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The Inhibition of Matrix Metalloproteases by Tissue Inhibitors of Metalloproteases Revealed by X-Ray Crystallography

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Key words: metalloproteases, tissue inhibitors, crystal structure

Tissue inhibitor of metalloproteinase I (TIMP I) is a member of a family of tissue inhibitors of metalloproteinases that are important in regulating the activity of a group of zinc dependent enzymes, the matrix metalloproteinases (MMP) by binding tightly with 1 1 stoichiometry The MMPs, including collagenase and gelatinase, are involved in the degradation and turnover of connective tissue in both normal and pathological conditions indeed an imbalance in the concentrations of TIMP and MMP has been observed in a number of degradative diseases In addition, changes in regulation of the 1 1 ratio are seen in neoplastic cells that have become metastatic

Crystals have been grown and data collected on native (to 1 8Å resolution) and platinum derivatives (to 2 3Å) The TIMP I protein used for the crystallisation experiments is a 27kDa protein that has been modified by site directed mutagenesis to yield a 21kDa deglycosylated form of the protein

The structure of a TIMP MMP complex has been determined (Gomis-Ruth *et al* 1997) and the coordinates from this structure have been used to build the structure of uncomplexed TIMP I

What is the role of the matrix metalloproteases (MMPs) and their inhibitors, TIMPs?

Degradation of the extracellular matrix (ECM) is effected by proteolytic enzymes from all classes of proteases, including the metalloproteinases (MMPs) MMPs are a family of well characterised zinc dependent endopeptidases including gelatinases, matrilysin, collagenases, stromelysin and thermolysin initially defined by their sequence similarity, their activity can be inhibited by metal chelators and by TIMPs that bind tightly to their target metalloproteinases Structural proteins are the most abundant proteins in the body and breakdown of these proteins and glycoproteins by MMPs is necessary for normal processes such as trophoblast implantation, bone modelling and wound healing MMP activity is also involved in pathologic processes and the results of uncontrolled degradation can be seen in inflammatory diseases such as rheumatoid arthritis and periodontal disease A more controlled pathologic degradation occurs at onset of metastasis in cancer when neoplastic cells become invasive MMP activity is regulated principally by members of the Tissue Inhibitors of Metalloproteinases (TIMP) family that bind tightly to their target MMP with 1 1 stoichiometry MMPs are extremely efficient enzymes and their potency has to be carefully regulated by TIMPs to maintain a balance between levels of connective tissue proteins and the metalloproteinases that degrade them

Structure and Function of TIMPs

TIMPs are a group of extremely stable proteins that specifically inhibit the zinc dependent metalloproteinases, binding both active and latent enzymes (Willenbrock and Murphy 1994) The TIMP family comprises TIMPs 1, 2, 3, 4, Imp a and b Human TIMP I and TIMP 2 have been sequenced and both the cDNA sequences code for a 21kDa protein, TIMP 1 has 184 residues with two glycosylation sites located at residues Asn30 and Asn78 and therefore migrates as a 28kDa protein on SDS PAGE gels TIMP 2 has 194 amino acid residues and does not contain a glycosylation site (Willenbrock and Murphy 1994) Despite similarities in sequence and function TIMP 1 and TIMP 2 are encoded by genes located on different chromosomes Removal of the carbohydrate moiety from the glycosylated sites of TIMP 1 does not affect the ability to inhibit MMPs therefore glycosylation may have a role in the turnover of TIMP or provide the ligand for binding to receptors in the extracellular matrix (Willenbrock and Murphy 1994) Sequence similarity between the TIMPs is approximately 40% with 25% identity between all three sequence homology between TIMP 1 and TIMP 2 is predominantly at the N terminus rather than the C terminus of the proteins

TIMP and MMP are co-expressed at a transcriptional level and this expression is tightly regulated to maintain the essential 1 1 ratio but gene expression of both can be modulated by various cytokines e g IFN-g upregulates MMP during wound healing

What happens if there is a change in the 1:1 ratio of MMPs and TIMPS?

Regulatory molecules such as cytokines can effect transitory imbalances when required in normal processes but more prolonged changes to the 1 1 ratio have been observed in a number of degradative diseases including multiple sclerosis, arthritisand rheumatism In addition deregulation of the balance between TIMPs and MMPs is thought to be important in the process of neoplastic cells acquiring metastatic potential Degradation of the extracellular matrix allows the metastatic cells to pass into lymph or blood circulatory systems for translocation to secondary sites *Secondary tumours are the major cause of*

death in cancer patients Structural information could provide information for therapeutic approaches to controlling metastasis in cancer and, of course, treatments for degradative diseases

Crystallographic Studies of TIMP – I

The TIMP-1 used for crystallographic studies in York was generously donated by Prof Gillian Murphy at the University of East Anglia

TIMP-1 crystals had already been grown in York but unfortunately were too small to collect x-ray data and microcrystals formed as the protein was being concentrated In order to prevent formation of microcrystals great care was taken during concentration of protein to a final concentration of no more than 2 0mg/ml Improved crystals were then grown by hanging drop method with the protein suspended over a 1ml well of mother liquor containing 500ml 50% PEG 2KME and 500ml Tris buffer pH8 4

Data Collection

X-ray data were collected on native crystals and crystals soaked in heavy atom compounds to provide phasing information and data were collected at S R S Daresbury EMBL Hamburg Outstation and Elettra Synchrotron, Trieste All data collected were under cryo conditions, native data were collected to 1 7Å and many heavy atom compound soaks were tried but the only heavy atom derivative was platinum Then the structure of TIMP 1 complexed with MMP 3 (stromelysin) was determined and the coordinates for the complexed TIMP were kindly passed on to us (1) so that the complexed TIMP 1 could be used to determine the structure of uncomplexed TIMP 1 by molecular replacement methods

Comparison between complexed and uncomplexed TIMP 1

Refinement of the uncomplexed TIMP 1 is still in progress nevertheless the apparent differences between the uncomplexed molecule and the TIMP 1/MMP 3 complex are predominantly at the interface between TIMP and MMP3 The complex described by Gomis Ruth et al (Gomis-Ruth *et al* 1997) shows the first four highly conserved TIMP residues together with Ser 68 and Val 69 bind on either side of the MMP catalytic zinc The Ala 65 to Cys 70 region of the MMP active site is involved in substrate interactions TIMP interactions with MMP3 occupy the entire length of the MMP active site cleft and other regions where the TIMP 1 molecule interacts with MMP include Val29 and Thr33 to Tyr 35, the cross bridged loop at Cys 132 Leu 133 and Ser 134 and Gln150 to Leu 152

When a superposition diagram of 'free' and complexed TIMP molecules was prepared it was discovered that there was a shift in the conformation of the 'free' TIMP not only at the first four residues but also at the Ala 65 to Cys 70 region and at Gln 150 to Leu 152 Other parts of the free' TIMP 1 model are at present not clear and have yet to be determined by further refinement but may be disordered due to residues having increased flexibility on exposure to solvent

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Characterization and Crystallisation of the Malate Dehydrogenase from *Streptomyces aureofaciens*

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Malate dehydrogenase (EC 1 1 1 37) is an enzyme that catalyzes conversion of malate to oxaloacetate reversibly using NAD (resp NADP) as a cofactor In eukaryotic cells several isoforms MDHs have been identified, differing in their subcellular localisation One form is a principal enzyme of the citric acid cycle operating within the mitochondria The other is found in the cytosol where it participates in the malate/aspartate shuttle This shuttle exchanges reducing equivalents across the mitochondrial membranes in the form of malate/oxaloacetate rather than as NAD/NADH (Hall *et al* 1992) A third isoenzyme can be found in the glyoxysomes of yeast and plants, where it function to convert malate that is produced from glyoxylate in the glyoxylate cycle (Minard and McAlister-Henn 1991) In prokaryotes there is only one form of the enzyme The enzymes share a common catalytic mechanism and their kinetic properties are similar although they are distinguishable by certain properties including differential inhibition by high concentrations of oxaloacetate (Banaszak and Bradshaw 1975)

Malate dehydrogenases are part of a well-studied family of NAD-dependent dehydrogenases (Branaszak and Bradshaw 1975) This family includes the enzymes lactate dehydrogenase (LDH), alcohol dehydrogenase, and glyceraldehyde-3-phosphate dehydrogrenase (Rossmann *et al* 1975) Characterization of these enzymes has included isolation from a large number of source, sequence determination, and functional characterization The two most closely related enzymes in this family are the MDHs and the well-studied