| ESRF | **Experiment title:** Structures of nucleotide complexes of human thymidylate kinase | **Experiment number**: LS-1139 |
|---|---|---|
| **Beamline**: ID14-EH3 | **Date of experiment**: from: 31.1.1999 to: 1.2.1999 | **Date of report**: 28.8.1999 |
| **Shifts:** 3 | **Local contact(s)**: Raimond Ravelli, Wim Burmester | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):
Nils Ostermann, Arnon Lavie*, Ilme Schlichting*, Thomas Veit, Jochen Reinstein, Roger S. Goody; Max Planck Institute for Molecular Physiology, Dept. of Physical Biochemistry, Otto-Hahn-Str. 11, 44227 Dortmund, Germany;
Manfred Konrad, Ralf Brundiers, Max Planck Institute for Physical Chemistry, Göttingen; Germany.
Chris Meier, Universität Hamburg, Hamburg, Germany

**Report:** The physiological reaction catalyzed by thymidylate kinase (TMPK) is the reversible transfer of the γ-phosphate of adenosine triphosphate (ATP) to thymidine monophosphate (dTMP) yielding the corresponding diphosphates ADP and dTDP. Its location at the junction of the *de-novo* and salvage pathways makes TMPK an essential enzyme for cell replication and thus an attractive target for the development of drugs against cancer or retro-viral infections. For the rational design of such targets a detailed understanding of the catalytic mechanism is required. Besides structures of the enzyme complexed with substrates or products, a complex of a transition state mimic such as $AlF_x$ is of special interest. Therefore we crystallized human TMPK in complex with TMP, ADP and $AlF_x$.

In addition to its physiological role TMPK is involved in the activation pathway of anti HIV prodrugs such as AZT (3'deoxy-3'azido-thymidine). This nucleoside analog must be phosphorylated three times by cellular enzymes to the activated metabolite AZTTP. The second phosphorylation step, the phosphorylation of AZT-monophosphate (AZTMP) to the diphosphate is catalysed by TMPK and has been shown to be the rate limiting step in the activation

pathway. This bottleneck in AZT activation results in an accomulation of millimolar concentrations of the toxic, partially activated metabolite AZTMP in cells treated with this drug. To understand the reasons for the poor activation of AZTMP by TMPK in detail, we crystallized human TMPK in complex with ATZMP and ATP. Crystallizing TMPK in complex with TMP and ATP in an analogous approach as used previously (unpublished) shows, that under appropriate crystallization conditions TMPK crystallizes preferably in complex with dTDP and ADP resulting in the product conformation of the protein.

Based on the kinetic and structural information from our previous work on yeast and *E.coli* TMPK [1-3], we started to design novel nucleoside analogs with improved properties in respect to TMPK phosphorylation. AZT-PCVMP is an acyclic derivative of AZTMP (the ribose is lacking the C2´ atom and O4´ is replaced by a methylene group). The structural characteristics of such an acyclic analog will give further guidance for the design of better substrates and in addition may result in a better understanding of the catalytic mechanism of TMPK. Therefore, we crystallized human TMPK in complex with ATZ-PCVMP and the ATP analog β,γ-imido-ATP (AppNHp). We collected three data sets at cryogenic temperature of the described complexes. The data collection statistics are summarized below. Refinement is in progress.

| **TMPK Complexes** | **TMP-ADP-AlF$_x$** | **AZTMP-ATP** | **AZTPCVMP-AppNHp** |
|---|---|---|---|
| Space group | $P4_32_12$ | $P4_32_12$ | $P4_32_12$ |
| Unit cell a,c (Å) | 101.59 / 49.29 | 100.76 / 49.47 | 101.30 / 49.50 |
| Resolution (Å) | 1.9 | 1.9 | 1.7 |
| # of observations measured/ unique | 106992 (20787) | 132856 (19506) | 202993 (28726) |
| Completeness (%) Overall (last shell) | 99.4(99.5) | 94.6(94.4) | 99.3(99.5) |
| $R_{sym}$ (%) Overall (last shell) | 7.5(46.3) | 8.6(59.8) | 7.3(33.3) |
| I/sigma Overall (last shell) | 11.5(2.1) | 13.2(2.3) | 13.7(3.7) |

[1] Lavie A, et al., *Structural Biology* **4**(8)**:** 601-4, 1997.

[2] Lavie A, et al., *Biochemistry* **37**(11)**:** 3677-86, 1998.

[3] Lavie A, et al., *Proc Natl Acad Sci U S A* **95**(24)**:** 14045-50, 1998.