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Comparison of SARS and NL63 Papain-Like Protease Binding Sites and Binding Site Dynamics: Inhibitor Design Implications

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The human severe acute respiratory syndrome coronavirus (SARS-CoV) and the NL63 coronaviruses are human respiratory pathogens for which no effective antiviral treatment exists. The papain-like cysteine proteases encoded by the coronavirus (SARS-CoV: PLpro; NL63: PLP1 and PLP2) represent potential targets for antiviral drug development. Three recent inhibitor-bound PLpro structures highlight the role of an extremely flexible six-residue loop in inhibitor binding. The high binding site plasticity is a major challenge in computational drug discovery/design efforts. From conventional molecular dynamics and accelerated molecular dynamics (aMD) simulations, we find that with conventional molecular dynamics simulation, PLpro translationally samples the open and closed conformation of BL2 loop on a picosecond–nanosecond timescale but does not reproduce the peptide bond inversion between loop residues Tyr269 and Gln270 that is observed on inhibitor GRL0617 binding. Only aMD simulation, starting from the closed loop conformation, reproduced the 180° ϕ–ψ dihedral rotation back to the open loop state. The Tyr–Gln peptide bond inversion appears to involve a progressive conformational change of the full loop, starting at one side, and progressing to the other. We used the SARS-CoV apo X-ray structure to develop a model of the NL63-PLP2 catalytic site. Superimposition of the PLP2 model on the PLpro X-ray structure identifies binding site residues in PLP2 that contribute to the distinct substrate cleavage site specificities between the two proteases. The topological and electrostatic differences between the two protease binding sites also help explain the selectivity of non-covalent PLpro inhibitors.

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Abbreviations used: SARS-CoV PLpro, severe acute respiratory syndrome coronavirus papain-like protease; nsp, non-structural protein; DUB, deubiquitinating; HCoV, human coronavirus; MSA, multiple sequence alignment; BtCoV, bat coronavirus; MD, molecular dynamics; cMD, conventional molecular dynamics; aMD, accelerated molecular dynamics; 3CLpro, chymotrypsin-like protease; 3D, three-dimensional; 2D, two-dimensional; PDB, Protein Data Bank.

Introduction

Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses.[1] The coronavirus responsible for severe acute respiratory syndrome (SARS-CoV) is probably the most studied human coronavirus (HCoV) and produces a unique pathogenesis because it causes both upper and lower respiratory tract infections and can also cause gastroenteritis.[2] Although containment of the first SARS epidemic in 2003 succeeded through epidemiological and quarantine measures, there is still no definitive therapy for SARS or other coronaviral infections. Shortly after the SARS outbreak, in 2003, researchers also identified HCoV-NL63[2,3] as another HCoV that causes respiratory infections and pneumonia. The virus is found worldwide and infects mainly young children, elderly, and immunodeficient patients. Both SARS-CoV and HCoV-NL63 use angiotensin-converting enzyme 2 as the cellular receptor to infect host cells.[4,5]

Coronaviral genomic RNA is released in the cell cytoplasm after infection, which then translates into two long polyproteins pp1a and pp1ab.[6] The replicase gene of coronaviruses often encodes two cysteine papain-like proteases, PLP1 and PLP2, and a cysteine chymotrypsin-like protease (3CLpro). SARS-CoV, avian infectious bronchitis virus, and some of the bat coronaviruses (BtCoVs) are distinct in that they encode only one papain-like protease domain.[7–9] In the case of SARS-CoV, autocatalytic processing of the polyproteins by PLpro and 3CLpro generates up to 16 non-structural proteins (nsps). 3CLpro is the main protease that processes multiples sites in the replicase polyprotein and has been targeted for therapeutic development.[10,11] PLpro cleaves pp1a at three sites[12] and has been shown to be essential for viral replication.[13–15] The resulting nsps coalesce with the endoplasmic reticulum membrane to form the multifunctional replicase complex. This complex is instrumental in sub-genomic RNA synthesis and, thus, proliferation of infection.[16,17]

In recent work, we introduced two classes of SARS-CoV PLpro-specific non-covalent inhibitors that exhibit significant SARS antiviral efficacy.[13–15] The crystal structure of inhibitor GRL0617 bound to the protein superimposed on the apo (open) X-ray structure (Fig. 1a) indicates that group I inhibitors

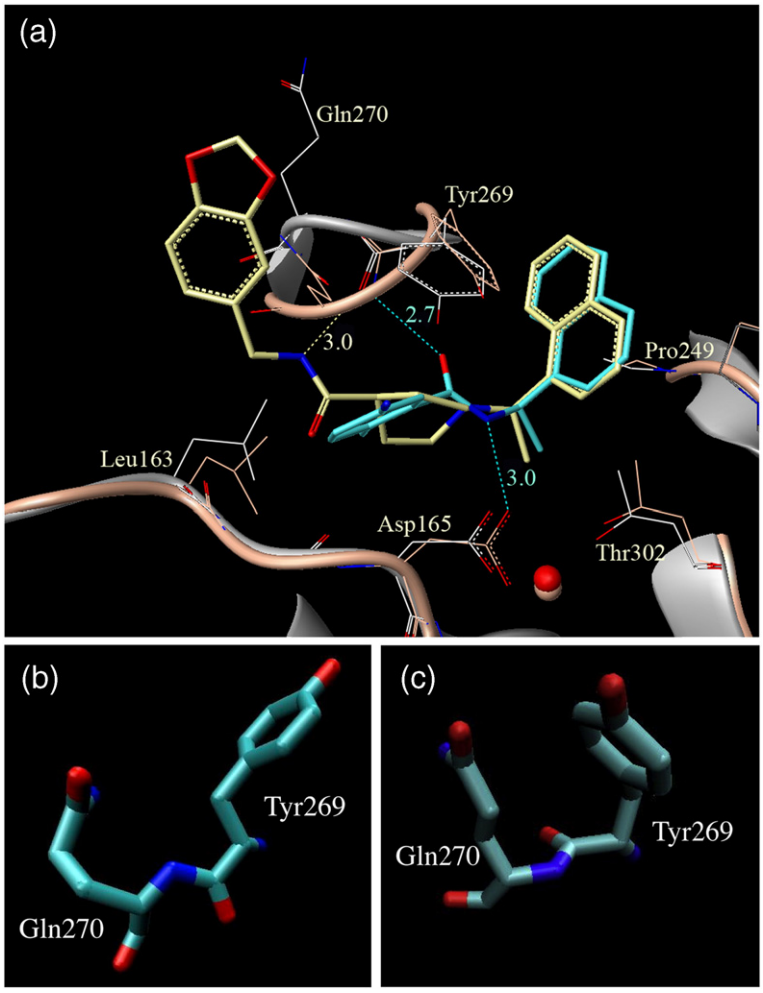

Fig. 1. Comparison of the BL2 loop from the three X-ray structures. (a) The purple ribbon diagram represents the 15g (shown in yellow stick figure)-bound crystal structure (PDB ID: 3mj5) conformation of SARS-CoV PLpro superimposed on the GRL0617S inhibitor (cyan stick figure) complex protein conformation (PDB ID: 3e9s) shown as gray ribbon. It also highlights the side-chain changes in some of the binding site residues and loop conformation differences between the two ligand-bound protein conformations. (b) A magnified image of the Tyr269–Gln270 peptide bond orientation (BL2 loop) in the apo (PDB ID: 2fe8) and 15g-bound protein X-ray crystal structure. (c) A magnified image of the same peptide bond between Tyr269 and Gln270 on the BL2 loop when bound to inhibitor GRL0617S. A 180° rotation of the peptide bond can be clearly observed between (b) and (c).