Identification of Prodomain Determinants Involved in ADAMTS-1 Biosynthesis*

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The metalloprotease ADAMTS-1 (a disintegrin and metalloprotease with thrombospondin type I motif), similarly to other members of the ADAMTS family, is initially synthesized as a zymogen, proADAMTS-1, that undergoes proteolytic processing at the prodomain/catalytic domain junction by serine proteinases of the furin-like family of proprotein convertases. The goals of this study were to identify residues of the prodomain that play an essential role in ADAMTS-1 processing and to determine the identity of the convertase required for zymogen processing. To gain insight into the putative roles of specific prodomain residues in ADAMTS-1 biosynthesis, we performed biosynthetic labeling experiments in transiently transfected human embryonic kidney 293 cells expressing wild-type and prodomain mutants of ADAMTS-1. Cells expressing wild-type ADAMTS-1 initially produced a 110-kDa zymogen form that was later converted to an 87-kDa form, which was also detected in the media. Although convertases such as PACE4 and PC6B processed proADAMTS-1, we found that furin was the most efficient enzyme at producing the mature ADAMTS-1 87-kDa moiety. Site-directed mutagenesis of the two putative furin recognition sequences found within the ADAMTS-1 prodomain (RRNR173 and RKKR235) revealed that Arg235 was the sole processing site. Use of the Golgi disturbing agent, Brefeldin A, and monensin suggests that the cleavage of proADAMTS-1 takes place in the Golgi apparatus prior to its secretion. Conserved residues within the prodomain of other ADAMTS members hinted that they might act as maturation determinants. Replacement with alanine of selected residues Cys106, Tyr108, Gly110, Cys125, and Cys181 and residues encompassing the 137–144 sequence significantly affected the biosynthetic profile of the enzyme. Our results suggest that conserved residues other than the furin cleavage site in the prodomain of ADAMTS-1 are involved in its biosynthesis.

Proteolysis of extracellular substrates by the ADAMTS1 (a disintegrin and metalloprotease with thrombospondin type I motif) family, which consists of at least 19 members, is an important mechanism regulating events such as cartilage biosynthesis, angiogenesis, and cell motility and growth (1). The first member, ADAMTS-1 (peptidase M12.222, Merops data base), identified as a cachexia-associated gene expressed in colon tumor cells (2), along with ADAMTS-4 and ADAMTS-5 (also called aggrecanases), degrades to different extents the cartilage proteoglycan aggrecan and lectican or aggrecan-like proteins such as brevican and versican. This suggested a significant participation of these enzymes in conditions such as arthritis (3–6). ADAMTS-1 is also anti-angiogenic, a property possibly explained by the recent finding that it sequesters vascular endothelial growth factor-1 (7). The phenotype of ADAMTS-1−/− mice revealed marked reduction in size with body weights of ∼70% of their wild-type or heterozygous littermates, and fertilization was impaired in females (8). ADAMTS-2, ADAMTS-14, and ADAMTS-3 are procollagen N-proteinases that proteolytically remove amino peptides in the processing of type I and type II procollagens to collagens (9–11). Deficiency of ADAMTS-2 led to an inherited connective tissue disorder called dermatosparaxis in animals and the Ehlers-Danlos syndrome (dermatosparactic-type) in humans. Reports have recently demonstrated that mutations in the ADAMTS-13 gene cause thrombotic thrombocytopenic purpura, a coagulation disorder, and that ADAMTS-13 is required for the processing of large von Willebrand factor multimers (12). The functions of most of the other ADAMTS members remain to be better clarified.

The structure of all of the ADAMTS members includes a signal peptide for access to the secretory pathway, a prodomain, a catalytic metalloprotease domain, a disintegrin-like domain, and a carboxyl-terminal ancillary domain having a conserved modular structure but containing a variable number of thrombospondin, type I repeat-like domains (2). Unlike the ADAM family, the ADAMTS members do not contain a transmembrane domain yet they may be located in the vicinity of the cell via binding to cell surface molecules or the pericellular matrix (13). Thus ADAMTSs are secreted proteins anchored to the cell surface or to the extracellular matrix (14).

ADAMTS proteases, similar to ADAM and MMPs, are synthesized aszymogens, which require activation via the proteolytic removal of a prodomain. The size of the prodomain varies, because it is in part determined by the location of the carboxyl-terminal most furin-processing site; however, in all of the ADAMTSs with the exception of ADAMTS-13, which has an overall atypical primary structure, the size is ∼200 residues. At least one sequence, but usually multiple furin recognition sequences, occurs in the prodomain of most members of the ADAMTS family. ADAMs, MMPs, and ADAMTS-1, ADAMTS-2, ADAMTS-4, ADAMTS-5, ADAMTS-9, and ADAMTS-12 (3, 4, 13, 15–17) have been shown to be processed by furin-like proprotein convertases. The convertase family is
comprised of seven calcium-dependent serine proteases (18) that recognize various sequence motifs containing permutations of basic residues in the P1, P2, P4, and P6 positions (19). One of the most studied convertases, furin, is concentrated in the trans-Golgi network and cycles between this compartment and the cell surface through the exocytic/endocytic pathway (20). The autoactivation and intracellular trafficking of furin and the cell surface through the exocytic/endocytic pathway are well characterized events (21).

Because zymogen activation is a critical post-translational regulatory step, we have addressed in this study, the critical determinant affecting this process. Although convertases like PACE4 and PC6B may process proADAMTS-1, we demonstrate that furin is the most efficient convertase at cleaving the proADAMTS-1 precursor intracellularly at Arg235 of the furin recognition consensus RKKE235. Moreover, we also identify different conserved residues within the prodomains of ADAMTS family members that could be involved in the maturation of proADAMTS-1. These observations could be of broad significance to understand the regulation of the ADAMTS proteases.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—QBI-293A cells (293A, Quantum Biotechnologies, Montréal, QC, Canada) derived from the human embryonic kidney-293 cell line were grown in complete Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, and 50 μg/ml streptomycin. CHO RPE.40 cells were cultured in RPMI 1640 medium containing 10% dialyzed fetal calf serum, 1 mM L-glutamine, and 1% SDS containing protease inhibitors (1 μM aprotonin, 10 μM pepstatin, 10 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Samples were centrifuged to remove insoluble material. Human ADAMTS-1 antisera, anti-furin (24), anti-PACE4 (Alexis Biochemicals), anti-PC6B (Alexis Biochemicals); or anti-PC7 was added, and samples were incubated overnight at 4 °C. Protein A/G Plus-agarose (Santa Cruz Biotechnology, Santa Cruz, CA) beads were added and incubated for 1 h at 4 °C. Beads were washed three times with 1 ml of radioimmunoprecipitation assay buffer, and labeled proteins were resolved by SDS-PAGE. Gels were treated with ENHANCE reagent (PerkinElmer Life Science), dried, and exposed for fluorography. Brefeldin A at 36 μM (Sigma) or monensin at 3.6 or 36 μM (Sigma) was added to the labeling mixture when indicated.

Western Blot—Transfected CHO RPE.40 cells were lysed in 1 ml of radioimmunoprecipitation assay buffer. 30 μl of each sample were subjected to SDS-PAGE and transferred to a nitrocellulose membrane. Immunoblot analysis was performed with monoclonal mouse anti-actin antibody (Chemicon). The antibodies were visualized with the horseradish peroxidase-coupled sheep anti-mouse immunoglobulin (Amersham Biosciences) using the Western Lighting Chemiluminiscence Reagent Plus according to the manufacturer’s instructions (PerkinElmer Life Sciences).

Deglycosylation—Deglycosylation of immunoprecipitated proteins from cell lysate and conditioned medium of ADAMTS-1 transfected cells was performed following immunoprecipitation using 10 units of N-glycosidase F (Roche Applied Science) for 3 h at 37 °C in 150 mM sodium phosphate, pH 7.4, 50 mM EDTA, 0.1% SDS, 1% 2-mercaptoethanol, and 0.5% Triton X-100 followed by SDS-PAGE and fluorography.

RESULTS

Biochemistry of ADAMTS-1—Previous work (22) describes how processing of proADAMTS-1 is required for its activation. To better characterize how the propeptide of ADAMTS-1 is involved in the biosynthesis of the enzyme, we transfected human kidney 293 cells with an expression vector containing the complete human ADAMTS-1 cDNA. Pulse-chase experiments were initially carried out to analyze the onset of ADAMTS-1 synthesis. ADAMTS-1 proteins were immunoprecipitated with a polyclonal anti-ADAMTS-1 recognizing the HDEQK&PFE-VTS sequence present in the metalloproteinase domain of ADAMTS-1. As seen in Fig. 1B, we detected in cell lysates after a 15-min pulse a 110-kDa form that corresponds well to the theoretical molecular mass (100 kDa, 916 residues) of unprocessed proADAMTS-1 (Fig. 1A). Following a 15-min chase period, a 87-kDa form corresponding to the proteolytically active ADAMTS-1 protein was present in the cells with a complete processing of proADAMTS-1 after 60 min of chase. The mature 87-kDa ADAMTS-1 is also detected in the media after 30 min. A 2-hour chase period enabled us to detect a doublet at 65 kDa in the media (Fig. 1C), a result of C-terminal processing by a metalloprotease (22).

Deglycosylation of ADAMTS-1—Longer pulse labeling and chase times enabled us to detect a doublet at 87 and 65 kDa. Because ADAMTS-1 possesses three N-glycosylation sites, we investigated the glycosylated state of the doublets. Deglycosylation with N-glycosidase F after metabolic labeling reveals that the upper band of the doublet at 87 kDa represents an N-glycosylated form as can be seen by the significant shift in electrophoretic mobility to an apparent molecular mass of 82 kDa, more in line with the calculated molecular mass of 79 kDa, which would correspond to mature ADAMTS-1 (residues 236–950, Fig. 1C). Moreover, the two forms at 65 kDa also appear to be N-glycosylated because both forms are shifted to lower molecular masses following N-glycosidase F treatment.

Processing of proADAMTS-1 by Convertases—Although furin is the better characterized proprotein convertase, PACE4, PC6B, and PC7 also cleave precursor proteins within the constitutive secretory pathway (25) but their precise function is still unclear. To investigate whether these convertases process
proADAMTS-1, we co-transfected ADAMTS-1 cDNA with the cDNA of these different convertases in the furin-deficient CHO RPE.40 cells (26). As can be seen in Fig. 2A, proADAMTS-1 is processed to 87 kDa in CHO RPE.40 cells by endogenous proteases but less effectively than in 293 cells as demonstrated by the presence of the 110-kDa zymogen form in the medium (compare Fig. 2A with Fig. 1 where the 110-kDa form is detected in the medium). Co-transfection of furin with ADAMTS-1 led to the complete processing of the 110-kDa form into the 87-kDa band and the 65-kDa doublet. PACE4 and

Fig. 1. Biosynthesis of ADAMTS-1 in QBI 293A cells. A, schematic representation of ADAMTS-1 forms. ADAMTS-1 is a mosaic protein containing different domains. Pro, prodomain; MP, metalloproteinase domain; Dis, disintegrin-like domain; TSP, thrombospondin, type 1 repeat; CRR, cysteine-rich region; and SP, spacer domain. Putative N-linked glycosylation motifs are shown (●). A peptide corresponding to residues 295–306 (HDEQK6PETS) was used to generate polyclonal anti-ADAMTS-1 antibodies. B, time course of ADAMTS-1 biosynthesis in QBI 293A cells. Cells transfected with pcDNA3.1MycHis/ADAMTS-1 or empty expression vector pcDNA3.1MycHis were pulse-labeled with [35S]Met/Cys for 15 min and chased in complete medium for the indicated periods of time. Labeled proteins from cell extracts prepared in radioimmunoprecipitation assay buffer or cultured medium were immunoprecipitated with anti-ADAMTS-1 (1/1000) polyclonal antibody and detected by fluorography. Arrow indicates the intracellular zymogen form. Arrowheads indicate the mature form, and the asterisk indicates the C-terminally processed forms in the medium. C, N-deglycosylation of ADAMTS-1. Pulse-chase was performed as in B with the exception that pulse was for 30 min and chase was for 2 h. Cells extracts (C) and media (M) were treated with 10 units of N-glycosidase F (PNGase F) for 3 h following immunoprecipitation.
PC6B also cleaved proADAMTS-1 as demonstrated by the reduced intensity of the 110-kDa zymogen form in both cells and media but not as efficiently as furin. Interestingly, PC7-dependent processing of proADAMTS-1 was very weak. Expression levels of the different convertases were verified by immunoprecipitating each convertase when co-transfected with ADAMTS-1 (Fig. 2B). Fig. 2B shows that mature furin (apparent molecular mass of 100 kDa) (27), PACE4 (103 kDa) (28), PC6B (theoretical molecular mass of 195 kDa), and PC7 (92 kDa) (29) are all expressed at similar levels. Sample loading was verified by Western blot using anti-actin (Fig. 2C). These results suggest that proADAMTS-1 is efficiently converted to the active 87- and 65-kDa forms by furin and, to a lesser extent, by PACE4, PC6B, and PC7. Because furin in the most ubiquitously expressed of all of the convertases, it is very likely to be the bona fide physiological zymogen convertase of the ADAMTS family. However, it is conceivable that other proteases of this family may also play a role in zymogen activation under certain conditions.

**Arg235 Is Required for ProADAMTS-1 Maturation**—Many ADAMTS family members possess more than one putative furin recognition sequence, RXRX, within their prodomain. There are two putative cleavage sites (RRNR173 and RKKR235) within the ADAMTS-1 prodomain. To assess the effect of abolishing either one of these sites on ADAMTS-1 biosynthesis, we performed site-directed mutagenesis on the P1 residue of each cleavage sequence by replacing Arg with Ala (ADAMTS-1/R173A and ADAMTS-1/R235A in Fig. 3A). Fig. 3B shows the result of metabolic labeling of 293A cells previously transfected either with ADAMTS-1/R173A or ADAMTS-1/R235A cDNA followed by immunoprecipitation with anti-ADAMTS-1 antibody. We observed that the biosynthetic profile of the ADAMTS-1/R173A mutant did not differ from that associated with wild-type ADAMTS-1. However, cells transfected with the ADAMTS-1/R235A cDNA did not process the 110-kDa form, which is also found intact in the media, indicating the necessity of Arg at position 235 for cleavage. No other bands were detected, suggesting that the alternative furin recognition site at position 173 was not used to produce other maturation fragments. Moreover, no additional processing was observed such as the C-terminal processing of the 87-kDa form into the C-terminally cleaved forms, indicating that these latter entities require the preliminary cleavage of the zymogen form. Thus, it...
is possible that the C-terminally processed forms observed at 65 kDa arise from the autocatalytic action of the proteolytically active forms of ADAMTS-1.

Because we had observed some processing of proADAMTS-1 in CHO RPE.40 cells, we investigated whether cleavage in these cells was also dependent on the furin recognition sites. Fig. 3C shows that proteolysis also required Arg235 for proper processing, because only the zymogen form (110 kDa) of ADAMTS-1/R235A was present in the cell extracts and in the media. Supplementing these cells with furin did not promote processing into the mature forms, suggesting the absolute requirement of Arg235 in the maturation process.

**Intracellular Localization of ADAMTS-1 Prodomain Cleavage**—To further define the intracellular compartment where proADAMTS-1 activation occurs, we treated cells expressing ADAMTS-1 with the Golgi-disturbing agents Brefeldin A and monensin (Fig. 4). Pulse-labeling analysis revealed that Brefeldin A, which inhibits protein transport between the endoplasmic reticulum and the Golgi apparatus, abolished the processing of the 110-kDa proADAMTS-1 form into the 87- and 65-kDa mature forms. The cells were also treated with monensin, a known inhibitor of post-Golgi transport (30). At 3.6 μM, monensin interfered with but did not completely abolish the production of the mature 87-kDa form that was detected in the
media, although this secretion was blocked at higher doses (36 μM). Taken together, these results identify the Golgi network as the major site of proADAMTS-1 processing.

Mutagenesis of Conserved ADAMTS-1 Prodomain Residues—To determine whether residues other than the furin recognition sequences are important in the maturation and secretion of ADAMTS-1, we aligned the prodomains of ADAMTS family members because conserved residues could potentially be required or implicated in these processes. Fig. 5 shows the alignment of the 19 human ADAMTS prodomains using hierarchical clustering. Only that portion of the prodomains with the highest sequence homology is shown, and identical amino acids found in >12 ADAMTS prodomains are termed “consensus” residues. This alignment identifies 15 conserved residues and motifs in the prodomain of ADAMTS besides the furin recognition sequences found at the C-terminal end of the prodomains.

Site-directed mutagenesis was performed to investigate the effect of replacing these conserved amino acids in the prodomain of ADAMTS-1. Fig. 6A illustrates the different Ala mutants of ADAMTS-1 used in the study. Pulse-chase analysis was performed in human embryonic kidney-293 cells transfected with nine different ADAMTS-1 prodomain mutants (Fig. 6B). First, we found that thezymogen of every mutant expressed was detected in cell extracts. The T111A, V112A, and P116A mutants essentially behaved similarly to the wild-type ADAMTS-1 with regards to their processing pattern. Replacing residues 137–144 by Ala (ADAMTS-1/8A) completely abolished the processing of the 110-kDa zymogen form into the 87-kDa active moiety, but this zymogen form failed to be detected in the media. For the C106A and C125A mutants, processing was greatly reduced and, similar to ADAMTS-1/8A mutants, no detectable forms were observed in the media. Conversely, significantly lower amounts of processed forms of Y108A, G110A, and C181A were found in the media. Intriguingly, although zymogen forms of these mutants were detected intracellularly, they were not observed as secreted products as was the furin recognition sequence mutant R235A (Fig. 3B), indicating that zymogen activation is not a prerequisite for secretion. Moreover, it can be seen that an overall reduction of immunoreactive material can be observed for these mutants, suggesting that a percentage of these proteins may undergo inefficient folding leading to degradation. Nevertheless, those immunoreactive moieties that resided intracellularly did not seem to act as good substrates for processing or for transiting through the secretory pathway. Finally, we examined the importance of Cys181, possibly involved in the cysteine switch process (see “Discussion”), an essential component in the biosynthesis of other metzrinendoproteinases (31). The results indicate that replacing Cys181 with Ala led to the reduced processing of proADAMTS-1 and reduced the levels of processed products in the media, suggesting that this particular

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**Fig. 4. ProADAMTS-1 is processed in the secretory pathway.** Transfected QB1 293A cells with pcDNA3.1MycHis/ADAMTS-1 or empty vector were pulsed for 3 h in the presence of 3.6 μM Brefeldin A (lanes 5–6), 3.6 μM monensin (lanes 7–8), and 36 μM monensin (lanes 9–10). C, control; M, media.

**Fig. 5. Alignment of ADAMTS prodomains.** ADAMTS-(1–20) prodomains were aligned using hierarchical clustering (43). Highest consensus motifs within the proregion of ADAMTS family members are represented. Amino acid numbering corresponds to ADAMTS-1 residues 104–152. Identical amino acids are **shadowed**, and residues of similar properties are in **gray**. Consensus residues are indicated at the **bottom line.** Underlined amino acids represent the three conserved motifs. **Asterisks** indicate those residues mutated to alanine.
cysteine may not be directly responsible for latency and activation as demonstrated for most of the MMPs.

**DISCUSSION**

Proteolysis of the extracellular matrix and cell surface proteins mediated by metalloproteases, including MMPs, ADAMs, and ADAMTSs, is of vital importance for tissue-remodeling processes during normal and pathological conditions such as tissue morphogenesis, wound healing, inflammation, and tumor cell invasion and metastasis (1, 32). Metalloproteases are synthesized as inactive proenzymes or zymogens. In most cases, to display any proteolytic activities, the prodomain located N-terminal to the catalytic domain must be removed from the zymogen. For proADAMTS precursors, members of the proprotein convertase family would be responsible for the proteolytic cleavage of the prodomain at the junction of the catalytic domain.

Here we report that the 110-kDa proADAMTS-1 precursor is rapidly processed intracellularly to a shorter form (87 kDa) that eventually gets secreted as a soluble entity. Moreover, although furin is the most efficient convertase at cleaving proADAMTS-1 within the constitutive secretory pathway PACE4, PC6B also process the zymogen. These convertases are, like furin, localized intracellularly within the trans-Golgi network/endosomal compartments (21), but it has been reported that PACE4 is secreted and binds to heparin in the extracellular matrix (33). Therefore, it is possible that PACE4 and PC6B may recognize and cleave proADAMTS-1 in different tissues or under various conditions of expression. The mechanism responsible for processing proADAMTS-1 in the furin-deficient CHO RPE40 cells may well be achieved by compensatory functions of enzymes such as PACE4 or PC6B. However, these proteases are dependent as is furin on the presence of a furin-like recognition sequence and Arg235 for proper cleavage of proADAMTS-1. The fact that PC7 processes ADAMTS-1 very weakly is intriguing because PC7 is the subtilisin-like PC with substrate specificity most similar to that of furin with which it is highly homologous (34).

Furin or furin-like PCs mediate prometalloprotease activation by cleaving at the C-terminal end of the conserved motif RXXR found between the prodomain and the catalytic domain. Rodriguez-Manzaneque and co-workers (15) have shown that a P4 Arg to Ala proADAMTS-1 mutant (AKKR235) did not exhibit
aggrecanase activity. Our data showed that the RKKA\textsuperscript{235} mutant, which also abolishes the furin recognition sequence, was not processed by furin and that only thezymogen form (110 kDa) was secreted. This may explain the lack of activity of these mutants. Moreover, it is important to note that, in metalloproteases such as ADAM19 and membrane type 1-MMP, alternative furin cleavage sites can be used when the primary site is abolished (35, 36). However, this is not the case for ADAMTS-1 because replacing the P1 Arg for Ala in the RKKA\textsuperscript{235} mutant did not yield to alternative processing at the RRNR\textsuperscript{173} site. A possible explanation for this is that the RRNR\textsuperscript{173} site is not in an area of the prodomain that is exposed to the aqueous environment and hence is not accessible to furin. In ADAMTS-9, multiple furin-like recognition sequences are found within the prodomain but only one is preferentially used (13).

Our results also show that maturation of proADAMTS-1 is an intracellular event. To further define the intracellular compartment where proADAMTS-1 activation occurs, we treated the cells with Brefeldin A, which blocks the trafficking between the endoplasmic reticulum and Golgi apparatus (37) but permits autoproteolysis of the furin zymogen (38). Brefeldin A inhibited the ADAMTS-1 activation and secretion, which suggests that the maturation of proADAMTS-1 by furin requires the specific microenvironment of the trans-Golgi network. Memonsin, a known inhibitor of post-Golgi transport (39), interfered with the secretion of ADAMTS-1 but not its maturation. Taken together, these results demonstrate that proADAMTS-1 processing takes place in the Golgi apparatus.

Alignment of the 19 ADAMTS family members revealed three conserved regions other than the furin recognition sequence at the C-terminal end of the proregron. ADAMTS-13, ADAMTS-17, and ADAMTS-19 proregrons showed poor homology with other members of the family. In one of these, ADAMTS-13, it has recently been determined that removal of its prodomain is not necessary for proteolytic activity toward the von Willebrand factor precursor (39). The short and poorly conserved ADAMTS-13 proregron thus seems to be dispensable for latency and activation.

Conserved residues within another metalloprotease proregron, membrane type 1-MMP, have been implicated in revealing crucial conformational constraints important for the folding and function of the enzyme (40). In a comparable fashion, we employed mutational analysis on the proregron of proADAMTS-1 to assess the consequence of replacing conserved residues on biosynthesis. We did not notice significant changes in the biosynthetic patterns of the less conserved residues such as T111A (found in 6 of 19 ADAMTS sequences), V112A (10 of 19 but all are hydrophobic in this position), and P116A (9 of 19) mutants, suggesting that these amino acids are not major determinants in ADAMTS-1 biosynthesis. Substitution of the more conserved residues such as Cys\textsuperscript{106} (found in 16 of 19 ADAMTS sequences), Tyr\textsuperscript{125} (12 of 19), Gly\textsuperscript{130} (16 of 19), Cys\textsuperscript{135} (15 of 19), Cys\textsuperscript{140}, and residues 137–144 had a more profound effect on the biosynthesis of the enzyme with processing and secretion being affected. However, it is important to mention that it is likely that substitution of these key residues resulted in a certain degree of folding defects as demonstrated by the lower overall levels of detected mutant proteins. This would imply that highly conserved residues in ADAMTS members are important for proper folding and/or are implicated as recognition motifs required for their maturation and secretion.

The cysteine switch model predicts that metalloproteinasises are kept latent by the interaction of a conserved Cys residue of the prodomain and a zinc atom in the catalytic domain that blocks the active site. Disruption of this interaction leads to the removal of the prodomain and activation of the enzyme (31). Most ADAMTS, similar to ADAM and MMP, possess a conserved asymmetric (or unpaired) cysteine and the catalytic zinc atom potentially implicated in a cysteine switch mechanism. The cysteine residue within the proregion sequence Gly-Thr-Cys\textsuperscript{181}–Gly-Val-Val-Asp is potentially the residue participating in the cysteine switch mechanism because of the similarity of the amino acids surrounding Cys\textsuperscript{181} to other cysteine switches found in MMPs and ADAMs (41). Impaired maturation and activation of the C181A mutant demonstrated that Cys\textsuperscript{181} is not crucial for latency. Moreover, the furin Arg\textsuperscript{235} mutant to Ala showed no maturation so the RKKR\textsuperscript{235} motif thus is clearly identifiable determinant for activation.

Importantly, abrogation of C-terminal post-secretory processing in the activation-deficient mutants also supports the likelihood that this could be autocatalytic, occurring either in cis or trans. Such a C-terminal processing event has been shown to have a regulatory role and now has been noted with several ADAMTS proteins (ADAMTS-1, ADAMTS-4, and ADAMTS-5) (22, 42). In conclusion, these studies shed light on the critical regulatory mechanisms of broad significance to the ADAMTS proteases. Many important features of the ancillary domain and catalytic site have been identified by engineered or naturally occurring mutations, yet there has not yet been a systematic analysis of the prodomain. Although the three-dimensional structure is not yet available for ADAMTSzymogens, we predict that the observations we have provided here will be crucial in understanding the structure of the zymogen and the role of the prodomain.

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REFERENCES

1. Iruela-Arispe, M. L., Carpio, D., and Luque, A. (2003) Ann. N. Y. Acad. Sci. 995, 183–190
2. Kann, K., Kanada, N., Nakashima, E., Fujiki, F., Ichimura, F., and Matsuhashi, K. (1997) J. Biol. Chem. 272, 556–562
3. Tortorella, M. D., Burn, T. C., Pratta, M. A., Abbaszade, I., Holdis, J. M., Liu, R., Rosenfeld, S. A., Copeland, R. A., Decieco, C. P., Wynn, R., Rockwell, A., Yang, F., Duke, J. L., Solomon, K., George, H., Bruckner, R., Nagase, H., Itoh, Y., Ellis, D. M., Ross, H., Wiswall, B. H., Murphy, K., Hillman, M. C., Jr., Holla, G. F., Newton, R. C., Magolda, R. L., Trzaskos, J. M., and Arner, E. C. (1999) Science 284, 1664–1666
4. Abbaszade, I., Liu, R. Q., Yang, F., Rosenfeld, S. A., Ross, O. H., Link, J. R., Ellis, D. M., Tortorella, M. A., Abbaszade, I., Holdis, J. M., Liu, R., Rockwell, A., Yang, F., Duke, J. L., Solomon, K., George, H., Bruckner, R., Nagase, H., Itoh, Y., Ellis, D. M., Ross, H., Wiswall, B. H., Murphy, K., Hillman, M. C., Jr., Holla, G. F., Newton, R. C., Magolda, R. L., Trzaskos, J. M., Arner, E. C., and Burn, T. C. (1999) J. Biol. Chem. 274, 23443–23450
5. Sandy, J. D., Westling, J., Kenagy, R. D., Iruela-Arispe, M. L., Verscharen, C., Rodrigues-Mazaneque, J. C., Zimmermann, D. R., Lemire, N. J., Fischer, J. W., Wight, T. N., and Cloves, A. W. (2001) J. Biol. Chem. 276, 13372–13378
6. Matthews, R. T., Gary, S. C., Zerillo, C., Pratta, M., Solomon, K., Arner, E. C., and Rockfield, S. (2000) J. Biol. Chem. 275, 22765–22770
7. Luque, A., Carpio, D., and Iruela-Arispe, M. L. (2005) J. Biol. Chem. 280, 23656–23665
8. Sando, T., Kunihara, H., Kuno, K., Yokoyama, H., Wada, T., Kunihara, Y., Imai, T., Wang, Y., Ogata, M., Nishimatsu, H., Moriya, N., Oh-hashi, Y., Morita, H., Ishikawa, T., Nagai, R., Yazaki, Y., and Matsushita, K. (2000) J. Clin. Investig. 106, 1345–1352
9. Colige, A., Sieren, A. L., Lu, S. W., Schwarze, U., Petty, E., Wetedelecky, W., Wilcox, W., Krakow, D., Cohn, D. H., Reardon, W., Byers, P. H., Lapiere, C. M., Prockop, D. J., and Nusgens, B. V. (1999) Annu. Hum. Genet. 65, 308–317
10. Colige, A., Vandenbergh, I., Thiry, M., Lambert, C. A., Van Beeumen, J., Li, S. W., Prockop, D. J., Lapiere, C. M., and Nusgens, B. V. (2002) J. Biol. Chem. 277, 5756–5766
11. Fernandes, R. J., Hirohata, S., Engle, J. M., Colige, A., Cohn, D. H., Eyre, D. R., and Apte, S. S. (2001) J. Biol. Chem. 276, 31592–31509
12. Liu, J.-C., Neuman, W. C. Jr., Ercan, F., Foroud, T., McClintick, J. N., McGee, B. M., Yang, A. Y., Siemieniak, D. R., Stark, K. R., Gruppo, R., Sarode, R., Shurin, S. B., Chandrasekaran, V., Stabler, S. P., Sabio, H., Bouhassira, E. E., Upshaw, J. D., Jr., Ginsburg, D., and Tsai, H. M. (2001) J. Biol. Chem. 276, 31509–31514
13. Somerville, R. P., Longere, J. M., Jungers, K. A., Engle, J. M., Ross, E., Evanko, S., Wight, T. N., Leduc, R., and Apte, S. S. (2003) J. Biol. Chem.
14. Kano, K., Terashima, Y., and Matsushima, K. (1999) J. Biol. Chem. 274, 18821–18826
15. Rodriguez-Manzaneque, J. C., Westling, J., Thai, S. N., Luque, A., Knauper, V., Murphy, G., Sandy, J. D., and Iruela-Arispe, M. L. (2002) Biochem. Biophys. Res. Commun. 293, 501–508
16. Cal, S., Arguelles, J. M., Fernandez, P. L., and Lopez-Otín, C. (2001) J. Biol. Chem. 276, 17892–17899
17. Wang, W. M., Lee, S., Steiglitz, B. M., Scott, I. C., Lebares, C. C., Allen, M. L., Brenner, M. C., Takahara, K., and Greenspan, D. S. (2003) J. Biol. Chem. 278, 19549–19557
18. Bergeron, F., Leduc, R., and Day, R. (2000) J. Mol. Endocrinol. 24, 1–22
19. Schechter, I., and Berger, A. (1967) Biochem. Biophys. Res. Commun. 27, 157–162
20. Moly, S. S., Thomas, L., Kameyama, C., Momba, M. C., and Thomas, G. (1998) J. Cell Biol. 142, 1399–1411
21. Thomas, G. (2002) Nat. Rev. Mol. Cell. Biol. 3, 753–766
22. Rodriguez-Manzaneque, J. C., Milchanowski, A. B., Dufour, E. K., Leduc, R., and Iruela-Arispe, M. L. (2000) J. Biol. Chem. 275, 33471–33479
23. Denault, J., Bissonnette, L., Longpre, J., Charest, G., Lavigne, P., and Leduc, R. (2002) FEBS Lett. 527, 309–314
24. Denault, J. B., Lazure, C., Day, R., and Leduc, R. (2000) Protein Expression Purif. 19, 113–124
25. Zhou, A., Webb, G., Zhu, X., and Steiner, D. F. (1999) J. Biol. Chem. 274, 20745–20748
26. Spence, M. J., Sucic, J. F., Foley, B. T., and Moehring, T. J. (1995) Somat. Cell Mol. Genet. 21, 1–18
27. Bissonnette, L., Charest, G., Longpre, J. M., Lavigne, P., and Leduc, R. (2004) Biochem. J. 379, 757–763
28. Tanguchi, T., Kuroda, H., Sakurai, K., Nagahama, M., Wada, I., Tsuji, A., and Matsuda, Y. (2002) Biochem. Biophys. Res. Commun. 296, 878–884
29. van de Loo, J. W., Creeners, J. W., Bright, N. A., Young, B. D., Roebroek, A. J., and Van de Ven, W. J. (1997) J. Biol. Chem. 272, 27116–27123
30. Dinter, A., and Berger, E. G. (1998) Histochem. Cell Biol. 109, 571–580
31. Van Wart, H. E., and Birkedal-Hansen, H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5578–5582
32. Chang, C., and Werb, Z. (2001) Trends Cell Biol. 11, S37–43
33. Tsuji, A., Sakurai, K., Kiyokage, E., Yamazaki, T., Kinde, S., Toida, K., Ishimura, K., and Matsuda, Y. (2003) Biochim. Biophys. Acta 1645, 95–104
34. Munzer, J. S., Basak, A., Zhong, M., Mamarbachi, A., Hamelin, J., Savaria, D., Lazure, C., Hendy, G. N., Benjannet, S., Chretien, M., and Seidah, N. G. (1997) J. Biol. Chem. 272, 19672–19681
35. Kang, T., Zhao, Y. G., Pei, D., Sucic, J. F., and Sang, Q. X. (2002) J. Biol. Chem. 277, 25580–25591
36. Yama, I., and Uizard, S. J. (2000) Mol. Biol. Cell 11, 2387–2401
37. Fujiwara, T., Oda, K., Yokota, S., Takatsuki, A., Ikehara, Y. (1988) J. Biol. Chem. 263, 18545–18552
38. Vey, M., Schofer, W., Berghof, S., Klenk, H. D., and Garten, W. (1994) J. Cell Biol. 127, 1829–1842
39. Majerus, E. M., Zheng, X., Tuley, E. A., and Sadler, J. E. (2003) J. Biol. Chem. 278, 46443–46448
40. Pavlaki, M., Cao, J., Hymowitz, M., Chen, W. T., Bahou, W., and Zucker, S. (2002) J. Biol. Chem. 277, 2740–2749
41. Massou, I., Kehla, L. F., Frisman, R., and Mobasher, S. (1998) FASEB J. 12, 1075–1085
42. Flannery, C. R., Zeng, W., Corcoran, C., Collins-Rae, L. A., Chockalingam, P. S., Hubert, T., Mackie, S. A., McDonagh, T., Crawford, T. K., Tomkison, K. N., LeValle, E. R., and Morris, E. A. (2002) J. Biol. Chem. 277, 42775–42780
43. Corpet, F. (1988) Nucleic Acids Res. 16, 10881–10890
