ESRF	**Experiment title: Structural studies on signal transduction pathways and cell cycle control.**	**Experiment number**: LS-1382 (LS-912)
Beamline: ID14-3 BM14	**Date of experiment**: from: 5th March to: 6th March, 1999 from: 30th April to: 1st May, 1999	**Date of report**: 16th February, 2000.
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Report: (In collaboration with Dr. D.Owen, LMB, Cambridge and the group of Dr. N. G. Oikonomakos, Athens, Greece).
(Biochem. (1999), 38: 14718-14730).

The catalytic mechanism of phosphorylase kinase probed by mutational studies.

The contributions to catalysis of the conserved catalytic aspartate (Asp149) in the phosphorylase kinase catalytic subunit (PhK; residues 1-298) have been studied by kinetic and crystallographic methods. Kinetic studies in solvents of different viscosity show that PhK, like cyclic AMP dependent protein kinase, exhibits a mechanism in which the chemical step of phosphoryl transfer is fast and the rate limiting step is release of the products, ADP and phosphoprotein, and possibly viscosity dependent conformational changes. Site directed mutagenesis of Asp149 to Ala and Asn resulted in enzymes with a small increase in K_m for glycogen phosphorylase b (GPb) and ATP substrates and dramatic decreases in k_{cat} (1.3×10^4 for Asp149Ala and 4.7×10^3 for Asp149Asn mutants, respectively). Viscosometric kinetic measurements with the Asp149Asn mutant showed a reduction in the rate-limiting step for release of products by 4.5×10^3 and a significant decrease (possibly as great as 2.2×10^3) in the rate constant characterising the chemical step. The date combined with the crystallographic evidence for the ternary PhK-AMPPNP-peptide complex (Lowe et al (1997) EMBO J. 6, 6646-6658) provide powerful support for the role of the carboxyl of Asp149 in binding and orientation of the substrate and in catalysis of phosphoryl transfer.
The constitutively active subunit PhK has a glutamate (Glu182) residue in the activation segment, in place of a phosphorylatable serine, threonine or tyrosine residue in other protein

kinases that are activated by phosphorylation. Site directed mutagenesis of Glu182 and other residues involved in a hydrogen bond network resulted in mutant proteins (Glu182Ser, Arg148Ala, Tyr206Phe) with decreased catalytic efficiency (approximate average decrease in k_{cat}/K_m by 20 fold). The crystal structure of the mutant Glu182Ser at 2.6 Å resolution (final statistics in Table 1) showed a phosphate dianion about 2.6 Å from the position previously occupied by the carboxylate of Glu182. There was no change in tertiary structure from the native protein but the activation segment in the region C-terminal to residue 182 showed increased disorder, indicating that correct localisation of the activation segment is necessary in order to recognise and present the protein substrate for catalysis.

Table 1. Data collection and refinement statistics for the Glu182Ser mutant PhK.

Figure 1. Final $2F_o$-F_c electron density map contoured at 1σ for the Glu182Ser PhK structure.

Data Collection	
Resolution 20-2.4 Å: (Highest resolution shell 2.53-2.4 Å)	
Number of Observations	40795
Number of Reflections	12745
Completeness	86.6% (78.5%)
Mean I/ Mean σ(I)	10.1 (2.9)
R_{sym}	4.8% (22.1%)
Refinement	
Number of atoms (mean B)	
Protein:	2279 (55.8 $Å^2$)
ATP/Mn/Phosphate	38 (44.2 $Å^2$)
Waters	104 (57.2 $Å^2$)
R-factor	25.1%
R-free	33.2%
RMS deviation from ideal bond lengths	0.013 Å
RMS deviation from ideal angles	2.7°