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Structural and biochemical characterization of SARS-CoV-2 main protease maturation process

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SARS-CoV-2 is the causative agent of COVID-19 and responsible for the global pandemic. The viral Main Protease (Mpro) is responsible for cleavage of the viral polyproteins, including its own N and C-terminus, therefore is a key enzyme for viral cycle and one of the most promising targets for drug development. (1) The main objective of this project is the elucidation of the Mpro maturation process. Three different constructs of the enzyme were obtained: an immature form (IMT Mpro), a native form (Mpro) and an inactive mutant (C145S Mpro). Both proteins were purified and crystallized. In addition, the constructs were characterized using SEC-MALS, FRET-based activity assays and native mass spectroscopy. Using C154S Mpro, a 3.5Å cryo-EM structure was also obtained. (1) The crystal structure of IMT Mpro revealed several structural changes compared with native protein, including differences in the substrate binding pocket. Also, the enzyme exhibited significant reduced activity and monomeric in solution, contrasting with the native form. Furthermore, we solved the crystal structure of C145S Mpro, which reveal to be in complex with both N and C-terminal peptides. This construct presented both monomeric, dimeric, trimeric and tetrameric oligomeric states. (2) Moreover, the cryo-EM structure of tetrameric C145S Mpro was obtained in complex between non cleaved N-terminal peptide, revealing details of N-terminal processing. Native MS results exhibited the presence of both cleaved and non-cleaved particles, showing that N-terminal cleavage is not required for dimerization. Lastly, we investigated how different classes of inhibitors affect Mpro oligomerization. Our data indicates that a non-covalent inhibitor MAT-POS-e194df51-1 hinders dimer formation, meanwhile the covalent inhibitor Nirmatrelvir enhance dimerization (3), suggesting that induced fit caused by covalent linkage is responsible for Mpro dimerization. The elucidation of this process can be used to propose specific inhibitors targeting intermediate immature forms of Mpro.

Palavras-chave: Mpro. Maturation. SARS-CoV-2.

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