Report on MX-1386 experiment.

Structural studies of MoMLV reverse transcriptase

Our aim were crystallographic studies of monomeric reverse transcriptase (RT) and we chose xenotropic murine leukemia virus-related virus (XMRV) RT which is nearly identical to well-studied mouse Moloney leukemia virus (MoMLV) enzyme. Replication of all retroviruses starts with the reverse transcription of the single stranded viral RNA genome into a double stranded DNA, which is subsequently integrated into host cell's chromosomal DNA. This stage of proviral DNA synthesis is catalysed by single enzyme - the viral reverse transcriptase (RT). The best studied RT is the heterodimeric HIV-1RT for which crystal structures with and without nucleic acid bound have been reported (1, 2). In contrast, for monomeric RT there are no available structures in functional complex with nucleic acid.

We obtained crystals of XMRV RT in complex with an DNA/RNA hybrid. They belong to $P4_32_12$ space group with unit cell parameters a=b=92.3 c=201.9 Å. By using the microfocus beamline 23-2 we were able to collect a dataset to 3.06 Å resolution. The structure was solved by molecular replacement using the reported apo MoMLV RT structure (PDB ID: 1RW3) (3) as a model. However, substantial parts of the model required rebuilding resulting from tracing errors in the original structure.

The structural model contains the polymerase subdomains – fingers, palm and thumb, in addition to the connection domain. A fragment of the RNA/DNA hybrid that interacts with those domains was also traced. The 3' of the DNA strand of the hybrid is located in the active site and properly positioned for a chain extension indicating that our structure corresponds to a catalytic complex. We did not observe any electron density for RNase H domain and a flexible linker preceding it, although the analysis of the content of the crystals clearly showed the full length protein on SDS-PAGE gels. We conclude that the RNase H domain is mobile in the structure. The overall protein structure resembles closely previously reported structures of XMRV RT but some global conformational changes occur upon substrate binding – in particular relocation of the thumb domain. Compared with HIV-1 RT, the structures are similar but we observed two additional α-helices in the thumb and connection domains of XMRV RT - the latter resulting from the monomeric nature of the XMRV RT. Based on our structure we identified residues involved in substrate binding in particular those responsible for DNA synthesis with concurrent displacement of downstream DNA.

In conclusion we solved the first structure of a monomeric RT with catalytically bound substrate, which allowed us to elucidate the mechanism of monomeric RT action and pinpoint the similarities and differences with well-characterized dimeric HIV-1 RT.

1. Huang H, Chopra R, Verdine GL, & Harrison SC (1998) Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science* 282(5394):1669-1675.
2. Sarafianos SG*, et al.* (2001) Crystal structure of HIV-1 reverse transcriptase in complex with a polypurine tract RNA:DNA. *The EMBO journal* 20(6):1449-1461.
3. Das D & Georgiadis MM (2004) The crystal structure of the monomeric reverse transcriptase from Moloney murine leukemia virus. *Structure* 12(5):819-829.