SALT ACTIVATION OF SARS 3C LIKE PEPTIDASE

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Severe acute respiratory syndrome (SARS) appeared first as a worldwide epidemy in November of 2002. Although the initial outbreaks of the deadly coronavirus that causes severe acute respiratory syndrome (SARS-CoV) were controlled by public health measures, the development of vaccines and antiviral agents for SARS-CoV is essential for improving control and treatment of future outbreaks. The SARS 3Cl peptidase is an attractive target since it is essential for viral replication. The enzyme is a cysteine peptidase but its structure is similar to that of chymotrypsin. The present study was carried out to express and purify SARS 3Cl peptidase in order to evaluate the effect of buffer composition, salts and glycosaminoglycans on its catalytic activity, using a FRET peptide related to a cleavable segment of the virus polyprotein. Recombinant SARS 3Clpro was expressed in *E. coli* Rosetta (DE3). The enzyme was purified to homogeneity by a two-step protocol including ion-exchange chromatography (Q-Sepharose) and gel filtration (Superdex 75). The eluted fractions were assayed for proteolytic activity and the purity of enzyme analyzed by SDS-PAGE. The proteolytic activity of SARS-3Clpro was found to be largely increased in the presence of 1 M Sodium Sulfate. This activation is anion-dependent, following the Hofmeister series ($citrate^{3-} > SO_4^{2-} > HPO_4^{2-} > acetate^- > HCO_3^- > Cl^- > Br^- > I^-$). Further assays will be performed to assess the structural and kinetic mechanisms of this activation. This work aim to the development of inhibitors compounds for this enzyme.