Abstract

Coronaviruses are enveloped viruses with a genome composed of a single-stranded positive-sense RNA molecule. With genome sizes, in some species, exceeding 30 kb, they represent the largest known RNA viruses. They infect birds and mammals, including humans, causing a wide spectrum of diseases. Most human coronaviruses predominantly cause mild respiratory infections. However, in the last two decades, there have been two extensive epidemics and a global pandemic of severe respiratory illnesses, often ending in fatalities, caused by coronaviruses. The most recent and extensive of these was caused by the coronavirus SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus). Within the coronavirus genome, there is a gene encoding a non-structural protein 14 (nsp14), which possesses 3'-5' exonuclease and methyltransferase enzymatic activities. The exonuclease nsp14 participates in the repair of misincorporated nucleotides during viral genome replication, and its presence is exceptionally rare among RNA viruses. The exonuclease activity of nsp14 is significantly enhanced by the binding of another viral protein, nsp10, which lacks enzymatic activity but acts as a critical cofactor for several enzymatically active coronavirus nsps. Both nsp14 and nsp10 are highly conserved and sequence-similar among various coronavirus species.

To characterize the exonuclease activity of nsp14 and its interactions with nsp10, mutant versions of the nsp14 and nsp10 proteins were created using PCR mutagenesis. Both wild-type and mutant versions of nsp14 and nsp10, as well as a truncated version of nsp10, were produced in a bacterial expression system utilizing *E. coli* bacteria. The exonuclease activity of each nsp14 variant was observed *in vitro* using activity assays with ssRNA, dsRNA, and dsRNA with mismatched base pair as substrates, in the presence or absence of nsp10 variants. The results of this study provided insights into the functioning of nsp14 exonuclease and its interactions with nsp10, which may serve as a basis for more detailed characterization in future research.

Key words: Coronaviruses, SARS-CoV-2, COVID-19, exonuclease, nsp14, nsp10