s8a.m1.p33 **Crystallographic study of recombinant TMPK from *Mycobacterium tuberculosis*, with substrates and inhibitors.** I. Li de la Sierra[§#], H. Munier-Lehmann[*], A.M. Gilles[*], O. Bârzu[*] and M. Delarue[§]. *[§] Unité de Biochimie Structurale and [*] Laboratoire de Chimie Structurale des Macromolecules, Institut Pasteur, 28 rue du Dr. Roux, 75724, Paris Cedex 1 .[#]Corresponding author at : e-mail: ines@pasteur.fr*
Keywords: kinase, enzyme inhibitor, tuberculosis.

In the presence of dTMP, $TMPK_{Mt}$ crystallises in the hexagonal space group $P6_522$ with one monomeric molecule (M.W. 24 kD) per asymmetric unit and with cell parameters of: ***a***=***b***=76.62 Å, ***c***=134.38 Å. Cryo-cooled crystals diffracted at 1.95 Å using synchrotron radiation (M.W. 24 kD). Resolution of the crystal structure of the complex was performed by the Multiple Isomorphous Replacement Method (MIR). The phases at 2.9 Å were obtained with five heavy atom derivatives, including the nucleotide analogue Iodo-dUMP. Refinement was performed between 20-1.95 Å resolution with REFMAC (R_{factor}= 20.64, R_{free}=24.81). These results were presented in a paper[2] and communication[3].

Up to now, this structure is the third one obtained for the TMPK family of proteins, after those of yeast and of *E. coli*. The global folding of the *M. tuberculosis* enzyme is similar to that of the two former ones, despite the low homology of their amino-acid sequences (25% with $TMPK_{yeast}$ and 26% with $TMPK_{Ec}$). It displays a core of five stranded parallel β-sheets surrounded by nine α-helices. The structures of the apo-enzymes are not known since in all three cases, the proteins were crystallised with at least the phosphate acceptor.

A detailed atomic comparison of the dTMP binding site in all three enzymes will be presented.

Attempts to characterize the transition state are under way.

$TMPK_{Mt}$ / AZTMP co-cristals were obtained very recently, its crystallographic study is under current investigation at the European Synchrotron Facility (ESRF) in Grenoble [4]. It should allow us to understand why this analogue is so efficient in inhibiting $TMPK_{Mt}$.

[1] This study is part of a larger EC project, which aimed at defining specific inhibitors of TMPKs.
[2] Li de la Sierra, I., Munier-Lehmann, H., Gilles, A.M., Bârzu, O. & Delarue, M. Crystallization and preliminary X-ray analysis of the Thymidylate Kinase from *Mycobacterium tuberculosis*, **2000**, *Acta Cryst.* D56 : 226-228.
[3] Li de la Sierra, I., "Etude Cristallographique de la TMPK de *M Tuberculosis*." GTBIO99 Réunion du Groupe Thématique de Biologie Structurale de la Soc. Fr. de Cristallographie (Hourtin, 1999).
[4] The project is supported by EEC grant CTBIO # 0354.

s8a.m1.p34 **Cellobiohydrolases and processivity.** A. Varrot*, S. J Charnock*, M. Schülein[¤], H. Driguez[¶] and G. J. Davies*. **Department of Chemistry, University of York, Heslington, York YO1 5DD, England, [¤]Novo-Nordisk A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark and [¶]Centre de Recherches sur les Macromolécules Végétales, CNRS, BP 53, 38041 Grenoble cedex 9, France*.
Keywords: cellulases, inversion mechanism, carbohydrate binding.

The cellobiohydrolase Cel6A from *Humicola insolens* belongs to family 6 of the glycoside hydrolases[1]. It hydrolyses the ß-1,4 glycosidic bond of cellulose with inversion of the configuration of the anomeric carbon. It is a modular protein with a catalytic module (CM) at its N-terminus to a cellulose-binding domain (CBD) via a flexible and highly glycosylated linker. The Cel6A CM has a distorted $(\alpha/\beta)_8$ barrel fold. The active-site is located within an enclosed tunnel formed by two large loops[2]. This tunnel topology is typical for cellobiohydrolase enzymes and may permit processive hydrolysis of cellulose. After an initial attack, cellobiohydrolases proceed from one chain end along the substrate releasing cellobiose without diffusing away. Their classification as *exo*-acting enzymes is under scrutiny since they exhibit some *endo*-acting properties. The latest could be explained by an occasional opening of the loop permitting an initial *endo*-attack of the substrate. A structure of the catalytic module in complex with cellotetraose identified six subsites and demonstrated the flexibility of the two active site loops[3]. Comparisons with a family 6 endoglucanase, Cel6B from *H. insolens*, which has an open substrate-binding cleft reveals that the active-site tunnel of cellobiohydrolases confers unique properties for the degradation of crystalline cellulose[4]. Kinetic and structural studies of mutant enzymes gave new insights into the processive mechanism.

[1] Henrissat B. "A classification of glycosyl hydrolases based on amino acid sequence similarities", Biochem. J., (1991), 280: 309-316.
[2] Varrot A. "Crystal structure of the catalytic core domain of the family 6 cellobiohydrolase II, Cel6A, from Humicolas insolens, at 1.92 A resolution.", Biochem J, (1999), 337: 297-304.
[3] Varrot A. "Structural changes of the active site tunnel of *Humicola insolens* cellobiohydrolase, Cel6A, upon oligosaccharide binding.", Biochemistry, (1999), 38: 8884-8891.
[4] Davies G. J. "Structure and function of *Humicola insolens* family 6 cellulases: Structure of the endoglucanase, Cel6B, at 1.6 Å resolution.", Biochem. J., (2000), in press.