Crystal Structure of HAV 3C Protease Complexed with a β-lactone Inhibitor: a New Crystal Form of HAV 3C and Its Application for Studying Enzyme-inhibitor Interaction

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We obtained a new crystal form of Hepatitis A virus (HAV) 3C protease in complex with a serine-derived β-lactone inhibitor (N-benzyloxycarbonyl-L-serine-β-lactone). The crystals, which diffract to the highest resolution known to date (1.4 Å) for HAV and related viral proteases, are of space group $P2_12_12_1$ and contain one molecule per asymmetric unit. Serendipitously, the inhibitor covalently binds to a histidine residue (His102) on the surface of the enzyme opposite to the active site and leaves the catalytic residue Cys172 unmodified. Moreover, this is the first time that a functional arrangement of the catalytic triad (Cys172, His44, Asp84) was observed in HAV 3C crystals, making them ideal for the study of enzyme-inhibitor interactions in atomic detail.

The new crystal form seems to result at least partly from the binding of the benzene moiety of the lactone inhibitor to a hydrophobic pocket of a neighboring molecule. Structural alignment with previously solved HAV 3C crystal structures indicates significant conformational changes occur beyond the site of chemical modification. The two anti-parallel β-strands (aa 139-158) between the N- and C-terminal domains of HAV 3C exhibit less flexibility than previously observed in other crystal forms, which may have an important role in the organization of a catalytically competent triad. In particular, the hydroxyl of Tyr143 forms a hydrogen bond with $O^{\delta 2}$ of Asp84, facilitating the latter to interact with $N^{\varepsilon 2}$ of His44, the general base in catalysis. We subsequently soaked HAV 3C-lactone crystals in solution containing another irreversible inhibitor N-iodoacetyl-Val-Phe-amide to explore the feasibility of using these new HAV 3C crystals for the study of interactions between the enzyme and active site-reacting inhibitors.

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