

Abstract

The SARS-CoV-2 virus (Severe acute respiratory syndrome coronavirus 2), the causative agent of the disease COVID-19 (coronavirus disease 2019), is an enveloped virus with a positive RNA genome. After the binding of the virus to the cell receptor and the release of the genome into the cytoplasm, the viral genome is immediately translated. It is translated into a polyprotein consisting of non-structural proteins necessary for viral replication. A part of this polyprotein is also protease 3-CL, which autocatalytically cleaves itself out from this polyprotein and further releases (and thus activates) individual proteins. Due to this key function in virus replication, 3-CL protease has become an important target for the design of specific antiviral drugs. As part of this thesis, we focused on the study of the precursor form of this protease, i.e. the protease that is still embedded in the polyprotein. The precursor form of the 3-CL protease was mimicked by attaching a 100 amino acid extramembrane C-terminal fragment of the nsp4 protein to the N-terminus of the mature form of the protease. The aim was to find out, how the sequence added to the N-terminus of the matured protease affects its enzymatic characteristics. As a part of this work, mutations of the N-terminal autoprocessing site were identified, which prevent the cleavage of the precursor into the mature form, which enables obtaining the purified precursor form of 3-CL protease and its further study. Enzyme activity of the precursor, substrate specificity, and thermostability were studied. This thesis contains information that can be applied for the development of antiviral drugs that would target the precursor form of protease 3-CL.

Key words

virus, SARS-CoV-2, protease 3-CL, Mpro, precursor, inhibition