Journal of Orthodontics/Vol. 27/2000/227–233

Scientific Section

Matrix Turnover

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Abstract. *This review concentrates on how the major component of extracellular matrix, collagen, is catabolized. This process is important in a number of aspects of orthodontics since matrix is constantly turning over, the rate of which differs in embryogenesis, ageing, disease, and physiological processes, such as orthodontic tooth movement. It is not the purpose of this review to consider each process in detail. The aim is to give a clear account of the matrix metalloproteinases (a major family of proteinases) including their classification, properties, and functions.*

Index words: Matrix Metalloproteinase, Collagenase, Gelatinase, Timp.

Introduction

The Extracellular Matrix

The extracellular matrix (ECM) is primarily a collection of fibrous proteins embedded in an hydrated polysacharide gel. The macro-molecules which make up the extracellular matrix are secreted at a local level by calls, in particular fibroblasts. If the matrix is more specialized, such as cartilage or bone, then the extracellular matrix is secreted by more highly differentiated cells, such as osteoblasts, which form bone, or chondrocytes, which make cartilage. The macro-molecules which make up the ECM fall into two groups:

- The collagens.
- The glycosaminoglycans (GAGs).

The GAGs usually link to a protein with a covalent bond and form proteoglycans. These glycosaminoglycan and proteoglycan molecules make a gel like 'ground substance', where other fibres can be embedded, such as collagen. The 'gel' allows diffusion of hormones and nutrients, whereas the collagen fibres strengthen the matrix, the characteristics of which differ, depending on functional requirements of the tissue. The variation in the combinations of these matrix macro-molecules leads to a diversity of tissue forms which can be adapted depending on the functional requirements. The spatial relationships between cells and their ECM also differs between tissues (Adams and Watt, 1993). The role of this matrix is to provide a physical framework for the cells that are responsible for its production, and to function as a medium which regulates cell identity, position, proliferation, and fate. The interaction between both soluble and insoluble components of the extracellular space with the cell surface are vital for the overall development, integrity and function of that tissue.

Cells within a matrix can modify their functions depending on the link which is made with the ECM. This is usually through the cell adhesion molecules which cross the cell membrane to connect ECM molecules to the cytoskeleton of a cell. The role of this class of molecule has been considered elsewhere (Kerrigan *et al*., 1998).

Apart from the collagens there are also elastin molecules, which form an extensive cross-linked network of fibres, and these are prominent within structures such as the periodontal ligament and any tissue which requires elasticity and functions of stress, as well as recoil of tissue. The fibronectin molecules within the matrix promote cell adhesion and exist as large aggregates within the extracellular space.

Extracellular Matrix Composition

Remodelling of the ECM is required during cellular migration (Corcoran *et al*., 1996a), a process which is essential during normal growth and development. In development, there are changes in ECM composition and configuration. The proteases responsible for ECM turnover may be implicated in many of the processes of normal craniofacial development.

During tissue remodelling, cells must detach from their local ECM environment, degrade components of that ECM, and then migrate to a new position through the proteolytically-modified matrix (Yu *et al*., 1996). In order to be successful, proteolytic degradation of the matrix must be regulated in space and in time for cell migration.

A number of proteolytic enzymes have been implicated in ECM catabolism, including aspartyl-, cystyl- metallo-, and serine-proteinases. The matrix metalloproteinases (MMPs) represent the major class of enzymes responsible for ECM metabolism and it is these enzymes that are described in greater detail within this review.

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0301-228X/00/030000+00$02.00

Matrix Metalloproteinases

The MMPs are a family of zinc- and calcium-dependent proteases, which are synthesized by endogenous connective tissue cells as well as some types of haemopoietic cell (Murphy, 1995). The first members of this family were discovered by Gross and Lapiere in 1962 when they demonstrated that a true collagenase was secreted by cultured tissue fragments obtained from resorbing tadpole tails during metamorphosis. Resorption and tail loss occurred at 37°C and neutral pH, a process later attributed to the actions of two MMPs: MMP-1 and MMP-13. Although essential for amphibian metamorphosis, Massova *et al.* (1998) suggested that MMPs are likely to be derived from a far more ancient source. They found a specific amino acid sequence for *Bacteroides fragilis* metalloproteinase toxin-2 with a 59 per cent identity to a continuous 27 amino acid stretch in human MMP-1. This portion is largely responsible for its activity and is clearly highly conserved across different species. A total of 16 MMPs have been sequenced from human sources and most of these show a high degree of amino acid conservation across several mammalian species (Kleiner and Stetler-Stevenson 1999). The recent discovery of a new human MMP (MMP-23) suggests that the members of this family are likely to increase in number as our abilities in molecular biology and biochemistry improve (Velasco *et al.*, 1999). The 16 human MMPs that are currently recognized are classified into five subgroups on the basis of their putative substrate specificity and internal homologies (Table 1). It is often difficult to identify individual MMP activity as several members of the MMP family can carry out identical enzymatic activities. Hence, if one enzyme is prevented from functioning then another may become more highly expressed to compensate for the loss.

Matrix Metalloproteinase Structure

Although the members of the MMP family possess different matrix substrate specificities, they all possess similar structural and functional features and demonstrate a similar mechanism of proteolysis (Birkedal-Hansen *et al.*, 1993). Most members of the MMP family are organized into three basic, distinctive, and well-conserved domains based on structural considerations:

- an amino-terminal propeptide
- a catalytic domain
- a haemopexin-like domain at the carboxy-terminal.

Figure 1 is a schematic diagram of the various MMP's with the relative proportions of regions responsible for the function of the enzyme. MMP-7 lacks the haemopexin-like domain and represents the smallest member of the MMP family. The amino acid sequence homologies between the MMP members is greatest at the aminio-terminal profragment 'activation region' and the zinc atom binding site of the central catalytic domain. There are also a variety of additional domains or short inserts attached to this common structure amongst members of the MMP family. For example, MMPs-2 and -9 each have a gelatin-binding domain inserted between the catalytic and active site domains and MMPs-14, -15, -16, and -17 all have a transmembrane domain added onto the C-terminal domain. Three of the membrane-associated MMPs (-14 to -16), along with MMP-11, are further distinguished by a short sequence inserted between the propeptide domain and the catalytic domain; a furin-sensitive cleavage site important in the intracellular activation of these enzymes. If the MMP structure is examined from the amino terminus, the following features may be seen:

1. *Signal peptide:* this sequence of 17–20 hydrophobic amino acids functions as a signal to facilitate secretion of

TABLE 1 *Classification of the matrix metalloproteinase family together with substrate specificity and molecular weights*

Subclass	Enzyme	MMP number	Substrates	Molecular weight (kDa)
Collagenases	Interstitial collagenase	MMP-1	Collagens I, II, III, VII, VIII, X, gelatin, proteoglycan core protein	54.0
	Neutrophil collagenase	MMP-8	As above	53.4
	Collagenase-3	MMP-13	As above and fibrillin	53.8
Gelatinases	Gelatinase-A	MMP-2	bFGF, collagens I, IV, V, VII, X, XI, elastin, entactin, fibrillin, fibronectin, galectin-2, gelatin, laminin-5, proteoglycan core protein of cartilage	71.0
	Gelatinase-B	MMP-9	Collagens I, IV, V, elastin, fibrillin, galectin-3, gelatin, proteoglycan core protein	Unglycosylated form: 78.4 Glycosylated form: 94
Stromelysins	Stromelysin-1	MMP-3	Casein, collagen II, III, IV, V, IX, X, XI, elastin, fibrillin, fibronectin, gelatin, laminin-5, pro-MMP-1, pro-MMP-8, pro-MMP-9, pro-MMP-13, proteoglycan core protein, tenascin, vitronectin	54.0
	Stromelysin-2	MMP-10	As above	54.2
	Stromelysin-3	MMP-11	Alpha-1-antiprotease	54.6
Membrane type	MT1-MMP	MMP-14	Collagen, I, fibrillin, fibronectin, gelatin, laminin-1, pro-MMP-2, pro-MMP-13, proteoglycan core protein of cartilage, tenascin	65.9
	MT2-MMP	MMP-15	Fibronectin, laminin, pro-MMP-2, pro-MMP-13, tenascin	75.8
	MT3-MMP	MMP-16	Collagen III, fibronectin, gelatin, pro-MMP-2	69.5
	MT4-MMP	MMP-17	Currently unknown	61.7
Other types	Matrilysin	MMP-7	Collagen IV, fibronectin, gelatin, laminin, nidogen, pro-MMP-1, proteoglycan core protein	29.7
	Metallo-elastase	MMP-12	Collagen IV, elastin, fibrillin, fibronectin, laminin, vitronectin	54.0
	RASI-1	MMP-18/19	Unknown	57.4
	Enamelysin	MMP-20	Amelogenin	54.4

Basic fibroblast growth factor (bFGF) and galectin-3 are both cell surface components. The molecular weights for the active form of the enzyme are lower than the full weight given.

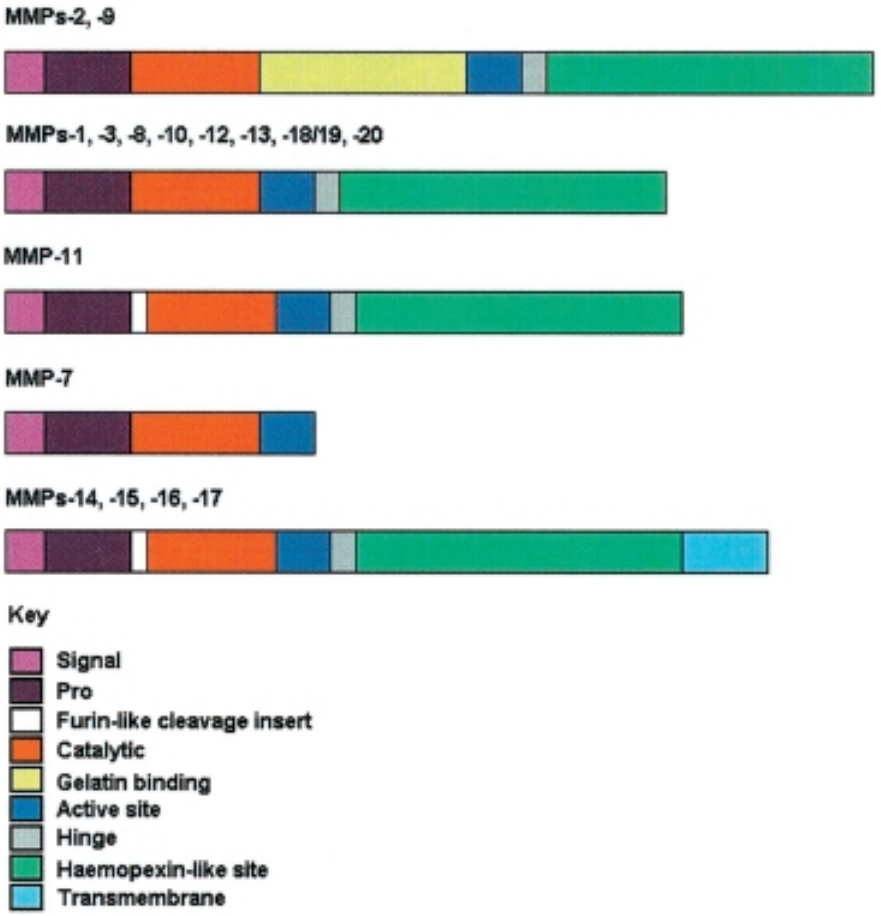

FIG. 1 A schematic diagram to show the structures of the various matrix metalloproteinases.

the MMP into the extracellular space via the endoplasmic reticulum. MMP-17 does not possess a signal peptide (Puente *et al*., 1996).

2. *Propeptide:* this portion is usually composed of approximately 80–90 amino acids, often with an N-terminal hydrophobic residue. Near the C-terminal portion of this segment is a highly conserved (…PRCGXPD…) sequence which provides the cysteine residue found in all MMPs (Massova *et al*., 1998). It has been suggested that the sulphydryl group of this cysteine residue is co-ordinated by the catalytic zinc ion and that interruption of this interaction by physical or chemical means initiates a cascade of events that results in cleavage of the amino-terminus of this enzyme. Activation can be triggered with sulphydryl-reactive compounds, such as organomercurials that interrupt the cysteine to zinc co-ordination, leading to autoproteolytic conversion of the pro-MMPs to the active form. Site-specific deletion of the arginine or cysteine residues in this sequence also results in autoactivation of the enzyme (Sanchez-Lopez *et al*., 1988). Physiologic activation of MMPs is probably initiated by proteases that cleave specific sites within the propeptide, but final processing to the active MMP that lacks the entire propeptide sequence often requires intermolecular, auto-proteolytic cleavage by the target MMP.

3. *Furin-like cleavage insert:* This sequence of approximately 25 hydrophobic amino acids is a characteristic of MMPs-11, -14, -15 and -16. MMP-17 is the only member of the membrane-associated MMPs that does not possess this site. Furin is an enzyme that is found associated with the Golgi apparatus within cells. The presence of this site in these MMPs enables intracellular activation by furin-like convertases. The active forms of other MMPs are produced in the extracellular environment by hydrolysis of the propeptide portion of the MMP structure, partially exposing the zinc and leading to autolytic cleavage of the remaining propeptide.

4. *Catalytic domain:* This includes the binding site of two zinc ions and at least one calcium ion co-ordinated to various amino acid residues (Massova *et al*., 1998). One zinc ion (the catalytic zinc) is present in the actives site, whilst the second zinc ion (the structural zinc) and the calcium ion reside together in a different site on the same domain. The catalytic zinc ion is essential for the proteolytic activity of the MMPs, but little is known about the roles of the structural zinc and the calcium ion. The catalytic domain is typically 160–170 amino acid residues in length, of which the 50–54 residues at the C-terminal end are responsible for the binding of the catalytic zinc. There is a highly conserved section of 11 amino acids within the zinc-binding portion of the catalytic domain (…VAAHEXGHXXG…), whilst the remaining amino acid residues can vary considerably.

The critical role of the calcium and zinc ions can be demonstrated by constructing assay systems where chelating

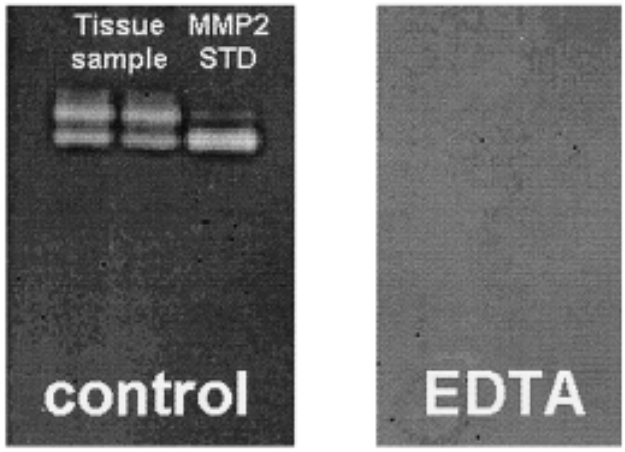

FIG. 2 A gelatin-polyacrylamide gel zymogram for detection of MMP-2 activity. The gel slice on the left (control) has been loaded with replicate samples of a tissue in the first two tracks and a MMP-2 standard in the third lane. The MMP-2 standard appears as a single band; the active form of the enzyme. In the tissue samples the lower band corresponds to the active form of the enzyme and the higher band, the heavier latent form of the enzyme. If the same system is incubated with a chelator such as ethylenediaminetetra—acetic acid (EDTA), the zinc and calcium ions are unavailable to activate the enzyme, and there is no evidence of enzyme activity (the gel is blank).

agents remove calcium and zinc. Figure 2 shows a gelatin-polyacrylamide gel zymogram being used to detect MMP-2 activity.

The gel slice on the left (control) has been loaded with replicate samples of a tissue in the first two tracks and a MMP-2 standard in the third lane. This MMP-2 standard appears as a single band, the active form of the enzyme. The double bands seen with the tissue samples have a lower band which corresponds to the active form of the enzyme and a higher band, the heavier latent form of the enzyme. If the same system is incubated with a chelator such as ethylenediaminetetra—acetic acid (EDTA), the zinc and calcium ions are unavailable to activate the enzyme and there is no evidence of enzyme activity (the gel is blank).

5. *Fibronectin-like repeats:* In the gelatinase members of the MMPs (MMPs-2 and -9) there are three repeats of the fibronectin type II domain inserted in the catalytic domain just ahead of the zinc-binding region. These residues aid in the binding of enzyme to gelatin (Strongin *et al.*, 1993).

6. *Hinge region:* The catalytic domain is connected to the adjacent haemopexin domain by a chain of anything from 0 to 75 residues called the hinge region. This region is absent in MMP-7.

7. *Haemopexin-like domain:* This COOH-terminal domain is composed of approximately 200 amino acid residues with four repeats resembling haemopexin and vitronectin. It is highly conserved among the MMPs, except for MMP-7 where it is absent. There is a cysteine residue at either end which join and, subsequently, fold into a four-bladed propeller-like structure around a central cavity occupied by a calcium ion. It does not appear to be essential for MMP catalytic activity although binding of the inhibitors of MMP activity (the Tissue Inhibitors of Metallo-Proteinases, or TIMPs) is facilitated by the presence of this domain. In the case of MMP-2 and MMP-9, TIMP will bind to this domain even when the enzymes are in their latent form. It is thought that the presence of this domain also contributes to the substrate specificity of MMP-1 and the stromelysins (Sanchez-Lopez *et al.*, 1993).

8. *Membrane insertion extension:* The four membrane-type MMPs (MMPs-14, -15, -16, and -17) have an extension that allows insertion of these proteases into the cell membrane. It is usually 80–110 residues in length of which 20 residues span the membrane.

Matrix Metalloproteinase Activation

MMPs-11, -14, -15, and -16 are activated prior to their secretion from the cell by furin-like proteases present on the cell Golgi apparatus (Nagase, 1997). The remaining MMPs are secreted as an inactive higher molecular weight proform (zymogen) with activation occurring in the extracellular space through limited proteolysis of the parent molecule resulting in a lower molecular weight species either via the plasminogen cascade system (Atkinson *et al.*, 1995) or by another member of the MMP family (Fridman *et al.*, 1992).

Specificity

Other major classes of enzymes include the serine and cysteine proteases. The specific actions of MMP's can be distinguished by using inhibitors to the cysteine and serine proteases. Figure 3 shows three gel slices to demonstrate this. The first gel slice (control) shows duplicate samples of tissue and a MMP-2 standard. The inclusion of a general inhibitor of MMP's (BB-3103) abolishes enzyme activity and gel lysis. A cysteine (NEM) and serine (AEBSF) inhibitor fails to have any effect on enzyme activity, and the areas of lysis are almost identical to the controls.

Functions of the Matrix Metalloproteinases

The MMPs have been implicated in a wide variety of normal physiological processes including bone remodelling, uterine resorption, trophoblast implantation, angiogenesis, and normal wound healing (Kleiner and Stetler-Stevenson, 1999). When present in excess, they are also thought to participate in the accelerated breakdown of ECM that is associated with a number of diseases including periodontitis (Reynolds and Meikle, 1997), arthritis, atherosclerosis, tissue ulcerations, tumour cell invasion, and metastasis (Birkedal-Hansen *et al.*, 1993).

As a group, the MMPs are able to cleave all of the structural components of the ECM in their native forms at neutral pH. Maintenance of ECM integrity appears to be their primary function.

It has also been shown that MMP action can have dramatic effects on cell adhesion. Ray *et al.* (1994) demonstrated increased adhesion of human melanoma cells in the presence of increased TIMP-2 and reduced adhesion with decreased TIMP-2 levels. These cells exhibited reduced motility in a chemotaxis assay, a process which could be prevented by the addition of MMP-2 or TIMP-2 to bring the enzyme/inhibitor ratio closer to that of the parent cell

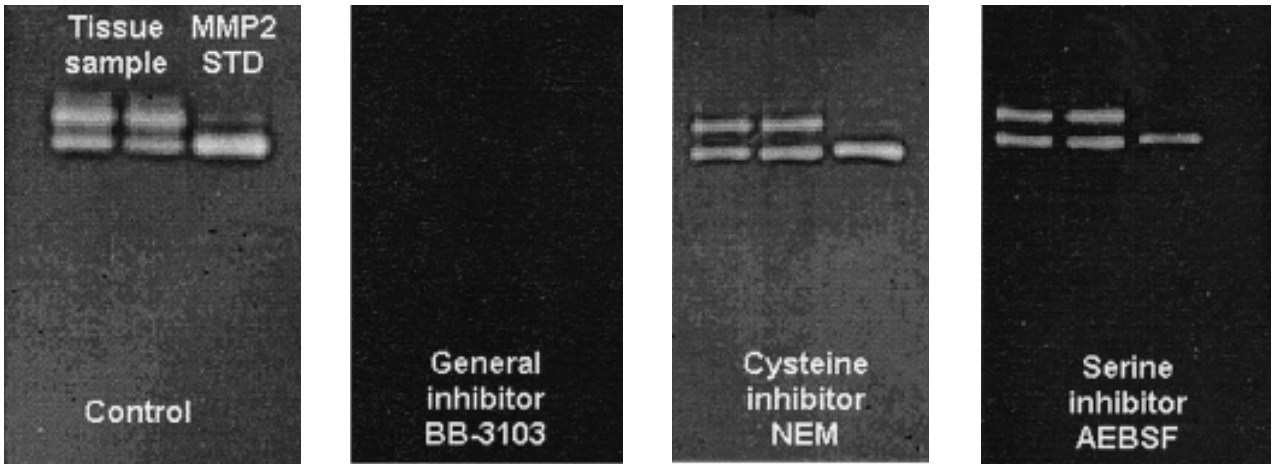

FIG. 3 Specificity of a gelatin gel zymography. The first gel (control) comprises replicate samples of tissue and a MMP-2 standard. The inclusion of a general inhibitor of MMP's (BB-3103) abolishes enzyme activity and gel lysis. A cysteine (NEM) and serine (AEBSF) inhibitor fails to have any effect on enzyme activity, and the areas of lysis are almost identical to the controls.

line. They concluded that the balance between active MMP-2 and TIMP-2 levels is critical for cell adhesion and motility within the ECM. If ECM attachment is too strong, then the cells will be unable to move through the ECM. Conversely, if they are loosely bound then it is probable that cell migration will be compromised.

Some members of the MMP family have been shown to uncover hidden properties of plasminogen and laminin-5. MMPs-3 (Lijnen *et al*., 1998), -7, -9 (Patterson and Sang, 1997), and -12 (Dong *et al*., 1997) have all been shown to degrade plasminogen into a protein fragment, angiostatin, that has no proteolytic activity, but is a powerful inhibitor of angiogenesis (O'Reilly *et al*., 1994). Hence, these MMPs may have a significant role to play in angiogenesis, although the precise mechanism by which they exert these effects remain unknown.

In addition, MMP-2 has also been found to stimulate chemoattraction by cleaving the structural ECM protein laminin-5. Cleavage of this protein may be critical in attracting inflammatory cells or in the autostimulation of tumour cell migration and metastasis. It appears, therefore, that MMPs may have functions well beyond their abilities to regulate the ECM.

Regulation of Matrix Metalloproteinase Expression and Activity

Most of the MMPs are not constitutively expressed by cells *in vivo*. Their expression is invariably rapidly induced in response to exogenous agents including cytokines, growth factors, and hormones. However, the TIMPs (Corcoran *et al*., 1996b) and the non-specific plasma proteinase inhibitors α_2-macroglobulin (α_2M) and α_1-antiprotease, are potent inducers of MMP expression. It is thought that TIMPs regulate MMP activity in the tissue or cell periphery, whereas α_2M regulates their activity in the interstitial fluid (Birkedal-Hansen *et al*., 1993).

The catalytic activity of the MMPs is regulated at multiple levels including transcription, secretion, activation, and inhibition. For most MMPs, including MMPs-1, -3, -7, and -9, serine proteases, such as plasmin and urokinase-type plasminogen activator have been found to initiate activation (Birkedal-Hansen *et al*., 1993; Kleiner and Stetler-Stevenson, 1993). Some MMPs may activate other members of the family; for example, the membrane-bound MMPs-14, -15 and -16 can activate MMP-2; and MMP-3 has been shown to be able to activate MMP-9 (Ogata *et al*., 1992). The interstitial collagenase MMP-1 can be activated by MMPs-3 and -10, and can itself activate latent MMP-2. Latent MMP-13 can be activated by MMPs-2, -3, -10, and -14, and can itself activate MMPs-2 and -9. Thus, the ability of MMPs to activate each other creates a complex network of proteases in the pericellular space. The MMPs with furin cleavage sites are likely to be activated by furin present on the Golgi apparatus prior to secretion from the cell or when presented to the cell surface (Sato *et al*., 1996).

TIMPs

The growth and repair of connective tissues is a delicately balanced process of ECM removal and replacement, with significant control by MMPs and their primary natural inhibitors, the TIMPs. An investigation of both MMPs and TIMPs is an essential part of any study, where ECM turnover is examined. The TIMPs regulate MMP function both at the level of their activation and in their ability to hydrolyse a particular substrate. They are small (21–28 kDa), multi-functional proteins of which there are currently four types (Table 2).

TIMP-1 inhibits all MMP's except MMP-14 and is the most efficient inhibitor of MMP-1. It shares a 42 per cent sequence homology with TIMP-2 and is inducible, particularly in bone. TIMP-2 can inhibit the activity of all the MMPs, although it is 10 times more effective at inhibiting the activity of MMP-2 (Kähäri and Saarialho-Kere, 1999). This arises in part because there are two MMP-2 binding sites within the TIMP-2 structure (Kleiner *et al*., 1992). In addition, TIMP-2 can also form a specific complex with the latent form of MMP-2 (Kleiner and Stetler-Stevenson, 1993). This, in turn, forms a cell surface trimolecular com-

TABLE 2 *Nomenclature, classification, and properties of the tissue inhibitors of matrix metalloproteinases (TIMPs)*

MMP inhibition	TIMP-1 All except MMP-14	TIMP-2 All	TIMP-3 MMPs-1, -2, -3, -9, -13 and (TNF)-—converting enzyme	TIMP-4 MMPs-1, -2, -3, -7, and -9
Most efficient inhibitory activity (compared to other MMPs)	MMP-1 (2× more efficient)	MMPs-2 and -9 (10× more efficient)	?	MMPs-2 and -7
Protein size	28.5 kDa	21.0 kDa	21.0 kDa	22.0 kDa
Amino acid sequence homology with other TIMPs	42% with TIMP-2		27% with TIMP-1	51% with TIMPs-2 and -3
Glycosylation	Yes	No	Yes	No
Localization	Diffusible	Diffusible	ECM bound	Diffusible
Gene location	Xp11.23–11.4	17q2.3–2.5	22q12.1–13.2	?
Expression	Inducible	Constitutive	Inducible	?
Major tissue sites	Bone, ovary	Placenta	Kidney, brain	Heart
Complex formation	pro-MMP-9	pro-MMP-2, MT1-MMP	pro-MMP-2, MT1-MMP, ECM	?

plex with MT1-MMP (MMP-14), a feature which may enable TIMP-2 to regulate MMP-2 activity on or near the cell surface (Itoh *et al*., 1998). TIMP-2 is also able to freely diffuse into the ECM, a characteristic not shared by all TIMP members. TIMP-3, which is ECM-bound, may also regulate MMP-2 activity by activating latent MMP-2 when associated with MT1-MMP (Sato *et al*., 1994).

Conclusions

This review has outlined in some detail crucial functions and actions of MMP's, and their inhibitors, the TIMPs. Orthodontists have a need to understand matrix turnover in the periodontal ligament and adjacent structures, embryology, and development. This complex area is under constant change and has an important role in many of the areas of interest to orthodontists involved in basic research.

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