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|---|---|---|
| ESRF | **Experiment title:**<br>Structural studies of 5'(3')-Deoxyribonucleotidase (DRNase). | **Experiment number**:<br>LS-2042 |
| **Beamline**:<br>ID 14-4 | **Date of experiment**:<br>from: 30 January 2002 to: 31 January 2002 | **Date of report**:<br>2003-02-27 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Dr. Monaco | *Received at ESRF:* |


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## Report:

**We collected 2 datasets with the human protein deoxyribonucleotidase which resulted in two articles, one published and the other submitted. Statistics of the data collection is summerized below.**

## -dNT-2 with Thymidine and Berylliumtrifluoride:

Rinaldo-Matthis, A., C. Rampazzo, et al. (2002). "Crystal structure of a human mitochondrial deoxyribonucleotidase." Nat Struct Biol 9(10): 779-87.

**5'-nucleotidases are ubiquitous enzymes that dephosphorylate nucleoside monophosphates and participate in the regulation of nucleotide pools. The mitochondrial 5'-(3') deoxyribonucleotidase (dNT-2) specifically dephosphorylates dUMP and dTMP, protecting mitochondrial DNA replication from excess dTTP.**

**We have solved the structure of dNT-2, the first determined structure of a mammalian 5'-nucleotidase. The structure reveals a relationship to the HAD family, with a phosphoserine phosphatase as the closest neighbor. A structure-based sequence alignment of dNT-2 with other 5'-nucleotidases also suggests a common origin for these enzymes.**

**The structure of dNT-2 has been studied in complex with bound phosphate and beryllium trifluoride plus thymidine as model for a phosphoenzyme product complex. Based on these structures, determinants for substrate specificity recognition and the catalytic action of dNT-2 are outlined.**


Rinaldo-Matthis, A., C. Rampazzo, et al. (2002). "Crystal structure of a human mitochondrial deoxyribonucleotidase." Nat Struct Biol 9(10): 779-87.

## -dNT with DPB-T

Crystal structure of the mitochondrial deoxyribonucleotidase in complex with two specific inhibitors

Agnes Rinaldo-Matthis[1)], Chiara Rampazzo[2)], Jan Balzarini[3)] Peter Reichard[2,4)], Vera Bianchi[2)] and Pär Nordlund[1)*] Submited to Molecular Pharmacology

Mono-phosphate nucleotidases are enzymes that dephosphorylate nucleotides to their corresponding nucleoside. They play potentially important roles in controlling the activation of nucleotide-based drugs and inhibitors in viral infections or cancer cells. The human mitochondrial deoxyribonucleotidase (dNT-2) dephosphorylates deoxy thymidine and uridine monophosphates. We here describe the high resolution structures of the dNT-2 enzyme in complex with two potent phosphonate inhibitors, DPB-T((S)-1-[2-Deoxy-3,5-O-(1-phosphono)benzylidene-β-D-*threo*-pentofuranosyl]thymine) at 1.6 Å resolution and PMcP-U (±-1-trans-(2-phosphonomethoxycyclopentyl)uracil) at 1.4 Å resolution respectively. The linear mixed competitive inhibitor DPB-T and the competitive inhibitor PMcP-U both bind in the active site of dNT-2 but in distinctly different binding modes. The pyrimidine part of the inhibitors binds with very similar hydrogen bond interactions to the protein but with their phosphonate groups in different binding sites when compared to each other, as well as to the previously determined position for phosphate binding to dNT-2. Together these phosphate/phosphonate binding sites describes what might constitute a functionally relevant phosphate entrance tunnel to the active site. The structures of the inhibitors in complex with dNT-2, being the first such complexes of any nucleotidase, might provide important information for the design of more specific inhibitors which could be useful for controlling the activation of nucleotide based drugs or to study the *in vivo* function of nucleotidases using chemical knockout.

**TABLE 1 Data statistics**

| Data set | $BeF_3$ | DPB-T |
|---|---|---|
| Wavelength (Å) | 0.94 | 0.94 |
| Space group | $P4_32_12$ | $P4_32_12$ |
| a=,b= | 74 Å | 73.5 |
| c= | 106 Å | 106.7 |
| Resolution (Å) | 30-1.9 | 20-1.6 |
| I/σ(I) | 25.4 | |
| Completeness(%)[1] | (98.1) | 100 |
| overall[1] | 99.7 | 100 |
| $R_{sym}$(%)[1,2] | 5.7(27) | 8.7 (16) |
| Avg. Redundancy | 10.0 | 8.9 |
| Rcryst/Rfree[3] | 16.9/21.5 | 16.5/19.9 |
| Residues | 193 | 193 |

## Report: MAD data collection on the Mercury L(III) edge and other datasets.

Peak, inflection point and remote datasets were collected on crystals of *Thermotoga maritima class* II ribonucleotide reductase.
The peak dataset had a resolution range of 30-2.5Å, completeness of 91%, redundancy 3.3 and Rmerge 5.7(30).
Inflection point dataset had a resolution range of 30-3Å, completenes of 98%, redundancy 2.0 and Rmerge 5.8(13.6).
The remote dataset had a resolution range of 30-3.5Å, completeness of 96.1 %, redundancy 1.7 and Rmerge 4.4(10.6).

At the remote wavelength a Au and Ir dataset was collected
Au: 30-2.7 Å, 94.8 % complete, redundaancy 1.7, Rmerge 4 (17.9)
Ir: 20-3Å, 98.5 % complete, redundancy 2.7, Rmerge 6.7 (14.1)

We also collected a dataset on crystals soaked with the effector dTTP:
Resolution 30-3Å, 97.8% complete, Redundancy 3.1, Rmerge 8.5(16.8)

**Rinaldo-Matthis, A., C. Rampazzo, et al. (2002). "Crystal structure of a human mitochondrial deoxyribonucleotidase." Nat Struct Biol 9(10): 779-87.**