the catalytic mechanism of CEP_C30, and figuring out the how ritonavir blocks this

procedure.

Supplemental Materials

Supplemental File S1. The structure model of CEP_C30.
Supplemental File S2. The structure model of PLVP.
Supplemental File S3. The structures of docking CEP_C30 and ritonavir at the optimal pose.
Supplemental File S4. The structures of docking CEP_C30 and lopinavir at the optimal pose.
Supplemental File S5. The structures of docking PLVP and ritonavir at the optimal pose.
Supplemental File S6. The structures of docking PLVP and lopinavir at the optimal pose.

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Declarations

There is no conflict of interest to declare.

Figure legends

Figure 1. The detail information, evaluation scores and Ramachandran plots of structure models of CEP_C30 and PLVP.

The structure model of CEP_C30 is a homo-dimer with no ligand, built by taking PDB: 4MDS.1.A as template. Each chain of the model of CEP_C30 is consist of 273 amino acid residues, with 95.60% sequence identity with the template. The structure model of PLVP is a monomer with a zinc ion ligand, built by taking PDB: 5TL7.1.B as template. The model of PLVP is consist of 317 amino acid residues, with 82.86% sequence identity with the template. The evaluation scores and Ramachandran plots of two models were automatically created by Structure Analysis and Verification server (version 5.0, https://servicesn.mbi.ucla.edu/SAVES/). There are three scores used to evaluate the models: Verify score, Errat score and Prove score. The higher Verify score and Errat score are, as well as the lower Prove score is, the more reasonably the model is built.

Figure 2. The interactions between components of Kaletra and proteases at the optimal poses.

(A) The interactions between ritonavir and CEP_C30; (B) the interactions between lopinavir and CEP_C30; (C) the interactions between ritonavir and PLVP; and (D) the interactions lopinavir and PLVP at the optimal poses. The main chains of CEP_C30 are shown in white, and of PLVP are shown in green. The side chains of CEP_C30 and PLVP which interact with ritonavir or lopinavir are shown in blue. Ritonavir and lopinavir are shown in red. The hydrogen bonds are shown in white interrupted lines.

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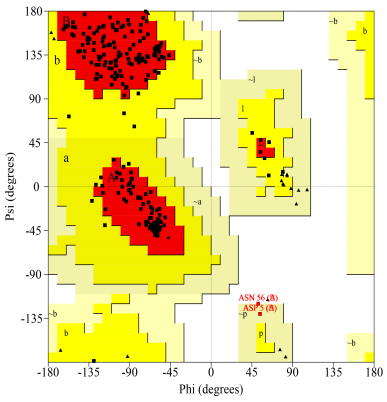
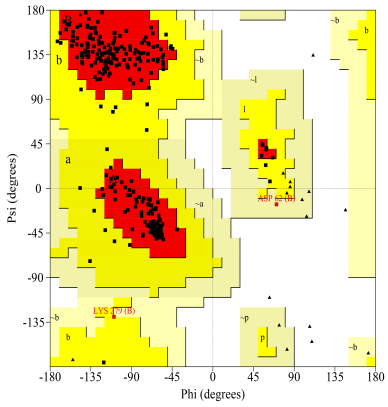
	CEP_C30	PLVP
Amino acid residues	273	317
Biounit oligo state	homo-dimer	monomer
Ligand	none	1 × zinc ion
Template PDB ID	4MDS.1.A	5TL7.1.B
Sequence identity with template	95.60%	82.86%
Verify score	91.03%	88.25%
Errat score	96.4143	91.2752
Prove score	2.5%	2.6%
Ramachandran plot		
	Residues in most favoured regions 91.2%	Residues in most favoured regions 92.2%
	Residues in additional allowed regions 7.9%	Residues in additional allowed regions 7.1%
	Residues in generously allowed regions 0.8%	Residues in generously allowed regions 0.7%
	Residues in disallowed regions 0%	Residues in disallowed regions 0%

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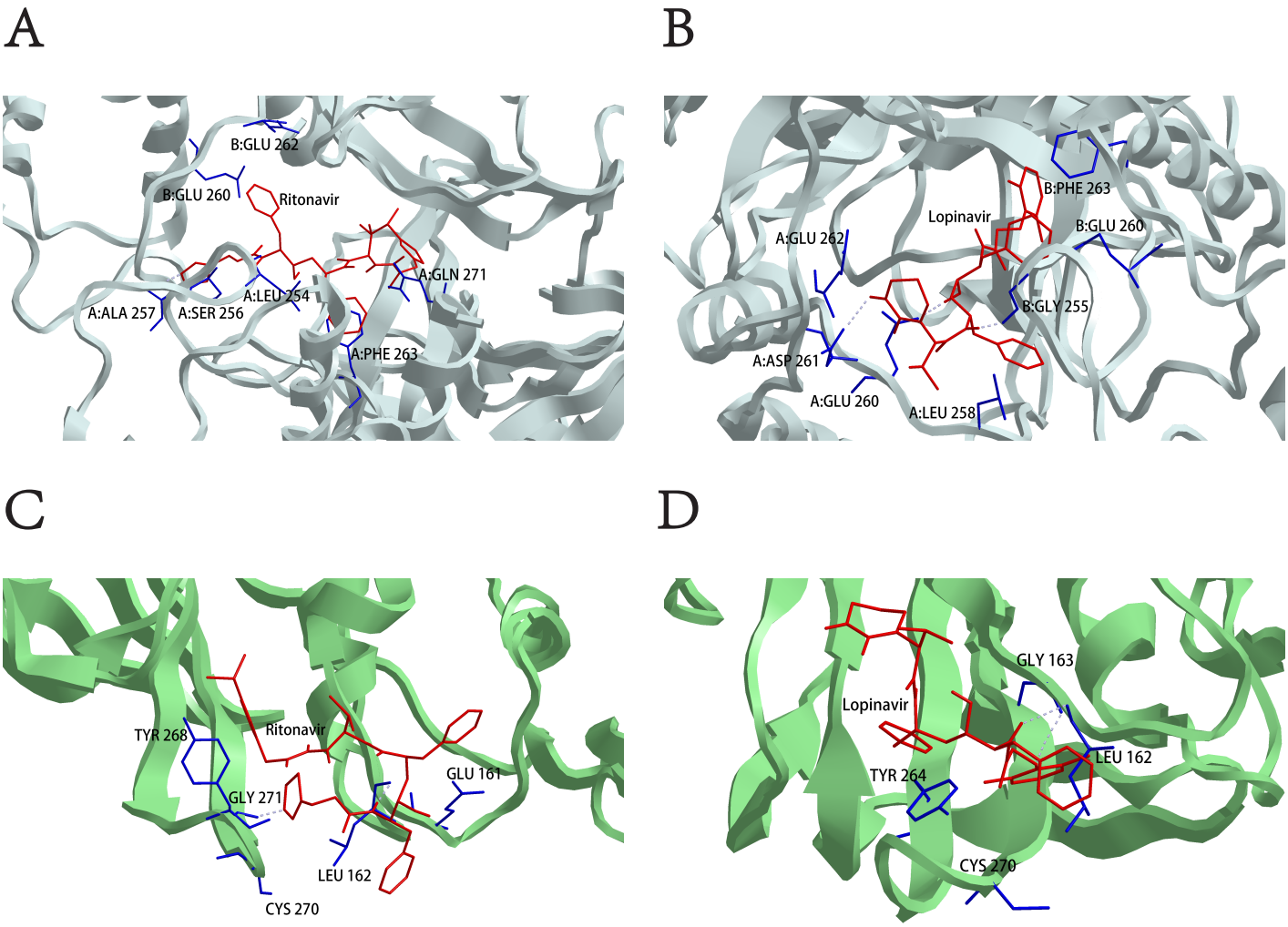

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