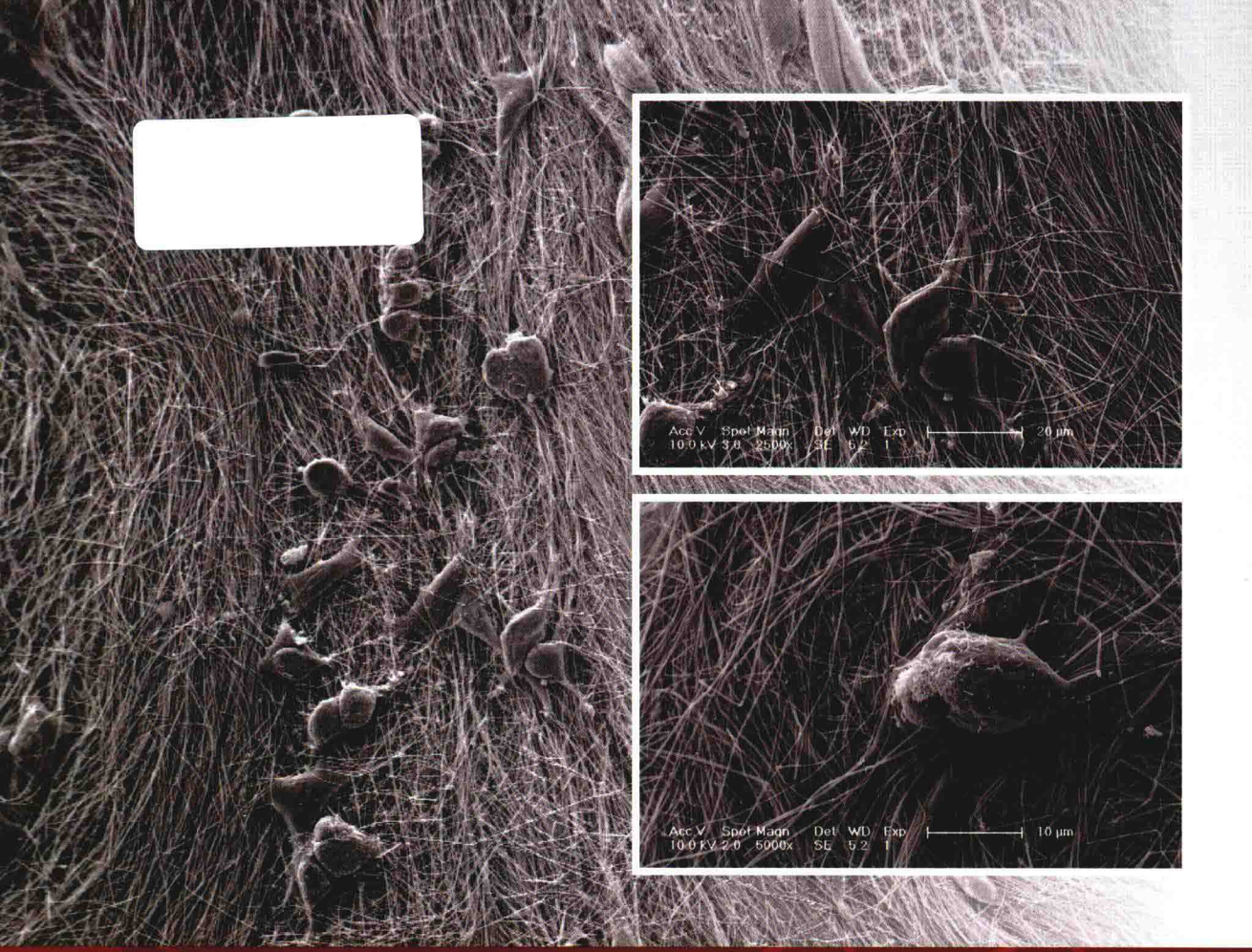

Edited by ***Irit Sagi and Jean P. Gaffney***

MATRIX METALLOPROTEINASE BIOLOGY

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Matrix Metalloproteinase Biology

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Contents

1 Matrix Metalloproteinases: From Structure to Function

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1.1 Introduction

Members of the matrix metalloproteinase (MMP) family are known to catalyze the hydrolysis of a great variety of biological macromolecules. Proteomic approaches have significantly expanded the number of known MMP substrates. However, the mechanisms by which macromolecular substrates are processed have often proved elusive. X-ray crystallography and NMR spectroscopy have yielded detailed information on structures of MMP domains and, in a few cases, full-length MMPs. As structures of MMPs and their substrates have been reported, examination of MMP•substrate complexes has provided insight into mechanisms of action. We examine the structures of MMPs and their substrates and consider how the various structural elements of MMPs contribute to the hydrolysis of biological macromolecules.

1.2 Structures of MMPs

1.2.1 General MMP structure and domain organization

MMPs belong to the M10 zinc metalloproteinase family [1]. All MMPs have the characteristic zinc binding motif HExxHxxGxxH in their catalytic domain. MMPs possess similar domain organizations. Most MMPs consist of a signal peptide followed by four distinct domains, the N-terminal prodomain (propeptide), catalytic (CAT) domain, linker (hinge) region, and C-terminal hemopexin-like (HPX) domain (Fig. 1.1). The membrane-type (MT) MMPs contain an additional transmembrane (TM) domain that anchors them to the cell membrane. Following the TM domain is a small cytoplasmic "tail".

There are several exceptions to this general domain organization. MMP-7 and MMP-26 (matrilysins) lack the linker region and HPX domain and thus are referred to as "minimal MMPs". MMP-2 and MMP-9 possess three repeats of fibronectin type

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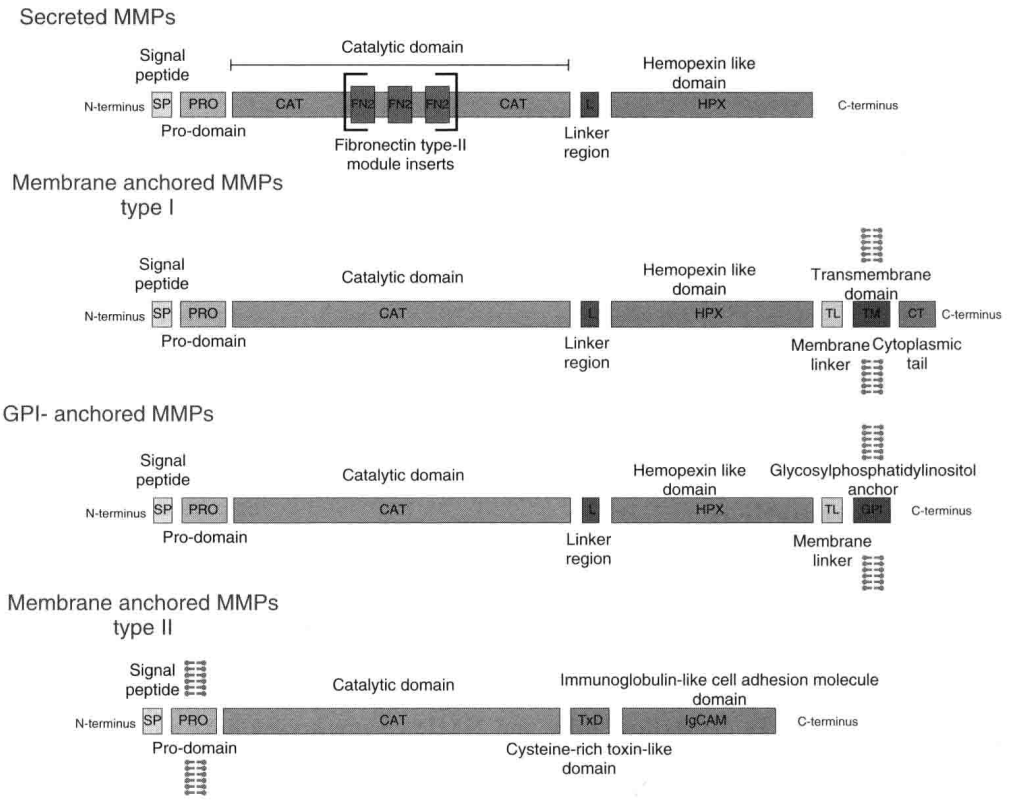

Figure 1.1 General domain organization of MMPs. (*See insert for color representation of this figure.*)

II-like motifs within the CAT domain. MMP-17 and MMP-25 are type I TM enzymes anchored to membranes through a C-terminal glycosylphosphatidylinositol (GPI) residue [2]. The N-terminal MMP-23 pro-domain contains a type II TM domain that anchors the protein to the plasma membrane. Instead of the C-terminal HPX domain common to other MMPs, MMP-23 contains a small toxin-like domain (TxD) and an immunoglobulin-like cell adhesion molecule (IgCAM) domain.

1.2.2 Catalytic domain

The topology of the CAT domain is similar among all MMPs. The CAT domain is composed of a five- stranded β-sheet which is interrupted by three α-helices (Fig. 1.2). Four of the five β-strands are aligned in a parallel fashion, while only the smallest "edge" strand runs in the opposite direction. Between strands III and IV there is an S-loop fixed by a structural Zn atom. The center of the catalytic site is located at helix B and the loop connecting it with helix C. This center helix provides the first and second His residues of the Zn-binding motif along with "catalytic" Glu residue. The loop behind this helix provides the third zinc binding His residue. Further down along this loop there is a 1,4 β-turn forming Met residue. This residue is highly conserved among metzincins and is believed essential for the structural integrity of the zinc-binding site. However, MMP-2 mutants where the conserved Met was replaced

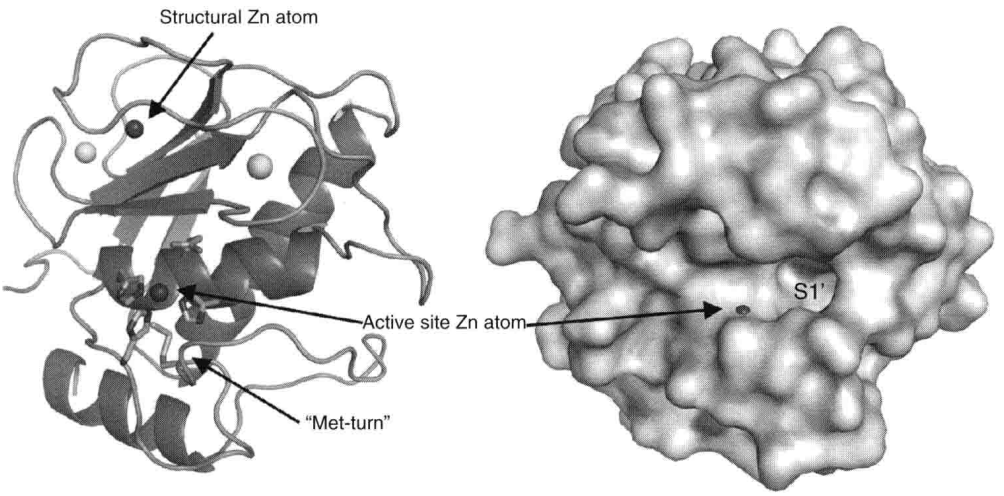

Figure 1.2 Typical structure of the CAT domain of MMPs. Characteristic structural elements are highlighted with arrows. Figure generated using MMP-8 structure (PDB 2OY2) [4]. (*See insert for color representation of this figure.*)

with Leu or Ser were able to cleave gelatin, type I collagen, and chemokine monocyte chemoattractant protein-3 with similar efficiency as wild-type MMP-2 [3].

1.2.3 Catalytic mechanism

On the basis of early structural information, a catalytic mechanism for MMPs was proposed (Fig. 1.3) [5, 6]. The carbonyl group of the scissile bond coordinates to the active site zinc (II) ion. A water molecule is hydrogen bonded to a conserved Glu residue and coordinated to the zinc (II) ion. The water molecule donates a proton to the Glu residue, allowing the generated hydroxide ion to attack the carbonyl at the scissile bond. This attack results in a tetrahedral intermediate, which is stabilized by the zinc (II) ion. The Glu residue transfers a proton to the nitrogen of the scissile amide, the tetrahedral intermediate rearranges, and amide bond hydrolysis occurs. During this catalytic process, the carbonyl from a conserved Ala residue helps to stabilize the positive charge at the nitrogen of the scissile amide.

1.2.4 Fibronectin type II-like inserts

Gelatinases (MMP-2 and MMP-9) bind to gelatin and collagen with significant contribution from their three fibronectin type II-like (FN2) repeats. MMP-2 and MMP-9 are unique among the MMPs in that the three FN2 modules (Col-1, Col-2, and Col-3) are inserted in their CAT domain in the vicinity of the active site [7]. More specifically, the FN2 modules of MMP-2 and MMP-9 are inserted between the fifth β-strand and helix B in the CAT domain (according to active enzyme domain organization). The basic fold of the FN2 module comprises a pair of β-sheets, each made from two antiparallel strands, connected by a short α-helix (Fig. 1.4). The two β-sheets form a hydrophobic pocket that is part of a hairpin turn, which orients the surrounding

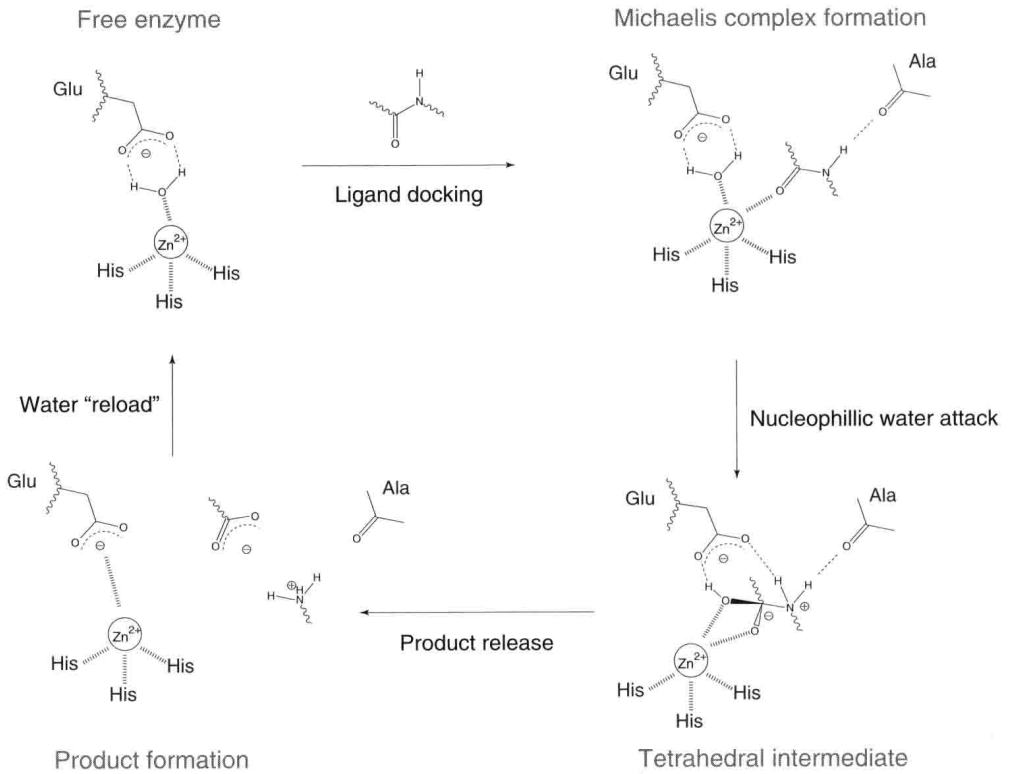

Figure 1.3 Mechanism of proteolysis catalyzed by MMPs. (Figure prepared based on mechanism proposed by Lovejoy et al. [5]). (*See insert for color representation of this figure.*)

aromatic side chains into the hydrophobic pocket. These pockets are the structural hallmark of the FN2 modules and contribute to substrate binding (see below) [8].

1.2.5 Linker region

The CAT domain is connected to the HPX domain via a linker (hinge) region. The length of this linker varies from 8 to 72 amino acids, depending on the enzyme (Fig. 1.5). The linker regions may be posttranslationally modified with sugar moieties. The conformational flexibility of the linker region contributes to MMP function. For example, in the case of MMP-9, it has been suggested that the long (72 residue), glycosylated, and flexible linker region mediates protein-substrate interactions by allowing the independent movement of the enzyme CAT and HPX domains [9]. Independent domain movements were also proposed to mediate enzyme translocation on collagen fibrils [10–12]. Domain flexibility may contribute to MMP activation via promoting long-range conformational transitions induced by the binding of activator proteins or ligand [13–15]. Finally, the linker region may help to re-orient the CAT domain with respect to the HPX domain during catalysis of collagen [16]. Domain flexibility may be rationalized for most MMPs by considering

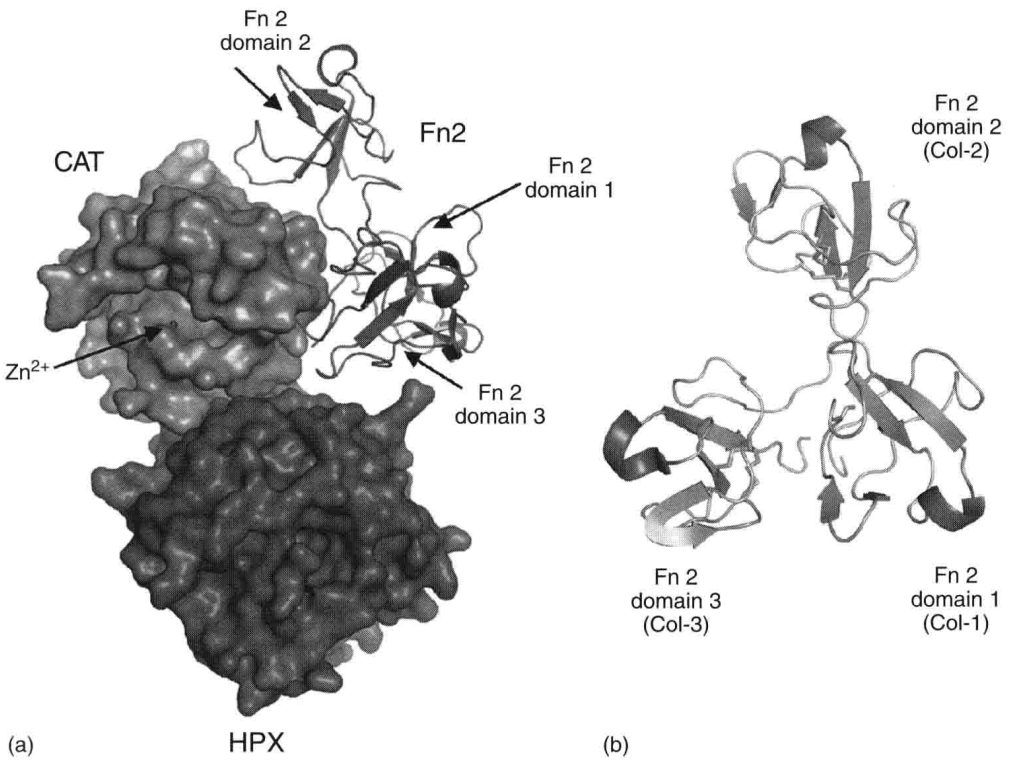

Figure 1.4 Fibronectin type II-like module structure and organization. (a) General orientation of FN2 modules of MMP-2. (b) Top view of FN2 modules. Figure prepared using MMP-2 structure (PDB 1CK7) [8]. (*See insert for color representation of this figure.*)

MMP	Linker region sequence	Length
MMP-26	GEK	3
MMP-23	GCLDRLFV	8
MMP-7	GKRSNSRKK	9
MMP-21	GSCEGSFDTAFDWI	14
MMP-1	GRSQNPVQPIGPQTPKA	17
MMP-13	GPGDEDPNPKHPKTPDK	17
MMP-8	GLSSNPIQPTGPSTPKP	17
MMP-27	GGLPKEPAKPKEPTIPHA	18
MMP-12	GDPKENQRLPNPDNSEPAL	19
MMP-2	GASPDIDLGTGPTPTLGPVTPEI	23
MMP-20	GPRKVFLGKPTLPHAPHHKPSIPDL	25
MMP-10	GPPPASTEEPLVPTKSVPSGSEMPAK	26
MMP-3	GPPPDSPETPLVPTEPVPPEPGTPAN	26
MMP-19	GKKSPVIRDEEEEETELPTVPPVPTEPSPMPDP	33
MMP-14	GGESGFPTKMPPQPRTTSRPSVPDKPKNPTYGPNI	35
MMP-11	GQPWPTVTSRTPALGPQAGIDTNEIAPLEPDAPPDA	36
MMP-17	GVRESVSPTAQPEEPPLLPEPPDNRSSAPPRKDVPHR	37
MMP-25	GKAPQTPYDKPTRKPLAPPPQPPASPTHSPSFPIPDR	37
MMP-28	GKPLGGSVAVQLPGKLFTDFETWDSYSPQGRRPETQGPKY	40
MMP-16	GPPDKIPPPTRPLPTVPPHRSIPPADPRKNDRPKPPRPPTGRPSYPGAKPNI	52
MMP-24	GPPAEPLEPTRPLPTLPVRRIHSPSERKHERQPRPPRPPLGDRPSTPGTKPNI	53
MMP-15	GTPDGQPQPTQPLPTVTPRRPGRPDHRPPRPPQPPPPGGKPERPPKPGPPVQPRATERPDQYGPNI	66
MMP-9	GPRPEPEPRPPTTTTPQPTAPPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLSPVDDA	72

Figure 1.5 Comparison of MMP linker lengths and sequences. Table was generated after alignment of human MMPs using sequences from the Uniprot database [19] and SeaView 4 [20] and Jalview [21] programs.

the amino acid composition (i.e., Gly and Pro residues) and the various lengths of linker regions (Fig. 1.5). The linker region and HPX domain of MT1-MMP and MMP-9 are proposed to offer allosteric control of enzyme dimer formation, which in turn modulates biological function [17, 18].

Glycosylation of MT1-MMP, which occurs in the linker region (residues 291, 299, 300, and 301), is required for the recruitment of tissue inhibitor of metalloproteinase 2 (TIMP-2) on the cell surface and subsequent formation of the MT1-MMP/TIMP-2/proMMP-2 trimeric complex and activation of proMMP-2 [22]. Glycosylation does not affect MT1-MMP collagen hydrolysis or autolytic processing [22].

1.2.6 Hemopexin-like domain

Except for MMP-7 and MMP-26, all vertebrate and human MMPs are expressed with a C-terminal HPX domain. The HPX domain is organized in four β-sheets (I to IV), arranged almost symmetrically around a central axis in a consecutive order (Fig. 1.6). The end result is a four-bladed propeller of pseudo-fourfold symmetry. Each propeller blade is formed by four antiparallel β-strands connected in a W-like topology, and is strongly twisted. The small C-terminal helix of the blade IV is tethered to the entering strand of blade I via a single disulfide bridge, stabilizing the whole domain. Within the central tunnel, up to four ions ($2Ca^{2+}$, $2Cl^-$) have been identified although their function is not clear [23].

The HPX domain mediates binding of MMP-1, MMP-8, MMP-13, MT1-MMP, and MMP-3 to collagen [24–28]. The HPX domain of MMP-2 was shown to possess critical secondary binding sites (exosites) required for the interactions of MMP-2 with fibronectin, and fibronectin was cleaved at a significantly reduced rate by an

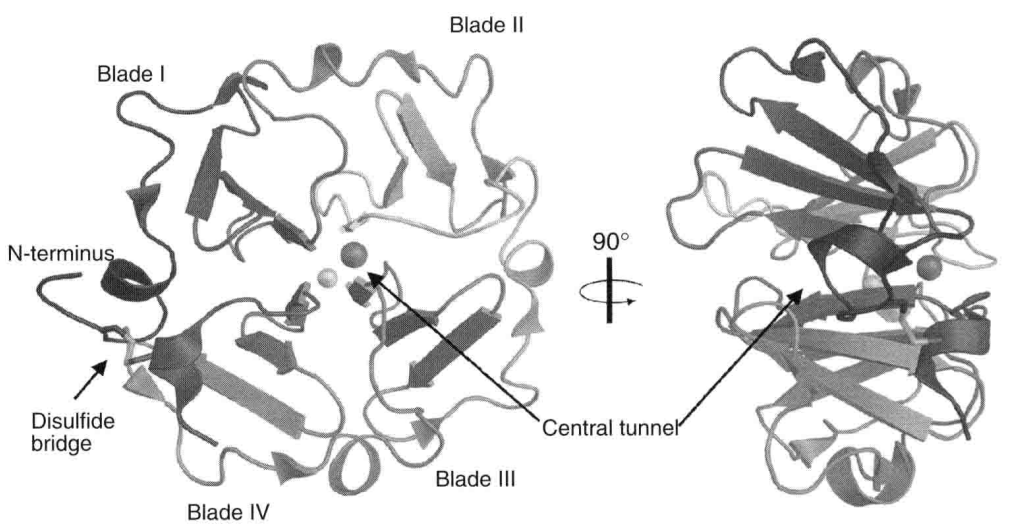

Figure 1.6 Typical structure of the HPX domain. The propeller-like structure is composed of four blades (I-IV) and stabilized by a single disulfide bridge, designated with an arrow. In the central tunnel, up to four different ions have been identified (here Ca^{2+} is orange and Cl^- is yellow). This figure was generated using the HPX domain of MT1-MMP (PDB 3C7X) [23]. (*See insert for color representation of this figure.*)

MMP-2 variant where the HPX domain was deleted [29]. In the case of MMP-2 and MMP-9, the HPX domain is important for interactions with TIMPs. The HPX domain of MMP-2 has also been shown to play a role in zymogen activation by MT1-MMP [30].

HPX domains modulate interaction of MMPs with cell-surface biomolecules. For example, the HPX of MMP-2 plays a role in the binding of the enzyme to the αvβ3 integrin [31, 32]. MT1-MMP has numerous cell surface binding partners, including tetraspanins (CD9, CD63, CD81, CD151, and/or TSPAN12), the α2β1 and αvβ3 integrins, and CD44 [33–39]. The HPX domain of MT1-MMP binds to CD63 and CD151 [35, 40]. Tetraspanins protect newly synthesized MT1-MMP from lysosomal degradation and support delivery to the cell surface [36].

CD44 also binds to MT1-MMP via the HPX domain of the enzyme, specifically blade I of the HPX domain [34, 41]. The association with CD44 leads to MT1-MMP localization to lamellipodia [34] [40]. The MT1-MMP/CD44 interaction promotes signaling through EGFR activation to the MAPK and PI3K pathways, enhancing cell migration [41]. CD44 also binds to MMP-9 via the HPX domain [40].

Highly efficient collagenolysis requires homodimerization of MT1-MMP, where association includes interactions of the HPX domain [42]. Homodimerization is symmetrical, involving residues Asp385, Lys386, Thr412, and Tyr436 in blades II and III of the HPX domain [43].

1.2.7 Transmembrane domain and cytoplasmic tail

On the basis of their method of attachment to the cell membrane, MT-MMPs may be classified into two groups, TM-type and glycosylphosphatidyl-inositol (GPI)-type. MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), and MT5-MMP (MMP-24) are type I TM proteins with a short cytoplasmic tail that is involved in the regulation of intracellular trafficking and activity of these proteases [44–46]. MT4-MMP (MMP-17) and MT6-MMP (MMP-25) are bound to the cell surface by a GPI-mediated mechanism [2, 47].

Although the structure of the TM domain has not been solved experimentally, a model has been generated (Fig. 1.7). Besides facilitating cellular localization, the TM domain allows MT-MMPs to process a unique set of substrates, interact uniquely with TIMPs, and participate in a non-conventional mechanism of regulation involving enzyme internalization, processing, and ectodomain shedding [48, 49].

The cytoplasmic tail of MT1-MMP is distinct from those of MT2-MMP, MT3-MMP, and MT5-MMP, and is well characterized. The cytoplasmic tail of MT1-MMP is important in the ERK activation cascade [52], S1P-dependent G_i protein signaling [53], and VEGF upregulation through Src tyrosine kinase pathways [54]. The multifunctional gC1qR proteins can bind to the cytoplasmic tail of MT1-MMP in a similar manner to the cytoplasmic portion of adrenergic receptor [55]. More recently, Uekita et al. [56] have identified a new 19 kDa MT1-MMP cytoplasmic tail binding protein-1 (MTCBP-1). MTCBP-1 is localized between three subcellular compartments (membrane, cytoplasm, and nucleus) that can regulate gene expression and may suppress the invasion and migration-promoting activity of MT1-MMP [56]. The cytoplasmic tail of MT1-MMP increases the expression of