



Molecular dynamic simulations analysis of ritonavir and lopinavir as SARS-CoV 3CL^{pro} inhibitors

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ABSTRACT

Since the emergence of the severe acute respiratory syndrome (SARS) to date, neither an effective antiviral drug nor a vaccine against SARS is available. However, it was found that a mixture of two HIV-1 proteinase inhibitors, lopinavir and ritonavir, exhibited some signs of effectiveness against the SARS virus. To understand the fine details of the molecular interactions between these proteinase inhibitors and the SARS virus via complexation, molecular dynamics simulations were carried out for the SARS-CoV 3CL^{pro} free enzyme (free SARS) and its complexes with lopinavir (SARS-LPV) and ritonavir (SARS-RTV). The results show that flap closing was clearly observed when the inhibitors bind to the active site of SARS-CoV 3CL^{pro}. The binding affinities of LPV and RTV to SARS-CoV 3CL^{pro} do not show any significant difference. In addition, six hydrogen bonds were detected in the SARS-LPV system, while seven hydrogen bonds were found in SARS-RTV complex.

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1. Introduction

Reported first in the Guangdong province of China, in late 2002, as an atypical pneumonia, severe acute respiratory syndrome (SARS) is of concern, since it subsequently rapidly spread to over 25 countries and displayed an estimated mortality rate of 3–6% in infected humans, leading to more than 700 human deaths (Donnelly et al., 2003). In March 2003, four months after the first case was reported, the causative agent of SARS was identified as a new coronavirus (CoV) (Kuiken et al., 2003; Peiris et al., 2003). The complete genomic sequence (29,751 nucleotides) of the +ssRNA virus was reported a month later (Marra et al., 2003), providing the opportunity for more in-depth studies and the identification of potential targets for drug and vaccine development. The genome of SARS-CoV contains 11–14 major annotated open reading frames including those predicted to encode for known viral proteins, including the replicase polyproteins, S (spike protein), polymerase, M (membrane protein), N (nucleocapsid protein) and E (small envelope protein). The viral proteinase and replicase are the preferred targets for searching and designing of antiviral compounds. Indeed, the SARS-CoV main proteinase,

3CL^{pro} (also called M^{pro}), exhibits a key role in proteolytic processing of the replicase polyproteins and, as an essential protein for viral replication and function, is viewed as a key target.

Whilst phylogenetic analysis of the SARS genome reveals little resemblance to any of the three known groups of coronavirus, several of the specific viral genes, including the main protease gene (SARS-CoV 3CL^{pro}), do show significant homology. Thus, sequence alignment of the SARS-CoV 3CL^{pro} with orthologues from other coronaviruses indicated that the enzyme is highly conserved, with 40% and 44% sequence identity, respectively, to human CoV (HCoV) 229E proteinase and porcine transmissible gastroenteritis virus (TGEV) proteinase (Anand et al., 2003). The crystal structure of the 3CL^{pro} protein has been determined (Yang et al., 2003; Hsu et al., 2005; Lee et al., 2005), revealing that it is similar to those of other CoV proteinases. SARS-CoV 3CL^{pro} forms a homodimer, comprised of three domains. Domains I (residues 8–101) and II (residues 102–184) are β -barrels and together resemble the structure of chymotrypsin, respectively, whereas domain III (residues 201–306) consists mainly of α -helices. Domains II and III are connected by a long loop (residues 185–200). The active site of the M^{pro} is comprised of a catalytic dyad that consists of the conserved residues H41 and C145, which are located at the cleft between domains I and II (Huang et al., 2004). The availability of protein structures and the known biological characteristics of 3CL^{pro} both encourage and help

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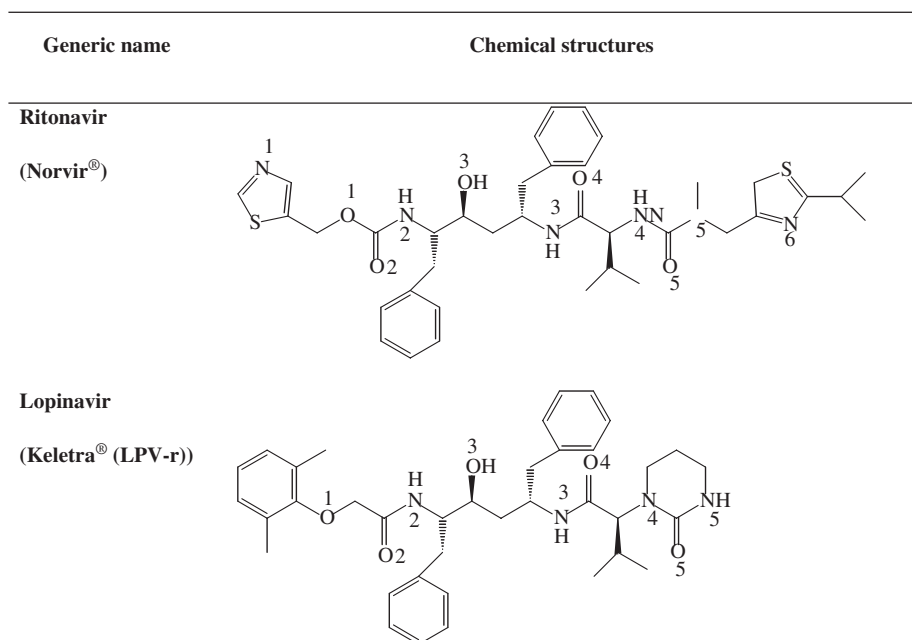


Fig. 1. Chemical formula of HIV-1 PR inhibitor, ritonavir and lopinavir, which exhibits signs of effectiveness against SARS.

facilitate it to be a key target for searching for drugs directed against SARS.

Although neither an effective antiviral drug nor a vaccine against SARS is currently available, several reports have indicated that HIV-1 protease inhibitors have potential for designing SARS-CoV proteinase inhibitors (Zhang and Yap, 2004; Yamamoto et al., 2004; Jenwitheesuk and Samudrala, 2003). In particular, the proteinase inhibitor Kaletra, which is a mixture of the proteinase inhibitors lopinavir and ritonavir (Fig. 1), exhibits encouraging signs of being partially effective against the SARS virus (Vastag, 2003). Therefore, a deeper understanding of known proteinase inhibitors as anti-SARS drugs can be used as a starting point to design and discover better or more specific inhibitors for the treatment of SARS, as well as any mutations by predicting resistance mechanisms and/or new drugs for such mechanisms in advance.

In the present research, the evidence for the potential benefits of HIV-1 protease inhibitors against the SARS coronavirus (SARS-CoV) is provided. Molecular dynamic simulations were performed in order to investigate the structure and dynamics behaviours of the free enzyme and its complexes with the lopinavir and ritonavir inhibitors.

2. Methods

2.1. Starting structure and protein preparation

In order to understand ligand–enzyme interactions as well as the effects of inhibitors on the structure and dynamic properties of the free enzyme, three molecular dynamics (MD) simulations were performed: SARS-CoV 3CL^{pro} free enzyme (free SARS) and its two complexes with the lopinavir (SARS–LPV) and ritonavir (SARS–RTV) inhibitors. The X-ray structure of the SARS-CoV 3CL^{pro} (PDB code 1UK3; 2.4 Å resolution) was used as the initial model to construct the SARS–RTV and SARS–LPV complexes (Fig. 2). Prior to the simulations, residues SerA1 and GlyA2, which had not been visible in the electron density maps, were modeled using the LEaP module in the AMBER 7 software package (Case et al., 2002). The modeled conformation at the beginning of each

simulation was similar to that of residues SerB1 and GlyB2 of the other polypeptide chain in the dimer, which exhibits a well-defined electron density.

2.2. Flexible docking of SARS and inhibitors

The flexible molecular docking (Muegge and Martin, 1999) with Genetic Algorithm was performed using BioMedCache 2.0 Software to find the most favorable binding interaction. Two drugs, ritonavir and lopinavir, were separately docked into the SARS-CoV 3CL^{pro} binding site. The residues associating in the binding pocket were defined by the conserved amino acid residues, namely H41 and C145, which are also known to be part of the catalytic site of the SARS-CoV proteinase (Anand et al., 2002, 2003; Yang et al., 2003). Additionally, their neighbor residues within a radius of 3 Å of these conserved amino acids were selected and considered as the members of the binding pocket.

The structure of SARS-CoV 3CL^{pro} was obtained from X-ray crystallographic structure (PDB ID:1UK3) (Anand et al., 2003). All missing hydrogen atoms were added into the enzyme using tleap in the AMBER 7 simulation package. The geometries of the inhibitors were generated from X-ray structured and all hydrogen atoms were added using Gaussian03, with the use of antechamber to assign atom types. The geometry of inhibitors was optimized by the semiempirical AM1 method. The PMF scores of the drugs were evaluated by the genetic algorithm with a population size of 50, crossover rate of 0.80, elitism of 5, mutation rate of 0.2, with the maximum generation cycle set to be 40,000. The size of the grid box was set at 25 Å × 25 Å × 25 Å. Since, as expected, no significant difference was found in terms of their orientations, among the complex structures within the low-energy clusters, the conformations with the lowest PMF energy score were used as the initial model for MD simulations. Finally, the complex structures with the lowest PMF energy score were constructed and used as an initial model for MD simulations.

2.3. Molecular dynamics simulations

Three MD simulations (free SARS, SARS–RTV and SARS–LPV) were carried out using the AMBER 7 simulation package. Energy

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