FEBS Letters 503 (2001) 158–162 FEBS 25131

Specific collagenolysis by gelatinase A, MMP-2, is determined by the hemopexin domain and not the fibronectin-like domain

Margaret L. Patterson, Susan J. Atkinson, Vera Knäuper[1], Gillian Murphy*

School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

Received 19 July 2001; accepted 19 July 2001

First published online 1 August 2001

Edited by Veli-Pekka Lehto

Abstract In view of the essential role of the hemopexin domain of the traditional interstitial collagenases, MMP-1, -8, -13 and MT1-MMP (MMP-14), in determining specific collagen cleavage we have studied the function of this domain in MMP-2, relative to that of the fibronectin-like domain that promotes gelatinolysis. Although the fibronectin-like domain promotes avid binding to collagen, our data demonstrate that the catalytic and hemopexin domains of MMP-2 are sufficient to effect the critical step in cleavage of rat type I collagen into 3/4 and 1/4 fragments. The mechanism of MMP-2 cleavage of collagen proceeds in two phases, the first resembling that of the interstitial collagenases, followed by gelatinolysis, promoted by the fibronectin-like domain.

Key words: Gelatinase A; Matrix metalloproteinase-2; Hemopexin; Fibronectin-like domain; Collagenolysis

1. Introduction

It has been shown previously that gelatinase A, MMP-2, could cleave both soluble and reconstituted fibrillar collagen [1], although this observation has been disputed [2]. Like all the collagenolytic MMPs, MMP-2 cleaves at a single site within the α1 and α2 chains of type I collagen (Gly^{775}-$Ileu^{776}$/Leu), which generate the classical 3/4 and 1/4 cleavage fragments [3]. However, it is notable that, whereas the specific collagenases have K_m values in the 1–2 μM range for type I collagens [4,5], the value for MMP-2 is around 8.5 μM [1].

Previous studies on the interaction of the collagenolytic MMPs with fibrillar collagen have established the importance of the hemopexin domain in both binding to and cleavage of this substrate [6–10]. However, MMP-3 (stromelysin 1) has a hemopexin domain that binds collagen but this does not support collagenolysis by MMP-3, or by a chimera constructed from the catalytic domain of MMP-1 and the hemopexin domain of MMP-3 [7,8]. The need for the specific interactions between catalytic and hemopexin domains of the collagenases has been discussed by Bode [11] and Murphy and Knäuper [12].

MMP-2 also binds tightly to native or denatured collagens and it has been shown that this is predominantly mediated by the three fibronectin type II-like repeats, the gelatin binding region, which are localised within the catalytic domain [13]. Deletion of this fibronectin-like domain apparently abolishes collagen binding and significantly abrogates the gelatinolytic capacity of MMP-2, without affecting the ability to cleave peptide substrates or to bind TIMP-1 or TIMP-2 [14]. Furthermore, the isolated recombinant forms of the fibronectin type II-like modules of MMP-2 were shown to bind gelatin [15]. Work by Steffensen et al. [16] identified at least two binding sites in the recombinant fibronectin-like domain for gelatin and native type I collagen. More recently synthetic triple helical peptides describing the cleavage site of type I collagen have been synthesised [17] and shown to bind wild-type MMP-1 and MMP-8 as well as MMP-2. However, the deletion mutant of MMP-2 lacking the fibronectin-like domain failed to bind to these synthetic triple helical peptides which indicates that this structural element of MMP-2 is essential for efficient binding [18]. The hemopexin domain of MMP-2 showed no apparent contribution to binding to type I collagen [19,20].

In view of these data it was interesting to speculate how the mode of interaction with MMP-2 to native collagens relates to its modest collagenolytic capacity. In this study we compare the relative collagenolytic activities of full length MMP-2, and two deletion mutants lacking either the fibronectin-like or the hemopexin domains. Our results indicate a novel biphasic collagenolytic mechanism of action of MMP-2, where the initial cleavage resembles that of the other specific collagenases, followed by further degradation of the 1/4 and 3/4 fragments.

2. Materials and methods

2.1. Preparation and activation of enzymes and active site titration

The following were expressed and purified from myeloma cell conditioned medium: human proMMP-2 [8], pro$\Delta_{191-364}$ MMP-2 (ΔGBR MMP-2) [14], pro$\Delta_{418-631}$ MMP-2 (N-terminal MMP-2) [8] and human MMP-1 [21]. Recombinant human MMP-3 was expressed and purified as described [22]. A transmembrane and cytoplasmic tail deletion mutant of human MT1-MMP was expressed in *Escherichia coli* and refolded as described previously [23].

MMP-1 was 'superactivated' by incubation with 10 μg/ml trypsin (Sigma, cat. No. T-8253) in the presence of active MMP-3 (at a 10:1 molar ratio) for 15 min at 37°C. MMP-3 was activated by incubation

*Corresponding author. Fax: (44)-1603-59 38 74.
E-mail address: g.murphy@uea.ac.uk (G. Murphy).

[1] Present address: Department of Biology, University of York, York YO10 5YW, UK.

Abbreviations: TIMP, tissue inhibitor of metalloproteinases; MMP, matrix metalloproteinase; ΔGBR MMP-2, pro$\Delta_{191-364}$ MMP-2; N-terminal MMP-2, pro$\Delta_{418-631}$ MMP-2; Mca, methoxycoumarin; Nva, norvaline; Dpa, diaminopropionyl; APMA, *p*-aminophenylmercuric acetate

PII: S0014-5793(01)02723-5

with 10 μg/ml trypsin for 15 min at 37°C. In both cases activation was stopped with of 100 μg/ml soya bean trypsin inhibitor.

MMP-2 and the two domain deletion mutants were activated by incubation with 2 mM APMA for 1 h at 25°C, followed by SDS–PAGE analysis [24] to ensure that minimal loss of the C-terminal domain had occurred.

All enzymes were active site titrated with varying known amounts of TIMP-1 (MMP-1 and MMP-3) or TIMP-2 (MMP-2 and the domain deletion mutants) [25] using Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 as a substrate for MMP-2 and the two domain deletion mutants. Mca-Pro-Leu-Ala-Nva-Dpa-Ala-Arg-NH_2 was employed for active site titrations of MMP-1 and MMP-3. The enzyme–TIMP complexes were added to 1 μM quenched fluorescent substrate in buffer (50 mM Tris–HCl pH 7.5, 200 mM NaCl, 5 mM $CaCl_2$, 0.05% Brij 35) and linear substrate hydrolysis was monitored using a Perkin Elmer LS50B fluorimeter.

2.2. Preparation of collagens and collagenolysis assays

Acid soluble rat skin type I collagen (both unlabelled and ^{14}C acetylated) was prepared as described previously [26]. Acid soluble bovine skin type I collagen (both unlabelled and ^{3}H labelled) was a generous gift from T. Cawston. In all experiments using type I collagen the substrate was incubated for 2 h at 35°C to allow formation of fibrils, thus the substrate is in the solid phase. In attempts to carry out incubations using soluble collagen it was not possible to prevent fibril formation, hence preformed fibrils gave some uniformity of substrate. Collagenolysis of radiolabelled collagen was assayed as follows: active enzymes of known concentrations were added to 100 μg radiolabelled triple helical type I collagen fibrils, 50 μl 2×Tris assay buffer (200 mM Tris–HCl pH 8.0, 60 mM $CaCl_2$) added, and made up to 300 μl with water. The samples were incubated at 35°C for 4 h and then centrifuged for 15 min at 12 000 rpm at 4°C to pellet the uncleaved collagen and radioactivity was counted from the supernatant. Collagen released (μg) was plotted against enzyme concentration, and units/nmol were calculated from the linear portions of the graphs. 15% of the rat skin type I collagen was buffer soluble and a further 2% was trypsin sensitive, while 30% of the bovine skin type I collagen was buffer soluble and a further 9% was trypsin sensitive. 60 μg unlabelled type I collagen was cleaved for 2 h at 25°C with active site titrated enzymes in a total volume of 200 μl in salt free buffer (50 mM Tris–HCl pH 7.6, 5 mM $CaCl_2$). For type II collagen 20 μg of substrate was cleaved in a total volume of 60 μl salt free buffer for 2 h at 25°C. The reactions were stopped with 50 mM EDTA (final concentration) and sufficient four times reducing sample buffer prior to boiling for 10 min. Samples were applied to 7.5% SDS–PAGE and all gels were stained with Coomassie brilliant blue G. Densitometry (using GelWorks 1D) was used to analyse the SDS–PAGE gels of cleavage of type II collagen. Samples were loaded in triplicate and the average rate of cleavage (μg collagen/min) was calculated for each enzyme and converted to specific activity (units/nmol).

2.3. Degradation of ^{14}C type 1 collagen films by purified recombinant MMPs

^{14}C type I collagen films were prepared according to the method of Atkinson et al. [27]. Active forms of purified recombinant MMP-1, MMP-2, ΔGBR MMP-2 and MT1-MMP, prepared as described above, or buffer alone were incubated on washed films at the concentrations indicated in 100 mM Tris–HCl pH 7.6, 30 mM $CaCl_2$, 0.05% Brij 35 for 18 h at 37°C. Supernatants were harvested, centrifuged at 10 000 rpm for 10 min to remove undigested collagen and aliquots were counted as described above. Collagen remaining in the wells was digested with 50 μg/ml bacterial collagenase and counted as before so that total counts could be estimated.

3. Results and discussion

A number of laboratories have studied the ability of MMP-2 to cleave native collagens, which has generated substantial debate and controversy [1,2,28]. The aim of this study was to investigate whether MMP-2 is a true collagenolytic MMP and, if so, how its mechanism of action is determined by the different domains of the enzyme and how this relates to the mechanism of the conventional interstitial collagenases, MMP-1, MMP-8, MMP-13 and MT1-MMP, which is dependent on the C-terminal hemopexin domain. Since collagenolysis of triple helical collagens is highly dependent on the quality of the substrate preparation and the amount of fibrils present, we used two highly trypsin resistant type I collagen preparations (of rat or bovine origin) for our comparative analysis of collagenolysis of wild-type MMP-2 and two deletion mutants lacking either the fibronectin-like (ΔGBR MMP-2) or the hemopexin domains of MMP-2 (N-terminal MMP-2), relative to the collagenolytic activity of 'superactivated' MMP-1. To ensure specificity, all assays were performed with preformed collagen fibrils and linearity of collagenolytic assays was established for all enzyme preparations over time.

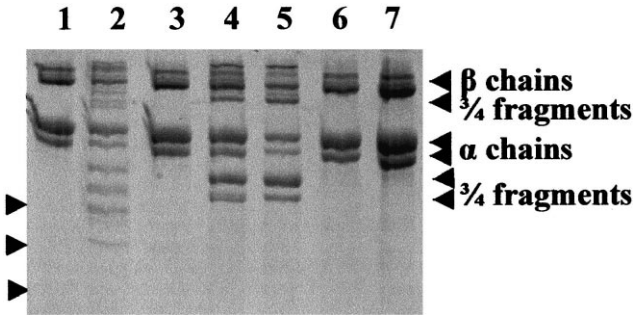

Fig. 1. Polyacrylamide gel electrophoretic analysis of rat skin type I collagen cleavage by MMP-1, MMP-3, MMP-2 and MMP-2 domain deletion mutants. Cleavage of native, acid soluble fibrillar rat skin type I collagen. The collagen was incubated for 2 h at 35°C to allow fibrils to form. 100 nM enzyme was added to 1 μM collagen and incubated at 25°C for 2 h. Lanes: 1, collagen incubated with buffer alone; 2, collagen incubated with MMP-2; 3, collagen incubated with N-terminal MMP-2; 4, collagen incubated with ΔGBR MMP-2; 5, collagen incubated with 'superactivated' MMP-1; 6, collagen incubated with MMP-3; 7, collagen incubated with 110 nM trypsin. The arrows on the left indicate further degradation products of the collagen cleaved by MMP-2.

3.1. Domain requirements of MMP-2 in collagenolysis: the role of the fibronectin-like and hemopexin domains of MMP-2 in determining specific type I collagen cleavage

In order to confirm that MMP-2 cleaved native collagen in the '1/4, 3/4 clip' fashion considered unique to the specific collagenases, we carried out incubations of preformed fibrils at 25°C followed by SDS–PAGE analysis of the respective MMP cleavage products (Fig. 1). Our results revealed that MMP-1 generated only 1/4 and 3/4 fragments (Fig. 1, lane 5), whilst MMP-2 produced comparable fragments but proceeded to degrade them further (Fig. 1, lane 2). While the N-terminal MMP-2 was unable to cleave collagen fibrils (Fig. 1, lane 3, Fig. 2), the mutant lacking the fibronectin type II repeats (ΔGBR MMP-2) displayed considerable collagenolytic activity with the generation of the classical 1/4 and 3/4 cleavage products of the interstitial collagenases (Fig. 1, lane 4, Fig. 2, Table 1). The ΔGBR mutant was also active against dried collagen films (Fig. 4), thus demonstrating that this activity is strongly dependent on the C-terminal hemopexin domain, a feature shared with the other collagenases (MMP-1, MMP-8, MMP-13 and MT1-MMP) [12,26]. Furthermore, the mutant lacking the fibronectin type II repeats (ΔGBR MMP-2) showed impaired ability to cleave the 3/4 fragments further, indicating that this activity of the wild-type enzyme is dependent on the fibronectin type II repeats (Fig. 1, lane 2). In the presence of the fibronectin-like domain, which binds tightly to

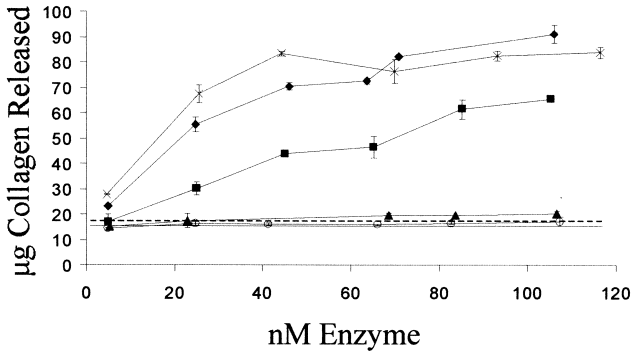

Fig. 2. Cleavage of rat skin type I collagen fibrils by MMP-1, MMP-3, MMP-2 and MMP-2 domain deletion mutants. Cleavage of preformed fibrils of native, acid soluble rat skin type I collagen as measured using ^{14}C labelled collagen. 100 µg ^{14}C acetylated rat skin type I collagen was incubated for 2 h at 35°C to allow fibrils to form. Enzymes were then added and the mixture made up to 300 µl, and incubated for 4 h at 35°C. The graph shows µg collagen released plotted against nM enzyme. ♦, MMP-2; ■, ΔGBR MMP-2; ▲, N-terminal MMP-2; ×, 'superactivated' MMP-1; ○, MMP-3. The solid line shows the amount of collagen that is buffer soluble and the dotted line shows the amount of collagen that is additionally susceptible to trypsin cleavage.

both collagen and gelatin [15,16,19], further breakdown of the 1/4 and 3/4 fragments occurs, even at 25°C, which is generally regarded as insufficient to denature the 3/4 fragment. This suggests that the fibronectin-like domain partially unfolds the specific 1/4 and 3/4 fragments allowing gelatinolysis to occur by MMP-2.

Our data thus confirm that MMP-2 can effect specific collagenolysis and compare favourably with the results of Aimes and Quigley [1] and Konttinen et al. [29]. Seltzer and Eisen [2] have suggested that MMP-2 is able to cleave any collagenous sequence in which the helicity is not perfect. It is clear that our preparations of collagen are highly trypsin resistant, but we have found that different forms (partially soluble or fibrillar) have differing susceptibilities to all proteinases, even the 'true' interstitial collagenases which are markedly more active on soluble collagen (data not shown). Our study has shown that specific 1/4 and 3/4 cleavage of collagen by MMP-2 does occur and it involves co-operation between the catalytic and hemopexin domains, comparable to the other collagenases, rather than between the catalytic and fibronectin-like domains, as occurs for gelatin or cleavage of the 3/4 collagen fragment (further discussed below). Thus the hemopexin domain of all collagenolytic MMPs discovered to date plays a unique role in triple helical substrate recognition and cleavage to the 1/4 and 3/4 fragments. Specific collagenolysis is also dependent on the catalytic domain [30,31] as well as the hinge region [32]. C-Terminal domain exchanges between collagenolytic MMPs do not result in the generation of efficient collagenolytic chimeric enzymes (data not shown), suggesting that these domains cooperate in cleavage of the triple helical substrates using sequence motifs which are not interchangeable between different MMPs of the collagenase subfamily.

Table 1
Specific activities of MMP-1, MMP-2 and mutants of MMP-2 against rat and bovine skin, acid soluble, type I collagen and bovine articular cartilage, acid soluble, type II collagen (units/nmol)

	Rat type I collagen	Bovine type I collagen	Bovine type II collagen
MMP-1	30	15	20
MMP-2	18	13	5
ΔGBR MMP-2	7	–	4
N-Terminal MMP-2	0	–	–
MMP-3	0	0	–
MT1-MMP	3	–	–

The assays were carried out as described in Section 2; type I collagen was incubated at 35°C for 4 h, type II collagen was incubated at 25°C for 2 h. Data are from representative experiments.

3.2. *Quantitative analysis of rat type I, bovine type I and bovine type II collagen degradation and comparison of the specific collagenolytic activity of wild-type and mutant MMP-2 and MMP-1*

For a quantitative analysis, ^{14}C labelled rat type I collagen fibrils were incubated with either MMP-2 or MMP-1 at 35°C and the specific collagenolytic activities were determined. MMP-2 had about 60% of the specific activity of MMP-1 (Fig. 2, Table 1). The comparison of the specific collagenolytic activities of ΔGBR MMP-2 and wild-type MMP-2 revealed that this mutant displayed only 38% of the activity of wild-type MMP-2 (Fig. 2, Table 1), implying that the fibronectin type II repeats potentiate activity of the wild-type enzyme in this assay. For comparison, MT1-MMP had a specific activity that was 10% of that for superactivated MMP-1 (Table 1). At comparable enzyme concentrations neither MMP-3 nor MMP-9 had any activity against rat type I collagen fibrils, thus demonstrating that our type I collagen preparation was resistant to non-specific cleavage, i.e. contained negligible amounts of denatured collagen, and that the other member of the gelatinase subgroup, MMP-9, does not belong to the collagenolytic MMPs.

In order to establish whether the observations made with collagen type I derived from rat skin were due to possible peculiarities of material from this species, we repeated our comparison of MMP-2 and MMP-1 using bovine skin derived acid soluble type I collagen. Fig. 3 shows that MMP-1 cleaved this collagen into the 3/4 fragment whilst MMP-2 rapidly degraded the 3/4 fragment into smaller fragments confirming our results obtained with rat type I collagen. In a quantitative fibrillar assay MMP-2 had 87% of the activity of MMP-1

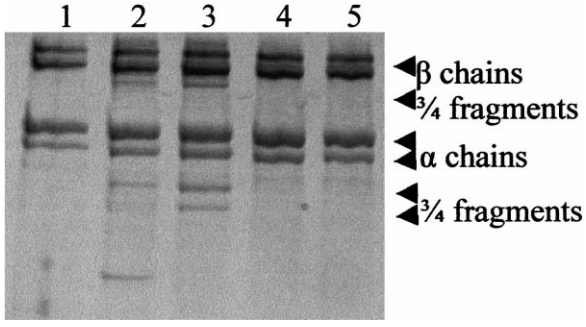

Fig. 3. Polyacrylamide gel electrophoretic analysis of bovine skin type I collagen cleavage by MMP-1, MMP-2 and MMP-3. Cleavage of native, acid soluble fibrillar bovine skin type I collagen. The collagen was incubated for 2 h at 35°C to allow fibrils to form. 100 nM enzyme was added to 1 µM collagen and incubated at 25°C for 2 h. Lanes: 1, collagen incubated with buffer alone; 2, collagen incubated with MMP-2; 3, collagen incubated with 'superactivated' MMP-1; 4, collagen incubated with MMP-3; 5, collagen incubated with 110 nM trypsin.

(Table 1). It is worthwhile noting that the specific activities vary for the different collagens from different species, but we were unable to produce values for kinetic constants due to the variation in the number of substrate cleavage sites for the two enzymes and the fibrillar status of the collagen.

We also compared the different enzymes in their ability to solubilise insoluble films of rat skin type I collagen at 37°C. MMP-1 was most efficient at low enzyme concentrations and MT1-MMP was far less active. In general, the data for MMP-2 and ΔGBR MMP-2 were similar to those obtained using collagen fibrils (Fig. 4). It is interesting to note that MMP-2 cleaves type I collagen more efficiently than MT1-MMP under these conditions.

The ability of MMP-2 to cleave bovine type II collagen was examined by SDS–PAGE analysis of cleavage of the soluble collagen at 25°C (Fig. 5). MMP-2 is capable of cleaving this collagen into the typical 1/4 and 3/4 fragments but to a much lesser extent than 'superactivated' MMP-1. Unlike MMP-1, MMP-2 cleaves the 1/4 and 3/4 fragments further, confirming the data obtained for type I collagen of rat or bovine origin. As a consequence, only specific activity values could be assigned to the MMPs for collagen II cleavage (Table 1).

Our quantitative analysis of type I and II collagen confirmed that the C-terminal region of MMP-2 was required for collagenolysis to occur. Furthermore, these assays revealed that the fibronectin-like domain was required for most efficient cleavage, since ΔGBR MMP-2 displayed only 38% of wild-type MMP-2 activity. This might suggest that the fibronectin type II repeats have a role in promoting efficient enzyme–substrate binding and cleavage or that the fibronectin type II repeats may participate in unfolding the fibrillar substrate. This was further analysed by incubating collagen fibrils with an equimolar amount or two-fold molar excess of an inactive proMMP-2 mutant (E375A MMP-2) in the presence of ΔGBR MMP-2 (data not shown). The effective presence of an excess of the fibronectin-like domain increased the collagenolytic activity of ΔGBR MMP-2, although pro-E375A MMP-2 alone has no activity. However, SDS–PAGE analysis of the 25°C cleavage products from this assay showed only the specific 1/4 and 3/4 cleavage products and no further degradation, as seen with wild-type MMP-2. It is therefore likely that the inactive gelatinase had a modest effect on the physical status or helicity of the collagen only allowing more rapid cleavage by ΔGBR MMP-2.

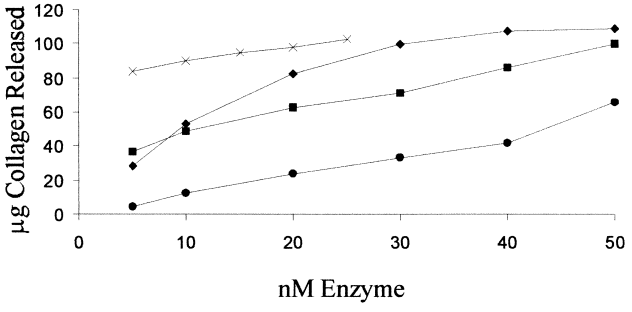

Fig. 4. Cleavage of radiolabelled rat skin type I collagen films by MMP-1, MMP-2, ΔGBR MMP-2 and MT1-MMP. Recombinant enzymes were incubated on dried ^{14}C-collagen films (150 μg) as described in the text. The amount of collagen released after 18 h is shown. The graph shows μg collagen released plotted against nM enzyme; each point represents the mean of triplicate wells. Data are taken from a representative experiment. ◆, MMP-2; ■, ΔGBR MMP-2; ×, 'superactivated' MMP-1; ●, MT1-MMP.

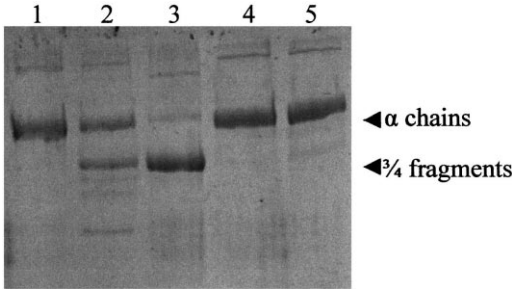

Fig. 5. Polyacrylamide gel electrophoretic analysis of bovine articular cartilage type II collagen cleavage by MMP-1, MMP-2 and MMP-3. 200 nM enzyme was added to 1 μM bovine type II collagen and incubated at 25°C for 2 h. Lanes: 1, collagen incubated with buffer alone; 2, collagen incubated with MMP-2; 3, collagen incubated with 'superactivated' MMP-1; 4, collagen incubated with MMP-3; 5, collagen incubated with 1.6 μM trypsin.

Thus from our analysis of the cleavage events it may be concluded that triple helical collagens are hydrolysed in a novel biphasic mechanism by wild-type MMP-2. The initial cleavage generating the 1/4 and 3/4 fragments is strongly dependent on the presence of the hemopexin-like C-terminal domain, while further hydrolysis is promoted by the fibronectin type II repeats, resulting in rapid clearance of the initial degradation products.

The importance of MMP-2 in the cleavage of triple helical collagen is not clear, since MMP-2 can be localised at the cell surface 'receptor' formed by MT1-MMP–TIMP2 complexes [23], it could play a role in focal collagenolysis, as MT1-MMP itself may also do. The collagenolytic capacity of MT1-MMP is relatively modest against fibrillar or insoluble collagen. A major function of MMP-2 in this situation could therefore still be in the potentiation of the initial cleavage and the further degradation of denatured collagen [33].

Acknowledgements: We thank S. Baker and M. Harrison for technical assistance and T. Cawston for the generous gift of bovine type I collagen. This study was funded by the Arthritis Research Council (M.L.P.), the Medical Research Council (S.J.A.) and the Wellcome Trust (V.K.).

References

[1] Aimes, R.T. and Quigley, J.P. (1995) J. Biol. Chem. 270, 5872–5876.
[2] Seltzer, J.L. and Eisen, A.Z. (1999) J. Invest. Dermatol. 112, 993–993.
[3] Jeffrey, J.J. (1998) in: Matrix Metalloproteinases (Parks, C.W. and Mecham, R.P., Eds.), pp. 15–38, Academic Press, San Diego, CA.
[4] Birkedal-Hansen, H., Moore, W.G.I., Bodden, M.K., Windsor, L.J., Birkedal-Hansen, B., DeCarlo, A. and Engler, J.A. (1993) Crit. Rev. Oral Biol. Med. 4, 197–250.
[5] Netzel-Arnett, S., Fields, G., Birkedal-Hansen, H. and Van Wart, H.E. (1991) J. Biol. Chem. 266, 6747–6755.
[6] Clark, I.M. and Cawston, T.E. (1989) Biochem. J. 263, 201–206.
[7] Allan, J.A., Hembry, R.M., Angal, S., Reynolds, J.J. and Murphy, G. (1991) J. Cell Sci. 99, 789–795.
[8] Murphy, G., Willenbrock, F., Ward, R.V., Cockett, M.I., Eaton, D. and Docherty, A.J.P. (1992) J. Biol. Chem. 267, 9612–9618.
[9] Knäuper, V., López-Otin, C., Smith, B., Knight, G. and Murphy, G. (1996) J. Biol. Chem. 271, 1544–1550.
[10] Knäuper, V., Cowell, S., Smith, B., López-Otin, C., O'Shea, M.,

Morris, H., Zardi, L. and Murphy, G. (1997) J. Biol. Chem. 272, 7608–7616.
[11] Bode, W. (1995) Structure 3, 527–530.
[12] Murphy, G. and Knäuper, V. (1997) Matrix Biol. 15, 511–518.
[13] Morgunova, E., Tuuttila, A., Bergmann, U., Isupov, M., Lindqvist, Y., Schneider, G. and Tryggvason, K. (1999) Science 284, 1667–1670.
[14] Murphy, G., Nguyen, Q., Cockett, M.I., Atkinson, S.J., Allan, J.A., Knight, C.G., Willenbrock, F. and Docherty, A.J.P. (1994) J. Biol. Chem. 269, 6632–6636.
[15] Banyai, L., Tordai, H. and Patthy, L. (1994) Biochem. J. 298, 403–407.
[16] Steffensen, B., Wallon, U.M. and Overall, C.M. (1995) J. Biol. Chem. 270, 11555–11566.
[17] Ottl, J., Battistuta, R., Pieper, M., Tschesche, H., Bode, W., Kühn, K. and Moroder, L. (1996) FEBS Lett. 398, 31–36.
[18] Ottl, J., Gabriel, D., Murphy, G., Knäuper, V., Tominaga, Y., Nagase, H., Kroger, M., Tschesche, H., Bode, W. and Moroder, L. (2000) Chem. Biol. 7, 119–132.
[19] Allan, J.A., Docherty, A.J.P., Barker, P.J., Huskisson, N.S., Reynolds, J.J. and Murphy, G. (1995) Biochem. J. 309, 299–306.
[20] Wallon, U.M. and Overall, C.M. (1997) J. Biol. Chem. 272, 7473–7481.
[21] Murphy, G., Allan, J.A., Willenbrock, F., Cockett, M.I., O'Connell, J.P. and Docherty, A.J.P. (1992) J. Biol. Chem. 267, 9612–9618.
[22] Koklitis, P.A., Murphy, G., Sutton, C. and Angal, S. (1991) Biochem. J. 276, 217–221.
[23] Butler, G.S., Butler, M.J., Atkinson, S.J., Will, H., Tamura, T., Van Westrum, S.S., Crabbe, T., Clements, J., D'Ortho, M.-P. and Murphy, G. (1998) J. Biol. Chem. 273, 871–880.
[24] Laemmli, U.K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599.
[25] Willenbrock, F., Crabbe, T., Slocombe, P.M., Sutton, C.W., Docherty, A.J.P., Cockett, M.I., O'Shea, M., Brocklehurst, K., Phillips, I.R. and Murphy, G. (1993) Biochemistry 32, 4330–4337.
[26] Cawston, T.E. and Murphy, G. (1981) Methods Enzymol. 80, 711–721.
[27] Atkinson, S.J., Patterson, M.L., Butler, M.J. and Murphy, G. (2001) FEBS Lett. 491, 222–226.
[28] Ohuchi, E., Imai, K., Fujii, Y., Sato, H., Seiki, M. and Okada, Y. (1997) J. Biol. Chem. 272, 2446–2451.
[29] Konttinen, Y.T., Ceponis, A., Takagi, M., Ainola, M., Sorsa, T., Sutinen, M., Salo, T., Ma, J., Santavirta, S. and Seiki, M. (1998) Matrix Biol. 17, 585–601.
[30] Chung, L., Shimokawa, K., Dinakarpandian, D., Grams, F., Fields, G.B. and Nagase, H. (2000) J. Biol. Chem. 275, 29610.
[31] Knäuper, V., Patterson, M.L., Gomis-Rüth, F.X., Smith, B., Lyons, A., Docherty, A.J. and Murphy, G. (2001) Eur. J. Biochem. 268, 1888–1896.
[32] Knäuper, V., Docherty, A.J., Smith, B., Tschesche, H. and Murphy, G. (1997) FEBS Lett. 405, 60–64.
[33] d'Ortho, M.-P., Will, H., Atkinson, S., Butler, G.S., Messent, A., Gavrilovic, J., Smith, B., Timpl, R., Zardi, L. and Murphy, G. (1997) Eur. J. Biochem. 250, 751–757.