Meeting report

Metalloproteinases: Role in pathology and development of inhibitors

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Mammalian cells produce a variety of zinc-dependent enzymes termed metalloproteinases. Two classes or families of these enzymes were discussed at this British Inflammation Research Association meeting at Bath University. The major part of the meeting was devoted to the connective tissue metalloproteinases (CTMPs). These enzymes are believed to be involved in the normal turnover of the extracellular connective tissue matrix. Evidence is accumulating that these enzymes are involved in the breakdown of tissues seen in diseases such as rheumatoid arthritis, periodontal disease and cancer metastases. Inhibitors of these enzymes are being developed by a number of companies for the treatment of destructive arthritic conditions. The latter part of this meeting discussed the cell surface associated metalloproteinases exemplified by the enzyme variously termed EC 24.11, neutral endopeptidase or membrane metalloendopeptidase. Potent, biologically active, inhibitors of these enzymes have been synthesized and tested.

This meeting was judged a success from the very beginning when a count of the audience revealed 140 people packed into a lecture theatre designed to seat 100. John Reynolds (Strangeways) and Gerry Higgs (Celltech) acted as chairmen for the morning and afternoon sessions respectively, and the lively discussions that followed each paper owed much to them.

Dr. John Reynolds (Strangeways) began the morning session with an overview of the role of the CTMPs: collagenases, stromelysins and gelatinases in tissue pathology. Starting with the hypothesis that proteinases are the major agents of tissue destruction, the evidence that the CTMPs are the proteinases of importance was established. The cell biology of the release of CTMPs and the complex interactions between positive (e.g. IL1) and negative (e.g. TGF/β) modulators of enzyme release was covered, as was the problem of enzyme activation. The interaction between the many inflammatory cytokines and growth factors present in a lesional site with the mesenchymal cells that synthesize the CTMPs is going to be extremely difficult to decipher. The point was stressed that the important parameter to measure was the ratio between the CTMPs and the natural inhibitors of these enzymes – the Tissue Inhibitors of Metallo Proteinases (TIMPs 1 and 2).

The various physiological and pathological states in which the CTMPs are believed to be involved were then briefly described. These include: morphogenesis/growth, implantation, uterine involution, bone remodelling, wound healing and – moving on to pathological states – joint destruction, tumour invasion and metastasis, tooth loss in periodontal disease and corneal ulceration. The techniques used to determine the participation of CTMPs in these various states include: tissue culture in various forms, biochemical analysis and immunocytochemistry. Most of these techniques, particularly the direct biochemical studies, are difficult to do because of the small amounts of enzymes being produced in pathological tissues. One recent study by Dean et al. (J. Clin. Invest., 1989) was described. Here the workers extracted neutral...
and acidic metalloproteinases from normal and osteoarthritic human cartilage and demonstrated a 150% rise in the diseased cartilage. In contrast, TIMP levels in the osteoarthritic cartilage were only increased by 50%.

Dr. Reynolds then went on to describe his own studies using cell culture and immunocytochemistry. The technique of growing cells such as fibroblasts, chondrocytes, osteoblasts, etc., on films of 14C-collagen to ascertain the mechanisms involved in the release and activation of the CTMPs was detailed with examples. The final part of the lecture was taken up with a detailed examination of the immunocytochemical localization of the CTMPs in various physiological and pathological conditions. The elucidation of the pattern of enzyme localization in the rabbit growth plate showed the power of this technique.

In a change to the programme, Dr. Andy Docherty from Celltech gave the second paper of the morning, on the molecular biology of the CTMPs. Dr. Docherty was responsible for the first reported cloning of TIMP and has been responsible for the cloning and expression of a number of the CTMPs. The details of the growing number of enzymes that make up the family of CTMPs was presented. There are now two collagenases, two stromelysins and a related enzyme termed PUMP1 (Putative MetalloProteinase) and two gelatinases in this family of metalloproteinases. With the exception of the polymorph collagenase, these enzymes have been cloned and comparisons of their structures have been made. The amino-acid sequences were shown to emphasize the homology between these enzymes and the fact that each enzyme shares 3 conserved cysteines. The gelatinases are of higher molecular weight than the mesenchymal collagenase or stromelysin. In both gelatinases, there is an insert of a 58 amino-acid repeating unit that is homologous to the collagen-binding domain of fibronectin. In the 92 kD gelatinase, there is an additional insert that has homology with type IV collagen. The role of these additional amino acid sequences is not immediately clear. The CTMPs are synthesized in a pro-enzyme form and activation involves the loss of the N-terminal sequence. The sites at which the various activators of these enzymes attack were described. At the present time, there is no X-ray crystallographic data on the CTMPs. The only zinc-dependent metalloproteinase that has been subject to high resolution crystallographic analysis is the bacterial enzyme thermolysin. The known structural details of the CTMPs were compared with the detailed model of thermolysin and it was concluded that this bacterial enzyme was a poor model of the mammalian counterparts.

A detailed account of the cloning systems used to express stromelysin and collagenase was presented. These enzymes are cloned in mammalian cells such as fibroblasts, chinese hamster ovary (CHO) cells or the NSO myeloma line. This allows the final protein to be glycosylated and properly folded. This is particularly important with TIMP1, which has 6 disulphide bonds and contains one third carbohydrate. A second TIMP has recently been cloned which is 40–45% homologous with TIMP1. The 12 cysteines are conserved but the molecule is not glycosylated. The position of the disulphide bonds in TIMP1 has recently been established.

The molecular biology of the CTMPs is starting to provide a wealth of structural detail about this family of enzymes and their inhibitors. With the associated ability to produce large amounts of these proteins in large-scale fermentors, it will also be possible to do all the functional studies that have been hampered up till now by lack of material.

Dr. Gill Murphy (Strangeways) then led on from the molecular studies to the enzymology of this family of CTMPs and to the mechanisms involved in the activation of these enzymes. The general properties of these enzymes are: (i) they are secreted in a latent proform; (ii) they cleave their substrates at the N-terminal side of hydrophobic residues and (iii) the active forms of the enzymes are inhibited by TIMP. The substrates for these enzymes include all the components of the connective tissue extracellular matrix, and collagenase and stromelysin cleave the a2-macroglobulin bait region. The need for good low molecular weight substrates of these enzymes was stressed.

As stated, the CTMPs are released from cells as pro-enzymes and require activation. The secrets of this activation are only now yielding to the experimentalist. Activation requires conformational changes in the enzyme and the loss of an N-terminal 80 amino-acid sequence. It is now clear that organomercurials, which have long been used to activate CTMPs, cause conformational changes. A mechanism of activation – the coiled spring hy-