Enzymes driving SARS-CoV-2 infection: Key biological targets for therapy

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Abstract

The SARS-CoV-2 virus is responsible for the global COVID-19 pandemic. Specific treatment or vaccine for cure against SARS-CoV-2 infection is yet to be released. It is widely understood that various enzymes present in the human body assist the growth of SARS-CoV-2. These enzymes play a pivotal role in mediating the virus' entry and replication which makes them an attractive biological target for therapeutic purposes. Analyzing the structure, binding region, catalytic site of these enzymes may help to identify high-throughput inhibitor candidates, which may help curtail the virus' life cycle and also arrest the infection. This review summarizes the role of enzymes in catalyzing cell infection by under SARS-CoV-2, and promising drugs aiming these enzymes for inhibition.

Introduction

Zoonotic viruses pose a serious threat to public health [1]. Belonging to this family of deadly viruses, SARS-CoV-2, is responsible for COVID-19, an infectious respiratory disease which has emerged into a global pandemic claiming millions of lives within a short span of two months [2-4]. SARS-CoV-2 has 86%, 50% and 96% similarity to the genome of the severely acute respiratory syndrome virus (SARS-CoV), the middle-east respiratory syndrome virus (MERS-CoV) and the horseshoe bat coronavirus RTG13, respectively [2]. The SARS-CoV-2 is a beta-coronavirus belonging to the family of Coronaviridae [5]. It consists of ~30,000 single stranded RNA nucleotides packaged inside the nucleocaspid protein (N) which are further wrapped inside the membrane protein (M), spike protein (S) and envelop protein (E) (Figure-1). The SARS-CoV-2 viral genome encodes for 29 proteins, out of which 16 are non-structural proteins (nsp), which aid virus' replication and infection, 4 of them are structural proteins (S, E, M, N) responsible for virus architecture and the rest are accessory proteins for countering the host immune response [6].

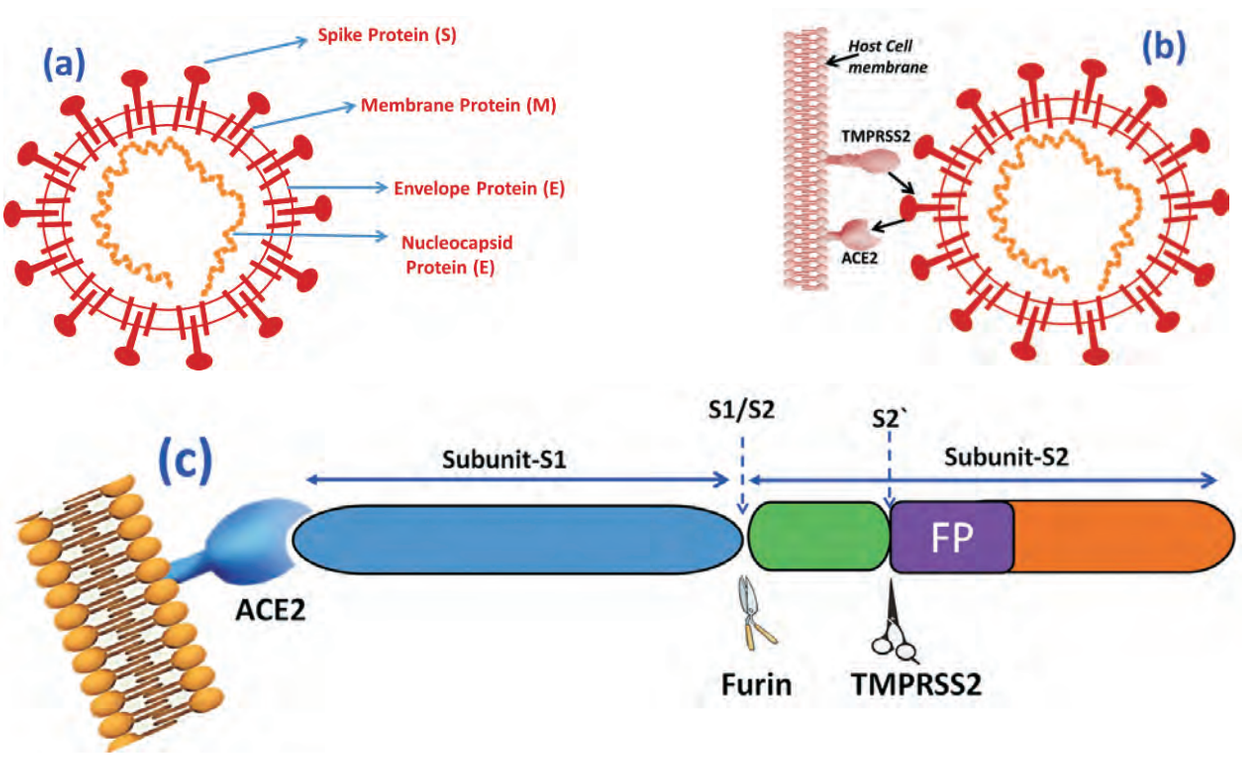

Fig. 1: (a) Structure of SARS-CoV-2. (b) Enzymes of host cell facilitating entry of virus in the cell. (c) (Cartoon representation of spike protein interaction) Interaction of spike protein with host cell enzymes (ACE2, Furin, TMPRSS2) to facilitate virus entry in human cell

The SARS-CoV-2 infection starts as soon as the virus enters the host/human cell. The spike (S) protein of the virus binds to angiotensin converting receptor enzyme 2 (ACE2), which is present on the surface of host cell and initiates fusion of its membrane with the cell membrane with the help of another host enzyme called transmembrane serine protease 2 (TRMPSS2) [7-8] (Figure-1). The ACE2 receptor is an immunomodulator which regulates the blood pressure, and is present in plenty in the cells of lungs, heart, kidneys etc. After entering host cell, the SARS-CoV-2 genomic RNA is released into the cytoplasm of the cell. The entire ~ 30,000 single stranded RNA nucleotides is translated by the host cell ribosomes. The translation products are called as polyprotein 1a (pp1a) and polyprotein 1ab (pp1ab), both having an overlapped polypeptide chain structure(Figure-2). These polypeptide chains contain multiple, distinct non-structural proteins (nsp 1–16), which regulate replication of viral RNA and assembly of newly generated copies and their maturation. However, the polypeptide needs to be cut into small functional proteins to carry out the replication and virion assembly. The enzymes, papain-like protease (PL_{pro}) and chymotrypsin like protease ($3CL_{pro}$) or main protease (M_{pro}), cuts these polyproteins to yield 16 small functional proteins (16 nsps). The role of each nsp is well defined. For example, the RNA-dependent RNA polymerase enzyme is encoded in nsp12 [9] which assist in RNA synthesis, genome and subgenomic RNA. Researchers are considering a number of potential drugs molecules which can bind to these key enzymes and inhibit their functioning and subsequently arrest the infection. However, this requires knowledge about enzyme structure, binding region, catalytic site, etc. In the following sections, the enzymes playing key role in SARS-CoV2 infections are discussed in detail.

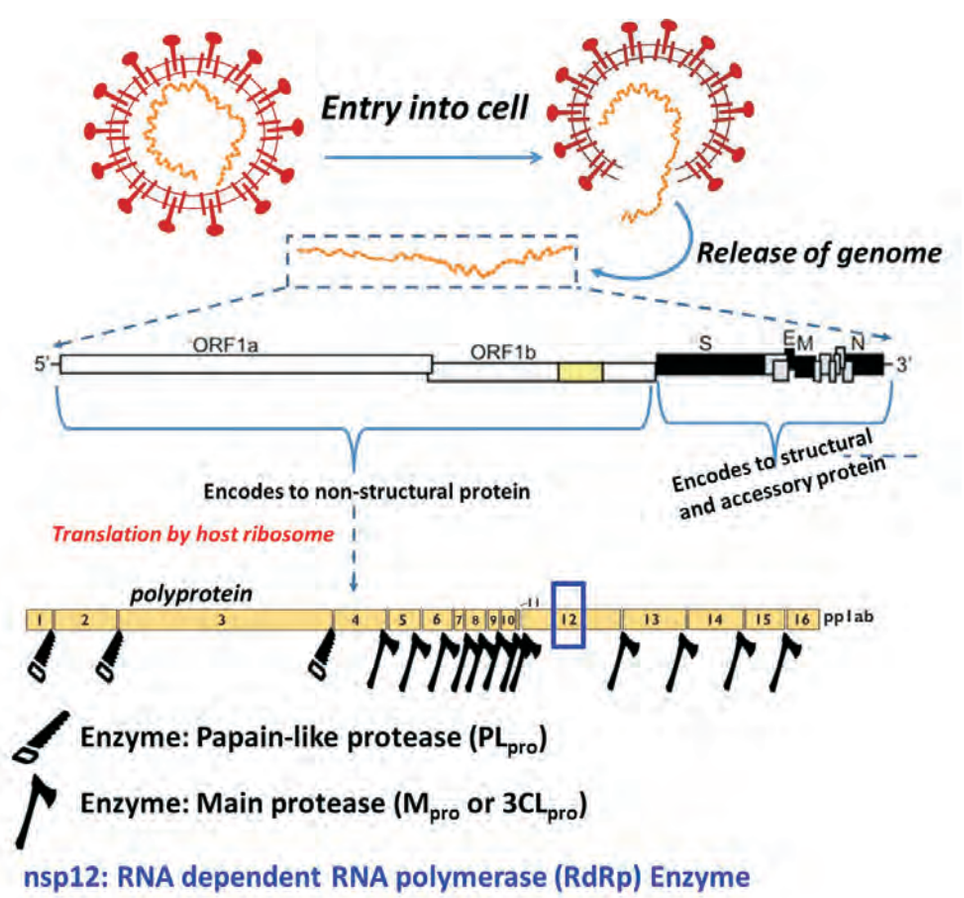

Fig. 2: Enzymes doing cleavage job to generate functional proteins essential for viral replication and assembly.

Host cell enzyme and entry of virus in the cell

Coronaviruses are named for the crown of protein spikes covering their outer membrane surface. All coronaviruses, including SARS-CoV-2, use the spike proteins (S) for binding with the host cell receptor for cell entry. The spike protein is a homotrimeric glycoprotein where each monomer is divided into S1 and S2 sub-units as shown in Figure 1 [10]. S1 sub-unit owns the domain for host cell attachment called receptor binding domain (RBD), which is a binding site with host cell receptor ACE2. On the other hand, S2 sub-unit contains fusion peptides responsible for fusion of virus membrane with the host cell membrane [10]. However, ensuing to virus-host cell binding (S1-hACE2), the fusion process of virus and host cell membrane cannot occur until and unless S protein is cleaved at S1/S2 site and fusion peptides are activated. These activation/priming functions are performed by host enzymes namely furin and transmembrane serine protease 2 (TMPRSS2). While furin is involved in the cleavage at S1/S2 site of S protein, the activation of fusion peptides is carried out by transmembrane serine protease 2 (TMPRSS2) by cleaving at S2' site (figure 1). Thus, TMPRSS2 and furin host proteases play an important role in priming the S protein of the SARS-CoV-2 [11]. It is also important to note that the receptor binding mode of SARS-CoV-2 S/RBD with hACE2 is similar to that of earlier SARS-CoV/RBD-hACE2 complex. However, SARS-CoV-2 RBD forms more atomic interaction with hACE2

than SARS-CoV RBD as inferred from structural studies carried out by Wrapp et al and Wang et al [12, 13].

Since binding of the S protein with hACE2 marks the beginning of viral infection which is well assisted by the furin and TMPRSS2 host enzymes, blocking the binding between S protein and hACE2 is the key strategy for therapeutics and vaccine development. Neutralizing antibodies are increasingly recognized as potential options to primarily target trimeric S protein [14] while there are some small drugs such as chloroquine, arbidol, etc., [15, 16] and peptide binders [17] which are effective in inhibiting the entry of virus. Moreover, there are phytochemicals like flavonoids and non-flavonoids which are effective in inhibiting the interaction between S protein and hACE2, owing to their high binding affinity towards S protein [18].

Enzymes facilitating protein cleavage, virus replication and assembly in host cell

Upon cell entry, viral RNA attaches to the host ribosome to yield two polyproteins pp1a and pp1ab that are essential for the production of new mature virions. As mentioned previously, the proteolytic cleavage of these two polyproteins is carried out by papain-like protease (Pl_{pro}) and the main proteinase (M_{pro} or $3CL_{pro}$). The X-ray structures of both $3CL_{pro}$ (PDB ID: 6W63) and PLpro (PDB ID: 6W9C) from SARS-CoV-2 (COVID-19) are shown in (Figure 3). PL_{pro} from SARS-CoV-2 and SARS-CoV, share about 83% sequence identity, with amino acid composition [9]. The multifunctional PL_{pro} crystallographic homotrimer has Cys–His–Asp catalytic triad in each monomer. The Zn ions help in connecting the three monomers. PL_{pro} domain has cysteine-protease that cleaves the replicase polyprotein at the N terminus of pp1a, releasing nsp1- nsp3 [9]. PL_{pro} is not

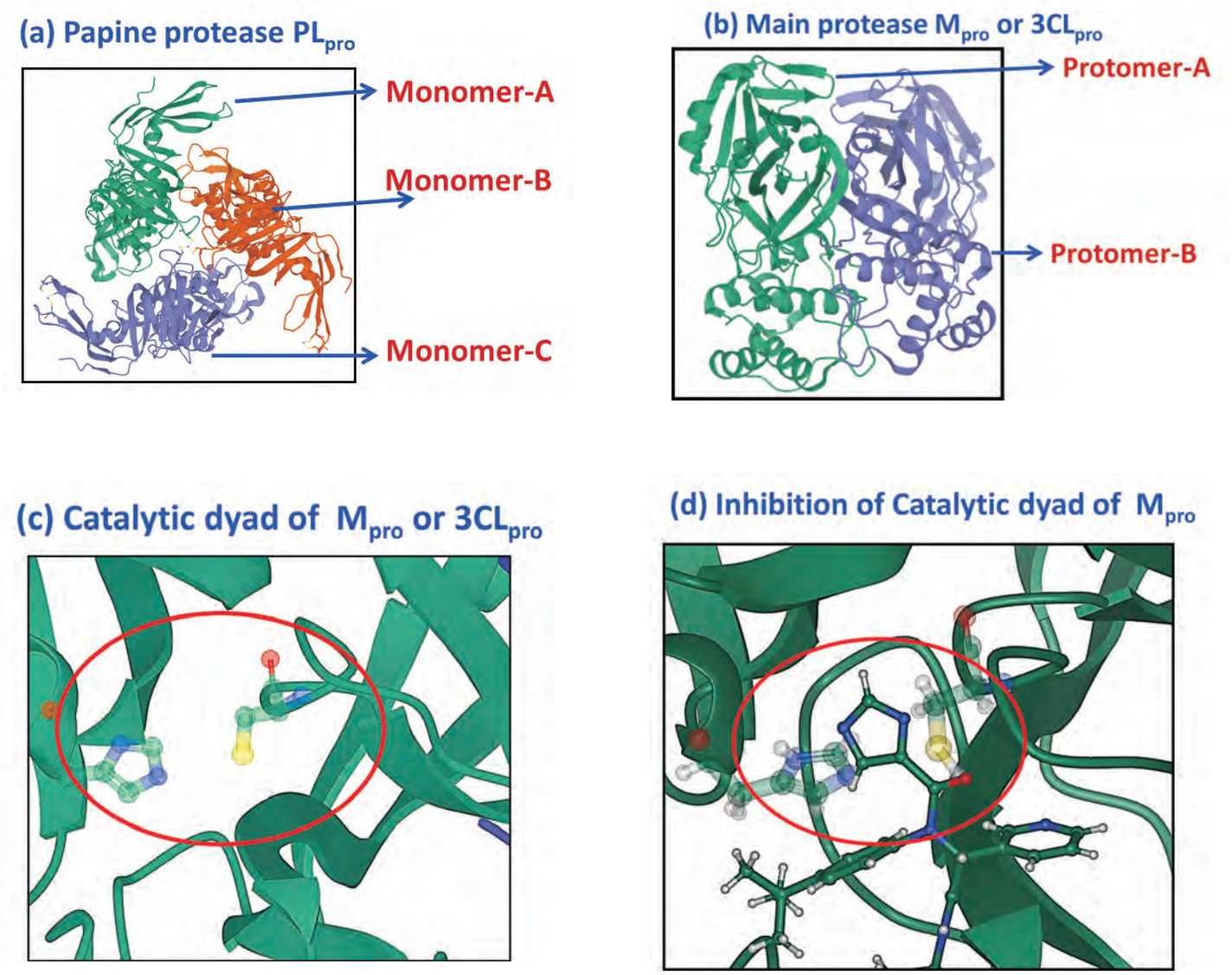

Fig. 3: Figure-3: (a) and (b) are the structure of enzyme PL_{pro} and M_{pro}. (c) depicts the catalytic dyad of M_{pro} and (d) shows interaction of drug with S atom of cysteine present in the catalytic dyad. Image is formulated at RCSB website

only is involved in cleaving the viral polyprotein, but it also is involved in removing cellular substrates like ubiquitin (Ub), termed deubiquitylation (DUB), and interferon-stimulated gene product 15 (ISG15) from host the cell proteins.

Like PL_{pro} homotrimer, the main protease $3CL_{pro}$ is a cysteine-protease but is active as a homodimer and utilizes a catalytic dyad (Cys-His) instead of a triad. Structure of M_{pro} deduced by Hilgenfeld et al [19] revealed that dimer form of M_{pro} is formed by linking of two protomers and each protomers has three domains. The catalytic dyad consisting of Cys145 and His41 residue is located in a cleft as shown in Figure 3. Proteolysis of polyproteins by M_{pro} is achieved by the nucleophilic attack of by S atom of cysteine molecule at the catalytic site. Therefore the drugs which can be effective against the M_{pro} must contain an electrophilic centre which can engage the nucleophilic S atom thereby inhibiting the proteolysis of polyproteins and in turn viral replication. The Figure 3d shows inhibition of catalytic dyad by a representative drug molecule. Drugs which showed promise in impeding the function of M_{pro} are combination of lopinavir and ritonavir, carmofur, ketomamides, N3 inhibitors and phytochemicals like alkaloids, terpenoids and polyphenolic compounds [19-24].

The 16nsp's generated from the proteolysis of polypeptide pp1a and pp1b finally form the viral replicase-transcriptase complex, which is responsible for the viral genome replication and subgenomic transcription. One of the key components/enzyme of this replicase-transcriptase complex is RNA dependent RNA polymerase (RdRp) enzyme, which is a domain of nsp12. RdRp is not a cleavage enzyme rather it is an enzyme that catalyzes the synthesis of RNA polymers. For SARS-CoV-2, RdRp enzyme

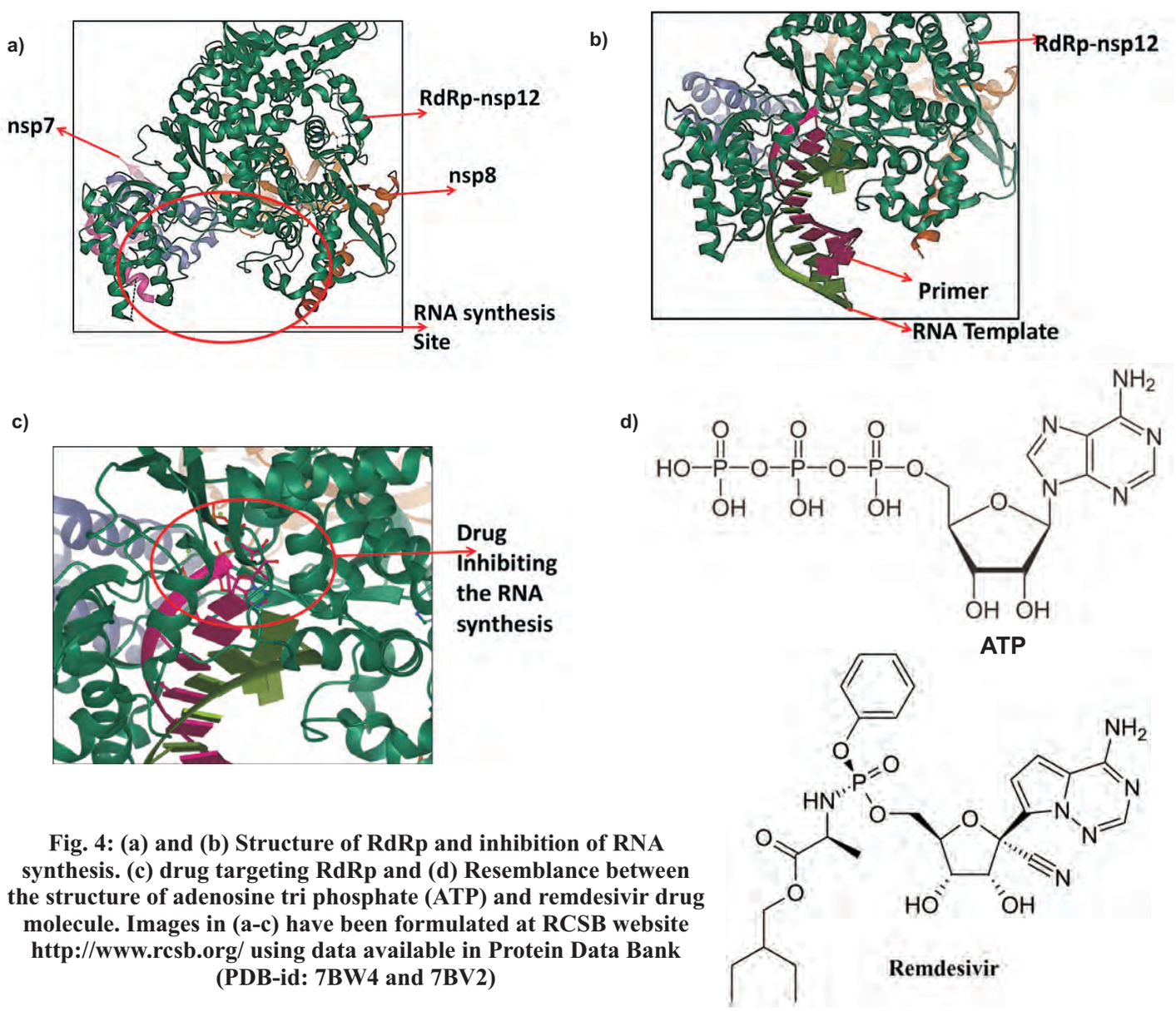

Fig. 4: (a) and (b) Structure of RdRp and inhibition of RNA synthesis. (c) drug targeting RdRp and (d) Resemblance between the structure of adenosine tri phosphate (ATP) and remdesivir drug molecule. Images in (a-c) have been formulated at RCSB website http://www.rcsb.org/ using data available in Protein Data Bank (PDB-id: 7BW4 and 7BV2)

catalyzes the synthesis of viral RNA from RNA templates or building blocks and thus plays a central role in replication and transcription cycle of SARS-CoV-2 [25].

Structure of RdRp as deduced by Gao et. al. [25] revealed that it contains various sub-domains namely finger, palm and the thumb. (subdomain). The palm subdomain consists of catalytic cavity where polymerization of RNA building blocks takes place as shown in Figure-4. The nucleotide entry and exit path of RdRp are positively charged, which can be easily accessed by the solvent molecules. Proper functioning of RdRp enzyme demands cooperative efforts from its co-factors nsp7 and nsp 8, which help in boosting the catalytic activity of RdRp [26].

From the above discussion, it is clear that RdRp is the central component of SARS-CoV-2 replication and transcription machinery. This makes RdRp also an attractive target for antiviral drugs such as remdesivir, galidesivir, ribavarine, favipiravir, etc [26-27]. Structural studies of these promising drugs gave an insight that molecules which mimic the structure of RNA building blocks like adenosine, guanine, etc. are effective in impeding the activity of RdRp (Figure 4). By mimicking the RNS building block like adenosine tri phosphate (ATP) it easily gets incorporated in nucleotide chain thus inhibiting the chain elongation process. In addition to the above drugs, there are phytochemicals like theaflavin was also found to be effective against RdRp [28].

Summary

The COVID-19 pandemic caused by highly transmissible SARS CoV-2 virus has strained the public health system besides seriously denting the prospects of global economic growth. This review outlines various important enzymes driving the (infection of) SARS-CoV-2 infection either by mediating in viral entry or assisting in replication and transcription process. Role of few salient enzyme like ACE2, Furin, TMPRSS2, PL_{pro}, M_{pro}, RdRp etc. has been discussed. These critical enzymes serve as attractive biological targets for drug development. The enzyme's structure, catalytic site and their role in infection has been discussed. This will indeed help in designing or repurposing the drug molecule which can be effective in blocking the entry or inhibit the replication of virus thereby terminating the infection. It is believed that review will provide the key learning points, and will serve as a primer for identifying novel therapeutic options.

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