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Crystal structure of MNV-1 RdRp-VPg(1-73) complex in the presence of RNA

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1 Introduction

Norovirus is the leading cause of epidemic acute, nonbacterial gastroenteritis, which is a potential threat to human health, but few antivirals or vaccines are effective [1]. This positive single strand RNA virus adopts *de novo* or primer-dependent replication by RNA-dependent RNA polymerase (RdRp). To understand the interaction between RdRp and VPg in replication of murine norovirus-1 (MNV-1), we determined the crystal structure of MNV-1 RdRp-VPg(1-73) complex in the presence of RNA.

2 Experiment

MNV-1 RdRp and different lengths of VPg including VPg(1-73) were cloned into pET14b or pET22b vectors and introduced into *Escherichia coli* ER2566 or BL21 (DE3) [2-3]. Protein expression was induced with 0.4-1.0 mM IPTG at 37˚C for 4 h or at 15˚C overnight. Proteins were purified by NI-NTA and Superdex 200 gel filtration chromatography. The homogenous fractions were concentrated to 1-5 mg ml^{-1}. MNV-1 RdRp protein (approximately 50 μM) was incubated with VPg in a molar ratio of 1:2 in the presence of 2 mM uridine triphosphate (UTP) or guanidine triphosphate (GPT), 10 nM oligo A ($A_{8\sim10}$) or oligo C ($C_{6\sim10}$), 2 mM $MnCl_2$, and 2 mM $MgCl_2$ at 4˚C overnight. Hanging drop vapour-diffusion method was used for crystallization by mixing 1.5 μl of complex protein and 0.5 μl of crystallization buffer containing 0.1 M cacodylate, pH 6.5, and 1.0 M sodium citrate. Triangular shaped crystals in the $P2_1$ space group appeared within a week. Diffraction data were collected with the crystals flash-cooled at 100 K in a stream of liquid N_2 in the mother liquor containing 25% glycerol using synchrotron radiation. Data were processed by HKL2000 and the complex model was built using Phenix and CCP4.

3 Results and Discussion

The crystal structure of MNV-1 RdRp-VPg(1-73) complex in the presence of RNA was very similar to that of MNV-1 RdRp [2]. The structure of RdRp resembled a right hand shape with fingers, palm and thumb domains (Fig. 1A). MNV-1 RdRp adopted an enclosed conformation where the extended N-terminal domain bridged across the fingers and thumb domains. A close analysis showed markedly different crystal packing between the RdRp and RdRp-VPg(1-73) complex structures in the presence of RNA. RdRp hexamers in the ternary complex are much more closely packed than those in the RdRp structure (Fig. 1B). VPg was bound to the base of the palm domain and the tip of the fingers domain of RdRp, but RNA template could not be modeled.

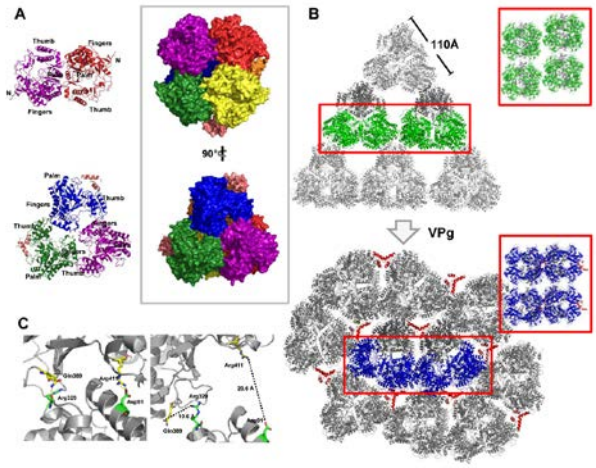

Fig. 1: Crystal structure of MNV-1 RdRp-VPg(1-73) complex in the presence of RNA. (**A**) Dimeric (upper panel) and trimeric (lower panel) RdRps are shown on the left. Six RdRp and two VPg molecules are shown in rainbow and tint pink color, respectively, in the box. (**B**) Crystal packings in RdRp natve (PDB ID 3QID) and RdRp-VPg(1-73) complex in the presence of RNA (PDB ID 3WFK) structures are shown at the 3-fold axis (on the left) and rotated views of red boxes are shown on the right. (**C**) RdRp-VPg(1-73) complex in the presence of RNA structure shows closer contacts between hexamers (on the left), compared to those in RdRp structure (on the right)

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