A Novel Mechanism of Latency in Matrix Metalloproteinases*

Mar López-Pelegrín 1, Miroslaw Ksiazek 5, Abdulkarim Y. Karim 61, Tibisay Guevara 1, Joan L. Arolas 2, Jan Potempa 4,5, and F. Xavier Gomis-Rüth 1,4

From the 1,2 Proteolysis Lab, Department of Structural Biology, Molecular Biology Institute of Barcelona, CSIC, Barcelona Science Park, c/Baldri Reixac, 15-21, 08028 Barcelona, Catalonia, Spain, the 3 Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Ul. Gronostajowa 7, 30-387 Kraków, Poland, and the 4 Oral Immunology and Infectious Disease, University of Louisville School of Dentistry, Louisville, Kentucky 40202

Background: Animal and plant matrix metalloproteinases (MMPs) are kept zymogenic through large prodomains and a cysteine-switch mechanism. Results: Bacterial MMP karilysin has only a short N-terminal peptide upstream of the catalytic domain, which lacks cysteines. Conclusion: This peptide inhibits through an aspartate-switch mechanism and also exerts other functions of authentic prodomains. Significance: Karilysin is kept latent by a novel mechanism for MMPs.

The matrix metalloproteinases (MMPs) are a family of secreted soluble or membrane-anchored multimodular peptidases regularly found in several paralogous copies in animals and plants, where they have multiple functions. The minimal consensus domain architecture comprises a signal peptide, a 60–90-residue globular prodomain with a conserved sequence motif including a cysteine engaged in “cysteine-switch” or “Velcro” mediated latency, and a catalytic domain. Karilysin, from the human periodontopathogen Tannerella forsythia, is the only bacterial MMP to have been characterized biochemically to date. It shares with eukaryotic forms the catalytic domain but none of the flanking domains. Instead of the consensus MMP prodomain, it features a 14-residue propeptide, the shortest reported for a metallopeptidase, which lacks cysteines. Here we determined the structure of a prokarilysin fragment encompassing the propeptide and the catalytic domain, and found that the former runs across the cleft in the opposite direction to a bound substrate and inhibits the latter through an “aspartate-switch” mechanism. This finding is reminiscent of latency maintenance in the otherwise unrelated astacin and fragilysin metallopeptidase families. In addition, in vivo and biochemical assays showed that the propeptide contributes to protein folding and stability. Our analysis of prokarilysin reveals a novel mechanism of latency and activation in MMPs. Finally, our findings support the view that the karilysin catalytic domain was co-opted by

...
strains (21). Structural studies supported the view that the catalytic domain of this MP is the result of horizontal gene transfer of a member of the ADAM/adamalysin family, which has 38 orthologs in humans (8, 22–25), from a mammalian host to this bacterium, which thrives in the intestinal tract (26, 27).

Returning to MMPs, karilysin from the human periodontopathogen Tannerella forsythia is the only bacterial family member to have been analyzed biochemically to date (9, 28–33). In addition to karilysin, only MmpZ from Bacillus anthracis has been functionally assessed at the genetic level through knock-out studies in B. anthracis cells, but it has not been isolated or characterized (34). Similarly to vertebrate MMPs, karilysin showed preference for medium-sized to bulky hydrophobic residues (leucine, tyrosine and methionine) in the specificity pocket, S1 (Ref. 30; for active-site cleft subsite nomenclature, see Ref. 35). It inactivates antimicrobial peptide LL-37 and integrins (see Ref. 35). It inactivates antimicrobial peptide LL-37 and integrants of the complement system, including ficolin-2, ficolin-3, C4, and C5, by proteolysis and may thus contribute to evasion of the innate host immune response (29, 31). Karilysin is sequentially and evolutionarily closer to MMPs from winged insects that are transmission vectors of human diseases (47% sequence identity with Dm1 from Aedes aegypti and Anopheles gambiæ; (9)) and mammals (44% identity with human MMP-11, -13, and -20 (9)) than to the few other bacterial sequences found in genomic sequences. Accordingly it was likewise suggested that it may be the result of horizontal gene transfer of an MMP gene from an animal to an intimate bacterial pathogen, which inhabits a biofilm on the tooth surface in humans (9).

The metzincins are characterized by a consensus sequence responsible for binding of the catalytic zinc ion (CSBZ), H-E-X-X-H-X-X-(G/N)-X-X-(H/D) (amino acid one-letter code; X stands for any residue), and a conserved methionine-containing turn, the “Met-turn” (1–5, 36). In MMPs, the CSBZ encompasses three histidine zinc ligands, the general base/acid glutamate for catalysis, and a structurally relevant glycine (3). In addition, the distinct MMP paralogs are multidomain proteins that display a disparate domain organization that is the result of successive polyplication, gene fusion, and exon shuffling (11). The only domains common to all animal and plant MMPs are a signal peptide, which is removed after secretion, a prodomain and a catalytic domain, as found, e.g. in human MMP-7 and MMP-26, and in plant MMPs (12, 16, 18).

Most peptidases are biosynthesized as zymogens containing prosegments, which are required for latency maintenance to prevent unbridled activity but also sometimes to assist in proper folding of the usually downstream catalytic moieties (37–40). Metzincin exceptions lacking prosegments include the archaemetzincins, for which no hydrolytic activity has so far been reported, i.e. they might not need to be kept latent (41, 42); the toxilysin EcxA from Escherichia coli, whose soluble expression requires co-expression with its cognate EcxB subunit, thus pointing to a chaperone-like function for this ancillary subunit (43–45); the cholerlysin StEcE from E. coli, for which an N-terminal immunoglobulin-like domain may assist the downstream catalytic moiety in proper folding (46); and igalyssins, where an all-β-domain of similar topology to immunoglobulin-like domains is likewise found at the N terminus of the catalytic moiety (see Protein Data Bank (PDB) access codes 4DF9 and 3P1V and Ref. 5).

MMP prodomains (see Table 1 in Ref. 47) span 60–90 residues and include a conserved sequence motif, P-R-C-G-(V/N)-P-D, engaged in a “cysteine-switch” or “Velcro” mechanism of latency (10, 16, 48–51). It has been suggested that this mechanism may be shared by variants within other metzincin families, for which conserved cysteines were described upstream of the catalytic domain, such as the ADAMs/adamalysins (motif P-K-M-C-G-V (8, 52–54)), leishmanolysins (motif H-R-C-I-H-D (2)), and pappalysins (motif C-G (55)). In contrast, the 472 residues encoded by the karilysin gene (see UniProt sequence database access code D0EM77) only comprise a short 14-residue potential propeptide, which lacks cysteines, between the 20-residue signal peptide and the 161-residue mature catalytic moiety (Fig. 1A). A C-terminal domain of 277 residues of unknown function and sequence unrelated to any domain found in eukaryotic MMPs completes the protein. This strongly suggests a potentially different mechanism of latency maintenance, hitherto unseen not only in MMPs but also in metzincins in general, as the shortest prosegments described to date are those of members of the astacin family, which span >34 residues (7, 56–58).

We had previously determined the structure of the catalytic domain of karilysin (termed Kly18 (9)). To shed light on the molecular determinants of the first mechanism of latency maintenance of a bacterial MMP, in this work we assayed the possible function of the propeptide in folding, stability, and activity inhibition of Kly18. We further solved the x-ray crystal structure of an active-site mutant of a construct spanning the propeptide and Kly18 affecting the catalytic glutamate, pKly18-E156A, to circumvent autolysis. The mechanism derived was supported by site-directed mutagenesis and it is discussed in the context of general MMP latency maintenance.

**EXPERIMENTAL PROCEDURES**

**Protein Production and Purification**—The gene coding for full-length wild-type T. forsythia prokarilysin without the 20-residue signal peptide (hereafter pKly; 52 kDa; residues Gln21–Lys7472 according to UP D0EM77, see also Fig. 1A) was cloned at BamHI and XhoI restriction sites into vector pGEX-6P-1 (GE Healthcare) as described elsewhere (30). The resulting vector, pKAR1 (see Table 1 for an overview of vectors and constructs used), confers resistance toward ampicillin and attaches an N-terminal glutathione S-transferase (GST) moiety followed by a human rhinovirus 3C proteinase (HR3CP) recognition site (L-E-V-L-F-Q-↓G-P; HR3CP cleavage leaves two extra residues, underlined, at the N terminus of the recombinant protein after digestion; three extra residues, L-G-S, are further present due to the cloning strategy). Single-residue point mutants pKly-Y35A and pKly-E156A (pKAR2 and pKAR3, respectively) were generated using the QuikChange Site-directed Mutagenesis Kit (Stratagene) according to the manufacturer’s instructions as described (30). Double mutant pKly-D25A/Y35A (pKAR4) was similarly generated using pKAR2 as a template. Genes coding for the E156A-mutated catalytic domain of karilysin, with and without the propeptide...
Structure of T. forsythia Prokarilysin

TABLE 1
Vectors and constructs

| Name       | Original vector       | Antibiotic resistance | Restriction sites | Insert                     | Fusion construct (N-terminal) | Additional N-terminal residues |
|------------|-----------------------|-----------------------|-------------------|----------------------------|-------------------------------|--------------------------------|
| pKAR1      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | wt Gln²¹-Lys⁶⁷² (pKly)     | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR2      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | Y35A mutant Gln²¹-Lys⁶⁷² (pKly-Y35A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR3      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | E156A mutant Gln²¹-Lys⁶⁷² (pKly-E156A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR4      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | D25A/Y35A mutant Gln²¹-Lys⁶⁷² (pKly-D25A/Y35A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR5      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | E156A mutant Tyr²⁵-Ser²⁰¹ (pKly-E156A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR6      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | E156A mutant Tyr²⁵-Ser²⁰¹ (Kly18-E156A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR7      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | wt Gln²¹-Ser²⁰¹ (pKly18)   | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR8      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | Y35A mutant Gln²¹-Ser²⁰¹ (pKly18-Y35A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR9      | pGEX-GP-1 (GE Healthcare) | amp                    | BamHI/Xhol        | D25A/Y35A mutant Gln²¹-Ser²⁰¹ (pKly18-D25A/Y35A) | GST + HR3CPr                   | G-P-G-S²                      |
| pKAR10     | pCR1-7a (59)           | kan                    | Ncol/Xhol         | wt Gln²¹-Ser²⁰¹ (pKly18)   | None                           | G-P-G-S²                      |
| pKAR11     | pCR1-7a (59)           | kan                    | Ncol/Xhol         | E156A mutant Gln²¹-Ser²⁰¹ (pKly18-E156A) | None                           | M-G                            |
| pKAR12     | pCR1-7a (59)           | kan                    | Ncol/Xhol         | Tyr²⁵-Ser²⁰¹ (Kly18)       | None                           | M-G                            |
| pKAR13     | pCR1-7a (59)           | kan                    | Ncol/Xhol         | E156A mutant Tyr²⁵-Ser²⁰¹ (Kly18-E156A) | None                           | M-G                            |

* a amp, ampicillin; GST, glutathione S-transferase; HR3CPr, recognition sequence for human rhinovirus 3C peptidase; kan, kanamycin; mut., mutant; wt, wild-type.
* After cleavage with HR3CP.

Proteins were produced by vectors pKAR10–pKAR13 were produced in E. coli BL21(DE3) cells, which were grown at 37 °C in Luria-Bertani medium supplemented with 30 μg/ml of kanamycin. Cultures were induced at an A₆₀₀ of 0.8 with 0.2–1 mM isopropyl β-D-thiogalactopyranoside and incubated either for 5 h at 37 °C or overnight at 18 °C. Cells were harvested by centrifugation at 7,000 × g for 30 min at 4 °C, washed in buffer B, and resuspended in the same buffer, and further lysed in an ice-bath using a digital sonifier (Branson). After centrifugation at 15,000 × g for 30 min at 4 °C, both cell debris and supernatant were analyzed by 15% Tricine-SDS-PAGE stained with Coomassie Blue.

Protein identity and purity were assessed by mass spectrometry using an Autoflex Bruker apparatus and N-terminal sequencing through Edman degradation at the Proteomics Facility of Centro de Investigaciones Biológicas (Madrid, Spain). Ultrafiltration steps were performed with Vivaspin 15 and Vivaspin 4 filter devices of 5-kDa cut-off (Sartorius Stedim Biotech). Approximate protein concentration was determined by measuring A₆₀₀ in a spectrophotometer (NanoDrop) using the calculated absorption coefficients E₀.1% = 2.32 and 2.42 for pKly18-E156A and Kly18-E156A, respectively.

**Autolytic Activation and Propeptide Inhibitory Activity Assays**—Mutants pKly-Y35A (from pKAR2), pKly-D25A/Y35A (pKAR4), and pKly18-Y35A (pKAR8) were incubated in buffer B at 37 °C and at 0.4 mg/ml final protein concentration for up to 120 h to assay autolysis. Reactions were stopped at specific time points by boiling aliquots in reducing/denaturing buffer, and samples were further analyzed by 10% or 15% Tricine-SDS-PAGE stained with Coomassie Blue. Kly18, obtained by autolysis from pKAR1-encoded protein, was incubated at 0.025 μg/ml of final protein concentration for 30 min with 0.1–10 mM pep tide Q-R-L-Y-D-N-G-P-L-T (purchased from GL Biochem Ltd.), which mimics the propeptide sequence. Proteolytic activity was subsequently measured at 37 °C in buffer C on substrate Mca-R-P-K-P-V-E-Nva-W-R-K(dnp)-NH₂ (Bachem; at 10 μM) in a microplate fluorimeter (Infinite M200, Tecan).

**Thermal Shift Assays**—Aliquots were prepared by mixing 7.5 μl of 300 Sypro Orange dye (Molecular Probes) and 42.5 μl of either pKly18-E156A (from pKAR5) or Kly18-E156A (pKAR6) at 1–2 mg/ml in buffer C in the absence and presence of 1–5 mM (hereafter pKly18-E156A and Kly18-E156A; 20 and 18 kDa; residues Gln²¹-Ser²⁰¹ and residues Tyr²⁵-Ser²⁰¹, respectively), were also cloned into vector pGEX-6P-1 (pKAR5 and pKAR6, respectively). Genes coding for pKly18 and its mutant proteins pKly18-Y35A and pKly18-D25A/Y35A were cloned into the same vector (pKAR7, pKAR8, and pKAR9, respectively) following a strategy previously described (59). Genes coding for pKly18, pKly18-E156A, Kly18, and Kly18-E156A were, furthermore, cloned at NcoI and XhoI restriction sites into vector pCRI-7a (59), which confers resistance toward kanamycin and does not attach fusion proteins (pKAR10–pKAR13, respectively). In these cases, the cloning strategy entailed that residues M-G were attached at the N terminus. All constructs were verified by DNA sequencing.

Proteins encoded by vectors pKAR1–pKAR9 were produced by heterologous overexpression in E. coli BL21(DE3) cells, which were grown at 37 °C in Luria-Bertani medium supplemented with 100 μg/ml of ampicillin. Cultures were incubated at an A₆₀₀ of 0.8 with 0.2 mM isopropyl β-D-thiogalactopyranoside and incubated overnight at 18 °C. Purification of wild-type and mutant pKly, and subsequent autolysis of the former to obtain Kly18, was achieved as described elsewhere (30). In turn, pKly18-E156A, Kly18-E156A, pKly18-Y35A, and pKly18-D25A/Y35A were purified as follows. After centrifugation at 7,000 × g for 30 min at 4 °C, the pellet was washed twice in 1× PBS, and resuspended in the same buffer supplemented with EDTA-free protease inhibitor mixture tablets and DNase I (both Roche Diagnostics). Cells were lysed using a cell disruptor (Constant Systems, Ltd.) at 1.35 Kbar, and the cell debris was removed by centrifugation at 40,000 × g for 1 h at 4 °C. The supernatant was filtered (0.22 μm pore size; Millipore), and incubated with glutathione-Sepharose 4B resin (GE Healthcare). The sample was washed first in 1× PBS and then in buffer A (50 mM Tris-HCl, 150 mM NaCl, pH 7.5), and eluted by incubation and cleavage with HR3CPr at a 1:20 enzyme:substrate (w/w) ratio for 48 h at 4 °C. The protein was concentrated by ultrafiltration, and finally purified by size-exclusion chromatography on 16/600 or 10/300 Superdex 75 columns (GE Healthcare) previously equilibrated with buffer B (20 mM Tris-HCl, pH 8.0) or buffer C (20 mM Tris-HCl, 150 mM NaCl, pH 7.5).
CaCl$_2$. Four replicates of each aliquot were analyzed in an iQ5 Multi-Color Real Time PCR Detection System (Bio-Rad) in 96-well PCR plates sealed with optical tape. Samples were heated from 30 to 95 °C at 0.5 °C/min, and the change in absorbance ($\lambda_{\text{ex}} = 490 \text{ nm}$; $\lambda_{\text{em}} = 575 \text{ nm}$) was monitored over time. The temperature of midtransition ($T_m$) was determined for both proteins from the inflection point of each curve using iQ5 software.

**Crystallographic data**

| Data | Value |
|------|-------|
| Space group | $P2_1/36.18, 121.69, 41.88$ |
| Cell constants (Å) | $a = 105.26$ |
| Wavelength (Å) | 0.97242 |
| No. of measurements/uniquereflexions | $104,406/22,975$ |
| Resolution range (Å)(outermost shell) | $60.8–2.01 (2.06–2.01)$ |
| Completeness (%) | 99.8 (96.2) |
| $R_{	ext{merge}}$ | 0.105 (0.534) |
| $R_{	ext{int}}$ (same reflections) | 0.116 (0.616)/99.6 (84.3) |
| Average intensity | 103 (2.5) |
| B-factor (Wilson) (Å$^2$/average multiplicity | 29.0/4.5 (4.0) |
| Resolution range used for refinement (Å) | $<2.01$ |
| No. of reflections used (test set) | 22,047 (741) |
| Crystallographic $R_{	ext{free}}$ (free $R_{	ext{free}}$) | 0.165 (0.194) |
| No. of protein atoms/solvent molecules/ligands/ions | 2 calcium |
| Root mean square deviation target values bonds (Å)/angles (°) | 0.010/0.98 |
| Overall average $R$-factor (Å) | 30.8 |
| Model validation | 345 (97.5%)/0/355 |
| Values in parentheses refer to the outermost resolution shell. Values in parentheses refer to the outermost resolution shell. |

**TABLE 2**

| Crystallographic data | Value |
|-----------------------|-------|
| Space group | $P2_1/36.18, 121.69, 41.88$ |
| Cell constants (Å) | $a = 105.26$ |
| Wavelength (Å) | 0.97242 |
| No. of measurements/uniquereflexions | $104,406/22,975$ |
| Resolution range (Å)(outermost shell) | $60.8–2.01 (2.06–2.01)$ |
| Completeness (%) | 99.8 (96.2) |
| $R_{	ext{merge}}$ | 0.105 (0.534) |
| $R_{	ext{int}}$ (same reflections) | 0.116 (0.616)/99.6 (84.3) |
| Average intensity | 103 (2.5) |
| B-factor (Wilson) (Å$^2$/average multiplicity | 29.0/4.5 (4.0) |
| Resolution range used for refinement (Å) | $<2.01$ |
| No. of reflections used (test set) | 22,047 (741) |
| Crystallographic $R_{	ext{free}}$ (free $R_{	ext{free}}$) | 0.165 (0.194) |
| No. of protein atoms/solvent molecules/ligands/ions | 2 calcium |
| Root mean square deviation target values bonds (Å)/angles (°) | 0.010/0.98 |
| Overall average $R$-factor (Å) | 30.8 |
| Model validation | 345 (97.5%)/0/355 |
| Values in parentheses refer to the outermost resolution shell. |

**RESULTS AND DISCUSSION**

**Roles of the Propeptide in Vitro and in Cellula—Wild-type karilysin is secreted as azymogen with a 14-residue N-terminal propeptide (31Q-R-L-Y-D-N-G-P-L-T-G-D-N-N$^{34}$), which is cleaved off at position Asn$^{34}$-Tyr$^{35}$ during maturation (Fig. 1A). This is the primary activation cleavage and it releases an active 48-kDa form (Kly48 (30)). In recombinant protein production, subsequent cleavages within the C-terminal domain give rise to Kly38 and, finally, to a stable form of 18 kDa (Kly18), which corresponds to the isolated mature catalytic domain (CD) (5, 28, 30, 33). These cleavages were shown to be autolytic as activation was repressed by general chelating MP inhibitors and in the inactive active-site variant, E156A, which ablated the catalytic glutamate of the CSBZ (1, 5, 30, 79, 80). In addition, cleavage-site mutant Y35A, which does not match the specificity of the enzyme, was activated only slowly when compared with the wild-type (30, 33).

To assess whether the propeptide had a chaperone-like function on the downstream catalytic moiety, we cloned the genes encoding pKly18-E156A and Kly18-E156A in a vector that does not attach a fusion protein at the N terminus that would assist...
in proper folding (pKAR11 and pKAR13, respectively; see “Experimental Procedures”). We found that the active-site mutant pKly18-E156A was successfully overexpressed in soluble form (Fig. 1B). In contrast to the zymogen, Kly18-E156A was produced only in insoluble form (Fig. 1B). Moreover, when expressed from the pKAR6 vector, which attaches an N-terminal glutathione S-transferase fusion protein (see Table 1), Kly18-E156A was obtained with a 10 times lower yield than the proprotein (vector pKAR5). We conclude that the propeptide plays a major role in proper folding of Kly18 as previously described for other MPs such as fragilysin (26, 27), funnelin metallocarboxypeptidases (79, 81, 82), and ADAMs/adamalysins (54) but not for mammalian MMPs (83).

We further examined the effect of the propeptide in response to denaturation by a thermal shift assay following the thermostability approach (60). Purified pKly18-E156A (pKAR5) showed two unfolding transitions compatible with unfolding of propeptide and CD, with a Tm value of 60 ± 0.5 °C (Fig. 1C). In contrast, the unfolding of purified Kly18-E156A (pKAR6) showed a single transition, with a Tm of 49 ± 2.2 °C. The addition of a physiological concentration of calcium resulted in a substantial increase in stability of both pKly18-E156A and Kly18-E156A. Accordingly, the former showed Tm values of 67.5 ± 1.7 and 76.5 ± 1.2 °C, and 69.5 ± 1.7 and 79 ± 2.2 °C in the presence of 1 and 5 mM CaCl2, respectively, whereas those of Kly18-E156A were 52.5 ± 1.2 and 54.5 ± 1 °C. This result is in agreement with the important role of calcium in Kly18 activity, as addition of 2–5 mM CaCl2 is reported to enhance activity about three times (30). Thus, regardless of calcium, the 14-residue propeptide redounded to a dramatic increase in Tm, underpinning that it plays a major role in the thermal stability of the zymogen. Finally, we assayed the effect of a decapeptide spanning propeptide sequence Gln21–Thr30 on the activity of purified mature Kly18 (from pKAR1) in the presence of a fluorogenic peptide substrate (Fig. 1D). We observed a weak but consistently concentration-dependent inhibitory effect as previously shown for other MPs when their propeptides or prodomains were added in trans, among others funnels (79, 81), ADAMs/adamalysins (84), and mammalian MMPs (85–87). Summarizing, the propeptide of karilysin is the shortest currently described for an MP and it exerts all roles, which collectively or selectively had been previously described for peptidase propeptides or prodomains: latency maintenance, folding assistance during biosynthesis, stability to thermal denaturation, and inhibition of peptidolytic activity (38, 39, 81).

Overall Structure of pKly18—Due to rapid autolytic processing of recombinant wild-type prokarilysin (30), crystals of pKly18 could only be obtained for an inactive variant affecting the catalytic glutamate (pKly18-E156A), as already reported for other MPzymogens (88–92). This protein crystallized as monoclinic crystals diffraction to 2 Å resolution with two molecules per asymmetric unit. These were essentially identical (Ca-atom
Structure of T. forsythia Prokarilysin

The 14-residue propeptide starts at the front right and runs in extended conformation across the active-site cleft, thus blocking access to the cleft, though in the opposite direction to a substrate, *i.e.* right to left (Fig. 2, A and C). This reverse orientation of the propeptide in the cleft may contribute to attenuate autolysis, as previously suggested for zymogens of cysteine peptidases and mammalian MMPs (39). The interaction with the CD buries a surface of $2,100 \pm 35 \text{ Å}^2$, which is much larger than the average of monomeric protein-protein domain intra-chain interfaces (1,193 Å$^2$ (96)) but is slightly lower than the range of typical MMP-protein inhibitor interaction surfaces (2,400–2,700 Å$^2$; see Ref. 97). The interaction includes 13 hydrogen bonds, a double salt bridge, one metalorganic bond, and hydrophobic carbon-carbon contacts between eight residues from the propeptide and 11 from the CD (see Table 3). Segments involved include almost the entire propeptide (Arg$^{22}$, Gly$^{31}$) and, from the CD, mainly Asn$^{111}$-Tyr$^{120}$ from the bulge-edge segment and the upper-ring strand, and Pro$^{175}$-Tyr$^{177}$ from the S$_1$'-wall forming segment. Further involved are Tyr$^{106}$, Ala$^{124}$, and Glu$^{138}$ and the zinc-liganding histidine side chains. Four inter-main chain hydrogen bonds form on the primed side of the cleft (two with the S$_1$'-wall forming segment and two with the bulge-edge segment and strand $\beta$IV) and three more on the upstream non-primed side (with $\beta$IV and L$\beta$IV$\beta$V; see Fig. 2C). In particular, Arg$^{22}$ contacts the base of the S-loop: it doubly salt bridges Glu$^{138}$, which is also one of the calcium ligands (see above, Table 3 and Fig. 2B), and hydrogen bonds three carboxyl oxygens of the S-loop, Asn$^{111}$, Gly$^{113}$, and Thr$^{112}$, which, again, is also a calcium ligand. In addition, the Arg$^{22}$ carbonyl oxygen binds the S$_1$'-wall forming segment and its side chain performs a hydrophobic interaction with Leu$^{115}$. Accordingly, this residue plays a major role in the stabilization of the Ca$^{997}$ site and, thus, the zymogen in general, which explains its enhanced stability in response to thermal denaturation (see above). In addition, superposition of pKly18-E156A onto mature Kly18 in complex with a tetrapeptidic cleavage product in the primed side (see below) and human MMP-8 with a modeled substrate traversing its cleft based on inhibitor structures (98) indicates that Arg$^{22}$ occupies the S$_1$' position of the cleft. However, the most important interaction of the propeptide with the CD is exerted by Asp$^{99}$, which approaches the catalytic zinc from the top and monodentately occupies through its O$_{61}$ atom the fourth position of the tetrahedral coordination sphere of the metal (2.00–2.04 Å apart; Fig. 2C) further to His$^{155}$, His$^{159}$, and His$^{165}$ O$_{62}$ atoms. The preceding carbonyl group of Tyr$^{24}$ binds strand $\beta$IV, and its aromatic side chain penetrates the deep hydrophobic S$_1'$ pocket, mainly interacting with the...
Structure of T. forsythia Prokarilysin

His\textsuperscript{155} ring face-to-face. The π-rings are \( \sim 3.5 \) Å apart and parallel but slightly displaced along the ring planes to form a half-overlapping sandwich, which gives rise to an optimal π-stacked structure (99). Downstream in the chain, Pro\textsuperscript{28} is in a pocket, probably S\textsubscript{2}, framed by His\textsuperscript{159}, Glu\textsuperscript{164}, and Tyr\textsuperscript{120}, the latter two interact through a tight hydrogen bond (Tyr\textsuperscript{120} O\textsuperscript{η}-Glu\textsuperscript{164} O\textsubscript{ε2},...
2.61 Å). Residue Leu29 is surrounded by the side chains of Tyr106, His117, and Phe119, which may feature S3 (Fig. 2C). After Gly31, the polypeptide abandons the active-site cleft moving outward to reach the primary activation cleavage point, Asn34-Tyr35 (Fig. 2A), after which the chain folds back toward the molecular moiety and enters strand β9252 of the NTS β-sheet.

**A Novel Activation Mechanism in MMPs—** Previous work had yielded three structures of mature wild-type Kly18 in complexes with tri- and tetrapeptidic cleavage products, as well as an inhibitory tetrapeptide in the non-primed side of the cleft (PDB 2XS3, 2XS4, and 4IN9 (9, 28)). These were obtained both in the presence and absence of magnesium and showed deviating chain traces for segment Asn53-His57 (L9252Iα) in the two molecules found in the asymmetric unit of the magnesium unbound structure (PDB 2XS3 (9)) and in the single molecules found in magnesium-bound (PDB 2XS4 (9)) and inhibitor-bound.

**FIGURE 2.** Overall structure of pKly18-E156A. A, ribbon-type plot of pKly18-E156A in standard orientation (35). Depicted are the propeptide (ribbon and carbon atoms in turquoise) and the mature enzyme moiety (β-strands in yellow labeled β-βV; α-helices in salmon labeled αA-αC; and coils and carbon atoms in tan). Further shown are the catalytic zinc ion (Zn999; bottom magenta sphere), the structural zinc ion (Zn998; top magenta sphere), and the structural calcium ion (Ca997; red sphere), as well as the side chains of the three catalytic zinc ligands (His155, His159, and His165), the Met turn methionine (Met173), the alanine replacing the catalytic glutamate (Ala156), and residues flanking the primary activation cleavage point, Asn34-Tyr35.

B, close-up of the window of A as stick model highlighting the structural zinc and calcium sites. Protein segments depicted are Asn101-Gly105 from the first part of the S-loop (carbons in gold), Asp109-Phe119 from the second part of the S-loop (carbons in sandy brown), His131-Glu138 from βV-βVαB (carbons in tan), and the side chain of Arg22 from the propeptide (carbons in turquoise). The zinc is bound by His102 N2, Asp104 O2, His117 N2, and His133 N1 at distances 1.99–2.06 Å, and the calcium is bound by Asp109 O1, Gly110 O, Thr112 O, Ile114 O, Asp135 O2, and Glu138 O2 at distances 2.34–2.39 Å. These distances agree with standard zinc- (1.99–2.09 Å; (109)) and calcium-binding (2.36–2.39 Å; (109)) distance values for oxygens and nitrogens. C, close-up of A in wall-eye stereo after a horizontal 30° rotation upwards. Selected hydrogen and ionic bonds (see also Table 3) are depicted as green lines. Residues and ions labeled in A are not labeled here for clarity.

D, superposition in wall-eye stereo of pKly18-E156A (ribbon in tan for the mature enzyme moiety and in brown for the propeptide, zinc ions in magenta, and calcium ion in red; stick model for the side chains of Ser12-Tyr35 with carbons in brown) and Kly18 (ribbon and zinc ions in pink; see PDB 2XS3, molecule A (9)), which was obtained in a product complex with peptide A-F-T-S bound to the primed side of the cleft (stick model with carbons in gold). Tyr35 is shown for both structures. E, detail of D in wall-eye stereo depicting the large rearrangement of the N terminus at Tyr35 after maturation cleavage at Asn34-Tyr35. The α-amino group of Tyr35 makes a salt bridge with the side chain of Asp187 in the mature enzyme. Aside from Tyr120 and Glu154 (significantly) and Pro122-Ala129 (slightly; see black arrows), maturation does not entail major conformational rearrangement of the rest of the structure.

---

**TABLE 3**

Direct interactions between the propeptide (PP) and the catalytic domain (CD)

| PP           | CD          | Hydrogen bonds (Å) | Hydrophilic carbon-carbon interactions |
|--------------|-------------|--------------------|----------------------------------------|
| R22 Nη2      | E138 Oe2    | 2.85 / 2.85        | D25 O61                                |
| R22 Nη1      | E138 Oe1    | 3.08 / 3.01        | Zn999                                 |
|              |             |                    | 2.00 / 2.04                            |

---

2.61 Å). Residue Leu29 is surrounded by the side chains of Tyr106, His117, and Phe119, which may feature S3 (Fig. 2C). After Gly31, the polypeptide abandons the active-site cleft moving outward to reach the primary activation cleavage point, Asn34-Tyr35 (Fig. 2A), after which the chain folds back toward the molecular moiety and enters strand β9252 of the NTS β-sheet.

**A Novel Activation Mechanism in MMPs—** Previous work had yielded three structures of mature wild-type Kly18 in complexes with tri- and tetrapeptidic cleavage products, as well as an inhibitory tetrapeptide in the non-primed side of the cleft (PDB 2XS3, 2XS4, and 4IN9 (9, 28)). These were obtained both in the presence and absence of magnesium and showed deviating chain traces for segment Asn53-His57 (L9252Iα) in the two molecules found in the asymmetric unit of the magnesium unbound structure (PDB 2XS3 (9)) and in the single molecules found in magnesium-bound (PDB 2XS4 (9)) and inhibitor-bound.
Structure of T. forsythia Prokarilysin

bound crystals (PDB 4IN9 (28)). In addition, significant differences were also found in the second half of the S-loop including the bulge-edge segment, which was metal-free in all structures, as the aforementioned magnesium, which coincides with a potassium site in the inhibitor-bound form, was found on the opposite surface of the CD (see Fig. 1, A and C, in Ref. 9, and Fig. 1A in Ref. 28), in a place that suggests little if any functional or structural relevance. In these structures, either an outward- or an inward-folded flap was found for the S-loop (Fig. 1E in Ref. 9 and Fig. 1D in Ref. 28), which suggests intrinsic flexibility of this protein segment to adapt to different substrates. Among the distinct mature Kly18 coordinates, molecule A of the magnesium-unbound structure (PDB 2XS3) was chosen here for comparison with pKly18-E156A as it showed the lowest divergence in the overall chain trace (Fig. 2, D and E).

Superposition revealed that the mature CD is preformed in the zymogen and, with some notable local exceptions (see below), is simply uncovered during maturation by removal of the propeptide, as found in mammalian MMPs (47) and other MPs such as funnels (79, 82). Removal occurs through cleavage near Gly162, which is solvent exposed on the molecular surface and thus readily accessible for processing (Fig. 2A). This explains why the wild-type zymogen undergoes rapid autolysis, so it cannot be isolated intact (see Ref. 30 and first section of “Results and Discussion”). This was the first cleavage observed in vitro, thus termed primary activation cleavage site, and no further cleavage was detected either within the propeptide or in the CD. The site is consistent with most vertebrate MMPs being activated at X-F/Y bonds, which are found at similar regions in all structures (10). Propeptide removal occurs under loss of a number of protein-protein interactions (see Table 3 and the preceding section), which explains why the mature enzyme is less stable to thermal denaturation (see first section of “Results and Discussion”). In particular, Arg22 plays a key role in structural relevance. In these structures, either an outward- or an inward-folded flap was found for the S-loop (Fig. 1E in Ref. 9 and Fig. 1D in Ref. 28), which suggests intrinsic flexibility of this protein segment to adapt to different substrates. Among the distinct mature Kly18 coordinates, molecule A of the magnesium-unbound structure (PDB 2XS3) was chosen here for comparison with pKly18-E156A as it showed the lowest divergence in the overall chain trace (Fig. 2, D and E).

As to further changes upon maturation, segment Pro122-Ala129 from Lβ1β/βV is slightly shifted downwards by ~2 Å and the side chains of Tyr210 and Glu164 rotate toward the zinc site (Fig. 2E). Activation only entails major rearrangement of the new N-terminal segment Tyr35-Ser40, on the left surface (Fig. 2, D and E), which is rotated downward around bonds C-Cα and Cα-N of Ser40. In this way, this segment nestles in a surface cavity framed by helix αC and the first segment of the CTS between Gly162, and the “family specific residue,” which is a serine in MMPs (1, 101) (here Ser166). This entails that the new α-amino group of Tyr25, which is translated 25 Å, establishes an intra-molecular salt bridge with Asp187 of αC, which is vaguely reminiscent of the activation of trypsin-like serine peptidases (102). Asp187, in turn, is itself further bound to Ser166 and is adjacent to a second aspartate, Asp185, which binds two main chain amides of the Met turn. This electrostatic network is characteristic of physiologically relevant mature MMPs, also referred to as “superactive forms” (47, 103). With the exception of the mature N-terminal fragment, the rest of this electrostatic network is already present in the zymogen (Fig. 2E).

Intensive studies of the activation of mammalian MMPs have produced the structures of pro-MMP-1 (PDB 1SU3 (90)), pro-MMP-3 (PDB 1SLM (104)), pro-MMP-9 (PDB 1L6J (105)), and pro-MMP-2 (PDB 1EAK (89)). These studies revealed that the mammalian MMP zymogens contain a pre-formed competent protease moiety and true prodomains, which span between 66 and 91 residues, as shown for pro-MMP-2 (Fig. 3A) (47). The prodomains include elongated N-terminal extensions that may interact with ancillary domains, such as the fibronectin type II insertions found in MMP-2 and MMP-9, followed by globular cores of ~55 residues. These are made up of three α-helices that are arranged around a 3-fold axis with a left-handed twist.

The prodomain globular core serves as a scaffold to place a downstream peptide, which runs in extended conformation in the opposite direction to a bound substrate and thus blocks the active-site cleft (Fig. 3, A and B). This peptide encompasses the conserved motif involved in cysteine-switch or Velcro latency characteristic of animal and plant MMPs (48–50), 100P-R-C-G-N-P-D106 (MMP-2 residues in italics; see PDB 1EAK and UP08253), which is equivalent to pKly18 segment 25L-Y-D-N-G-P-L29 (Fig. 3, C and D). Both the cysteine- and aspartate-switch motif show an intricate electrostatic network producing a unique scaffold to interact with the mature catalytic domain moiety. In contrast to pKly18, where the first cleavage occurs in the primary activation cleavage site, however, classical mammalian pro-MMPs are activated by conformational changes in the prodomain induced by cleavage in a so-called “bait region”...
by several peptidases such as trypsin, plasmin, and other MMPs. Activation follows a “stepwise activation” process to eventually yield the final cleavage site X-F/Y accessible for processing and dissociation of cysteine and zinc to generate a functional active site. Residues of the conserved motif (Pro100-Asp106) key for structural integrity of the inhibitory segment are depicted for their side chains. B, close-up of A after removal of prodomain segment Pro43-Asn66 to provide insight into the interactions of the conserved motif. Key electrostatic interactions are shown as green lines. The catalytic glutamate, Glu404, is replaced by a glutamine, the histidines from the CSBZ are His403, His407, and His413. C and D, scheme depicting the interaction modi of the propeptides of pro-MMP-2 through a cysteine-switch mechanism (C) and pKly18 through an aspartate-switch mechanism (D). The catalytic zinc ions are shown as magenta spheres and relevant interactions are shown as yellow dashed lines.

Conclusions—This examination of the structure and function of the zymogen of the first bacterial MMP to be studied biochemically has uncovered several features of the activation mechanism of pKly18, which are shared with animal and plant MMPs: (i) the relevant cleavage site is X-F/Y; (ii) the scissile bond is located in similar regions of the structure; (iii) activation entails rearrangement of the segment equivalent to Tyr110-Ser109 to yield a salt bridge between the new α-amino group and the first of two conserved aspartates in helix αC; (iv) this aspartate is bound to the family-specific serine; (v) the aspartate immediately downstream binds two main chain amides of the Met turn; (vi) the inhibitory segments run across the cleft in the opposite direction to a genuine substrate and metal blockade occurs through the side chain of an intervening residue, not through a chain terminus; and (vii) the catalytic moiety is largely preformed in the zymogen. All these features are related to the highly conserved CD itself. In contrast, all features of the mechanism related to the segment preceding this conserved CD diverge: (i) in pKly the propeptide spans just 14 residues and does not contain repetitive secondary structure elements, whereas eukaryotic MMPs feature a true protein prodomain that folds into a pseudosymmetric three-helix bundle followed by a segment in extended conformation; (ii) no relevant sequence similarity is found between the proregions; (iii) in eukaryotic MMPs activation occurs through a cysteine-switch mechanism exerted by residues from a conserved sequence motif, whereas in pKly18 this motif is absent and activation follows an aspartate-switch mechanism; (iv) multiple cleavages are apparently required in eukaryotic MMPs to liberate the CD, whereas a single cleavage suffices in pKly; and (v) the prodomain is not required for (re)folding of the catalytic moieties in eukaryotic MMPs, whereas it is in karilysin. In addition, pKly shares parts of its mechanism of latency with otherwise unrelated MPs from the astacin and fragilysin families. Accordingly, this overall novel mechanism unveiled for MMPs supports previous hypotheses, according to which Kly18 originated from an animal MMP CD co-opted through horizontal gene transfer by T. forsythia. This transfer was fostered by the intimate coexistence of the latter with the human blood-irrigated gingival crevice. Subsequently, Kly18 would have evolved in a bacterial envi-
structure of T. forsythia Prokarilysin

environment, where it was furnished with unique flanking domains that contribute to a mechanism of zymogenicity similar to distantly related MPs only (9).

Acknowledgments—We are grateful to the joint IBMB/IRB Automated Crystallography Platform for assistance during crystallization experiments and Robin Rycroft for very helpful contributions to the manuscript. We acknowledge the help provided by local contacts at the ESRF synchrotron. The clone for production of human rhinovirus 3C proteinase was a generous gift from Arie Gerlof (EMBL, Hamburg). Funding for data collection was provided in part by ESRF. The Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University is a beneficiary of structural funds from the European Union (POSG.02.01.00-12-064/08).

REFERENCES
1. Bode, W., Gomis-Ruth, F. X., and Stoeker, W. (1993) Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the “metzincins”. FEBS Lett. 331, 134–140
2. Gomis-Ruth, F. X. (2003) Structural aspects of the metzincin clan of metalloendopeptidases. Mol. Biotechnol. 24, 157–202
3. Gomis-Ruth, F. X. (2009) Catalytic domain architecture of metzincin metalloproteinases. J. Biol. Chem. 284, 15353–15357
4. Stöcker, W., Grams, F., Baumann, U., Reinemer, P., Gomis-Ruth, F. X., McKay, D. B., and Bode, W. (1995) The metzincins: topological and sequential relations between the astacins, adamasmins, serralysins, and matrixins (collagenases) define a superfamiliy of zinc- peptides. Protein Sci. 4, 823–840
5. Cerda-Costa, N., and Gomis-Ruth, F. X. (2014) Architecture and function of metalloendopeptidase catalytic domains. Protein Sci. 23, 123–144
6. Balaban, N. P., Rudakova, N. L., and Sharipova, M. R. (2012) Structural and functional characteristics and properties of metzincins. Biochemistry 77, 119–127.
7. Stöcker, W., and Gomis-Ruth, F. X. (2013) Astacins:proteases in development and tissue differentiation, in Proteases: Structure and Function (Brix, K., and Stöcker, W., eds) pp. 235–263, Springer Verlag, Vienna
8. Gomis-Ruth, F. X. (2013) Zinc adamasmins. in Encyclopedia of Metalloproteins (Uversky, V. N., Krebsinger, R. H., and Permyakov, E. A., eds) pp. 2345–2349, Springer Verlag, Heidelberg
9. Cerda-Costa, N., Guevara, T., Karim, A. Y., Ksiazek, M., Nguyen, K. A., Arolas, J. L., Potempa, J., and Gomis-Ruth, F. X. (2011) The structure of the catalytic domain of the Tannerella forsythia matrix metalloproteinase karilysin in complex with a tetrapeptidic inhibitor. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 69, 472–476
10. Jusko, M., Potempa, J., Karim, A. Y., Ksiazek, M., Rieszbeck, K., Garred, P., Eick, S., and Blom, A. M. (2012) A metalloproteinase karilysin present in the majority of Tannerella forsythia isolates inhibits all pathways of the complement system. J. Immunol. 188, 2338–2349
11. Karim, A. Y., Kulczycka, M., Kanytka, T., Dubin, G., Jabaiah, A., Daugherty, P. S., Thogersen, I. B., Enghild, J. J., Nguyen, K. A., and Potempa, J. (2010) A novel matrix metalloproteinase-like enzyme (karilysin) of the periodontal pathogen Tannerella forsythia ATCC 43037. Biol. Chem. 391, 105–117
12. Koziel, J., Karim, A. Y., Przybyszewska, K., Ksiazek, M., Rapala-Kozik, M., Nguyen, K. A., and Potempa, J. (2010) Proteolytic inactivation of LL3 by karilysin, a novel virulence mechanism of Tannerella forsythia. J. Invertebr. Pathol. 2, 288–293
13. Potempa, J., Gomis-Ruth, F. X., and Karim, A. Y. (2013) Karilysin in Handbook of Proteolytic Enzymes (Rawlings, N. D., and Selvesen, G. S., eds) 3rd Ed., pp. 887–891, Academic Press, Oxford
14. Guevara, T., Ksiazek, M., Skottrup, P. D., Cerda-Costa, N., Trillo-Muyo, S., de Diego, I., Riise, E., Potempa, J., and Gomis-Ruth, F. X. (2013) Structure of the catalytic domain of the Tannerella forsythia matrix metalloproteinase karilysin in complex with a tetrapeptidic inhibitor. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 69, 472–476
15. Jusko, M., Potempa, J., Karim, A. Y., Ksiazek, M., Rieszbeck, K., Garred, P., Eick, S., and Blom, A. M. (2012) A metalloproteinase karilysin present in the majority of Tannerella forsythia isolates inhibits all pathways of the complement system. J. Immunol. 188, 2338–2349
16. Karim, A. Y., Kulczycka, M., Kanytka, T., Dubin, G., Jabaiah, A., Daugherty, P. S., Thogersen, I. B., Enghild, J. J., Nguyen, K. A., and Potempa, J. (2010) A novel matrix metalloproteinase-like enzyme (karilysin) of the periodontal pathogen Tannerella forsythia ATCC 43037. Biol. Chem. 391, 105–117
17. Koziel, J., Karim, A. Y., Przybyszewska, K., Ksiazek, M., Rapala-Kozik, M., Nguyen, K. A., and Potempa, J. (2010) Proteolytic inactivation of LL3 by karilysin, a novel virulence mechanism of Tannerella forsythia. J. Invertebr. Pathol. 2, 288–293
18. Potempa, J., Gomis-Ruth, F. X., and Karim, A. Y. (2013) Karilysin in Handbook of Proteolytic Enzymes (Rawlings, N. D., and Selvesen, G. S., eds) 3rd Ed., pp. 883–886, Academic Press, Oxford
19. Skottrup, P. D., Sorensen, G., Ksiazek, M., Potempa, J., and Riise, E. (2012) A phase display selected 7-mer peptide inhibitor of the Tannerella forsythia metalloproteinase-like enzyme karilysin can be truncated to Ser-Trp-Ph-Pro. Plos One 7, e48537
20. Pomerantsve, A. P., Pomerantsva, O. M., Moayeri, M., Fattah, R., Talant, C., and Leppla, S. H. (2011) A Bacillus anthracis strain deleted for six proteases serves as an effective host for production of recombinant proteins. Protein Expr. Purif. 80, 80–90
21. Gomis-Ruth, F. X., Botelho, T. O., and Bode, W. (2012) A standard orientation for metalloproteinases. Biochim. Biophys. Acta 1824, 157–163
22. Stöcker, W., and Bode, W. (1995) Structural features of a superfamily of zinc-endopeptidases: the metzincins. Curr. Opin. Struct. Biol. 5, 383–390
23. Stroud, R. M., Kossiakoff, A. A., and Chambers, J. L. (1977) Mechanisms of zymogen activation. Annu. Rev. Biophys. Bioeng. 6, 177–193
Structure of T. forsythia Prokarilysin

78. Karplus, P. A., and Diederichs, K. (2012) Linking crystallographic model and data quality. Science 336, 1030–1033
79. Gomis-Ruth, F. X. (2008) Structure and mechanism of metallocarboxypeptidases. Crit. Rev. Biochem. Mol. Biol. 43, 319–345
80. Matthews, B. W. (1988) Structural basis of the action of thermolysin and related zinc peptidases. Acc. Chem. Res. 21, 333–340
81. Vendrell, J., Querol, E., and Aviles, F. X. (2000) Metallocarboxypeptidases and their protein inhibitors. Structure, function and biomedical properties. Biochim. Biophys. Acta 1477, 284–298
82. Arolas, I. L., and Gomis-Ruth, F. X. (2003) Zinc metallocarboxypeptidases. in Encyclopedia of Metalloproteins (Uversky, V. N., Kretsinger, R. H., and Permyakov, E. A., eds) pp. 2473–2479, Springer Verlag, Heidelberg, Germany
83. Wetmore, D. R., and Hardman, K. D. (1996) Roles of the propeptide and metal ions in the folding and stability of the catalytic domain of stromelysin (matrix metalloproteinase 3). Biochemistry 35, 6549 – 6558
84. Gonzales, P. E., Solomon, A., Miller, A. B., Leesnitzer, M. A., Sagi, I., and Mills, M. E. (2004) Inhibition of the tumor necrosis factor-α converting enzyme by its prodomain. J. Biol. Chem. 279, 31638–31645
85. Fotouhi, N., Lugo, A., Visnick, M., Lusky, W., Ralph, J., and Hanglow, A. C. (1994) Potent peptide inhibitors of stromelysin based on the prodomain region of matrix metalloproteinases. J. Biol. Chem. 269, 30227–30321
86. Stetler-Stevenson, W. G., Talano, J. A., Gallagher, M. E., Krutzsch, H. C., and Liotta, L. A. (1991) Inhibition of human type IV collagenase by a highly conserved peptide sequence derived from its prosegment. Am. J. Med. Sci. 302, 163 – 170
87. Woesner, J. F., Jr., and Nagase, H. (2000) Matrix metalloproteinases and TIMPs (Protein Profile Series) (Sheterline, P., ed) Oxford University Press, New York
88. Guevara, T., Yiallouros, I., Kappelhoff, R., Bissdorf, S., Stöcker, W., and Gomis-Ruth, F. X. (2010) Proenzyme structure and activation of astacian metalloproteinase. J. Biol. Chem. 285, 13958–13965
89. Morogunova, E., Tuuttila, A., Bergmann, U., Isupov, M., Lindqvist, Y., Schneider, G., and Tryggvason, K. (1999) Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed. Science 284, 1667–1670
90. Jozic, D., Bourenkov, G., Lim, N. H., Visse, R., Nagase, H., Bode, W., and Maskos, K. (2005) X-ray structure of human pro-MMP-1: new insights into proteolysis activation and collagen binding. J. Biol. Chem. 280, 9578–9585
91. DeMidyuk, I. V., Gromova, T. Y., Polyakov, K. M., Melik-Adamyan, W. R., Kuranova, I. P., and Kostrov, S. V. (2010) Crystal structure of proteolysin precursor: insights into propeptide function. J. Biol. Chem. 285, 2003–2013
92. Gao, X., Wang, J., Yu, D. Q., Bian, F., Xie, B. B., Chen, X. L., Zhou, B. C., Lai, I. H., Wang, Z. X., Wu, J. W., and Zhang, Y. Z. (2010) Structural basis for the autoprocessing of zinc metalloproteases in the thermolysin family. Proc. Natl. Acad. Sci. U.S.A. 107, 17569–17574
93. Bode, W., and Maskos, K. (2003) Structural basis of the metalloproteinases and their physiological inhibitors, the tissue inhibitors of metalloproteinases. Biocatal. Biotechnol. 384, 863–872
94. Chothia, C., Levitt, M., and Richardson, D. (1977) Structure of proteins: packing of α-helices and pleated sheets. Proc. Natl. Acad. Sci. U.S.A. 74, 4130–4134
95. Schechter, I., and Berger, A. (1967) On the size of active site in protease.. I. papain. Biochem. Biophys. Res. Commun. 27, 157–162
96. Jones, S., Marín, A., and Thornton, J. M. (2000) Protein domain interfaces: characterization and comparison with oligomeric protein interfaces. Protein Eng. 13, 77–82
97. Batra, J., Soares, A. S., Mehner, C., and Radisky, E. S. (2013) Matrix metalloproteinase-10/TIMP-2 structure and analyses define conserved core interactions and diverse exosite interactions in MMP/TIMP complexes. PLoS One 8, e75836
98. Grams, F., Reimer, P., Powers, J. C., Kleine, T., Pieper, M., Tschesche, H., Huber, R., and Bode, W. (1995) X-ray structures of human neutrophil collagenase complexed with peptide thiol inhibitors: implications for substrate binding and rational drug design. Eur. J. Biochem. 228, 830–841
99. Hunter, C. A., and Sanders, J. K. M. (1990) The nature of π–π interactions. J. Am. Chem. Soc. 112, 5525–5534
100. Gomis-Ruth, F. X., Kress, L. F., and Bode, W. (1993) First structure of a snake venom metalloproteinase: a prototype for matrix metalloproteinases/collagenases. EMBO J. 12, 4151–4157
101. Jiang, W., and Bond, J. S. (1992) Families of metalloendopeptidases and their relationships. FEBS Lett. 312, 110–114
102. Bode, W. (1979) Aktivierung, Aktivitat und Inhibierung des Rindertrypsin. Die Naturwissenschaften 66, 251–258
103. Reinemer, P., Grams, F., Huber, R., Kleine, T., Schnierer, S., Pieper, M., Tschesche, H., and Bode, W. (1994) Structural implications for the role of the N terminus in the “superactivation” of collagenases: a crystallographic study. FEBS Lett. 338, 227–233
104. Becker, J. W., Marcy, A. I., Rokosz, L. L., Axel, M. G., Burbaum, J. J., Fitzgerald, P. M., Cameron, P. M., Esser, C. K., Hagemann, W. K., Hermes, J. D., and Springer, J. P. (1995) Stromelysin-1: three-dimensional structure of the inhibited catalytic domain and of the C-truncated proenzyme. Protein Sci. 4, 1966–1976
105. Elkins, P. A., Ho, Y. S., Smith, W. W., Janson, C. A., D’Alessio, K. J., McQueney, M. S., Cummings, M. D., and Romanic, A. M. (2002) Structure of the C-terminally truncated human ProMMP9, a gelatin-binding matrix metalloproteinase. Acta Crystallogr. D Biol. Crystallogr. 58, 1182–1192
106. Cha, J., Pedersen, M. V., and Auld, D. S. (1996) Metal and pH dependence of heptapeptide catalysis by human matrilysin. Biochemistry 35, 15831–15838
107. Nagase, H. (1997) Activation mechanisms of matrix metalloproteinases. Biol. Chem. 378, 151–160
108. Nagase, H., Enghild, J. I., 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
109. Harding, M. M. (2006) Small revisions to predicted distances around their relationships. J. Biol. Chem. 281, 17569–17574
110. Weiss, M. S. (2001) Global indicators of X-ray quality. Acta Crystallogr. D Biol. Crystallogr. 57, e75836
111. Evans, P. (2006) Scaling and assessment of data quality. Acta Crystallogr. D Biol. Crystallogr. 62, 72–82