	Experiment title: H-Pr kinase-phosphatase BAG - CNRS gif sur Yvette	**Experiment number**: LS 1798
Beamline: ID14-H2 ID14-H4 ID14-H1	**Date of experiment**: from: 20/09/00 to: 21/09/00 from: 23/09/00 to: 24/09/00 from: 07/02/01 to: 08/02/01	**Date of report**: 26/02/01
Shifts: 2,3,1	**Local contact(s)**: Gordon Leonard, E. Mitchell	*Received at ESRF:*

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Report:

The HPr-kinase/phosphatase HprK/P of *L. casei* is a bacterial Ser/Thr kinase without sequence similarity with eukaryotic kinases nor any other protein known of known 3D structure. It is implicated in catabolite repression and carries out the ATP or GTP-dependent phosphorylation of the small protein HPr on residue Ser-46 by the HPr-kinase, and also its dephosphorylation. Phosphorylated HPr interacts with the transcription regulator CcpA to activate gene expression of the carbon catabolite repression signalisation pathway. The HPr kinase activity is enhanced by fructose bis-phosphate, whereas inorganic phosphate enhances the reverse reaction..

The aim of this study was to elucidate the structure of this new family of protein kinases and to understand its mechanism. We obtained crystals of a truncated form of the protein that has full enzymic activity. ..

On ID14-H2 Sept 20-21, 2000, several crystal forms were tested, a native data set was collected at 3Â resolution.and a heavy atom derivative search was performed. It failed, and we returned on Sept. 23-24 with crystals of seleniated protein. Four data sets were collected at ID14-H4, three for phase determination at 3 Å, one for extending the native data to 2.8 Å. These data led to structure solution. A complete model of the protein has now been refined to 2.8 Å resolution and submitted for publication.

Collection statistics	Lambda 1(inflection)	Lambda 2(remote)	Lambda 3(peak)
	0.9792 Å	0.9393 Å	0.9393 Å
Resolution (Å)	3.0	3.0	3.0
Completeness	99.6	99.3	99.7
I/sigma	9.9	10.7	10.4
Rsym	5.3	4.5	5.3

Additional data was very recently (Feb 7-8, 2001) collected on ID14-H1 with crystals of putative complexes of the *L. casei* protein with substrates and of the protein from *Neisseria.*

Publication

X-ray structure of HPr kinase : a bacterial protein kinase with a P-loop nucleotide binding domain.
S. Fieulaine, S. Morera, S. Poncet, V. Monedero, A. Galinier, J. Janin, J. Deutscher & S. Nessler (2001)
Submitted to *EMBO Journal*

Abstract

HPr kinase/phosphatase (HprK/P) is the major regulator of carbon metabolism in Gram-positive bacteria. It catalyses the ATP-dependent phosphorylation of Ser46 in HPr, a phosphocarrier protein of the phosphotransferase system, and also its dephosphorylation. HprK/P is unrelated to eukaryotic Ser/Thr protein kinases, but contains the Walker motif A characteristic of nucleotide binding proteins. We report here the X-ray structure of a truncated active fragment of *Lactobacillus casei* HprK/P at 2.8 Å resolution, solved by the MAD method on a seleniated protein. The protein is a hexamer with each subunit containing an ATP-binding domain similar to nucleoside/nucleotide kinases, and a putative HPr binding domain unrelated to other substrate binding domains. The Walker motif A forms a typical P-loop which binds inorganic phosphate in the crystal. We modelled ATP binding by comparison with adenylate kinase, and built a tentative model of the complex with HPr based on a docking simulation. The results confirm that HprK/P represents a new family of protein kinases, first identified in bacteria, but which may also have members in eukaryotes.