Structure, Mechanism and Specificity of FMDV 3C Protease

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Foot-and-mouth disease virus (FMDV) causes a widespread and economically devastating disease of domestic livestock. The viral RNA genome is translated as a single polypeptide precursor that must be cleaved into functional proteins by virally-encoded proteases. Ten of the thirteen cleavages are performed by the highly conserved 3C protease ($3C^{pro}$), making the enzyme an attractive target for anti-viral drugs. We have developed a soluble, recombinant form of FMDV $3C^{pro}$, determined the crystal structure to 1.9 Å resolution and analysed the cleavage specificity of the enzyme. The structure indicates that FMDV $3C^{pro}$ adopts a chymotrypsin-like fold and possess a Cys-His-Asp catalytic triad in a similar conformation to the Ser-His-Asp triad conserved in almost all serine proteases. This observation suggests that the dyad-based mechanisms proposed for this class of cysteine proteases need to be re-assessed. Peptide cleavage assays revealed that the recognition sequence spans at least four residues either side of the scissile bond (P4-P4´) and that FMDV $3C^{pro}$ discriminates only weakly in favour of P1-Gln over P1-Glu, in contrast to other $3C^{pro}$ enzymes that strongly favour P1-Gln. The relaxed specificity may be due to the unexpected absence in FMDV $3C^{pro}$ of an extended β–ribbon that folds over the substrate binding cleft in other picornavirus $3C^{pro}$ structures. Collectively these results establish a valuable framework for the development of FMDV $3C^{pro}$ inhibitors.

Keywords: viral protease, catalytic mechanism, antivirals