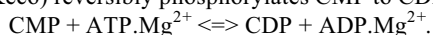


[s8a.m1.p3] Phosphorylation or oxydation level of the phosphate acceptor nucleotide modifies crystal structure of *E. coli* CMP kinase : correlation with kinetics and site-directed mutagenesis. T. Bertrand^{1*}, A.M. Gilles², P. Briozzo³. ¹ *Laboratoire d'enzymologie et de biochimie structurales, UPR 9063 du CNRS, 91198 Gif-sur-Yvette Cedex, France;* ² *Laboratoire de chimie structurale des macromolécules, URA 1129 du CNRS, Institut Pasteur, 75724 Paris Cedex 15, France.* ³ *Laboratoire de chimie biologique, Institut national agronomique Paris-Grignon, 78850 Thiverval-Grignon, France.* *e-mail: bertrand@lebs.cnrs-gif.fr
Keywords: bacterial CMP kinases, ligand-induced fit, specificity.

Cytidine monophosphate kinase from *E. coli* (CMPKeco) reversibly phosphorylates CMP to CDP :



CMPKeco, like other known bacterial CMP kinases, is very specific for CMP. This is in contrast with UMP/CMP kinases from eukaryotes which phosphorylate the two pyrimidine nucleotides with comparable efficiency (1).

We have published the first structures of a bacterial CMP kinase, CMPKeco, alone and in complex with CDP. This enlightened the particular specificity of the enzyme, and a new type of induced-fit movement of the CMP binding domain (2).

Beside CMP specificity, the second feature of the enzyme is its ability to phosphorylate 2'deoxyCMP with a rate similar to that for CMP. We present new structures of CMPKeco in complex with CMP or 2'dCMP (we also got diffraction data for crystals obtained with the competitive inhibitor 2'3'dideoxyCMP). The phosphorylation level of the nucleotide, as well as the oxydation level of its sugar, induce a different network of interactions with the enzyme. They also change the conformation of the bound nucleotide sugar. These structural informations are confronted with the observed kinetic parameters of CMPKeco and of its mutant D185A - a residue which side chain can interact with both 2' and 3'OH of the ribose.

The different structures of CMPKeco emphasize important residues of the enzyme, which are found conserved in other bacterial CMP kinases. Thus, the observed structural features of CMPKeco could be general properties of CMP kinases from prokaryotes.

[1] Bucurenci N., Sakamoto H., Briozzo P., Palibroda N., Serina L., Sarfati R.S., Labesse G., Briand G., Danchin A., Bärzu O. & Gilles A.M. *J. Biol. Chem.* **271**: 2856-2862, 1996.

[2] Briozzo, P., Golinelli-Pimpaneau, B., Gilles, A.M., Gaucher, J.F., Burlacu-Miron, S., Sakamoto, H., Janin, J. & Bärzu, O. *Structure* **6**: 1717-1527, 1998.

[s8a.m1.p4] Crystallographic studies of gluconate kinase. L. Kraft, G. Schneider, Y. Lindqvist, *Molecular Structural Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 77 Stockholm, Sweden.*

Keywords: gluconate, kinase, MAD.

Gluconate kinase, GntK from *Escherichia coli* is an ATP-binding protein of 19 kD and is assumed to function as a dimer. Gluconate is taken up into cells and subsequently phosphorylated by gluconate kinase to gluconate-6-phosphate, which is further catabolized by the Entner-Doudroff and pentose phosphate pathways¹. The initial uptake and phosphorylation can be achieved through the Gnt1 or the Gnt2 systems and the thermoresistant gluconate kinase is a part of the Gnt1 system². There is also a thermosensitive gluconate kinase and it is a part of the Gnt2 system. The two systems have led to much confusion, but the Gnt2 system is thought to be a subsidiary system for gluconate uptake and metabolism³.

The sequence does not show any significant overall sequence homology to any known protein but it has the ATP-binding motif similar to those found in other kinases (GXXXXGK[TS]). The goal is to solve the three dimensional structure of gluconate kinase and elucidate the catalytic mechanism by studying mutations in the active site and complexes as a part of a larger metabolic engineering program.

We have worked out the crystallization conditions of the protein and we have also collected a native data set to high resolution at the synchrotron, MAXII in Lund, Sweden. The use of MIR methods to solve the structure failed because of non-isomorphism of the crystals. Because of this lack of isomorphism we have expressed, purified and crystallized selenomethionine substituted gluconate kinase. We recently collected data at three different wavelengths at the EMBL outstation at DESY in Hamburg and are now evaluating the results.

[1] Izu H., Adachi O., Yamada M. "Gene Organization and Transcriptional Regulation of the *gntR* Operon Involved in Gluconate Uptake and Catabolism of *Escherichia coli*.", *Journal of Molecular Biology*, (1997), 267: 778 - 793.

[2] Tong S., Porco A., Isturiz T., Conway T. "Cloning and Molecular Genetic Characterization of the *Escherichia coli gntR, gntK* and *gntU* Genes of GntI, the Main System for Gluconate Metabolism.", *Journal of Bacteriology*, (1996), 178: 3260 - 3269.

[3] Bausch C., Peekhaus N., Utz C., Blais T., Murray E., Lowary T., Conway T. "Sequence Analysis of the GntII (Subsidiary) System for Gluconate Metabolism Reveals a Novel Pathway for L-Ionic Acid Catabolism in *Escherichia coli*.", *Journal of Bacteriology*, (1998), 180: 3704 - 3710.