<table>
<tr><td> </td><td>Experiment title: Cytidine Monophosphate kinase
BAG - CNRS gif sur Yvette</td><td>Experiment number:
LS 1798</td></tr>
<tr><td>Beamline:
ID14-2</td><td>Date of experiment:
from: 20 to 22 September 2000</td><td rowspan="2">Date of report:
02-27-01

Received at ESRF:</td></tr>
<tr><td>Shifts:
2</td><td>Local contact(s):
Andreas Bracher</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
B atrice Golinelli, CNRS*
Pierre Briozzo, INRA*</td></tr>
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Report:

Cytidine monophosphate kinase (CMPK) catalyses a key step in the pyrimidine biosynthesis pathway, the phosphorylation of CMP by ATP. CMPK from Escherichia coli (CMPKeco), in contrast to eukaryotic UMP/CMP kinases, phosphorylates efficiently 2'-deoxy-CMP but not dideoxyCMP. In order to explain the structural basis of these differences, we solved the crystal structure of CMPKeco complexed with ddCMP and compared it to those in complex with CMP or 2' deoxyCMP. This showed that the direct interactions between Arg 181 and Asp 185 of CMPKeco and the 3'OH of the sugar are fundamental for phosphorylation.

Data statitics

Complex with dideoxyCMP

space group P212121, a=72.6, b=75.3, c=78.0. The completion is 93 % (96.1 % in the last shell), Rsym=7.7 % (39.6 % in the last shell).
The structure has been solved by molecular replacement and refined to R= 22.1 %, Rfree=25.3 %.

Publication :

Thomas Bertrand, Pierre Briozzo, Liliane Assairi, Augustin Ofiteru, Nadia Bucurenci, H l ne Munier-Lehmann, B atrice Golinelli, Octavian B rzu & Anne-Marie Gilles (2001) Substrate specificty of bacterial CMP kinases enlightened by crystal structures and site-directed mutagenesis of the Escherichia coli enzyme, **submitted**

Abstract : Cytidine monophosphate kinase from *Escherichia coli* (CMPKeco), in contrast to eukaryotic UMP/CMP kinases, phosphorylates efficiently 2 -deoxy-CMP (dCMP). In order to explain the structural basis of this difference we solved the crystal structures of CMPKeco complexed either with CMP or with dCMP. Both structures showed original interactions involving the 3 hydroxyl from the pentose. These interactions are lost in our structures with the nucleotide analogues Ara-CMP, a poorer substrate, or 2 ,3 -dideoxy-CMP, a very weak substrate. All crystals contain two molecules per asymmetric unit. With CMP, these molecules are essentially identical. With the other substrates, one molecule is in a closed conformation similar to that with CMP; the second one is more open and in comparison lacks several enzyme-substrate interactions. This suggests that in solution CMPKeco always binds tightly its best natural substrate CMP in a catalytically reactive mode, but can bind weaker its alternate substrates in less productive modes. The

structures also show the contribution of pentose hydroxyl interactions in ligand-induced movements of enzyme domains. Furthermore, we substituted by site-directed mutagenesis the two residues interacting with the 3 hydroxyl of the pentose (Asp185 and Arg181), which decreased significantly the phosphorylation activity of both CMP and dCMP. Arg181 is conserved in all nucleoside monophosphate (NMP) kinases, and its role in the stabilisation of the transition state has been reported (Schlichting, 1997). As for other NMP kinases, this residue has probably a major role in catalysis by CMP kinases. On the other hand, Asp185 is probably a catalytic residue typical of bacterial CMP kinases. When Ser101, which is hydrogen bonded to Asp185, was substituted the phosphorylation of CMP was only moderately reduced. The same substitution provokes, however, a dramatic decrease in the phosphorylation rate of dCMP. Ser101 is the only strictly conserved residue of the characteristic NMP-bind insert of bacterial CMP kinases. This is the first experimental evidence of the catalytic role of this insert.