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L-Methionine may modulate the assembly of SARS-CoV-2 by interfering with the mechanism of RNA polymerase

Maximo A. Benavides

Bio Medical Science World Corp., Houston, TX 77030, USA

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ABSTRACT

Coronaviruses have received worldwide attention following several severe acute respiratory syndrome (SARS) epidemics. In 2019, the first case of coronavirus disease (COVID-19) caused by a novel coronavirus (SARS-coronavirus 2 [CoV-2]) was reported. SARS-CoV-2 employs RNA-dependent RNA polymerase (RdRp) for genome replication and gene transcription. Recent studies have identified a sulfur (S) metal-binding site in the zinc center structures of the RdRp complex. This metal-binding site is essential for the proper functioning of the viral helicase. We hypothesize that the use of essential nutrients can permeabilize the cell membranes. The oxidation of the metal-binding site occurs via analogs of the essential S-containing amino acid, L-Methionine. L-Methionine can operate as a carrier, and its binding would cause the potential disassembly of RdRp via the S complex and drive methyl donors via a possible countercurrent exchange mechanism and electrical-chemical gradient leading to SARS-CoV-2 replication failure. Our previously published hypothesis on the control of cancer cell proliferation suggests that the presence of a novel disulfide/methyl-adenosine triphosphate pump as an energy source would allow this process.

The S binding site in L-Methionine serves as a potential target cofactor for SARS-CoV RdRp, thus providing a possible avenue for the future development of vaccines and antiviral therapeutic strategies to combat COVID-19.

Background

The novel coronavirus (COV) SARS-CoV-2 is responsible for the COVID-19 pandemic in 2020, and vaccines and antivirals can be used to prevent and treat it, respectively [1,2]. Viral genome replication and gene transcription are carried out using various viral machinery comprising a set of nonstructural proteins (NSPs). Viral polyproteins [3] assemble to ensure replication fidelity and gene transcription for proper SARS-CoV-2 propagation in the human body. One complex of proteins required for this process is the catalytic subunit of an RNA-dependent RNA polymerase (RdRp) [4], an enzyme that also has helicase capabilities, as observed by cryo-electron microscopy [5,6].

RdRp functioning requires the binding of metal ions [7,8]. Metals such as selenium, sulfur (S), and zinc (Zn) can be used as metal cofactors [9]. The S binding site of the protein is vulnerable to metal ion dislocation and subsequent protein degradation by oxidants (e.g., oxygen, superoxide, and nitric oxide) [10], biological redox reactions [11], proteins, and DNA and RNA synthesis [12–14].

Sulfur is present in proteins in intra- or intermolecular disulfide bridges that determine the properties and functions of proteins and enable reactions via disulfide exchange. This exchange can modify or disassemble protein configurations and elicit an immune response in cells [15]. One essential S-containing amino acid of interest is methionine, which has unique thiol properties and functions [15]. Thiol-mediated uptake is an efficient cellular uptake of substrates attached to thiol-reactive groups, specifically disulfides or covalent thiol-disulfides. This thiol-disulfide exchange causes a thiolate group (-S) to attack an S atom to form a disulfide bond (-S-S). The novel disulfide bond is fragmented, while another -S atom is discharged as a new thiolate carrying a negative charge [16,17]. Methionine is an antioxidant because it acts as a cofactor in the glutathione peroxidase process. This oxidizing mechanism determines the antiviral status. The proposed mechanisms of L-Methionine cellular entry include those similar to the cellular entry of phenols, hypochlorite [17], or the Ebola virus [18]. Cellular access may also occur via a mechanism similar to diphtheria toxin entry [19]. In vitro research has shown that L-Methionine controls cellular proliferation and the cell cycle and silences p53 mRNA in cancer

Abbreviations: COV, coronavirus; COVID-19, coronavirus disease 2019; NSPs, nonstructural proteins; RdRp, RNA-dependent RNA polymerase; S, sulfur; Zn, zinc; SARS, severe acute respiratory syndrome.

E-mail address: maxbenav@b-msw.com.

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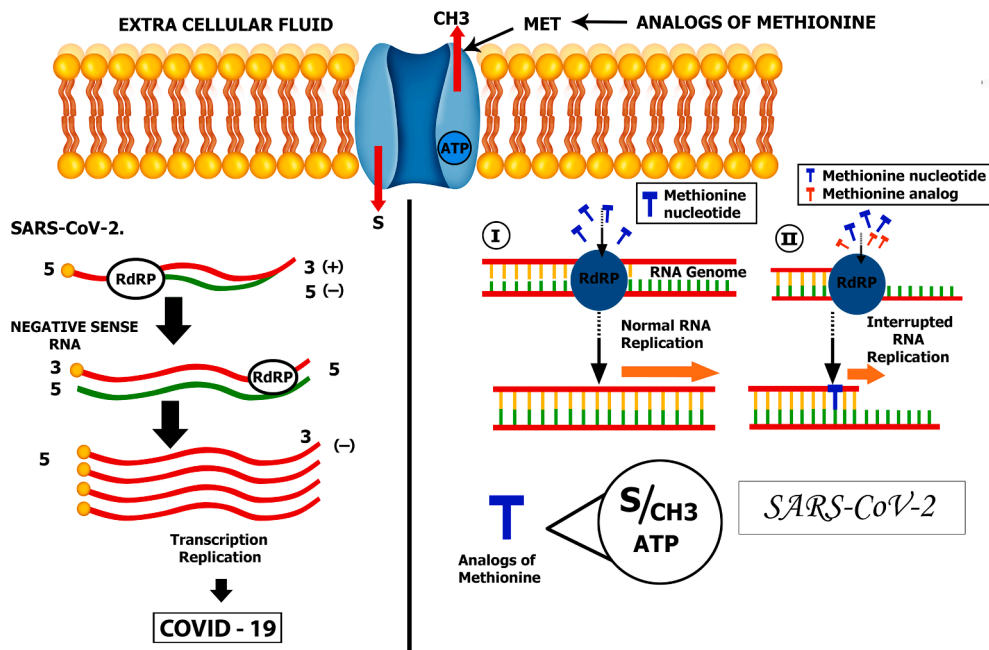


Fig. 1. Show the intervention of methionine analog nucleotide, L-Methionine, as an inhibitor targeted on the catalytic mechanism of RNA replication, RNA-dependent RNA polymerase (RdRp). S/CH₃-ATP pump is the supplier of energy to develop with success this mission.

cells, but not in benign cells, without activating an immune response [20–24].

The published hypotheses of virus destabilization [25,26] try to interfere with the assembly of RNA polymerase. The critical points are the cellular internalization and the complexity of the cell membrane, as well as the difficulty level for penetration into the Cell. Our proposal recognizes these crucial points and considers the use of a carrier and an energy source to achieve our aim. Previously, we hypothesized in cancer cells [27] that the mechanism is controlled by a possible sulfur/methyl pump, given uniquely via methionine analogs. We tried to introduce sulfur into the RNA polymerase through an essential amino acid, a nutrient, which would lead to possible incorporation and consequent instability in the assembly of SARS-CoV-2 RNA-dependent RNA polymerase (See Fig. 1).

Methionine is the first amino acid, encoded by the codon AUG, that is incorporated in any new protein and is repeatedly exchanged/removed during the transcription-translation process. We believe that the use of L-Methionine can transport sulfur into the RNA polymerase via a counter-current mechanism of electrical-chemical gradient, which has not yet been investigated. Therefore, the use of methionine analogs could repeatedly manipulate the incorporation of sulfur in this area. Studies have demonstrated that the N-terminal methionine excision plays a crucial role in controlling protein turnover [28]. In addition, methionine aminopeptidase catalyzes the removal of the initiator methionine and, subsequently, affects the functioning of the methionine salvage pathway, resulting in the observation that the redundant methionine inhibits cell growth [29]. Analogous amino acids can function as competitive inhibitors and modify the cellular mechanism via misincorporation. We believe that methionine analogs modulate multiple biological mechanisms and possible structural changes in proteins *in vivo*. This phenomenon is being analyzed by linking immune symptoms [30]. Methionine can help identify a new immunologic target to control COVID-19. It is recognized to be important in the methylation of RNA and DNA that leads to T cell differentiation and proliferation [31].

The proposed novel pump is uniquely expressed in cells that are unknown to the immune system. We believe that it provides the energy needed by the cells for invading the human body. Studies of ion channels have linked the control of cell proliferation and migration [32]. The S incorporated from methionine induces us to think that it may act as a

covalent inhibitor, and this concept is gaining acceptance within the scientific community [33].

The results described above and in other published findings are significant. They suggest that L-Methionine could potentially disassemble SARS-CoV-2 RdRp, thus providing a potential antiviral target for the formulation of vaccines and antiviral therapies against COVID-19.

Hypothesis

Recent research into COV polyproteins, such as ORF1a, ORF1ab, and NSPs, revealed that proteolytic cleavage facilitates transcription and, ultimately, viral replication. It has been demonstrated that nsp12, a catalytic subunit of RdRp, in combination with nsp7 and nsp8, an exonuclease and an accessory factor, respectively, processes the synthesis of the virus and propagates the transmission of SARS-CoV-2.

SARS-CoV-2 RdRp contains a Zn ion in a position that is conserved across similar viruses, and further studies must confirm that S-binding metal ions are indispensable for maintaining the structural integrity of the viral RdRp complex. Sulfur clusters (inorganic cofactors) can trigger redox reactions and affect the proteins involved in DNA and RNA synthesis. Therefore, our research aims to investigate whether S-metal cofactors can link directly to the -S cluster and consequently cause the destabilization and degradation of structural proteins by oxidants.

Our *in vitro* research on -S amino acid analogs [20–23] led us to hypothesize that the oxidation of -S metal-binding sites via analogs of -S-containing amino acids (e.g., L-Methionine) inhibits the RdRp complex and consequently blocks SARS-CoV-2 replication in cells. Methionine can affect cellular function when the carbon-nitrogen-sulfur complex and methyl donors from L-Methionine replace oxygen, mimicking the cellular entry mechanism of an alcohol group. If these complexes attach and donate methyl groups to the Zn centers of the RdRp complex, the metal-binding site would be unable to activate the viral helicase, thereby leading to viral disassembly. It could be due to a possible counter-current exchange mechanism and electrical-chemical gradient proportioned by a novel disulfide/methyl-adenosine triphosphate pump as an energy source, which would allow the process.

The -S metal-binding site on the Zn centers could serve as a target cofactor for SARS-CoV-2 RdRp and inform the formulation of vaccines and antiviral therapies targeting COVID-19. The mechanism of the

primogeniture of methionine, the start codon, is not yet understood and deserves to be investigated. It may be the key to understanding the common mechanism that involves the immunology-cancer-aging target.

Therefore, further studies on the effects of L-Methionine on viral replication are warranted.

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Consent statement/ethical approval

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Declaration of Competing Interest

The author declare that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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