Protonation states of the active site in dNTPase, a target of anticancer drugs

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1. Introduction

Human dNTPase hydrolyzes oxidized nucleoside triphosphates with its broad substrate specificity, and prevents transversion mutations caused by misincorporation of the oxidized nucleotides into DNA. On the other hand, human dNTPase was highlighted as a potential anticancer target because it highly expresses in cancer cells and avoids the misincorporation of oxidized nucleotides that result in DNA damage and cell death. Therefore, blockade of human dNTPase was suggested as a novel strategy for anticancer therapeutics. Recently, we carried out high resolution X-ray crystallographic analysis and mutational analysis on human dNTPase, and suggested that human dNTPase recognizes the different substrates by changing the protonation states of its substrate binding site. In order to validate the mechanism, it is essential to observe hydrogen atoms by neutron crystallography using large high quality crystals.

Here we grew large crystals of the human dNTPase/oxidized nucleotide complex and carried out neutron diffraction experiments using these crystals.

2. Experiment

We optimized and established the crystallization method for growing large high-quality crystals of human dNTPase using micro seeding, and grew five large crystals for neutron diffraction experiments (Fig. 1). The length of the longest side of these crystals is approximately 2 mm. These crystals were soaked for 2 weeks at 293 K in a crystallization solution containing D_2O instead of H_2O. These crystals were scooped up in a cryoloop and flash-frozen in a nitrogen stream at 100 K after stepwise soaking using a cryoprotectant with glycerol.

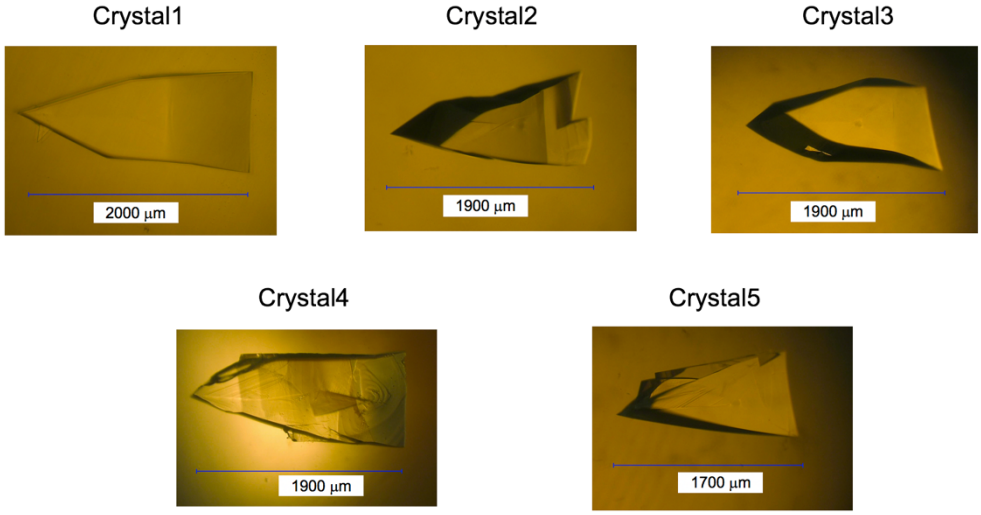

Figure 1 Large crystals of human dNTPase

The crystals were mounted on the iBIX diffractometer at BL03 and test measurements (500 kW) were carried out using crystal1, 2, and 3. The results of test measurements (30 min exposure) showed that crytal3 was the best crystal which diffracted to 2.7 Å resolution. After an overnight measurement using crystal 3, a diffraction

spot at 1.8 Å resolution was observed. Neutron diffraction measurement (500 kW) for structure refinements using crystal3 was carried out for 10 days.

3. Results

A total of 28 data sets were collected (Fig. 2). The TOF neutron data were indexed, integrated, scaled, and processed with STARGazer, and 2.1 Å resolution neutron data for structure refinements was obtained. Data collection statistics were listed in Table 1.

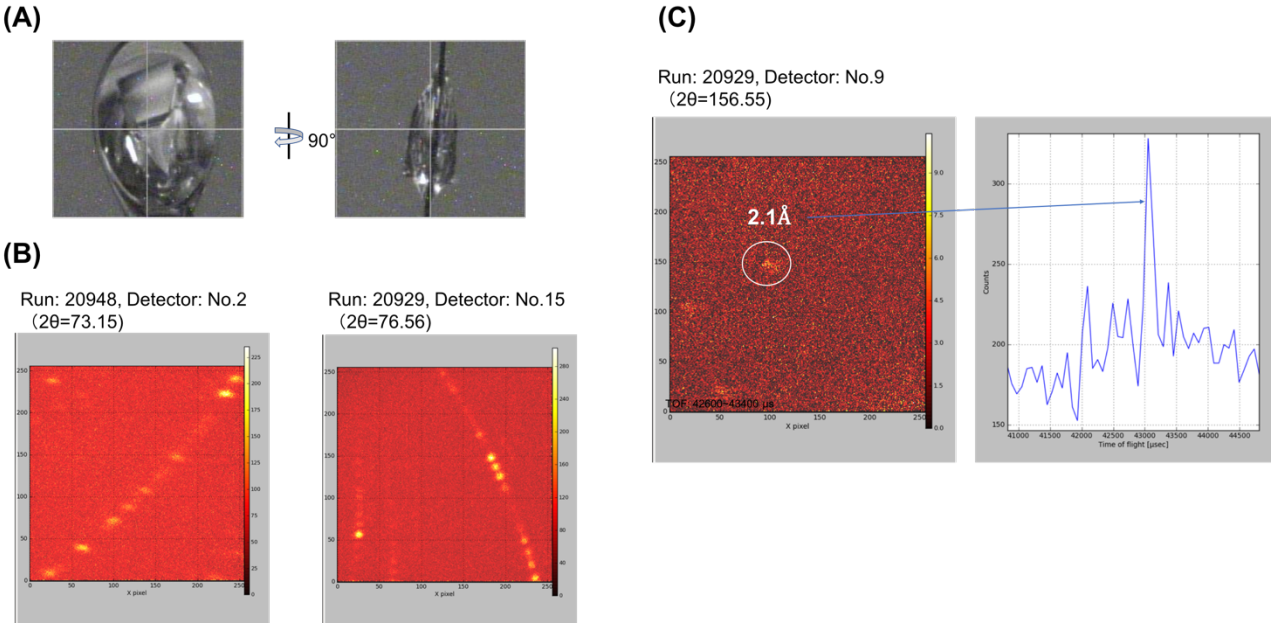

Figure 2 (A) Crystal photo. (B) Laue images. (C) TOF image.

Table 1 Data collection statistics

Data Collection	
Space group	$P2_12_12_1$
Cell *a*,*b*,*c* (Å)	46.33, 47.60, 124.29
Resolution (Å)	2.1
Total No. of refs	88,449
No. of unique refs	16,382
Completeness (%)	98.5 (99.1)
Redundancy	5.4
$I/\sigma(I)$	7.8 (2.8)
R_{merge} (%)	21.7 (53.3)

4. Conclusion

We also carried out X-ray diffraction measurement using the same crystal and obtained 1.5 Å resolution X-ray data. Joint refinements using neutron and X-ray data using PHENIX are in progress.