Abstract

Coronaviral methyltransferases participate in the modification of the 5'-end of viral RNA. Their enzymatic activity not only ensures efficient mRNA translation, but also allows the virus to escape the recognition of the innate immune system. This work is focused on the SARS-CoV-2 methyltransferases (the methyltransferase domain of the nonstructural protein 14, MT14, the nonstructural protein 16 and its cofactor – the nonstructural protein 10, nsp16/10), which represent attractive molecular targets for therapeutic intervention. The aim of this work was to structurally characterize the coronaviral methyltransferases in complex with various small molecules. The recombinantly prepared proteins were purified and subsequently subjected to crystallization trials. The obtained crystals of the nsp16/10 heterodimer in complex with sinefungin were soaked in a solution containing a S-adenosyl-L-homocysteine analogue. Crystals suitable for X-ray crystallography of MT14 in complex with two different inhibitors were obtained by optimizing the identified primary crystallization conditions. The aquired structural data of the MT14 inhibitory complexes will serve as a basis for the design of new small molecule inhibitors targeting the S-adenosyl-L-methionine binding site.

Keywords: methyltransferase, nsp14, nsp16, coronavirus, SARS-CoV-2, recombinant expression, protein crystallization

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