Crystal structures of MNV-1 RdRp-complexes

Intekhab Alam, Ji-Hye Lee, and Kyung Hyun Kim*
Department of Biotechnology and Bioinformatics, Korea University

Introduction

Norovirus (NV) is the leading cause of acute, nonbacterial gastroenteritis. Murine norovirus-1 (MNV-1) which can be grown in macrophage cell line RAW 264.7 serves as a surrogate model system for studying human norovirus (HuNoV) biology. MNV-1 RNA dependent RNA polymerase (RdRp) which is the main enzyme responsible for virus replication requires a protein primer VPg (Virion protein genome linked). The understanding of NV biology is important for the development of drug and vaccine against caliciviruses. In this study we have solved the crystal structureS of MNV-1 RdRp in complex with some inhibitors as 5-fluorouracil (5FU), ribavirin, and 2-thiouridine (2TU) [1, 2]. In order to gain further insight in NV replication attempts are made to solve the crystal structure of MNV-1 RdRp in complex with its protein primer VPg. Such structures will be helpful for better understanding of NV biology and will aid in designing some novel inhibitors against them.

Experiment

MNV-1 RdRp proteins were overexpressed in *E. coli,* and purified by affinity and size-exclusion chromatography. The final concentrations of RdRps were 5 mg/ml. Crystals were screened by hanging drop vapour diffusion method using commercial kits (Emerald biosystems, Bainbridge Island, USA). 1.5 µl of protein was mixed with 0.5 µl of buffer and two different temperatures 4˚C and 22˚C were tested for the initial screening. Crystals were observed at 4˚C in 1.26 M $(NH_4)_2SO_4$ and 0.1 M cacodylate (pH 6.5) and they were further refined with respect to salt and pH, to give diffraction quality cubic crystals in 1 M $(NH_4)_2SO_4$ and 0.1 M cacodylate (pH 6.5). For ribavirin and 2TU concentrated RdRp was incubated separately with 20 mM 2TU or ribavirin at 4˚C overnight and crystals were screened by sitting drop method using commercial kits (Emerald biosystems, Bainbridge Island, U.S.A). Good quality crystals for ribavirin were observed in 2-3 days after incubation at room temperature in 1.26 M $(NH_4)_2SO_4$, 0.1 M CHES (pH 9.5) and 0.2 M NaCl, which was further refined by hanging drop method with varying pH range (9.0-10.0) and $(NH_4)_2SO_4$ concentrations (0.8-1.4 M). For 2TU diffraction quality crystals were found in 2.0 M $(NH_4)_2SO_4$, 0.1 M cacodylate pH 6.5 and 0.2 M sodium chloride. To obtain crystals in complex with 5FU, crystals were soaked in 10 mM 5FU for 30-60 min prior to data collection. Diffraction data were collected with the crystals flash-cooled at 100 K in a stream of liquid N_2 using a synchrotron radiation source, BL-17A at Photon factory, Japan. Crystals diffracted to resolutions of 2.2-2.5 Å for the native and ligand complexed data. The native and ligand complexed crystals were of space group C2 and contained three monomers in the asymmetric unit.

Results

The MNV-1 RdRp structure was solved using CCP4 for molecular replacement, employing HuNoV RdRp structure (PDB ID 1SH2) with all its side chains retained as a search model. Molecular replacement gave a single prominent solution after the rotation and translation functions were employed. Difference Fourier maps with coefficients $2|F_o|-|F_c|$ and $|F_o|-|F_c|$ were used to model 5FU, 2TU and ribavirin interacting with amino acid residues at the active site. The overall structure with the ligand binding site is shown in figure 1.

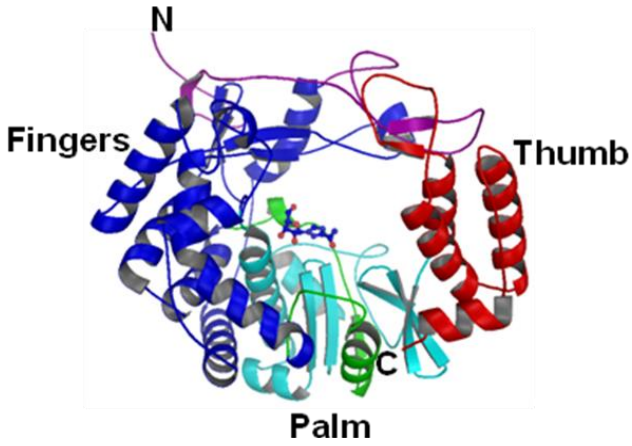

Fig.1 The overall structure of MNV-1 RdRp with palm (green and cyan), fingers (blue) and thumb (red) domains. N terminal is shown in pink while C is also in red. Ribavirin is represented as a ball and stick model (blue) in the active site.

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References

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* khkim@korea.ac.kr