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

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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 COV disease 2019 (COVID-19). COVID-19 infection rates rose to a global 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 intramolecular 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 (-SH) 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.
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 -Methionine can transport sulfur into the RNA polymerase via a countercurrent 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 -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., i-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 i-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 countercurrent 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 immunity-cancer-aging target. Therefore, further studies on the effects of t-Methionine on viral replication are warranted.

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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.

**References**

[1] Chen N, Zhou M. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan. China: a descriptive study. Lancet 2020; 395:507–13.

[2] Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579(7798): 265–9.

[3] V'kovski P, Kratze J, Steiner S, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 2020;18(3):155–70.

[4] Ahn D-G, Choi J-K, Taylor DR, Oh J-W. Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. Arch Virol 2012;157(11):2995–104.

[5] Gao Y, Yan L, Huang Y, Liu F, Zhao Y, Cao L, et al. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Science 2020;368(6492):779.

[6] Chen J, Malone B, Llewellyn E, Grasso M, Shelton PMM, Olinares PDB, et al. Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex. Cell 2020;182(6):1560–1573.e13.

[7] Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp7 and nsp8 cofactors. Nat Commun 2019;10:2342.

[8] Shimberg GD, Michel SJL. Iron-sulfur cluster in zinc finger proteins. Methods Enzymol 2018;599:101–37.

[9] Netz DJ, Pierik JA. Eukaryotic DNA polymerases require an iron-sulfur cluster for the formation of the active complexes. Nat Chem Biol 2011;8:125–32.

[10] Imlay JA. Iron-sulfur clusters and the problem with oxygen. Mol Microbiol 2006;59(4):1073–82.

[11] Klinge S, Pellegrini L. An iron-sulfur domain of the eukaryotic primase is essential for RNA primer synthesis. Nat Struct Mol Biol 2007;14:875–7.

[12] Weiner BR, Chazin WJ. An iron-sulfur cluster in the C terminal domain of the p58 subunit of human DNA primase. J Biol Chem 2007;282:23344–5.

[13] Girbig M, Muller CW. Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states. Nat Struct Mol Biol 2021;28:210–9.

[14] Lipinski B, Egyud IG. Thiol-induced crosslinking of human blood proteins. Implication for tumor immunity. Bioorg Med Chem Lett 1992;2:919–24.

[15] Corcoran A, Cotter TG. Redox regulation of protein kinases. FEMS J 2013;280(9): 1944–55.

[16] Poole LB. The basics of thiols and cysteines in redox biology and chemistry. Free Radic Biol Med 2015;80:148–57. https://doi.org/10.1016/j.freeradbiomed.2014.11.013.

[17] Kampf G, Steinmann E. Persistence of coronavirus on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020;104(3):246–51.

[18] Lipinski B. Can selenite be an ultimate inhibitor of EBOLA and other viral infections? Br J Med Res 2015;6:319–24.

[19] Ryser HJ. Cell surface sulphhydril are required for the cytotoxicity of diphtheria toxins but not of ricin in Chinese hamster ovary cells. J Biol Chem 1991;266(28): 1843942.

[20] Benavides MA, Oeschlager DR, Zhang H-G, Stockard CR, Vital-Reyes VS, Katzoori VR, et al. Methionine inhibits cellular growth dependent on the F53 status of cells. Am J Surg 2007;193(2):274–83.

[21] Benavides MA, Hagen KL, Fang W. Suppression by L-Methionine of cell cycle progression in LNCaP and MCF-7 but not benign cells. Anticancer Res 2010;30: 1891–6.

[22] Benavides MA, Rosland MC, da Silva CP, Gomes Sares CF, Cerqueira de Oliveira AM, Kemp R, et al. L-Methionine inhibits growth of human pancreatic cancer cells. Anticancer Drugs 2014;25(2):200–3.

[23] Benavides MA, Hu D, Barzoidan MK, Bruno A, Du P, Lin S, et al. L-methionine-induced alterations in molecular signatures in MCF-7 and LNCaP cancer cells. J Cancer Res Clin Oncol 2011;137(3):441–53.

[24] Tripoli F, Goccetti P. Methionine supplementation affects metabolism and reduces tumor aggressiveness in liver cancer cells. Cells 2020;9(11):2491. https://doi.org/10.3390/ce101102491.

[25] Kieliszek M, Lipinski B. Selenium supplementation prevents coronavirus infections (COVID-19). Med Hypotheses 2020;143:109878. https://doi.org/10.1016/j.mehy.2020.109878.

[26] Kieliszek M, Lipinski B. Pathophysiological significance of protein hydrophobic interactions: an emerging hypothesis. Med Hypotheses 2018;110:15–22. https://doi.org/10.1016/j.mehy.2017.10.021.

[27] Benavides MA. Novel inhibitors of disulfide/methyl-ATP pump inhibit the proliferation of cancer cells: Analogs of Methionine. Med Hypotheses 2022;158: 110743. https://doi.org/10.1016/j.mehy.2021.110743.

[28] Giglione C, Boulant A, Meimel T. Protein N-terminal methionine excision. Cell Mol Life Sci 2004;61(12):1455–74. https://doi.org/10.1007/s00018-004-3466-8.

[29] Dunmott B, Micka WS, Chang YH. N-terminal methionine removal and methionine metabolism in Saccharomyces cerevisiae. J Cell Biochem 2003;89(5):964–74. https://doi.org/10.1002/jcb.10566.

[30] Rodgers KJ, Shiozawa N. Missincorporation of amino acid analogues into proteins by biosynthesis. Int J Biochem Cell Biol 2008;40(8):1452–66. https://doi.org/10.1016/j.biocel.2008.01.009.

[31] Geltink RIK, Pearce EL. The importance of methionine metabolism. Elife 2019;8: e47221. https://doi.org/10.7554/eLife.47221.

[32] Li M, Xiong ZG. Ion channels as targets for cancer therapy. Int J Physiol Pathophysiol Pharmacol 2011;3(2):156–66.

[33] Ghosh AK, Samanta I, Mondal A, Liu WR. Covalent inhibition in drug discovery. ChemMedChem 2019;14(9):898–906. https://doi.org/10.1002/cmdc.201900107.