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ADAMTS-4 (aggrecanase-1): N-Terminal activation mechanisms

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Abstract

ADAMTS-4 (aggrecanase 1) is synthesized as a latent precursor protein that may require activation through removal of its prodomain before it can exert catalytic activity. We examined various proteinases as well as auto-activation under a wide range of conditions for removal of the prodomain and induction of enzymatic activity. The proprotein convertases, furin, PACE4, and PC5/6 efficiently removed the prodomain through cleavage at Arg^{212}/Phe^{213}, generating an active enzyme. Of a broad range of proteases evaluated, only MMP-9 and trypsin were capable of removing the prodomain. In the presence of mercuric compounds, removal of the prodomain through autocatalysis was not observed, nor was it observed at temperatures from 22 to 65 °C, at ionic strengths from 0.1 to 1 M, or at acidic/neutral pH. At basic pH 8–10, removal of the prodomain by autocatalysis occurred, generating an active enzyme. In conclusion, the pro-form of ADAMTS-4 is not catalytically active and only a limited number of mechanisms mediate its N-terminal activation.

Keywords: ADAMTS-4; Aggrecanase; Aggrecan; MMP; Proprotein convertase; Activation

ADAMTS[1]-4 (aggrecanase 1) and ADAMTS-5 (aggrecanase 2), members of the "a disintegrin and metalloproteinase with thrombospondin motifs" (ADAMTS) family, are thought to be responsible for aggrecan degradation in arthritic disease [1–3]; reviewed in [4]. These proteins are glutamyl endopeptidases that cleave the aggrecan core protein at the carboxyl terminus of Glu^{373}, Glu^{1545}, Glu^{1714}, Glu^{1819}, and Glu^{1919} (human sequence) [5,6].

ADAMTS-4 is synthesized as a protein containing a signal peptide$^{1–51}$, prodomain$^{52–212}$, catalytic domain$^{213–439}$ with the zinc-binding motif HELGHVFNMLH, disintegrin-like domain$^{440–520}$, thrombospondin Type I (TSP) motif$^{521–571}$, and a spacer region$^{572–837}$ [7]. In addition to aggrecan, ADAMTS-4 cleaves other aggregating proteoglycans such as versican-1, versican-2, and brevican at Glu^{441}, Glu^{405}, and Glu^{393}, respectively [8–10]. The TSP-1 motif of ADAMTS-4 is important for substrate recognition and cleavage, as a truncated form of ADAMTS-4 lacking the TSP-1 motif is inefficient in cleaving aggrecan, several peptides spanning the TSP-1 motif effectively block ADAMTS-4 cleavage of aggrecan and ADAMTS-4 shows poor activity against GAG-free aggrecan [11].

More recently, it has been demonstrated that ADAMTS-4 is not exclusively a glutamyl endopeptidase and not limited to cleavage of proteoglycans. ADAMTS-4 cleaves α2-macroglobulin (α2M) within the bait region following Met^{690}. In addition, this cleavage of the macroglobulin molecule is not dependent on the TSP-1 motif, as a truncated form of the enzyme$^{213–431}$ lacking the C-terminal domains$^{431–837}$ is equally active against α2M [12]. Other reported substrates include COMP [13], TIMP-4 [14], matrilin-2 [15], and transferrin, decorin and fibromodulin

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[1] *Abbreviations used:* ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; TSP, thrombospondin type I; α2M, α2-macroglobulin; IL-1, interleukin-1; TNF, tumor necrosis factor; TIMP-3, tissue inhibitor of metalloproteinase-3; ECM, extracellular matrix; PC, proprotein convertase; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; CMK, chloromethylketone; APMA, *p*-aminophenylmercuric acetate; HMBA, *p*-hydroxymercuricbenzoic acid; PVDF, polyvinylidene difluoride; IP, immunizing peptide.

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[16], although cleavage of the latter three required removal of the spacer domain$^{688–837}$ within ADAMTS-4. Finally, it has been shown that ADAMTS-4 is capable of autocatalytic C-terminal truncation through cleavage at Lys^{694} and Thr^{581} [17].

Regulation of ADAMTS-4 occurs at multiple levels, including transcription, translation, endogenous inhibitors and substrate accessibility. Expression of ADAMTS-4 mRNA and protein in cartilage can be induced by interleukin-1 (IL-1), tumor necrosis factor (TNF), and fibronectin fragments [18–20], while oncostatin M in combination with IL-1 [21], transforming growth factor β [22], and interleukin-17 [23] have been reported to induce ADAMTS-4 mRNA. Only two naturally occurring inhibitors of ADAMTS-4 have been identified to date, α 2-macroglobulin (α2M) and tissue inhibitor of metalloproteinase-3 (TIMP-3). α2M is found at high levels in synovial fluid and inhibits ADAMTS-4 with a second-order rate constant on the order of 10^6 M^{-1} s^{-1} [12]. TIMP-3, on the other hand, is present in the cartilage extracellular matrix (ECM) and is a potent inhibitor with a K*i*(app) value of 3.30 nM [24]. In order for ADAMTS-4 to cleave aggrecan, access to the substrate is required. Cartilage aggrecan is surrounded by and interacts with many ECM proteins that may interfere with this accessibility and could therefore act as ADAMTS-4 antagonists. For example, relatively high levels of thrombospondin-1 are present in the matrix (≈0.1–1 μM) and bind to the GAG chains of aggrecan, thus preventing aggrecan cleavage by aggrecanases (unpublished observations).

A final means of controlling ADAMTS-4 activity is through post-translational processing. Like all mammalian metalloproteinases, ADAMTS-4 is synthesized as a full-length precursor protein. With a bulky prodomain and a putative cysteine-switch at Cys^{194}, the proenzyme is unlikely to be catalytically active. The mechanisms/agents that mediate N-terminal activation through removal of the prodomain in vivo are poorly understood. When ADAMTS-4 was first purified from IL-1-stimulated bovine nasal cartilage, only one active species was present and had the N-terminus ^{213}FASL, suggesting cleavage between the pro- and catalytic domains at RAKR^{212}–^{213}FASL [7]. Likewise, when recombinant full-length ADAMTS-4 is expressed in insect cells or mammalian cells, only active enzyme with the N-terminus ^{213}FASL is detected, once again suggesting removal of the prodomain through cleavage at RAKR^{212}–^{213}FASL. The P1, P2, P3, and P4 amino acids encode a recognition motif, RX(K/R)R, for members of the proprotein convertase (PC) family, which have been shown to activate several metalloproteinases in vitro, including MT1-MMP, ADAM-10, and ADAM-17 reviewed in [25,26]. This site of cleavage, RAKR^{212}–^{213}FASL, may also be susceptible to cleavage by trypsin-like proteinases such as plasmin and plasminogen activator, as well as several metalloproteinases that have been show to have a preference for Phe at P1′, including MMP-1, -2, -3, -7, -8, and -9. In addition, it has been shown that ADAMTS-4 can cleave itself in the spacer region at Thr^{581}/Phe^{582} and Lys^{694}/Phe^{695} [17], raising the possibility that removal of the prodomain through cleavage at Arg^{212}/Phe^{213} is mediated by intra-/intermolecular autocatalysis.

The objectives of the current studies were to determine whether ADAMTS-4 activity is dependent on removal of the prodomain and to explore which agents/mechanisms mediate N-terminal activation. We examined the ability of (1) PCs, including furin, PC1/3, PC2, PC5/6B, and PACE4, as well as other serine proteinases such as trypsin, chymotrypsin and plasmin; (2) metalloproteinases including MMP-1, -2, -3, -7, -8, -9, -13, -14, -15, -16, -17, and -24, as well as ADAM-17; (3) the aspartyl proteinase, cathepsin D; and (4) autocatalysis in the presence of mercuric compounds, *p*-aminophenylmercuric acetate and *p*-hydroxymercuribenzoic acid, as well as under various conditions including different pH, ionic strength, and temperature to remove the prodomain of ADAMTS-4.

Materials and methods

Materials

Recombinant human MMPs were expressed and purified at Pfizer. Full-length MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and catalytic domain MMP-7, MMP-8, and MT1-MMP were utilized. Enzymes were purified by standard chromatography protocols [27]. Recombinant enzymes expressed as full-length species were activated by 4-aminophenylmercuric acetate except MMP-9, which was activated by human MMP-3. All enzymes were evaluated for purity and approximate molecular weight by polyacrylamide gel electrophoresis under reducing conditions in the presence of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and were found to be at least 95% pure by Coomassie brilliant blue staining. Stock concentrations of the active enzymes were determined by titration against synthetic hydroxamate-based inhibitors. These inhibitors are known to react in a 1:1 stoichiometric fashion with the corresponding target enzyme. MT2-MMP (MMP-15) (catalytic domain), MT3-MMP (MMP-16) (catalytic domain), MT4-MMP (MMP-17) (catalytic domain), and MT5-MMP (MMP-24) were purchased from Chemicon International (Temecula, CA); ADAM-17 was purchased from Oncogene Science. Recombinant human furin, human PC1/3, mouse PC2, human PC5/6B, and rat PACE4 were purchased from Enzyme System Products (Livermore, CA). Recombinant PC4 was expressed in house in HEK293 cells. Activity and concentration of each PC was confirmed by active site titration using the PC-inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethylketone (CMK) from Bachem (King of Prussia, PA). Trypsin, chymotrypsin, plasmin, and cathepsin D were purchased from Sigma (St. Louis, MO). The monoclonal neoepitope antibody (Ab) BC-3 was licensed from Dr. B. Caterson (University of Wales, Cardiff, UK). Polyclonal Abs against the N-terminus or the C-terminus of ADAMTS-4 were purchased from Triple Point Biologics (Forest Grove, OR). These antibodies