Rational drug designs based on crystal structures of the Hepatitis C Virus NS3 helicase-inhibitor complexes

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Introduction

Hepatitis C virus (HCV), a positive-stranded RNA virus of the *Flaviviridae* family, is the major causative agent of transmitted non-A, non-B hepatitis and currently infects approximately 3% of the world's population. The infection easily results chronic hepatitis, and then may lead to liver cirrhosis and hepatocellular carcinoma. Unfortunately, no effective treatments for hepatitis C virus are available. Nonstructural protein 3 (NS3), one of the virus proteins, is a 631-residue bifunctional enzyme with the N-terminal 180-residue serine protease domain and the C-terminal 450-residue helicase domain. Here we report crystal structures of NS3 helicase domain from a Taiwanese HCV strain, complexed with various inhibitors, which would provide more knowledge in further rational drug design.

Materials and methods

To identify potential inhibitors for HCV helicase, the structural-based searches through available chemical databases were performed using the DOCK program. Following DOCK screening, more than ten potential inhibitors were selected and purchased for the enzymatic assay and structural studies. The NS3 helicase domain from a Taiwanese HCV strain was expressed, isolated, and crystallized as described previously. The crystals belong to the space group $P3_121$, with the unit cell dimensions of a = b = 92 Å, and c = 105 Å at the cryotemperature. The crystals soaked in various amounts of inhibitors for different length times, and the diffraction data were measured at 100 K. The crystal structures were solved by using the program AMORE in the CCP4 suite, and refined using *CNS* and *TURBO*.

Results and discussion

As the previously solved structures, the helicase domain consists of three structural domains forming a Y-shaped configuration [1]. Domains 1 and 2 have similar topologies, composed of a large central parallel β-sheet flanked by α -helices, whereas domain 3 is predominantly α-helical. In this study, the inhibitors bind at the interdomain cleft between domains 1 and 2.

The Fo – Fc density maps revealed that blue HT has the highest affinity to the enzyme among the inhibitors. In consistence with, the activity assay also showed that blue HT strongly inhibits the enzyme activity. The sulfate group interacts with five consecutive main chain NH groups of residues 207-211 (GSGKS), the side chain of Ser 211, and an additional water-mediated contact to Asp 290. The benzyl ring has close contact with Tyr 241 and Phe 238, and the amino group interacts with the backbone carbonyl group of G237 (Fig. 1).

In short, the negatively charged sulfonate group of blue HT contributes the major interaction, while the *para*-conformation avoids steric hindrance [2]. Further rational drug design could be performed by using the blue HT as a lead compound. Potential inhibitors containing the *para*-chain in blue HT will be searched through the databases, verified by the DOCK program, and selected for further characterization.

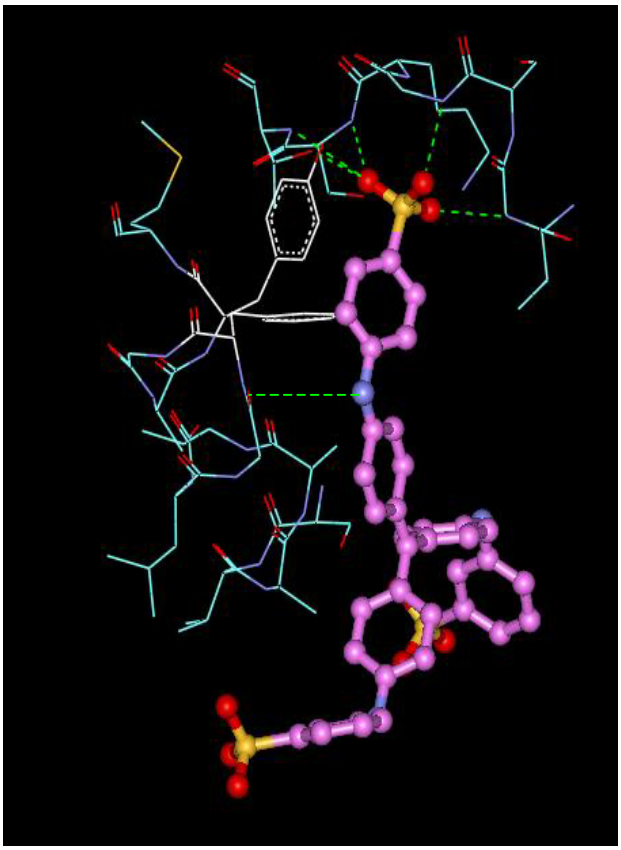

Fig. 1 The detailed interaction between BlueHT and NS3 helicase.

References

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