

Enzymes

MS04.01.01 STRUCTURAL BASIS FOR PHOSHOPEPTIDE RECOGNITION AND CATALYSIS BY PROTEIN PHOSPHATASES. David Barford, Amit Das, Marie-Pierre Egloff, Laboratory of Molecular Biophysics, University of Oxford, Rex Richards Building OX1 3QU, UK.

Protein phosphatases are signal transduction enzymes that catalyse protein dephosphorylation reactions. In eukaryotic cells, serine, threonine and tyrosine (and to a small extent histidine) residues are subject to reversible phosphorylation by protein kinases and phosphatases. Although protein kinases belong to a single gene family, four gene families encode protein phosphatases, two of which encode protein tyrosine phosphatases (PTPs) while the other two encode serine/threonine specific protein phosphatases. We have determined the structures of representative members of several classes of protein phosphatases including protein tyrosine, phosphatases and Ser/Thr specific protein phosphatases, in some instances in complex with phosphorylated peptides.

The structures of these enzymes will be discussed and the structural information will be used to explain differences in overall structure. The protein tyrosine phosphatases and Ser/Thr protein phosphatases catalyse dephosphorylation reactions by different mechanisms. PTPs utilise thio-phosphate intermediates whereas the Ser/Thr protein phosphatases catalyse single step reactions. The structures of the catalytic sites of protein phosphatases will be discussed in order to understand the nature of the catalytic mechanisms and the basis for substrate specificity. Particular attention will be paid to PTP1B and PP1. The crystal structure of PP1 was solved using multiple wavelength anomalous dispersion methods with tungstate as a heavy atom derivative.

MS04.01.02 MAP KINASES AND THEIR ACTIVATING ENZYMES. Elizabeth J. Goldsmith, Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX 75235-9050

The ras-activated protein kinase cascades are transducers of extracellular signals and differentially participate in the signaling of transformation, proliferation, and differentiation. Each cascade is a module consisting of three enzymes, a MAP kinase and two upstream activating enzymes. The MAP kinases are tightly regulated by dual phosphorylation events, one on a tyrosine and one a threonine residue. We have determined the structure of both the low activity unphosphorylated form and the high activity doubly phosphorylated form. The results show that regulation of activity is conferred by the conformation of the phosphorylation lip near the active site and the relative orientation of the two domains of the molecule. The phosphorylation site tyrosine is found buried in the low activity enzyme structure. Apparently conformational changes take place as the MAP kinase is bound to its activating enzyme, MEK. Analysis of the structures of MAP kinase mutants shows that the phosphorylation lip becomes disordered when negative charges are introduced in place of the phosphorylation site residues. Apparently, this region is relatively unstable, thus allowing small binding energies, derived from interaction with the activating enzyme, to dislodge the lip for phosphorylation.

We also have undertaken to determine the structures of several other protein kinases belonging to MAP kinase pathways, and have determined the structure of the MAP kinase homolog activated in response to cytokines, P38.

MS04.01.03 ENZYMATIC C5-CYTOSINE METHYLATION OF DNA. Xiaodong Cheng, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

C5-cytosine DNA methylation has been found in at least some members of almost every major biological species. The methylation pro-

ceeds through the formation of a transient, covalent protein-nucleotide complex. A key step is nucleophilic attack by a cysteine thiol at C6 of the target cytosine. The formation of this Cys-Cyt covalent bond potentiates a nucleophilic attack by the activated C5 on the methyl group, of S-adenosyl-L-methionine (AdoMet). During this reaction, AdoMet is converted to S-adenosyl-L-homocysteine (AdoHcy).

M.HhaI, a 327-amino acid protein, methylates the first cytosine of its recognition sequence 5'-GCGC-3'. The structure of *M.HhaI* was solved, in a binary complex with AdoMet (Cheng *et al.*, *Cell* 74:299-307, 1993), and in a covalent ternary complex with a 13-mer oligonucleotide containing 5-fluoro-2'-deoxycytidine at the site of methylation and AdoMet (Klimasauskas *et al.*, *Cell* 76:357-369, 1994). Two additional ternary structures were recently solved: *M.HhaI* complexed with both a 13-mer unmethylated substrate DNA and the cofactor product, AdoHcy, and *M.HhaI* complexed with both final products of the reaction, a 13-mer DNA methylated on both strands and AdoHcy (M. O'Gara, S. I. Klimasauskas, R. J. Roberts, and X. Cheng, submitted, 1996).

The complexes containing 2'-deoxycytidine, 5-fluoro-2'-deoxycytidine, or 5methyl-2'-deoxycytidine at the target represent the pre-methylation complex, the dihydrocytosine intermediate, and the methylation product, respectively. In these three ternary structures, the target flips out from the DNA helix and into the target nucleotide-binding pocket, near AdoMet. Because the functional amino acids revealed by the *M.HhaI* structures are so well conserved among the family of C5-cytosine methyltransferases, they likely all have similar structures and use the same mechanism. Many of these residues are conserved in aminomethyltransferases as well (Malone *et al.*, *J. Mol. Biol.* 253:618-632, 1995), and it will be interesting to see if they play analogous roles in the quite different reaction mechanisms used by those enzymes.

MS04.01.04 STRUCTURE AND MECHANISM OF SLT70, A BACTERIAL MURAMIDASE Bauke W. Dijkstra, Andy-Mark W. H. Thunnissen, Erik van Asselt, BIOSON Research Institute and Laboratory of Biophysical Chemistry Groningen University, Nijenborgh 4, 9747 AG Groningen, the Netherlands

The main component of the bacterial cell wall is murein or peptidoglycan, a polymer built up of linear glycan strands consisting of alternating N-acetylglucosamine (GlcNAc) and N-acetyl muramic acid (MurNAc) residues, which are cross-linked by short peptides. Inhibition of the enzymes involved in murein-synthesis and degradation by e.g. antibiotics is often lethal to the bacterium¹.

The 70 kDa soluble lytic transglycosylase from *E. coli* (SLT70) catalyzes the cleavage of the beta-1,4-glycosidic bond between MurNAc and GlcNAc. Its 3D-structure has been elucidated at 1.65 Å resolution. The unusually doughnut-shaped SLT70 molecule is built up of three distinct domains, which are all very rich in α -helices. The C-terminal domain (residues 451 to 618) contains the active site; this domain resembles the fold of lysozyme^{2,3}. To confirm a lysozyme-like reaction mechanism crystallographic binding studies were carried out with bulgecin A⁴, (GlcNAc)₃, and a natural 1,6-anhydro-muropeptide product. All compounds bind in the active site of SLT. Bulgecin A is a glucosamine containing molecule that enhances the bulging activity of many beta-lactam inhibitors. Its glucosaminyl part is bound at subsite C, close to the position of the GlcNAc residue in the active sites of lysozymes. The (GlcNAc)₃ is also bound in the active site of SLT, and extends over subsites B, C and D. The two saccharide moieties (GlcNAc-beta(1->4)-1,6-anhydro-MurNAc) of the muropeptide product are bound near subsites E and F in the SLT70 active site groove. Combination of the results obtained with these three substrate analogues allows a detailed picture of how a natural peptidoglycan polymer binds in the active site.

¹Thunnissen, A.-M.W.H. & Dijkstra, B.W. *Nature Struct. Biol.* 3, 218-221 (1996)

²Thunnissen, A.-M.W.H. *et al.* *Nature* 367, 750-753 (1994)

³Thunnissen, A.-M.W.H. *et al.* *PROTEINS: Struct. Funct. Genet.* 22, 245-258 (1995)

⁴Thunnissen, A.-M.W.H. *et al.* *Biochemistry* 34, 12729-12737 (1995)