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Investigating the Monomer-Dimer alterations of SARS-CoV-2 3-CL Protease

Recently the world has been ravaged by the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 belongs to the Coronaviridae family, Coronaviruses (CoVs) are positive-sense single-stranded RNA viruses with a 5′ Cap and a 3′ poly-A tail that may infect both human beings and animals. SARS-CoV-2 maturation, replication, and invasion all rely on the viral polyproteins (pp1a and pp1ab) being cleaved by a cysteine-rich 3C-like protease(3CLpro), as that is its primary function. It has been reported that 3CLpro is catalytically active as a dimer and each protomer contains a catalytic dyad (His41/cys145). It is well known that the N- and C-terminal domains were crucial in regulating the monomer/dimer equilibrium of 3CLpro and its catalysis. Unconventional approaches have been explored to design therapeutic agents against 3CLpro as it has highly conserved sequences: Direct inhibition of the catalytic site by employing compounds targeting the substrate binding pocket; and Reduction of the catalytic activity by targeting the dimerization interface. The elucidation of dimerization pathways is crucial for the structure-based development of novel therapeutics for infectious diseases caused by coronaviruses. Our research intends to identify the crucial function of long-distance interactions within the protease as well as to clarify whether dimerization is unquestionably required to activate 3CLpro catalysis. Our study demonstrates the mechanism by which the enzyme alters its structure in response to the loss of dimerization and the structural modifications that result in the inactivation of the catalytic activity. Based on the structural investigation, we mutated amino acids at crucial regions of the dimer interface to test their relevance in oligomerization and activity. We checked the oligomerization by Size Exclusion Chromatography and Native-PAGE. The result for the oligomerization was also confirmed by the enzyme activity. Also, compare the effect of mutants on the thermal stability by Differential scanning calorimetry and Circular Dichroism thermal. From our initial results, a certain single amino acid is a crucial part of sustaining dimerization; as a result, its mutation abruptly converted the enzyme into an inactive monomeric form. The structural study of the mutants will demonstrate a specific amino acid's crucial function in retaining the active site's internal structure and in aligning important residues associated with binding at the dimer interface and substrate catalysis.

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