

Structural Studies of Nucleic Acid Complexes with Moloney Murine Leukemia Virus Reverse Transcriptase

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Beamline(s): **X25, X4A**

Introduction: Reverse transcriptase (RT) is an essential retroviral enzyme and is responsible for replicating the single-stranded RNA genome of the retrovirus producing a double-stranded DNA copy. Moloney murine leukemia virus reverse transcriptase (MMLV RT) is functionally similar but architecturally distinct from HIV-1 RT and is currently the only other reverse transcriptase amenable to structural studies. Our goals for these studies include understanding the structural basis of the mechanistic properties of this enzyme and comparing the structure of MMLV RT to the related human immunodeficiency virus type-1 (HIV-1) RT.

Methods and Materials: Crystals of an N-terminal fragment of MMLV RT and the full-length MMLV RT complexed with nucleic acid have been obtained. Synchrotron radiation was required in order to measure complete native data sets suitable for the structural analysis. Prior to obtaining time at X25, it was not possible to successfully index and integrate X-ray diffraction images from crystals of the MMLV RT-DNA complex. Although both of these problems can potentially be solved by molecular replacement, additional heavy atom phasing may be required for the structure determination of the full-length MMLV RT complex. We have initiated studies to find a potential heavy atom derivative for this problem.

Results: Data have been measured for the nucleic acid complex with the N-terminal fragment to 1.9Å resolution at X4A. A molecular replacement solution has been obtained for this problem, and we are currently completing the crystallographic refinement of this structure. For the complex with the full-length MMLV RT, native data were measured to 3.2Å at X25. These crystals belong to space group $P 2_12_12_1$ with cell dimensions of $a=52.1\text{Å}$, $b=238.2\text{Å}$, and $c=94.2\text{Å}$. We have obtained a preliminary molecular replacement solution for this problem and are analyzing this solution. In addition, we have established that crystals soaked in mercuric acetate still diffract and may prove useful should derivative phasing be required.

Conclusions: Structural studies on the N-terminal fragment of MMLV RT complexed with DNA have already provided new insights regarding functionally important interactions of nucleic acid with the enzyme. We expect that completion of the structural analysis of the full-length MMLV RT-DNA complex will provide further insights into the structure-function relationships in reverse transcriptases as well as related polymerases.

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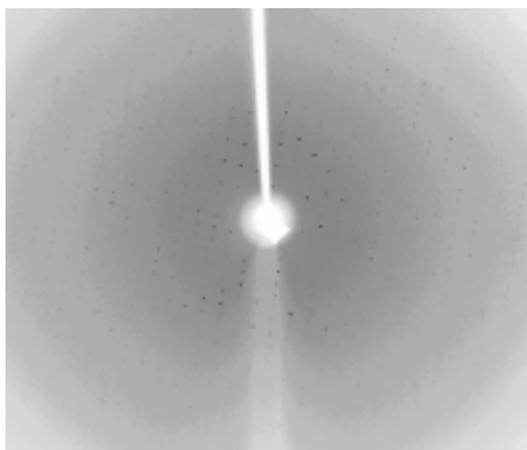


Figure 1. A diffraction image measured for crystals of the full-length MMLV RT at X25. The diffraction pattern extends to 3.2 Å resolution in this image.