# Membrane-type matrix metalloproteinases (MT-MMPs) in tumor metastasis

メタデータ	言語: eng
	出版者:
	公開日: 2017-10-05
	キーワード (Ja):
	キーワード (En):
	作成者:
	メールアドレス:
	所属:
URL	http://hdl.handle.net/2297/14434

## Membrane-Type Matrix Metalloproteinases (MT-MMPs) in Tumor Metastasis<sup>1</sup>

#### Hiroshi Sato<sup>2</sup> and Motoharu Seiki

Department of Molecular Virology and Oncology, Cancer Research Institute, Kanazawa University, 13-1 Takaramachi, Kanazawa 920

Received for publication, September 27, 1995

Activated gelatinase A is reportedly associated with tumor spread. We identified novel matrix metalloproteinases that localize on the cell surface and mediate the activation of progelatinase A. Thus, these progelatinase A activators were named membrane-type matrix metalloproteinase-1 and -2 (MT-MMP-1 and -2, respectively). MT-MMP-1 is overexpressed in malignant tumor tissues, including lung and stomach carcinomas that contain activated gelatinase A. This suggests that MT-MMP-1 is associated with the activation of progelatinase A in these tumor tissues. The expression of MT-MMP-1 also induced binding of gelatinase A to the cell surface by functioning as a receptor. The cell surface localization of proteinases has advantages over pericellular proteolysis. MT-MMP-1 and its family may play a central role in the cell surface localization and activation of progelatinase A and *via* this mechanism, tumor cell use exogenous progelatinase A to mediate the proteolysis associated with invasion and metastasis.

Key words: gelatinase A, gelatinase A activator, matrix metalloproteinase, MT-MMP, tumor metastasis.

A major characteristic of malignant tumor cells is their ability to invade and form metastatic foci at distant sites in the body. The high frequency at which matrix metalloproteinase (MMP) transcripts or proteins is detected in invasive tumor cells and tissues suggests that these enzymes are closely associated with tumor invasion and metastasis (1, 2). The degradation of the extracellular matrix should be an essential step to allow the spread of the tumor cells.

Extracellular matrix has dynamic roles not only in supporting tissue structures but also in regulating cellular functions. For example, cell proliferation, differentiation, adhesion, and motility are tightly controlled by surrounding environment extracellular matrix. A number of studies have provided evidence for the involvement of MMPs and their inhibitors in developmentally regulated processes. These include ovulation (3), embryogenic growth and differentiation (4), and the development of organs (5). Thus, the expression of MMPs involved in the degradation of components of extracellular matrix must be tightly regulated and the overexpression of MMPs should be associated with various pathological events (6, 7).

The expression of MMP function is regulated mainly by 3 processes: transcription, activation of latent MMP, and

inhibition by tissue inhibitor of MMPs (TIMP) (8). In addition to the overexpression of various MMPs in tumors, the close correlation between gelatinase A activation and metastatic progression in various tumors suggests that gelatinase A activator is a key enzyme triggering tumor spread (9-12). In this review we focus on the membranetype MMP associated with activation of progelatinase A.

#### Modular structure of MMPs

The MMP gene family encodes 11 metal-dependent endopeptidases with activity against most, if not all extracellular matrix macromolecules. The enzymes have a similar structure composed of the five modular domains (Fig. 1A). The hydrophobic signal sequence is followed by a propeptide that is important to maintain the latency of the enzyme and which is cleaved when the enzyme is activated. The catalytic domain contains the two Zn<sup>2+</sup>-binding sites that are essential for the catalytic function. The prolinerich hinge region marks the transition to the  $\sim 200$  residue hemopexin- or vitronectin-like COOH-terminal domain that appears to play a role in encoding substrate specificity. Matrilysin/PUMP-1 does not possess this last domain and therefore is considerably smaller than the other members of the family. The two gelatinases have an insertion which has sequence similarity to the collagen-binding domains of fibronectin in the catalytic domain (13, 14). The fibronectin domain is involved in the substrate-binding properties of the molecules.

#### Activation mechanism of MMP

MMP is secreted in a proenzyme form which is enzymatically inactive. Van Wart's group has proposed a cysteine switch model for the mechanism of maintaining

<sup>&</sup>lt;sup>1</sup>This work was supported by the Special Coordination Fund for Promoting Science and Technology from the Ministry of Science and Technology of Japan; by a Grant-in-Aid from the Ministry of Education, Science, and Culture of Japan.

 $<sup>^2</sup>$  To whom correspondence should be addressed. Fax: +81-762-60-7840

Abbreviations: aa, amino acid; APMA, aminophenyl-mercuric acetate; MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; PCR, polymerase chain reaction; TIMP, tissue inhibitor of MMP; TPA, 12-O-tetradecanoyl-phorbol acetate.

## A

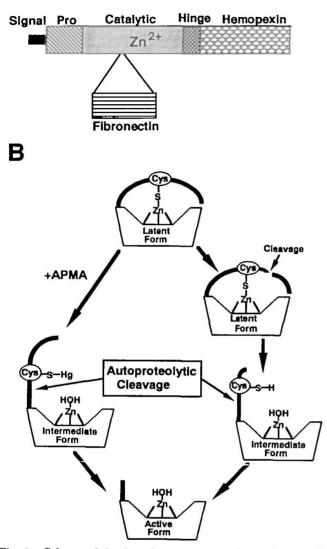


Fig. 1. Scheme of the domain structures and cysteine switch mechanism for MMP activation. A: The domain structure. Signal, signal peptides; Pro, the pro-peptide domain that contains PRC-GVPD, the most conserved sequence in the pro-peptide domain and is necessary to mask the active site of enzymes; Catalytic, core domain essential for enzymatic activity;  $Zn^{2+}$ ,  $Zn^{2+}$ -binding site; Fibronectin, a fibronectin-like domain of two gelatinases (gelatinases A and B); Hinge, proline-rich sequence potentially functioning as a hinge region; Hemopexin, hemopexin-like repeat. B: Cysteine switch mechanism. Proteolytic enzymes cleave the propeptide, ahead of the cysteine to generate intermediate forms. Alternatively, reagents that react with sulfydryl groups, such as organomercurials (e.g. aminophenyl-mercuric acetate, APMA), will modify the cysteine. In a second step, these intermediate forms can be autoproteolytically cleaved to remove the propeptide and confer permanent activity.

the latency that is shown in Fig. 1B (15). The "Pro" domain of the latent molecule is folded around so that the cysteine residue in the conserved PRCGVPD region can form a complex with the zinc molecule (16). Proteinases (trypsin, plasmin, chymotrypsin, neutrophil elastase, and plasma kalikrein) that attack a short basic sequence exposed on the surface of the molecule are thought to trigger the activation process of proMMPs in vivo (17). This initial cleavage causes a conformation change in the molecule that disrupts the cysteine- $Zn^{2+}$  interaction and frees the  $Zn^{2+}$  to participate in the proteolytic cleavage. The mechanism is thus called "cysteine switch mechanism." The enzyme can then attack the peptide sequence downstream of the PRCGVPD in an autolytic manner and cleave it (by both intra- and intermolecular mechanisms). An alternative activation of the enzyme with mercurial compounds such as aminophenyl-mercuric acetate (APMA) causes chemical modification of the cysteine and generates free zinc at the catalytic center by the cysteine switch mechanism.

Gelatinase A is unique among MMPs in that it does not possess sequences susceptible to proteolytic activation by the above serine proteinases. The mechanism of progelatinase A activation has been attracted attention of many tumor biologists, because a close association between the expression of activated gelatinase A and tumor spread has been found in various types of tumors (10, 11).

#### Identification of membrane-type MMP

Activated gelatinase A is released in vitro from fibrosarcoma HT1080, breast tumor MDA-MB-321, and normal fibroblast cells stimulated with TPA or concanavalin A (18-21). The activation activity resides in the plasma membrane fraction and it is sensitive to EDTA and TIMP-2 which are inhibitors of MMP, thus suggesting that the activator might be a membrane-bound MMP.

All the known MMPs are in a soluble form and none of them is the membrane-type. The complexity of extracellular matrix components suggests that more MMPs are involved in the efficient turnover of extracellular matrix during tissue remodeling. However, conventional biochemical approaches are no longer useful for identifying unknown enzymes. Polymerase chain reaction (PCR) using a set of degenerate primers for conserved amino acid sequences is a powerful means with which to identify new members of a gene family. Amino acid sequences for the cysteine switch (PRCGVPD) and binding sites for zinc molecule are highly conserved among all known MMPs. Reverse-transcription PCR amplification of MMP gene fragments with degenerate primers for these sequences using mRNA from various sources as a template has amplified not only known MMP genes but also unique fragments homologous to MMP (22). One of these was derived from human placenta and the other from melanoma tissue. Two cDNAs have been isolated from a human placenta cDNA library using these fragments as probes (22-24). Long open reading frames encoding 582 and 604 amino acids (aa) have been identified; both of these were aligned with known MMP family members but they were most closely related to each other (Fig. 2). While these proteins have a common MMP domain structure, they have three unique insertions. First there is an insertion of 11 aa between the pro-peptide and the catalytic enzyme domains. Stromelysin-3/MMP-11 has a similar insertion at the same position (25). The conserved RXKR sequences precede the potential processing sites of these two MMPs. The peptide bond following RXKR is the processing site of many secretory proteins by the eukaryotic KEX2 family of endopeptidase (26). Stromelysin-3 is activated by furin, one of intracellular KEX2 family endopeptidases (27). The MT-MMPs have a second insertion of 8 aa in the catalytic enzyme domain which is not present in other MMPs. The

### Membrane-Type Matrix Metalloproteinases

	Signal peptide Pro-peptide
MMP-1	MSFPPLLLLLFNG
HHP-2	MSITFELITION CONTRACT TO A CONTRACT AND A CONTRACT
04P~3	MSLPTILLLCVA
DHCP7 DHCP8	HR-LTVLCAVCLL
	HTSLATLPFLLLLHVQISKAPPVSSKEKNTKTVQDYLEKFYQLFSXQYQSTR-KNGTNVIVEKLKEHQRFFGLNVTGKP
HP-9	KSLMQPLVLVLLVLGCCPFAPRQRQSTLVLFFGDLRTNLTDRQLAEETLTRTGTTRVAEDRGESKSLGPALLLQKQLSLPETGEL
MP-10 MP-11	WRILAPLVLLCLPVCSAIPLSGAAKEEDSNKDLAQQYLEKIINLEKDVKQFRRX-DSNLIV-KKIQGAQKFLGLEVTGKL
	HAPANILRSAARALLPPHLLLLLQPPPLLARALPPUHHLHAERGCPQPHHALPSSPAPAPATOE
MP-12	MCPLLILLLQ-ATASQLPLNSSTSLEKNNVLFGERILEKFIGLEINKLPVTKYKSGNLWKEKIQEMQHFIGLKVTGQL
T-HHP-1	HSPAPRPSRCLLLPLLTLGTALASLGSAQSSSFSP
T-104P-2	MILLTFSTGRRLDFVHHSGVFFLQTLLWILCATVCGTEQYFNVEVWLQKYGTLPPTSPRMSVVRSAETHQ-SALAAMQQFYGINMTGKV M.L.L.LLL
onsensus	
	Pro-peptide Catalytic
MP-1	DASTLKVHKQPRCGVPDVAQSEQQADIM
H2P-2	DONTIETHRAPRCGNPDVANYNFFPRKWNDKNQITYRIGITPDLDPETVDDAFARAFQVWSDVTPLRFSRIHDGEADIH
HP-3	DSDTLEVMRKPRCGVPDVGHFRTPGIPKWRKTHLTYRIVNITPDLPKDAVDSAVEKALKVWEEVTPLTFSRLYEGEADIM
£₽-7	NSRVIEIMONPRCGVPDVAEYSLPPNSPKWTSKVVTYRIVSYTRDLPHITVDRLVSKALNMWGKEIPLHFRKVVWGTADIM
£₽-8	NEETLDHNKKPRCGVPDSGGFMLTPGNPKWERTNLTYRIRNYTPQLSEAEVERAIKDAFELWSVASPLIFTRISQGEADIN
£P~9	DSATLKAMRTPRCGVPDLGRFQTFEGDLKWHHHNITWIQNISEDLPRAVIDDAFARAFALWSAVTPLTFTRVYGRDADIV
<b>P-10</b>	DTDTLEVHRKPRCGVPDVGHFSSPPGHPKWRKTHLTYRIVNYTPDLPRDAVDSAIEKALKVWEEVTPLTFSRLYEGEADIM
HP-11	APRPASSLRPPRCGVPDPSD-GLSARNRQKRFVLSGGRWEKTDLTYRILRPPWQLVQEQVRQTHAEALKVWSDVTPLTFTEVHEGRADIM
HTP-12	DTSTLENGHAPRCGVPDLHHFREMPGGPVWRKHIITYRINNYTPDMNREDVDYAIRKAPQVWSNVTPLKFSKINTGMADIL
r-MMP-1	DADTMKAMRRPRCGVPDKFGAEIKANVRRKRIAIQ-G-LKWQHNEITFCIQNYTPKVGEYATYEAIRKAFRVWESATPLRFREVPYAYIREGHEKQADIM
1-MMP-2	DRNTIDWHKKPRCGVPDQTRGSSKFHIRRKRYALTGQKWQHKHITYSIKNVTPKVGDPETRKAIRRAFDVWQNVTPLTFEEVPISELENGK-RDVDIP
onsensus	DTLHRKPRCGVPDPPG.PKWTYRI.NYTPDLVD.AI.KAF.VWS.VTPLTFVG.ADIM
	♦IS-1 Catalytic ◆IS-2
œ-1	ISFVRGDHRDNSPFDGPGGNLAHAFQPGPGIGGDAHFDEHERWIN-NFTEYN
£P−2	INFGRWEHGDGYPFDGKDGLLAHAFAPGTGVGGDSHFDDDELWTLGEGQVVRVKIGNADGEICKFPFLPNGKEINSCTDTGRSDGFLWCSTTINFEKDGK
MIP-3	ISFAVREHGDF1PFDGPGNVLAHA1APGPGINGDAHFDDDEOWTK-DTTGTN
·œ~7	IGFARGAHGDSYPFDGPGNTLAHAFAPGTGLGGDAHFDEDERWTDGSSLGIN
£	IAFYQRDHGDNSPFDGPNGILAHAFQPGQGIGGDAHFDAEETNTN-TSANYN
œ-9	IQFGVAEHGDGYPFDGKDGLLAHAPPFCPGIQGDAHFDDDELNSLGKGVVVPTRFGNADGAACHFPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTDDR
(P-10	ISPAVKEHGDFYSFDGPGHSLAHAYPPGPGLYGDIHFDDDEKWTE-DASGTN
(P-11	IDFAR WOGDDLPFDGPGGILAHAFPFNHREGDVIFDVDETWIIGDDQCTD
(P-12	WFARGAHGDFHAFDGKGGILAHAFGPGSGIGGDAHFDEDEFWTT-HSGGTN
C-MMP-1	IFFAEGHCDSTPFDCEGGFLAHAIFPCPNIGCDTHFDSAEPWTV-RNDLN
-104P-2	IIFASGFHCDSSPFDGEGGFLAHAYFPGPGIGGDTHFDSDEPWTLGNPNHDG
nsensus	I.FAHDDPFDCPGG.LAHAF.PGPGIGGDAHFD.DD.WTN
	Catalytic
œ−1	
œ-2	IGFCPHEALFTMGGNAEGOPCKFPFRFOGTSIDSCTTEGRTDGIRMCGTIEDIDRKKIGFCPETAMSTV-GGNSEGAPCVFPFTFLGNKYESCTSAGRS
412-14	
£P-9	FGFCPSERLYTRDGNADGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATTANYDRDKLFGFCPTRADSTVMGGNSAGELCVFPFTFLGKEYSTCTSEGRG
12-9 12-10	
1P-9 1P-10 1P-11	
1P-9 1P-10 1P-11 1P-12	
4P-8 4P-9 4P-10 4P-11 4P-12 F-MMP-1	
4P-9 4P-10 4P-11 4P-12 F-MMP-1 F-MMP-2	
4P-9 4P-10 4P-11 4P-12 5-MMP-1 5-MMP-2	
127-9 127-10 127-11 127-12 1-114 1-112 1-114 1-112 1-114 1-112	
42-9 42-10 42-11 42-12 5-MMP-1 5-MMP-2 onsensus	Catalytic Hinge
IP-9         IP-10         IP-12         IP-12         IP-12         IP-12         IP-12         IP-12         IP-12         IP-13         IP-14         IP-15         IP-16         IP-17         IP-18         IP-19         IP-19         IP-19         IP-19         IP-19         IP-19         IP-11         IP-11	Catalytic Hinge
4P-9 4P-10 4P-11 4P-12 4P-12 4P-12 4P-1 4P-1 4P-1 4P-2	Catalytic Hinge
4P-9 4P-10 4P-11 4P-12 7-M04P-1 7-M04P-2 onsensus 4P-1 4P-2 4P-3	Catalytic Hinge
42-9 42-10 42-11 42-12 4-MHP-1 MHP-2 0nsensus 42-1 42-2 42-3 42-7	Catalytic Hinge
4₽-9 4₽-10 4₽-11 4₽-12 -₩4₽-1 -₩4₽-2 500560505 4₽-1 4₽-2 4₽-3 4₽-7 4₽-8	Catalytic Hinge 
P-9 P-10 P-11 P-12 M4P-1 M4P-2 msensus P-1 P-2 P-3 P-3 P-7 P-8 P-9	Catalytic Hinge Catalytic Hinge Catalytic LHRVAA-HELGHSLGLSHSTDIGALMIPSY-TFSGDVQLAQDD-IDGI7AIYG GKMWCATTANYDDDRKWGFCPDQGYSLFLVAA-HEFGHAWGLEHSQDPCALMAPIY-TYT-KNTRLSQDD-IKGIQELYG
IP - 9         IP - 10         IP - 11         IP - 12         I-MUP - 1         I-MUP - 1         I-MUP - 1         I-MUP - 1         IP - 10	Catalytic Hinge 
IP-9         IP-10         IP-11         IP-12         I-MMP-1         I-MMP-2         Insensus         IP-1         IP-2         IP-3         IP-7         IP-8         IP-9         IP-10         IP-11	Catalytic Hinge 
IP - 9         IP - 10         IP - 11         IP - 12         I-MOHP-1         I-MOHP-2         Donsensus         IP - 1         IP - 2         IP - 2         IP - 3         IP - 7         IP - 8         IP - 9         IP - 10         IP - 12	Catalytic Hinge 
IP - 9         IP - 10         IP - 11         IP - 12         I-MOHP-1         I-MOHP-2         Donsensus         IP - 1         IP - 2         IP - 2         IP - 3         IP - 7         IP - 8         IP - 9         IP - 10         IP - 12	Catalytic       Hinge
₽ -9         1P -10         P -11         P -12         r-M0P-1         r-M0P-2         onsensus         P -1         P -2         P -3         P -8         P -9         IP -10         IP -11         PP-12         *-H04P-1         *-H04P-1	Catalytic Hinge 
PP-9 PP-10 PP-11 PP-12 -MMP-1 -MMP-1 -MMP-2 msensus PP-1 PP-2 PP-3 PP-3 PP-3 PP-3 PP-9 PP-9 PP-10 PP-11 PP-12 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 PP-10 P	Catalytic       Hinge
PP-9 PP-10 PP-11 PP-12 -MMP-1 -MMP-2 msensus PP-1 PP-3 PP-3 PP-7 PP-8 PP-9 PP-11 PP-11 PP-12 -MMP-1 -MMP-2	Catalytic       Hinge
₽ -9         1P -10         P -11         P -12         r-M0P-1         r-M0P-2         onsensus         P -1         P -2         P -3         P -8         P -9         IP -10         IP -11         PP-12         *-H04P-1         *-H04P-1	Catalytic       Hinge         DCKMWCATTANYDDDRKWGFCPDQCYSLFLVAA-HEFGHAMGLEHSQDFGALMAPII-TYTKNFRLSQDD-IKGIQELYG
PP-9 PP-10 PP-11 PP-12 -MMP-1 -MMP-2 msensus PP-1 PP-3 PP-3 PP-3 PP-3 PP-7 PP-8 PP-9 PP-11 PP-11 PP-12 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-1 -MMP-2 -MMP-1 -MMP-	Catalytic       Hinge         DCKMWCATTANYDDDRKWGFCPDQCYSLFLVAA-HEFGHAMGLEHSQDFGALMAPII-TYTKNFRLSQDD-IKGIQELYG
P-9 P-10 P-11 P-12 MUP-1 MUP-1 MUP-2 onsensus P-1 P-2 P-3 P-7 P-8 P-9 P-9 P-10 P-10 P-11 P-12 MUP-1 MUP-2 MUP-2 MUP-1 MUP-2 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 MUP-1 	Catalytic       Hinge
IP - 9         IP - 10         IP - 11         IP - 12         -MOHP-1         -MOHP-2         onsensus         IP - 1         IP - 2         IP - 7         IP - 8         IP - 9         IP - 10         IP - 12         -HOHP - 1         -HOHP - 1         -HOHP - 1         PH - 10         IP - 12         -HOHP - 2         onsensus         IP - 1         IP - 2	Catalytic       Hinge         DGKMNCATTANYDDDRKWGPCPDQGYSLFLVAA-HEFGHAMGLEHSQDPGALMAPIY-TYTKNFRLSQDD-IKGIQELYG
P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-2 msensus P-2 P-3 P-7 P-8 P-9 P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-1 -MMP-1 P-12 P-12 P-12 P-2 P-3 P-1 P-2 P-3 P-3 P-1 P-12 P-2 P-2 P-2 P-2 P-2 P-2 P-2 P-	Catalytic       Hinge
P-9 P-10 P-11 P-12 MUP-1 MUP-1 MUP-2 onsensus P-1 P-2 P-3 P-7 P-8 P-9 P-10 P-10 P-11 P-12 MUP-2 MUP-2 MUP-2 MUP-2 P-3 P-7 P-3 P-7 P-3 P-7 P-7	Catalytic       Hinge
P-9 P-10 P-12 -MMP-1 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-1 -MMP-2 -MMP-1 -MMP-2 P-1 P-12 P-2 P-3 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7	Catalytic       Hinge         DGKMNCATTANYDDDRKWGFCPDQCYSLFLVAA-HELGHANGLEHSQDPGALMAPII-TYTKNFRLSQDD-IKGIQELYG
P-9 P-10 P-11 P-12 MMP-1 MMP-1 MMP-2 msensus P-2 P-3 P-3 P-7 P-8 P-1 P-1 P-2 P-3 P-7 P-7 P-8 P-9	Catalytic Hinge Catalytic Hing
P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-2 maensus P-1 P-2 P-3 P-10 P-11 P-12 -MMP-2 -MMP-1 -MMP-2 maensus P-1 P-2 P-3 P-7 P-8 P-7 P-8 P-1 P-12 -MMP-1 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-2 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-1 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-3 P-7 P-8 P-7 P-7 P-8 P-12 P-7 P-7 P-8 P-12 P-7 P-7 P-7 P-8 P-12 P-7 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-8 P-7 P-7 P-8 P-9 P-10	Catalytic         Hinge           LHRVAA-HELGHSLGLSHSTDIGALMYPSY-TFSGDVQLAQDD-IDGI7AIYG         LHRVAA-HELGHSLGLSHSTDIGALMYPSY-TFSGDVQLAQDD-IDGI7AIYG           DGXMNCATTANYDDDRXWGFCPDQGYSLFLVAA-HEFGHAWGLENSQDPGALAMPIT-TYTKNFRLSQDD-IKGI0ELYG         LFLVAA-HEFGHAWGLENSQDPGALAMPIT-TYTKNFRLSQDD-IKGI0ELYG          FLIYAATHELGHSLGLFHSANTEALMYPLYHSLTDLTRFRLSQDD-IKGI0KLYG        FLIYAATHEFGHSLGLAMSSDPGALMYPNYA-FRETSNYSLPQDD-IKGIQKLYG          FLIYAATHECHSLGUHTSANTHANYPYYRCH-GPPGHKADOD-IKGIQKLYG        FLIYAA-HEFGHSLGLAMSSDPGALMYPNYA-FRETSNYSLPQDD-IKGIQKLYG          FLIYAA-HEFGHSLGLGHSSDPAAVMYPTYCN-GPPGHKADO-VNGIGSLYG        FLIYAA-HEFGHALGLDHSSDPEALMYPNYA-FRETSNYSLPQDD-IKGIQKIGG           DGRLMCATTSNFDSDKXWGFCPDQGYSLFLVAA-HEFGHXLGLQHSSDPEALMYPNYA-FRETSNYSLPQDD-VNGIQSLYG        FLIYAA-HEFGHXLGLQHSDPAAVMPPTYK-YTDINTFRLSADD-VNGIQSLYG
P-9 P-10 P-12 -MMP-1 -MMP-2 -MMP-2 -MMP-2 -MMP-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-2 -MMP-1 -MMP-2 -MMP-1 P-7 P-8 P-9 P-10 P-7 P-8 P-9 P-10 P-7 P-8 P-9 P-10 P-1 P-12 P-7 P-8 P-9 P-10 P-1 P-12	Catalytic Hinge Catalytic Hing
P-9 P-10 P-12 -MMP-1 -MMP-1 -MMP-2 msensus P-1 P-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-1 P-3 P-7 P-8 P-9 P-10 P-9 P-9 P-10 P-11 P-12 P-9 P-10 P-11 P-12	Catalytic         Hinge
P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-2 maensus P-1 P-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-2 maensus P-1 P-2 P-3 P-7 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-1 P-12 -MMP-1 -	Catalytic         Hinge
P-9 P-10 P-12 -MMP-1 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-2 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-9 P-10 P-12 -MMP-1 -MMP-2 -MMP-1 -MMP-2	Catalytic Hinge  Hinge  Catalytic Hinge  Catalytic Hinge  Catalytic Hinge  Catalytic Hinge Hemopexin  Catalytic Hinge  Catalytic Hinge Hemopexin  Catalytic Hinge  Hinge  Hinge  Catalytic Hinge Hemopexin  Catalytic Hinge Hinge  Catalytic Hinge  Catalytic Hinge Hinge  Catalytic Hinge  Catalytic Hinge Hinge  Catalytic Hinge Hinge  Catalytic Hinge Hinge  Catalytic Hinge  Hinge  Catalytic Hinge  Cat
P-9 P-10 P-11 P-12 -MMP-1 -MMP-2 nsensus P-1 P-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-1 -MMP-2 nsensus P-1 P-7 P-8 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-7 P-8 P-7 P-7 P-7 P-7 P-11 P-12 P-12 P-12 P-10 P-11 P-12 P-12 P-10 P-11 P-12 P-7 P-8 P-11 P-12 P-10 P-11 P-12 P-7 P-8 P-10 P-11 P-12 P-7 P-8 P-7 P-8 P-11 P-12 P-10 P-11 P-12 P-10 P-11 P-12 P-7 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-10 P-11 P-12 P-10 P-11 P-12 P-10 P-11 P-12 P-10 P-11 P-12 P-10 P-11 P-12 P-10 P-11 P-12 P-7 P-8 P-10 P-11 P-7 P-8 P-7 P-7 P-8 P-10 P-11 P-7 P-8 P-7 P-7 P-8 P-7 P-8 P-10 P-11 P-7 P-7 P-8 P-7 P-7 P-8 P-10 P-12 P-7 P-8 P-7 P-7 P-8 P-7 P-10 P-12 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-7 P-7 P-7 P-8 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7	Catalytic Hinge Catalytic Hinge Catalytic Hinge CXMWCATTANYDDDRXWGFCPDQCJELVUA-HELGHSLGLSHSTDIGALMYPSY-TFSGDVQLAQDD-IDCI7AIYG CXMWCATTANYDDDRXWGFCPDQCJELVUA-HEGGHSLGLSHSDDFCALMAPIY-TYTKNFRLSQDD-IKCIQELYG
P-9 P-10 P-12 -MMP-1 -MMP-2 -MMP-2 -MMP-2 -MMP-2 -MMP-2 P-3 P-7 P-8 P-9 P-10 P-11 P-12 -MMP-2 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-7 P-8 P-9 P-10 P-12 -MMP-1 -MMP-2 -MMP-1 -MMP-2	Catalytic Hinge  Hinge  Catalytic Hinge  Catalytic Hinge  Catalytic Hinge  Catalytic Hinge Hemopexin  Catalytic Hinge  Catalytic Hinge Hemopexin  Catalytic Hinge  Hinge  Hinge  Catalytic Hinge Hemopexin  Catalytic Hinge Hinge  Catalytic Hinge  Catalytic Hinge Hinge  Catalytic Hinge  Catalytic Hinge Hinge  Catalytic Hinge Hinge  Catalytic Hinge Hinge  Catalytic Hinge  Hinge  Catalytic Hinge  Cat
IP -9         IP -10         IP -11         IP -12         I-MUP-1         IP -12         IP -1         IP -2         IP -1         IP -2         IP -8         IP -7         IP -1         IP -1         IP -1         IP -2         IP -3         IP -1         IP -2         IP -1         IP -10         IP -11         IP -12         IP -10	Catalytic         Hinge
P-9 P-10 P-11 P-12 M0P-1 M0P-1 M0P-2 msensus P-1 P-2 P-3 P-7 P-8 P-9 P-10 P-12 M0P-1 M0P-1 M0P-2 M0P-1 P-2 P-3 P-7 P-8 P-7 P-8 P-9 P-10 P-12 P-12 P-12 P-12 	Catalytic         Hinge
P-9         (P-10)         (P-11)         (P-12)        MO(P-1)        MO(P-2)         onsensus         P-1         P-2         P-3         P-10         (P-11)         P-10         (P-11)         P-12        MO(P-1)        MO(P-1)         P-7         P-8         P-7         P-8         P-9         P-10         P-11         P-10         P-11         P-12         -MO(P-1)	Catalytic         Hinge
P-9 P-10 P-11 P-12 -MMP-1 -MMP-2 msensus P-1 P-2 P-3 P-7 P-9 P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-2 P-3 P-7 P-3 P-7 P-8 P-9 P-10 P-2 P-3 P-7 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-10 P-2 P-3 P-10 P-10 P-2 P-3 P-10 P-10 P-10 P-2 P-10 P-2 P-3 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7	Catalytic         Hinge
P-9 P-10 P-11 P-12 -MMP-1 -MMP-2 msensus P-1 P-2 P-3 P-7 P-9 P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-2 P-3 P-7 P-3 P-7 P-8 P-9 P-10 P-2 P-3 P-7 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-10 P-2 P-3 P-10 P-10 P-2 P-3 P-10 P-10 P-10 P-2 P-10 P-2 P-3 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7	Catalytic         Hinge
P-9 P-10 P-12 P-14 P-12 P-14 P-12 P-14 P-2 P-3 P-7 P-8 P-9 P-10 P-12 P-12 P-12 P-12 P-12 P-2 P-3 P-10 P-11 P-12 P-2 P-3 P-12 P-2 P-3 P-12 P-12 P-12 P-12 P-12 P-2 P-3 P-12 P-2 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-12 P-3 P-7 P-3 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7	Catalytic Hinge LHRVAA-HELGHSLGLSHSTDIGALHYPSY-TFSGDVQLAQOD-IDGI7AIYG CKNCATTANYDDDRKWGPCPDQGYSLFLVAA-HEPGHAKGLSHSQDPCALMAPIY-TYTKNFRLSODD-IKGIQKLGG FLYAA-HELGHSLGLHSANTEALHYPLYHSLTDLTRFRLSODD-IKGIQKLGGENE FLYAAHHELGHSLGLHSSDPCALMAPIY-TYTCH-GDPOTKGIQOD-IKGIQKLGGENE FLYAAHHELGHSLGLHSSDPCALMYPHYHSLTDLTRFRLSODD-IKGIQKLGGENE FLYAAHHELGHSLGLHSSDPCALMYPHY-RFTEGPPLHKDD-VNGIRKLYGPREPEPRPPTTTTPOPT 
P-9         P-10         (P-11)         (P-12)         r-M0(P-1)         r-M0(P-2)         onsensus         P-1         P-2         P-3         P-10         (P-10         (P-11         P-12         r-M0(P-1)         r-M0(P-1)         P-10         P-11         PP-2         Pmsensus         PP-1         PP-7         PP-8         PP-10         PP-11         PP-2         msensus         PP-1         PP-3         PP-7         PP-8         PP-1         PP-2         PP-3         PP-1         PP-3         PP-1         PP-3         PP-4	Catalytic Hinge LHRVAA-HELCHSLGLSHSTDIGALMYPSY-TFS-GDVQLAQDD-IDGI7AIYG LHRVAA-HELCHSLGLSHSTDIGALMYPSY-TFS-GDVQLAQDD-IDGI7AIYG CKNMCATTANYDDDRXWGFCCPQQTSLFLVAA-HEFCHAKGLSHSQDPCALMAPIT-TTT-KNRLSQDD-ING105LYG 
HP-9         HP-10         HP-11         HP-12         HP-14         HP-12         HP-14         HP-15         HP-16         HP-17         HP-18         HP-19         HP-10         HP-11         HP-12         HP-10         HP-11         HP-12         HP-10         HP-11         HP-12         HP-14         HP-15         HP-16         HP-17         HP-18         HP-19         HP-10         HP-11         HP-2         HP-3         HP-10         HP-11         HP-12         HP-10         HP-12         HP-10         HP-12         HP-10         HP-12         HP-10         HP-12         HP-10         HP-12         HP-12         HP-3         HP-3         HP-3         HP-3         HP-3         HP-3	Catalytic Hinge Catalytic Hing
P-9         PP-10         PP-11         PP-12         PD-12         PD-12         PD-12         PP-12         PP-13         PP-14         PP-15         PP-16         PP-17         PP-18         PP-19         PP-111         PP-2         PP-30         PP-101         PP-12         PP-101         PP-111         PP-12         PP-101         PP-101         PP-101         PP-102         PP-101         PP-101         PP-101         PP-102         PP-103         PP-104         PP-105         PP-304         PP-310         PP-310	Catalytic Hinge Catalytic Hinge Catalytic Hinge Catalytic Hinge Connections of the second sec
P-9         PP-10         (P-11         (P-12        MO(P-1        MO(P-2)         onsensus         P-1         P-2         P-10         P-11         P-12         P-14         P-1         P-2         P-10         P-111         P-12         P-104         P-11         P-2         P-3         P-7         P-8         P-9         P-10         P-11         P-12         -MOP-1         -MOP-2         onsensus         P-10         P-11         P-10         P-3         P-7         P-8         P-9         P-7         P-8	Catalytic Hinge Catalytic Hing
P-9 P-10 P-12 P-14 P-12 P-12 P-12 P-12 P-20 P-3 P-7 P-7 P-7 P-3 P-10 P-12 P-10 P-12 P-3 P-7 P-8 P-9 P-10 P-12 P-9 P-10 P-12 P-12 P-12 P-7 P-8 P-9 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-12 P-10 P-2 P-3 P-10	Catalytic Hinge  LHWWA-HELCHSLGLSKSTDIGALMYPSY-TFSGDVQLAQDD-IDGIYAIYG  GGWMCATTANYDDDRKWGFCPDQGYSLFUVA-HEGGKAMGLEHSQDPGALMYPIY-TYKYFLSQDD-INGIGALYGG  LFUVA-HEGKLGLGHSSDPGALMYPIY-TYKYFLSQDD-INGIGALYGEN  LFUVA-HEGKLGLGHSSDPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLDHSSVPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLDHSSVPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLDHSSVPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLGHSSDPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLGHSSDPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLGHSSDPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLGHSSDPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLGHSSDPGALMYPIY-RETSISJCDD-DUGGALYGENEPPPTTTPQPT  LFUVA-HEGKLGLGHSSDPSAIMYPIY-NPUTTFLSADD-RGIQSLG  GNIFUVA-HEGKLGLEHSSDPSAIMAPFIY-MPUTTPLSDD-RGIQSLG  GNIFUVA-HEGKLGLEHSSDPSAIMAPFIY-MDTTNLJDD-RGUGULG  HInge Hamopexin
P-9 P-10 P-11 P-12 -MMP-1 -MMP-1 -MMP-2 nsensus P-1 P-3 P-7 P-8 P-9 P-10 P-12 -MMP-1 -MMP-2 nsensus P-1 P-2 P-3 P-7 P-8 P-9 P-10 P-11 P-2 P-10 P-12 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-11 P-2 P-7 P-8 P-10 P-12 P-12 P-7 P-8 P-9 P-10 P-11 P-12 P-7 P-8 P-9 P-10 P-11 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-7 P-7 P-8 P-7 P-7 P-8 P-9 P-10 P-12 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-7 P-7 P-8 P-9 P-10 P-12 P-7 P-7 P-8 P-7 P-7 P-8 P-9 P-10 P-12 P-7 P-7 P-8 P-9 P-10 P-12 P-7 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-9 P-10 P-11 P-12 P-7 P-8 P-9 P-10 P-11 P-12 P-7 P-8 P-9 P-10 P-11 P-12 P-12 P-10 P-11 P-12 P-7 P-8 P-9 P-10 P-11 P-12 P-7 P-8 P-9 P-10 P-12 P-7 P-8 P-9 P-10 P-11 P-12 P-7 P-8 P-10 P-12 P-7 P-7 P-8 P-9 P-10 P-12 P-7 P-7 P-7 P-8 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7 P-7	Catalytic Hinge LHRWAA-HELGHSLGLSHSTDIGALHTPSY-TFS-GDVQLAQDD-IGGTANTG GCMMCATTANYDDDRKGCPCPQGTSLFUVAA-HEGGHSLGLSHSTDIGALHTPSY-TFS-GDVQLAQDD-IGGTANTG LFUVAA-HEIGHSLGLHSSDTGALHAPHT-TYTNTFRLSQDD-IKGTQSLTG LFUVAA-HEIGHSLGLHSSDTGALHTPHY-TYTNTFRLSQDD-IKGTQSLTG LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGKSNSSRK LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGKSNSSRK LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGGKSNSSRK LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGGKSNSSRK LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGG LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGG LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETSHSPLODD-IKGTQKLTGG LFUVAA-HEIGHSLGLHSSDTGALHTPHY-RETS-GPPLHKDD-KNGTKKLTGGRPEPEPEPEPTTTTPQT LFUVA-HEIGHSLGLHSSDTGALHTPHY-MEDTSFVLODD-IKGTQKLTGG LFUVA-HEIGHSLGLHSSDTGALHTPHY-MEDTSFVLODD-IKGTQKLTGG LFUVA-HEIGHSLGLHSSDTGALHTPHY-MDTENFVLODD-IKGTQKLTGG LFUVA-HEIGHSLGLHSSDTGALHTPTY-MEDTSFVLOPDC-RAGIQQLTGG NDLFUVA-HEIGHSLGLHSSDTGALHTPTY-MEDTSFVLOPDC-RAGIQQLTGG NDLFUVA-HEIGHSLGLHSSDTGALHTPTY-NTY-FYL-PEVELN NDLFUVA-HEIGHSLGLHSSDTGALHTPTY-PEVELN SNDLFUVA-HEIGHSLGLHSSDTGALHTYPY-PEVELTTTIRGE-VHFFKDRFYRTHPTY-PEVELN NDLFUVA-HEIGHSLGLHSSDTGALHTYPY-PEVELTTTIRGE-VHFFKDRFYRTHPTY-PEVELN SNDLFUVA-HEIGHSLGLHSSDTGALHTYPY-PEVELTTTIRGE-VHFFKDRFYRTHRTYTPY-PEVELN NDLFUVA-HEIGHSLGLHSSDTGALTTYPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEP
P-9         (P-10)         (P-11)         (P-12)         ->>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>	Catalytic Hinge Catalytic Hing
P-9         PP-10         PP-11         PP-12         PD-12         PD-12         PD-12         PP-12         PP-13         PP-14         PP-15         PP-16         PP-17         PP-18         PP-19         PP-111         PP-2         PP-30         PP-101         PP-12         PP-101         PP-111         PP-12         PP-101         PP-101         PP-101         PP-102         PP-101         PP-101         PP-101         PP-102         PP-103         PP-104         PP-105         PP-304         PP-310         PP-310	Catalytic Hinge LHRWAA-HELGHSLGLSHSTDIGALHTPSY-TFS-GDVQLAQDD-IGGTANTG GCMMCATTANYDDDRKGCPDQGTSLFUVAA-HEGGHNGLEHSQDFGLAMAPIT-TYTKNFRLSQDD-IKGIQKICGC

Fig. 2. (continued on next page)

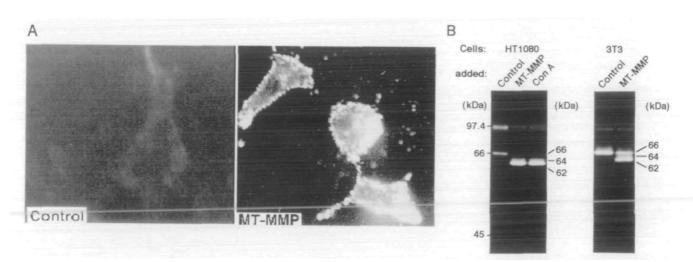
		ле	mopexi				
HHP-1	MIAHDPPGIGHKVDAVPHKDGFFYFFHGTRQY	KFDPKT-	KRILTL-QK	ANS-WFNCRKN	469		
MMP-2	LIADAWNAIPDNLDAVVDLOGGGHSYFFKGAYYL	KLENQS-	LKSVKF-GS	IKSDWLGC	660		
MMP-3	QIAEDFPGIDSKIDAVFEEFGFFYFFTGSSQL	EFDPNA-	KKVTHT-LK	SNS-WLNC	477		
HMP-7					267		
10-CP-8	SISGAFPGIESKVDAVFQQEHFFHVFSGPRYY	AFDLIA-	ORVTRV-AR	GNK-WLNCRYG	467		
HMP-9	EVDRMFPGVPLDTHDVFQTREXAYFCQDRF IWRVSSRSELNOVDOVG IVT ID ILOCPED						
MMP-10	LIADDFPGVEPKVDAVLQAFGFFYFFSGSSQF	EFDPNA-	RHVTHI-LK	SNS-WLHC	476		
MHP-11	R-ATDWRGVPSEIDAAFQDADGYA-YFLRGRLYW	KFDPVK-	VKALEGFPR	LVGPDFFGCAEPANTFL	488		
MMP-12	LITKNFQGIGPKIDAVFYSKNKY-YYFFQGSNQF	EYDFLL	ORITKT-LK	SNS-WFGC	470		
HT-HHP-1	NIKVWE-GIPESPRGSFHGSDEVFTYFYKGNKYW	KFNNOKL.	<b>KVEPGIPKS</b>	ALRDWNGCPSGGRPDEGTEEETE-VIIIEVDEEGGGAVSAAAVVLPVLLL	549		
HT-HHP-2	PITVWK-GIPESPOGAFVHKENGFTYFYKEGVLEIQTTRYSRLEPGHPRSILKDLSGCDGPTDRVKEGHSPPDDVDIVIKLDNTASTVKAIAIVIPCILA .IF.GIDAVFYFF.GFDW.C						
Consensus	.1F.GIDAVFIFF.G	. FD	•••••		800		
				<b>▲</b> IS-3			
HHP-1		469					
HOM2 - 2		660					
MMP-3		477					
HO-7		267					
HHP-8		468					
HHP-9		708					
HHP-10		476	-				
HHP-11		489	F1g. 2	Alignment of MMP as sequences. The reported human	1 MM		

470

582

604

Homopovin



arrows (IS-1 to IS-3)

Fig. 3. Expression of MT-MMP. A: Cell surface localization of MT-MMP expressed in COS-1 cells. COS-1 cells transfected with control or MT-MMP-1 expression plasmid were stained by an immunofluorescent antibody staining method using a monoclonal antibody against MT-MMP-1. B: Processing of progelatinase A by the

LLVLAVGLAVFFFRRHGTPRRLLYCORSLLDKV

LCLLVLVYTVFOFKRKGTPRHILYCKRSMOEWV

expression of MT-MMP-1 Culture supernatants from HT-1080 or NIH3T3 cells transfected with control or MT-MMP-1 expression plasmid or treated with concanavalin A were analyzed by gelatin zymography.

Fig. 2 Alignment of MMP as sequences. The reported human MMPs are

listed and aligned with the deduced MT-MMP-1 and MT-MMP-2 proteins.

Specific insertions characteristic to MT-MMPs are indicated by the upward

third insertion at the C terminus contains a hydrophobic amino acid stretch which can pass through the plasma membrane and act as a potential transmembrane domain. Therefore, they were named membrane-type MMP (MT-MMP-1 and MT-MMP-2, respectively) (23, 24). MT-MMP proteins were localized in the membrane by the immunofluorescent staining of transfected cells with monoclonal antibodies raised against MT-MMP-1 and MT-MMP-2 peptides (Fig. 3A). These antibodies recognized 63 and 64 kDa proteins for MT-MMP-1 and MT-MMP-2, respectively.

#### Activation of progelatinase A by MT-MMP expression

The expression of MT-MMP on the cell surface fits the requirements for an activator for progelatinase A. The expression of MT-MMP-1 in cells secreting 68 kDa progelatinase A and 92 kDa progelatinase B converted only progelatinase A to the 62 kDa fully active form through the 64 kDa intermediate as demonstrated by gelatin zymography (Fig. 3B) (24). MT-MMP-2 expression also induced progelatinase A activation (23). The MT-MMP-1 mRNA transcript and protein were expressed in MDA-MB-231 cells stimulated with concanavalin A and HT1080 cells exposed to TPA, indicating that the gelatinase A activator in these cells is MT-MMP-1 (28, 29).

#### **Expression of MT-MMP genes in human tissues**

MT-MMP-1 and MT-MMP-2 mRNA transcripts (4.5 and 12 kb, respectively) are expressed in various tissues, but they are distributed quite differently (22, 23). MT-MMP-1 mRNA expression is predominant in the lungs, kidneys, and placenta where extracellular matrix remodeling is relatively active, and lowest in the brain. In contrast, MT-MMP-2 mRNA expression is high in the heart, brain, and placenta, and is undetectable in the lungs, kidneys, liver, spleen, and muscle.

HHP-11 HHP-12 HT-HHP-1

T-MMP-2

Consensue

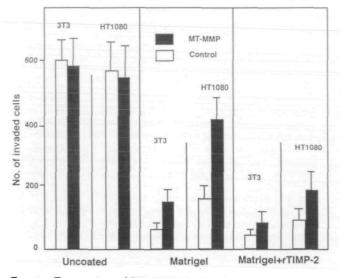


Fig. 4. Expression of MT-MMP-1 stimulates the invasion of cells in vitro. NIH3T3 and HT1080 cells were transfected with MT-MMP-1 (MT-MMP) or control plasmids, then the invasion of the reconstituted basement membrane in the presence (Matrigel + rTIMP-2) or absence of  $10 \,\mu g/ml$  recombinant TIMP-2 (Matrigel) was assayed Motility was analyzed on uncoated filters (Uncoated).

#### Elevated expression of MT-MMP-1 in tumor tissues

A significant statistical association between the expression level of activated gelatinase A in tumor tissue and tumor spread has been reported. Therefore, attempts have been made to identify the gelatinase A activator in tumor tissues (24, 30, 31). MT-MMP-1 expression is elevated in various tumor tissues including lung, gastric, colon, and breast cancers, in which activated gelatinase A is expressed. Detailed statistic analyses have demonstrated a close correlation between MT-MMP-1 expression and gelatinase A activation in lung carcinoma, indicating that MT-MMP-1 is involved in the activation of gelatinase A in tumor tissue. Although the MT-MMP-2 cDNA fragment has been obtained from melanoma tissue, elevated expression of MT-MMP-2 is not often observed in tumor tissues.

According to immunohistochemical studies of gastric carcinomas, MT-MMP-1 is predominantly localized in and on carcinoma cells (30). On the other hand, gelatinase A is immunolocalized only on the cell membranes of carcinoma cells. Almost all gelatinase A-positive tumors expressed MT-MMP-1 in the cells as well. In most of these, fibroblasts and vascular endothelial cells in the advanced carcinoma tissue were also immunostained for MT-MMP and gelatinase A and the staining was weak or negative in the normal tissue remote from the carcinoma. It seems that MT-MMP-1 is expressed in the stromal cells in pathophysiological conditions, where the extracellular matrix macromolecules are undergoing rapid remodeling. Colocalization of gelatinase A with MT-MMP-1 on the tumor cell membrane raised the question as to how gelatinase A, of which the mRNA transcript was detected in stromal cells around tumor cells, localizes on the tumor cell membrane. This is consistent with the in vitro finding that activated gelatinase A is enriched in the plasma membrane of cancer cells. Although the specific binding of gelatinase A to the surface of tumor and normal fibroblast cells has been described, a membrane protein that functions as a gela-

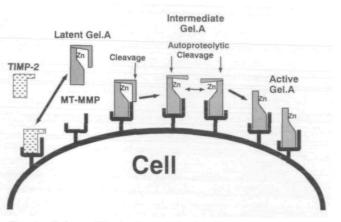


Fig 5. Scheme illustration of the binding and activation of progelatinase A by MT-MMP and its inhibition by TIMP-2 on the cell surface.

tinase A receptor has not been identified.

#### MT-MMP-1 also functions as a receptor of gelatinase A

The fact that activated gelatinase A is enriched in the plasma membrane of cancer cells in which MT-MMP localizes, suggests that MT-MMP is involved not only in the activation but also in the binding of gelatinase A (32, 33). The specific binding of gelatinase A to the cells expressing MT-MMP was demonstrated by a study using <sup>125</sup>I-labeled progelatinase A that was processed to the intermediate form (Sato, H., Takino, T., Kinoshita, T., Imai, K., Okada, Y., Stetler-Stevenson, W.G., and Seiki, M., manuscript submitted). Complex formation between MT-MMP and gelatinase A was demonstrated by immunoprecipitation using monoclonal antibodies against MT-MMP and gelatinase A. These results indicated that MT-MMP serves not only as an activator but a receptor of gelatinase A. Processing from the intermediate to fully active form was dependent on the gelatinase A concentration. Unlike the wildtype progelatinase A, a mutant defective in catalytic function was processed only to the intermediate form by co-expression with MT-MMP. Thus, the processing of progelatinase A to intermediate form is catalyzed by the function of MT-MMP and the binding with MT-MMP on the cell surface concentrates the intermediate form locally to allow autoproteolytic processing to the fully active form.

#### Blocking of gelatinase A binding by TIMP-2

TIMP-2 but not TIMP-1 binds to the surface of normal fibroblast cells and inhibits the activation of progelatinase A by these cells (34; Sato, H., Takino, T., Kinoshita, T., Imai, K., Okada, Y., Stetler-Stevenson, W.G., and Seiki, M., manuscript submitted). This suggests direct interaction between TIMP-2 and MT-MMP. Indeed, TIMP-2 bound to cells expressing MT-MMP and interfered with the binding and activation of progelatinase A. Immunoprecipitation has shown that TIMP-2 forms a complex with MT-MMP. Therefore, TIMP-2 is thought to competitively inhibit binding of gelatinase A to MT-MMP.

## Expression of MT-MMP stimulates the invasion of cells

As gelatinase A degrades various extracellular matrix macromolecules, including type IV collagen and laminin, we determined whether or not progelatinase A activated by MT-MMP expression enhances the invasiveness of cells producing progelatinase A. We found that MT-MMP expression in HT-1080 and NIH3T3 cells more than doubled the number of invasive cells compared with controls when analysed using a modified Boyden Chamber (Fig. 4) (24). The invasion was sensitive to TIMP-2, which

inhibits binding and activation of gelatinase A by MT-MMP

and also the proteolytic activity of activated gelatinase A.

Implications

Tumor spread reportedly correlates with increased levels of activated gelatinase A (10, 11). This activation occurs in association with the plasma membrane and activated gelatinase A binds specifically to the tumor cell surface, although the molecular features of these interactions remain to be elucidated. MT-MMP-1 fulfills the criteria for a plasma membrane associated activator and receptor of gelatinase A. Via this mechanism, MT-MMP localizes matrix digestion to the vicinity of the tumor cell surface (Fig. 5). The cell surface binding of gelatinase A not only promotes enzyme activation but also regulates enzyme activity by increasing the rate of substrate cleavage (35). The cell surface localization of proteinases is a common cellular strategy for regulating pericellular proteolysis, as exemplified by urinary-type PA (uPA), the activity of which is localized predominantly on the cell surface via binding to the specific uPA receptor (36). Co-expression of MT-MMP and gelatinase A in osteoblasts during mouse embryonic development and the efficient activation of progelatinase A in the tissue indicate that the MT-MMPgelatinase A system plays an important role in tissue remodeling during organogenesis (Kinoh, A., Sato, H., Tsunezuka, Y., Okada, Y., Kawashima, A., and Seiki, M., manuscript submitted). Thus, MT-MMP and its family play a central role in the activation and cell surface localization of gelatinase A not only in tumor invasion and metastasis but also during normal development. Identification of the proteinase that triggers the proteolytic cascade of human cancers should be valuable both as an index of prognosis and as a guide in the design of antiproteolytic strategies aimed at controlling the progression of the disease.

Note Added in Proof—While this manuscript was being prepared, the new MT-MMP was reported [Will, H. and Hinzmann, B. (1995) Eur. J. Biochem. 231, 602-608]. Thus, MT-MMP-2 in this paper will be renamed as MT-MMP-3.

#### REFERENCES

- 1. Liotta, L.A., Steeg, P.S., and Stetler-Stevenson, W.G. (1991) Cancer metastasis and angiogenesis: An imbalance of positive and negative regulation. *Cell* 64, 327-336
- Tryggvason, K., Hoyhtya, M., and Pyke, C. (1993) Type IV collagenases in invasive tumors. Breast Cancer Res. Treat. 24, 209-218
- Brannstrom, M., Woessner, J.F., Jr., Koos, R.D., Sear, C.H., and LeMaire, W.J. (1988) Inhibitors of mammalian tissue collagenase and metalloproteinases suppress ovaluation in the perfused rat ovary. *Endocrinology* 122, 1715-1721
- Brenner, C.A., Adler, R.R., Rappolee, D.A., Pedersen, R.A., and Werb, Z. (1989) Genes for extracellular matrix-degrading

metalloproteinases and their inhibitor, TIMP, are expressed during early mammalian development. *Genes Dev.* 3, 848-859

- Nakanishi, Y., Sugiura, F., Kishi, J.-L., and Hayakawa, T. (1986) Collagenase inhibitor stimulates cleft formation during early morphogenesis of mouse salivary gland. *Dev. Biol.* 113, 201-206
- Sato, H., Kida, Y., Mai, M., Endo, Y., Sasaki, T., Tanaka, J., and Seiki, M. (1992) Expression of genes encoding type IV collagendegrading metalloproteinases and tissue inhibitors of metalloproteinases in various human tumor cells. Oncogene 7, 77-83
- Tsuchiya, Y., Endo, Y., Sato, H., Okada, Y., Mai, M., Sasaki, T., and Seiki, M. (1994) Expression of type-IV collagenases in human tumor cell lines that can form liver colonies in chick embryos. Int. J. Cancer 56, 46-51
- 8. Matrisian, L.M. (1990) Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet.* 6, 121-125
- Brown, P.D., Bloxidge, R.E., Anderson, E., and Howell, A. (1993) Expression of activated gelatinase in human invasive breast carcinoma. *Clin. Exp. Metastasis* 11, 183-189
- Brown, P.D., Bloxidge, R.E., Stuart, N.S.A., Gatter, K.C., and Carmichael, J. (1993) Association between expression of activated 72-kilodalton gelatinase and tumor spread in non-small-cell lung carcinoma. J. Natl. Cancer Inst. 85, 574-578
- Azzam, H.S., Arand, G., Lippman, M.E., and Thompson, E.W. (1993) Association of MMP-2 activation potential with metastatic progression in human breast cancer cell lines independent of MMP-2 activation. J. Natl. Cancer Inst. 85, 1758-1764
- Yamagata, S., Yoshii, Y., Suh, J.G., Tanaka, R., and Shimizu, S. (1991) Occurrence of an active form of gelatinase in human gastric and colorectal carcinoma tissues. *Cancer Lett.* 59, 51-55
- Collier, I.E., Wilhelm, S.M., Eisen, A.Z., Marmer, B.L., Grant, G.A., Seltzer, J.L., Kronberger, A., He, C., Bauer, E.A., and Goldberg, G.I. (1988) H-ras oncogene-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloproteinase capable of degrading basement membrane collagen. J. Biol. Chem. 263, 6579-6587
- Wilhelm, S.M., Collier, I.E., Marmer, B.L., Eisen, A.Z., Grant, G.A., and Goldberg, G.I. (1989) SV-40 transformed human lung fibroblasts secrets a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophage. J. Biol. Chem. 264, 17213-17221
- 15. Van Wart, H.E. and Birkedal-Hansen, H. (1990) The cystein switch: A principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl. Acad. Sci. USA* 87, 5578-5582
- Springman, E.B., Angleton, E.L., Birkedal-Hansen, H., and Van Wart, H.E. (1990) Multiple modes of activation of latent human fibroblast collagenase: Evidence for the role of a Cys73 active-site zinc complex in latency and a "cysteine switch" mechanism for activation. Proc. Natl. Acad. Sci. USA 87, 364-368
- Nagase, H., Enghild, J.J., Suzuki, K., and Salvesen, G. (1990) Stepwise activation mechanisms of the precursor of matrix metalloproteinase 3 (stromelysin) by proteinases and (4-aminophenyl)mercuric acetate. *Biochemistry* 29, 5783-5789
- Strongin, A.Y., Marmer, B.L., Grant, G.A., and Goldberg, G.I. (1993) Plasma membrane-dependent activation of the 72-kDa type IV collagenase is prevented by complex formation with TIMP-2. J. Biol. Chem. 268, 14033-14039
- Overall, C.M. and Sodek, J. (1990) Concanavalin A produces a matrix-degradative phenotype in human fibroblasts. Induction and endogenous activation of collagenase, 72-kDa gelatinase, and Pump-1 is accompanied by the suppression of the tissue inhibitor of matrix metalloproteinases. J. Biol. Chem. 265, 21141-21151
- Ward, R.V., Atkinson, S.J., Slocombe, P.M., Docherty, A.J., Reynolds, J.J., and Murphy, G. (1991) Tissue inhibitor of metalloproteinases-2 inhibits the activation of 72 kDa progelatinase by fibroblast membranes. *Biochim. Biophys. Acta* 1079, 242-246
- Atkinson, S.J., Ward, R.V., Reynolds, J.J., and Murphy, G. (1992) Cell-mediated degradation of type IV collagen and gelatin films is dependent on the activation of matrix metalloproteinases. *Biochem. J.* 288, 605-611

- 22. Takino, T., Sato, H., Yamamoto, E., and Seiki, M. (1995) Cloning of a gene potentially encoding a novel matrix metalloproteinase having a trans-membrane domain at the C-terminus. *Gene* 115, 293-298
- Takino, T., Sato, H., Shinagawa, A., and Seiki, M. (1995) Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library: MT-MMP form a unique membrane-type subclass in the MMP family. J. Biol. Chem. 270, 23013-23020
- 24. Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. (1994) A matrix metalloproteinase expressed on the surface of invasive tumor cells. *Nature* 370, 61-65
- Basset, P., Bellocq, J.P., Wolf, C., Stoll, I., Hutin, P., Limacher, J.M., Podhajcer, O.L., Chenard, M.P., Rio, M.C., and Chambon, P. (1990) A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature* 348, 699-704
- Fuller, R.S., Brake, A.J., and Thorner, J. (1989) Intracellular targeting and structural conservation of a prohormone-processing endoprotease. *Science* 246, 482-486
- Pei, D. and Weiss, S.J. (1995) Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature 375, 244-247
- Strongin, A.Y., Collier, I., Bannikov, G., Marmer, B.L., Grant, G.A., and Goldberg, G.I. (1995) Mechanism of cell surface activation of 72-kDa type IV collagenase—Isolation of the activated form of the membrane metalloprotease. J. Biol. Chem. 270, 5331-5338
- 29. Yu, M., Sato, H., Seiki, M., and Thompson, E.W. (1995) Complex regulation of membrane-type metalloproteinase expression and matrix metalloproteinase-2 activation by concanavalin A in MDA-MB-231 human breast cancer cells. *Cancer Res.*

**55**, 3272-3277

- Nomura, H., Sato, H., Seiki, M., Mai, M., and Okada, Y. (1995) Expression of membrane-type matrix metalloproteinase in human gastric carcinomas. *Cancer Res.* 55, 3263-3266
- Tokuraku, M., Sato, H., Murakami, S., Okada, Y., Watanabe, Y., and Seiki, M. (1995) Activation of the precursor of gelatinase A/ 72 kDa type IV collagenase/MMP-2 in lung carcinomas correlates with the expression of membrane-type matrix metalloproteinase (MT-MMP) and with lymph node metastasis. Int. J. Cancer 64, 355-359
- Monsky, W.L., Kelly, T., Lin, C.-Y., Yeh, Y., Stetler-Stevenson, W.G., Mueller, S.C., and Chen, W.-T. (1993) Binding and localization of M<sub>r</sub> 72,000 matrix metalloproteinase at cell surface invadopondia. *Cancer Res.* 53, 3159-3164
- Zucker, S., Moll, U.M., Lysik, R.M., DiMassimo, E.I., Stetler-Stevenson, W.G., Liotta, L.A., and Schwedes, J.W. (1990) Extraction of type-IV collagenase/gelatinase from plasma membranes of human cancer cells. *Int. J. Cancer* 45, 1137-1142
- 34. Ward, R.V., Atkinson, S.J., Reynolds, J.J., and Murphy, G. (1995) Cell surface-mediated activation of progelatinase A: Demonstration of the involvement of the C-terminal domain of progelatinase A in cell surface binding and activation of progelatinase A by primary fibroblasts. *Biochem. J.* 304, 263-269
- Young, T.N., Pizzo, S.V., and Stack, M.S. (1995) A plasma membrane-associated component of ovarian adenocarcinoma cells enhances the catalytic efficiency of matrix metalloproteinase-2. J. Biol. Chem. 270, 999-1002
- 36. Pyke, C., Kristensen, P., Ralfkiaer, E., Grondahl-Hansen, J., Eriksen, J., Blasi, F., and Dano, K. (1991) Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invasive foci in human colon adenocarcinoma. Am. J. Pathol. 138, 1059-1067