Mechanism of RNA stimulated ATP hydrolysis by tick-borne encephalitis virus NS3 helicase

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Tick-borne encephalitis virus is the cause of tick-borne encephalitis, an important arboviral disease affecting population within European and north-eastern Asian countries. There is currently no specific treatment available although it is preventable by vaccination [1, 2]. The lack of specific antiviral together with low vaccination coverage allowed the expansion of the virus within the Europe in recent years.

In the lifecycle of TBEV, NS3 helicase (NS3H) domain plays an essential role in viral genome replication. This domain carries out three enzymatic activities: RNA 5'-triphosphatase, RNA helicase and ATP hydrolysis. The latter activity is coupled to and provides energy for the RNA helicase activity during unwinding of the double-stranded RNA replication intermediate [3]. To understand the coupling between ATP hydrolysis and NS3H activity, we determined several crystal structures of NS3H, either the apo form or in complex with non-hydrolyzable ATP-analogue (AMPPNP), ADP or ADP-Pi (post-hydrolysis state). These represent structural snapshots of the key stages in ATP hydrolysis and nucleotide exchange. We also demonstrated that the ATP hydrolysis is stimulated in the presence of ssRNA but not ssDNA, both of which bind but the latter acts as a competitive inhibitor. Thus, RNA selectivity is not due to specific binding but is encoded in the coupling mechanism.

The obtained structures served as basis for molecular dynamics simulations of NS3H in complex with ssRNA. RNA binding in the post-hydrolysis state leads to an allosteric change which forces opening of the ATP binding site and allows release of the resulting inorganic phosphate ion, P_i. The allosteric change is commensurate with movement of ssRNA, suggesting that this step plays a key role in the tight coupling between helicase and ATPase activities.

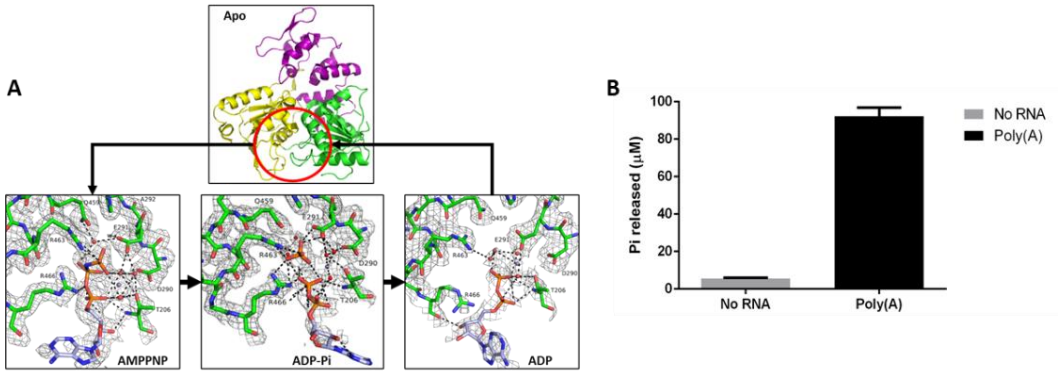

Figure 1. (A) ATP hydrolysis cycle captured in crystal structures. Upper panel: Structure of apo NS3H (PDB: 7AY4; 1.83Å). ATPase site is highlighted in red circle. Lower panel, from left to right: NS3H -AMPPNP–Mn^{2+} (PDB: 7BM0; 1.90Å), NS3H-ADP–Pi–Mn^{2+} (PDB: 7NXU; 2.10Å) and the NS3H-ADP–Mn^{2+} (PDB: 7BLV; 2.21Å). Residues from Walker B and motif VI are shown in sticks representation. An anomalous Fourier map (grey matrix) was calculated and displayed at a level of 1σ, the Mn^{2+} (purple spheres) and the highly coordinated waters (red spheres). Hydrogen bonds are displayed as black dashed lines. (B) Stimulation of ATP hydrolysis in the presence of ssRNA (poly(A)) determined using phosphate release assay.

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Keywords: ATP hydrolysis; crystal structures; helicase; MD simulation; TBEV

We would like to acknowledge the help and support of Dr. Manfred S. Weiss and Prof. Pavlina M. Rezacova in diffraction data collection at BL14.1 of BESSY II electron storage ring operated by the Helmholtz-Zentrum. Presented work was supported by the European Regional Development Fund-Project No. CZ.02.1.01/0.0/0.0/15_003/0000441.