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ADAMTS1 cleaves aggrecan at multiple sites and is differentially inhibited by metalloproteinase inhibitors☆

Juan Carlos Rodríguez-Manzaneque,[a] Jennifer Westling,[b] Shelley N.-M. Thai,[a] Alfonso Luque,[a] Vera Knauper,[c] Gillian Murphy,[c] John D. Sandy,[b,d] and M. Luisa Iruela-Arispe[a,*]

[a] *Department of Molecular, Cell and Developmental Biology, Molecular Biology Institute, University of California at Los Angeles, 611, Charles E. Young Drive, Los Angeles, CA 90095, USA*

[b] *Shriners Hospital for Children, Tampa, FL 33612, USA*

[c] *School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK*

[d] *Department of Pharmacology and Therapeutics, University of South Florida, Tampa, FL 33620, USA*

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Abstract

ADAMTS1 is a secreted protein that belongs to the recently described ADAMTS (a disintegrin and metalloprotease with thrombospondin repeats) family of proteases. Evaluation of ADAMTS1 catalytic activity on a panel of extracellular matrix proteins showed a restrictive substrate specificity which includes some proteoglycans. Our results demonstrated that human ADAMTS1 cleaves aggrecan at a previously shown site by its mouse homolog, but we have also identified additional cleavage sites that ultimately confirm the classification of this protease as an 'aggrecanase'. Specificity of ADAMTS1 activity was further verified when a point mutation in the zinc-binding domain abolished its catalytic effects, and latency conferred by the prodomain was also demonstrated using a furin cleavage site mutant. Suppression of ADAMTS1 activity was accomplished with a specific monoclonal antibody and some metalloprotease inhibitors, including tissue inhibitor of metalloproteinases 2 and 3. Finally, we developed an activity assay using an artificial peptide substrate based on the interglobular domain cleavage site (E^{373}–A) of rat aggrecan.

Keywords: ADAMTS; Aggrecan; Extracellular matrix; Metalloproteinase; Proteoglycan

Proteolytic degradation and/or activation of extracellular matrix (ECM) components is continuously occurring during morphogenesis, tissue repair, and pathological processes [1]. Enzymes responsible for these activities include matrix metalloproteinases (MMPs), bone morphogenetic protein 1 (BMP1)/tolloid family of metalloproteinases, tissue serine proteinases, and *adamalysin*-related membrane proteinases that contain disintegrin and metalloproteinase domains (ADAMs). More recently, a new family of proteins, ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs), has been added to this list. The first member of this family to be identified was procollagen I N-proteinase (ADAMTS2). It was shown to release the amino-terminal propeptide of type I and II procollagens, a requirement for the final generation of collagen fibrils [2]. Later, aggrecanases 1 and 2 were both purified and recognized as members of the ADAMTS family (named ADAMTS4 and ADAMTS5, respectively) [3,4]. These enzymes were identified as the proteinases responsible for cleaving the matrix proteoglycan aggrecan at multiple positions; this activity is considered as a landmark of cartilage degradation during inflammatory joint diseases [5]. Mouse ADAMTS1 was also found to cleave aggrecan but only at the most C-terminal of the five known aggrecanase sites [6]. Additional studies showed cleavage of other proteoglycans closely related to aggrecan: versican by ADAMTS1 and

☆ *Abbreviations:* ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs; ECM, extracellular matrix; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases

* Corresponding author. Fax: +1-310-794-5766.
E-mail address: arispe@mbi.ucla.edu (M.L. Iruela-Arispe).

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4 [7] and brevican by ADAMTS4 [8]. The identification of specific substrates for some ADAMTS members, together with the analysis of *adamts1* and *adamts2* null mice [9,10], suggested a relevant role of this family of proteases on extracellular matrix remodeling with implications on morphogenesis.

To best ascertain the biological role of ADAMTS proteins, a complete characterization of their catalytic activity is required. In this study, we evaluated proteolysis by the human recombinant ADAMTS1 (previously named METH-1 [11]) on a large spectrum of extracellular matrix proteins, tested the effects of broad metalloproteinase inhibitors, identified a specific neutralizing antibody, and derived an assay for assessment of proteolytic activity.

Material and methods

Materials. Aggrecan (high buoyant density fraction D1) was isolated from normal mature human cartilage and rat chondrosarcoma by established methods [12]. Versican was obtained as described [7]. Amino-procollagen type I and type III were extracted from dermatosparactic calf skin and purified as described [13,14] (a generous gift of Dr. A. Colige, from Universite de Liege, Belgium). Type I collagen and fibronectin were purchased from Sigma. Type II, IV, and V collagens, laminin, and vitronectin were purchased from Chemicon. Pepsinized type VIII collagen was a generous gift from Dr. H. Sage (Heart Hope Institute, Seattle, WA). Type XVIII collagen was a generous gift from Dr. R. Heljasvaara (University of Oulu, Finland). Thrombospondin 1 was purified from human platelets as described [15]. ADAMTS2 was a generous gift of Dr. A. Colige, from Universite de Liege, Belgium. MMP1, MMP8, MMP9, and MMP14 were purchased from Chemicon. Thrombin was purchased from Roche. Human TIMP1 and TIMP2 were purchased from Calbiochem. TIMP3 (human) was prepared by expression in NSO myeloma cells [16]. TIMP4 (bovine) was expressed as inclusion bodies from *E. coli* and refolded as described for N-TIMP2 [17].

Construction of plasmids and production of recombinant proteins. Generation of full-length human ADAMTS1 and METH-1^{934Myc} constructs have been previously described [18]. To obtain the zinc-binding site mutant ($E^{385} > A$)-METH-1, a single-base substitution ($A^{1154} > C$) was introduced by PCR using forward primer Z1F (535-556): 5′GGCACGTGCGGGGTCGTGGACG, and reverse primer Z2R (1167–1143): 5′CA CGTGGCCTAATGCATGGGCTGTG. The resulting 633 bp product was subcloned into pCR-Blunt II-TOPO vector (Invitrogen), sequenced and digested with *Bbr*PI restriction enzyme. This mutated fragment was substituted into *Bbr*PI-digested METH-1^{934Myc} plasmid. The furin-cleavage site mutant ($R^{232} > A$)-METH-1 was obtained by introducing a two-base substitution ($AG^{695} > GC$) using forward primers Z1F (see above) and F1F (685–704): 5′GGAAGCATAGCAAAGAA GCG, and reverse primer F2R (1167–1143): 5′CACGTGGCCTAATACATGGGCTGTG. Further subcloning of the 633 bp fragment was pursued as above.

Stable 293T cell lines expressing each of these constructs were obtained by co-transfection with hygromycin-resistance vector. Purification of recombinant protein included heparin-affinity chromatography and chelating-affinity chromatography [18].

Proteolytic digestions. For aggrecan digestion, 80 μg substrate (about 60 μg glycosaminoglycan) was incubated with 4.7 μg recombinant human ADAMTS1 in 200 μl 100 mM NaCl, 20 mM Tris, 10 mM $CaCl_2$, pH 7.5 for 16–20 h at 37 °C. The product was deglycosylated in 50 mM sodium acetate, 50 mM Tris, 10 mM EDTA, pH 7.6 with 1.8–15 mU chondroitinase ABC (protease-free) at 37 °C for 1 h followed by 0.3 mU endobetagalactosidase and 0.3 mU keratanase II at 37 °C for 2 h. This last treatment was not required for rat aggrecan digestions. Evaluation of inhibitors on ADAMTS1 activity was achieved by previous incubation with metalloprotease inhibitors or antibodies for 1 or 3 h, respectively, at room temperature.

Digestion of type I procollagen was performed using 10 μg substrate, 50 mM sodium cacodylate, pH 7.5, 200 mM NaCl, 2 mM $CaCl_2$, 2.5 mM *N*-ethylmaleimide, 0.5 mM phenylmethylsulfonyl fluoride, and 0.02% Triton X-100, during 18 h at 26 °C.

Other substrates were tested under the same incubation conditions as used for aggrecan digestion.

Generation of monoclonal antibodies for human ADAMTS1. 3E4C6B4 and 5C6D5 monoclonal antibodies against human ADAMTS1 were generated by immunizing Balb/c mice with purified p87-ADAMTS1 [18]. Mice were first injected intraperitoneally with purified ADAMTS1 (10 μg) and complete Freund coadjuvant (300 μl). Successive injections of purified ADAMTS1 (10 μg) and incomplete Freud coadjuvant (300 μl) were done intraperitoneally at day 15 and 33, and intravenously at day 45. At day 48, spleenocytes were obtained from immunized mouse and fused with SP2 myeloma cells at a 4:1 ratio following established techniques [19]. Conditioned media from hybridoma culture were screened by ELISA against purified p87-ADAMTS1 coated to plastic. Further characterization of positive clones was done by immunoprecipitation using conditioned media from ^{35}S-radiolabeled 293T cells transfected with full-length ADAMTS1 or pcDNA vector as negative control [18] and by Western blot of conditioned media. Positive cells were cloned by repeated limiting dilution.

Immunoblot analysis. Deglycosylated products resulting from aggrecan digestions were analyzed as previously described [20]. Production and specificity analysis of all the antisera used for diagnosis of aggrecan digestion have been described previously [21]. Antibodies used included: G1 (to human and rat aggrecan G1 domain), G3 (LEC-7, to human and rat aggrecan G3 domain), LGQRPP (to the N-terminal neo-epitope at human L^{1920}), TFKEEE (to the C-terminal neo-epitope at human E^{1714}), TASELE (to the C-terminal neo-epitope at human E^{1539} or rat E^{1274}), NITEGE (to the C-terminal neoepitope at human and rat E^{373}). The VDIPEN antiserum (to the C-terminal neoepitope at human and rat N^{341}) was affinity-purified antiserum 1319 from Dr. John Mort. When more than one antiserum was used on a single blot (Figs. 1 and 2), the chemiluminescent signal from the first probe was eliminated (or reduced) by washing the membrane with 200 mM Tris base, 1.37 M NaCl, 0.1% (v/v) Tween20, pH 7.6 for 1 h, prior to incubation with the subsequent antiserum.

Fluorescent peptide assay. Peptides were synthesized based on the primary sequence at the ADAMTS cleavage site at Glu^{373}–Ala in rat aggrecan. Substrate peptides (190 μM) were incubated overnight in 20 mM Tris, pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$ at 37 °C in the presence or absence of ADAMTS1 (500 nM). The digests were then passed through a Hypersil C18 column in 0.1% TFA/water with a 10–80% gradient of 0.1% TFA/water and 70% acetonitrile. The substrate and products were detected using UV at 214 nm and fluorescence at excitation 334 nm, emission 514 nm. All product peaks were collected and mass verified by MALDI-TOF mass spectrometry (University of Florida Protein Chemistry Core).

Results

To further expand our current understanding of the substrates for ADAMTS1, we tested its proteolytic activity on a large spectrum of purified extracellular matrix proteins (Table 1). None of the major collagens, procollagens, or glycoproteins tested were cleaved by

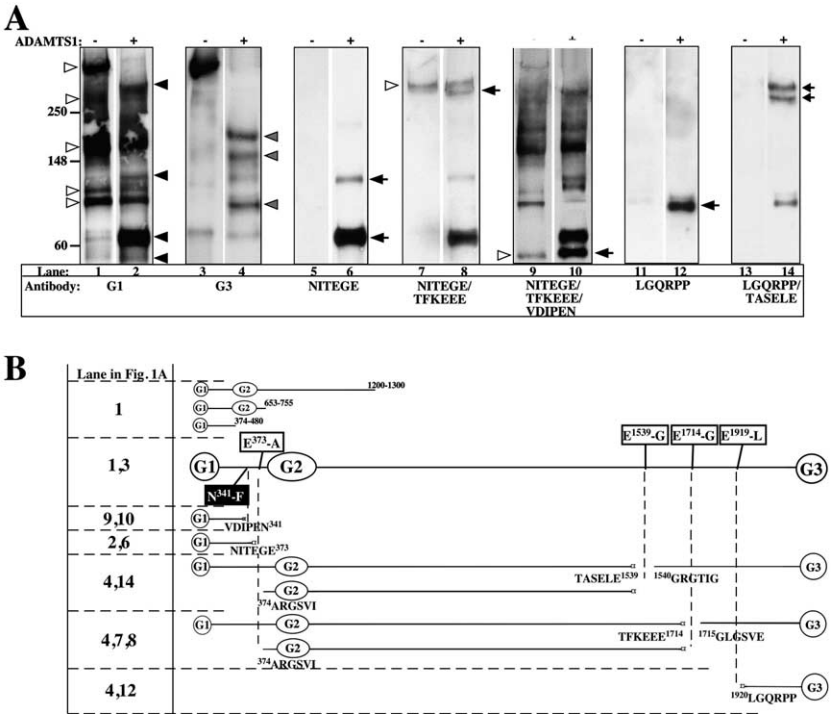

Fig. 1. Analysis of human aggrecan and products generated after incubation with ADAMTS1. (A) Deglycosylated samples were subjected to SDS–PAGE (gradient 4–12%) and immunoblotted with G1 (lanes 1–2), G3 (lanes 3–4), NITEGE (lanes 5–6), NITEGE, and TFKEEE (lanes 7–8), NITEGE, TFKEEE, and VDIPEN (lanes 9–10), LGQRPP (lanes 11–12), and LGQRPP and TASELE (lanes 13–14) antisera. Untreated material showed full-length aggrecan and at least four major G1-bearing core species (open arrowheads in lane 1). Trace amounts of cleavage products were identified as G1-TFKEEE^{1714} and G1-VDIPEN^{341} (open arrowheads in lanes 7 and 9). (B) Schematic description of aggrecan fragments and location of neo-epitopes generated by aggrecanases and MMPs. Residue numbers indicated are for human aggrecan.

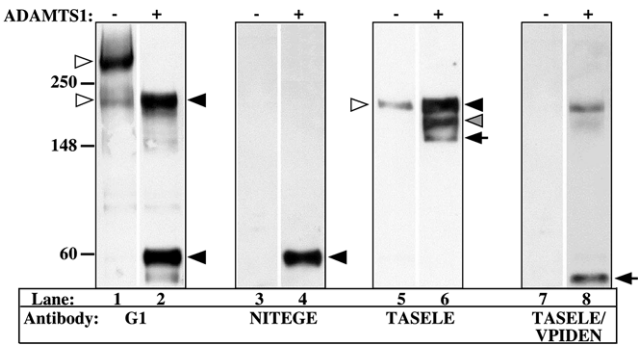

Fig. 2. Analysis of rat aggrecan and products generated after incubation with ADAMTS1. Deglycosylated samples were subjected to SDS–PAGE (gradient 4–12%) and immunoblotted with G1 (lanes 1–2), NITEGE (lanes 3–4), TASELE (lanes 5–6), and TASELE and VDIPEN (lanes 7–8) antisera.

ADAMTS1, including type I procollagen, a recognized substrate for ADAMTS2. Conditions used to provide adequate opportunity for recognition and cleavage included substrate:enzyme molar ratios 1:1 to 1:20 and buffers compatible with its ability to digest aggrecan, which was used as a positive control. Extracellular proteins found to be cleaved by ADAMTS1 were restricted to two proteoglycans tested: aggrecan and versican. Full characterization of versican cleavage has recently been described by us [7]. Regarding ADAMTS1 activity on aggrecan, we used both human and rat

Table 1
Substrates tested for proteolytic activity

Substrates	Cleavage by	
	ADAMTS1	Control protease
Pro-collagen type I	No	Yes (ADAMTS2)
Pro-collagen type III	No	Yes (MMP1)
Collagen type I	No	Yes (MMP1)
Collagen type II	No	Yes (MMP1)
Collagen type IV	No	Yes (MMP9)
Collagen type V	No	Yes (MMP14)
Collagen type VIII	No	Yes (MMP9)
Collagen type XVIII	No	ND
Aggrecan	Yes	Yes (MMP8)
Versican	Yes[a]	ND
Fibronectin	No	Yes (MMP14)
Laminin	No	ND
Vitronectin	No	ND
Thrombospondin 1	No	Yes (Thrombin)

Activity of ADAMTS1 on a variety of substrates was evaluated at 1:1 and 1:20 ratios. Other proteases (indicated between parenthesis) were used as positive control. ND: Not determined.

[a] Described in [7].

aggrecan, and the degree of cleavage was determined with seven different antisera (described under Material and methods and [21]) that recognize N-terminal globular domain of aggrecan (G1), C-terminal globular domain (G3), or different neo-epitopes known to be produced by the action of aggrecanases and/or MMPs in vivo.

Digestion of aggrecan with ADAMTS1 resulted in several previously uncharacterized fragments. Immunoblotting with G1 antibody showed increased amounts of G1-bearing products (Fig. 1A, black arrowheads in lane 2). G3 antiserum revealed the presence of three G3-bearing fragments upon digestion with ADAMTS1 (Fig. 1A, gray arrowheads in lane 4). The generation of species by cleavage at aggrecanase sites was confirmed using specific neo-epitope antisera. NITEGE antiserum identified two species, the expected G1-NITEGE^{373} and a novel related product that appears to represent a dimeric form (Fig. 1A, arrows in lane 6). ADAMTS1 treatment did not significantly increase reactivity of the fragment G1-TFKEEE^{1714} already present, but this antiserum showed a new species which results from the N-terminal deletion of this fragment (Fig. 1A, arrow in lane 8). In agreement with previous data for mouse ADAMTS1 activity on bovine aggrecan [6], only one major fragment was identified containing the sequence L^{1920} GQRPP (Fig. 1A, arrow in lane 12). TASELE antiserum also identified a doublet that corresponds to G1-TASELE^{1539} and the fragment resulting from its N-terminal deletion (Fig. 1A, arrows in lane 14). Finally, an unexpected increase in the G1-VDIPEN^{341} fragment was also confirmed by its uniquely strong reactivity with the neo-epitope antiserum (Fig. 1A, arrow in lane 10).

The ability of ADAMTS1 to function as a typical 'aggrecanase' was additionally supported by the digestion of rat aggrecan (Fig. 2). Rat aggrecan substrate contained the full-length core and trace amounts of a cleavage product (Fig. 2, open arrowheads in lane 1). This weak secondary product was identified as endogenous G1-TASELE^{1274} (rat numbering) as revealed by its reactivity with TASELE antiserum (Fig. 2, open arrowhead in lane 5). Digestions with ADAMTS1 eliminated the full-length core and produced two major G1-reactive products (Fig. 2, black arrowheads in lane 2). Identification of these species was confirmed by their reactivity with NITEGE and TASELE antisera (Fig. 2, black arrowheads in lanes 4 and 6, respectively). In addition, much as with the human aggrecan substrate, we detected a significant amount of a 50 kDa G1-bearing fragment (Fig. 2, lane 2) which reacted specifically with the VDIPEN antiserum (Fig. 2, arrow in lane 8). TASELE antiserum showed reactivity with another product that corresponds to the N-terminal deletion of the G1-TASELE fragment (Fig. 2, gray arrowhead in lane 6), as was shown above for the human form (Fig. 1A, lane 14). A minor TASELE-reactive product (Fig. 2, arrow in lane 6), which was not identified by the anti-G1 serum, was also detected; the nature of this fragment will be discussed later.

The specificity of ADAMTS1 proteolytic activity was further confirmed with an inactive mutant form. Specific mutations in the zinc-binding domain have been shown to suppress the activity of metalloproteinases [22]. Single substitution of residue E^{385} to A at the zinc-binding site disabled the catalytic activity of ADAMTS1 (Fig. 3A). In addition, the presence of the prodomain is known to cause latency of the protease activity in MMP and ADAM proteases [22]. In ADAMTS1, release of the prodomain is mediated by furin that cleaves the consensus sequence RKKR^{235} [18]. A single amino acid substitution in the furin-cleavage site resulted in a recombinant form in which the prodomain was no longer released by furin. When this form was tested for activity on aggrecan, no cleavage products were observed (Fig. 3B), confirming the need for an N-terminal processing event to activate ADAMTS1.

Inhibition of the proteolytic activity of ADAMTS1 was approached using monoclonal antibodies raised against recombinant human ADAMTS1 protein and recognized metalloproteinase inhibitors. In contrast to broad-spectrum metalloproteinase inhibitors, the use of antibodies as catalytic inhibitors has the advantage of specificity [23]. Monoclonal antibodies were raised against p87-ADAMTS1 recombinant protein. From several antibodies tested, the clone 3E4C6B4 displayed inhibitory activity (Fig. 4A). Clone 5C6D5, which also recognized ADAMTS1 but was not neutralizing, was used as negative control. Characterization of clone 3E4C6B4 indicated that the recognition epitope was located within the metalloprotease-spacer cassette (positions 235–717). Clone 5C6D5 recognized an epitope located in the carboxyl-terminal end of ADAMTS1 protein. These antibodies did not present any cross-reactivity with ADAMTS4 nor ADAMTS5 (data not

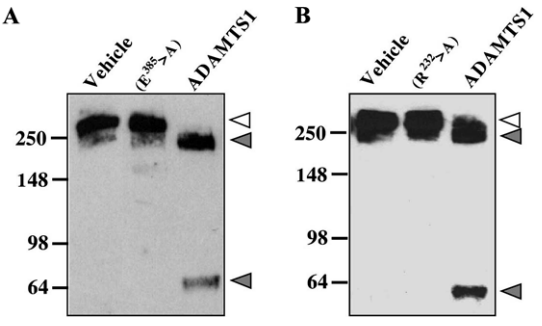

Fig. 3. Aggrecanase activity of mutated ADAMTS1. Rat aggrecan was incubated in the presence of vehicle, wild type ADAMTS1, or the mutants: zinc-binding site mutant (E^{385} > A) (panel A), and furin-cleavage site mutant (R^{232} > A) (panel B). Deglycosylated samples were resolved by SDS–PAGE (8%) and immunoblotted with G1 antiserum. Undigested aggrecan (open arrowheads), aggrecan fragments (filled arrowheads).

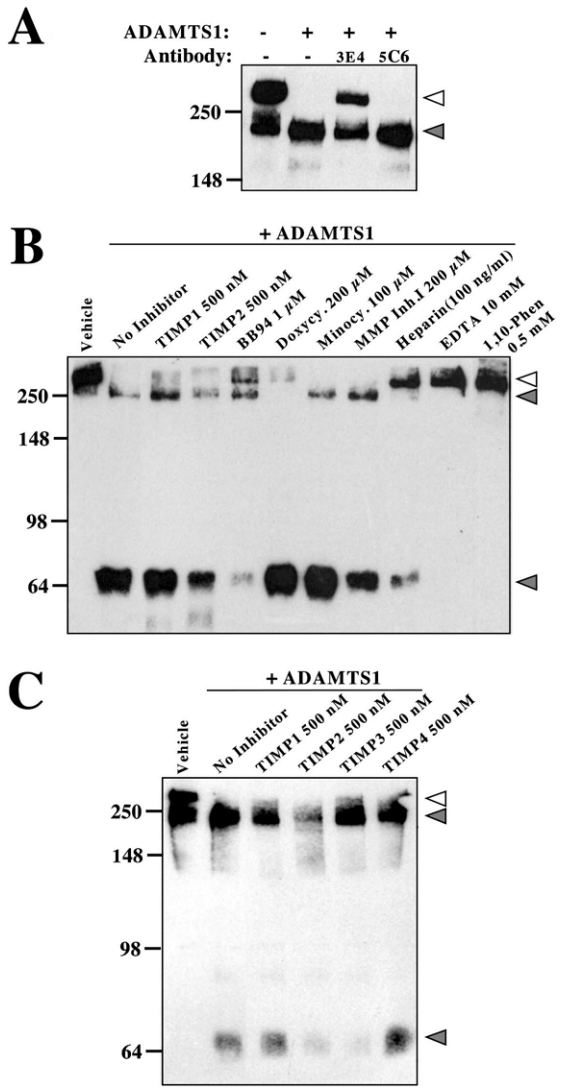

Fig. 4. Aggrecanase activity of ADAMTS1 in the presence of monoclonal antibodies and metalloproteinase inhibitors. Rat aggrecan was digested in the presence of ADAMTS1 or vehicle, previously incubated with specific monoclonal antibodies for 3 h at room temperature (panel A), or with metalloproteinase inhibitors at the indicated concentrations for 1 h at room temperature (panels B and C). Deglycosylated samples were resolved by SDS–PAGE (8%) and immunoblotted with G1 anti-serum. Undigested aggrecan (open arrow heads), aggrecan fragments (filled arrowheads).

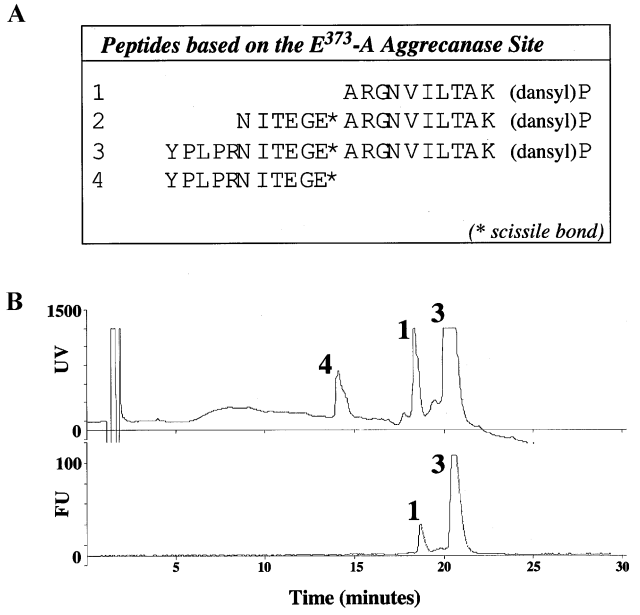

Fig. 5. Separation of products of ADAMTS1 dependent cleavage of a peptide substrate. (A) Peptides based on the E^{373}–A aggrecanase site. (B) Dansylated substrate (peptide 3) was digested with ADAMTS1 and products (peptides 1 and 4) were separated on Hypersil C-18 with detection at 214 nm (upper panel) and fluorescence at excitation 334 nm, emission 514 nm (bottom panel).

shown). Recognized metalloproteinase inhibitors displayed various degrees of inhibition. Total inhibition was observed with EDTA and 1,10-phenanthroline (Fig. 4B). Among several synthetic MMP inhibitors tested, BB94 also blocked aggrecan cleavage by ADAMTS1, MMP inhibitor I, and doxycycline showed only partial inhibition, and minocycline did not have any effect. In addition, heparin was able to block ADAMTS1 catalytic activity, suggesting that heparin binding could be an important modulator for ADAMTS1 activity in vivo. Finally, the physiological inhibitors of matrix metalloproteinases, TIMP1, 2, 3, and 4, were also tested. We observed no significant inhibition with TIMP1 or TIMP4 at 500 nm (Fig. 4B and C). However, TIMP2 and TIMP3 were able to inhibit ADAMTS1 activity, but not completely, at the same concentration (Fig. 4B and C). These results are in contrast to the inhibitory effects of TIMPs in other ADAMTS family members. For example, TIMP1 and TIMP3 had been shown to inhibit ADAMTS4 activity completely at 500 nm [24–26].

The ability of ADAMTS1 to cleave peptides based on the E^{373}–A aggrecan cleavage site was further investigated with the aim to establish an activity assay for the enzyme. Four peptides were synthesized (Fig. 5A) and their structure and purity was determined on HPLC with fluorescence and UV detection. ADAMTS1 cleaved peptide 3 into two major products that eluted in the positions expected for the products of cleavage at the target E–A bond only (Fig. 5B). Confirmation of the structures as peptides 1 and 4 was made by MALDI-TOF mass spectrometry of the collected peaks. The material in the peak labeled 1 had a mass-to-charge ratio $(m/z) = 1289.53$ corresponding to peptide 1, the C-terminal product ARGNVILTAK(dansyl)P, plus one positive charge. The material in the peak labeled 4 gave a $m/z = 1373.51$ consistent with the structure of peptide 4, the N-terminal product YPLPRNITEGE, plus one positive charge. In other incubations we found that peptide 2 was not cleaved by ADAMTS1 even after extended digestion with excess enzyme, suggesting that proteolysis was highly dependent on the presence of residues in the P7–P11 positions [27] for these substrates.

Discussion

These results indicate that human ADAMTS1 displays all of the features attributed to aggrecanases previously ascribed to ADAMTS4 [28] and ADAMTS5 [4]. Unlike other mammalian metalloproteinases, ADAMTS1 appears to have a restricted spectrum of substrates, which at present is confined to the large proteoglycans aggrecan ([6] and this report) and versican [7].

ADAMTS1 has been shown to cleave bovine aggrecan at the equivalent of the human E^{1919}–L bond [6]; here we extend this observation and show that this protease can cleave effectively at four 'aggrecanase' sites in both human and rat aggrecans. These cleavage sequences all possess a glutamic acid in the upstream position and a non-polar or uncharged polar residue (A, L, and G) in the downstream position; indeed, these proteases have been described as glutamyl-endopeptidases [7]. In addition, we show that ADAMTS1 cleaves only at the expected bond in an artificial peptide substrate based on the interglobular domain cleavage site (E^{373}–A) of rat aggrecan.

Based on these results, the degradation of aggrecan in vivo may result from the individual or combined action of ADAMTS1, 4, and 5. Aggrecan is a major component of cartilage and its degradation by aggrecanases is considered as an important prognosis factor during the development of degenerative joint disease as arthritis [5,29]. In addition, aggrecan fragments generated by aggrecanase activity are also found in the rat spinal cord throughout development and after injury [30]. Interestingly, ADAMTS1 has been found in human articular cartilage [31], and more recent findings also verified the presence of ADAMTS1 transcripts in a subset of neurons during murine development (S.N.M. Thai and M.L. Iruela-Arispe, submitted). Furthermore, induction of ADAMTS1 expression was reported in adult rat injured neurons [32]. Despite the described presence of ADAMTS1 in locations where aggrecan fragments are found, its physiological contribution to this proteolytic activity is still to be confirmed in vivo.

Development and characterization of specific inhibitors of the catalytic activity is valuable to assess the relative contribution of these metalloproteases in pathological/physiological processes that involve degradation of aggrecan. Here, we generated a monoclonal antibody against human ADAMTS1 (clone 3E4C6B4) that does not recognize ADAMTS4 or ADAMTS5 and that inhibits the aggrecanase activity of ADAMTS1. Further studies on the in vivo distribution of ADAMTS1 and other ADAMTS proteins, along with gene-deletion approaches, are now required to establish specific roles for each of these proteinases in processes such as rheumatoid arthritis.

The detailed characterization of aggrecan digestion by ADAMTS1 also revealed three novel cleavage products. One of these, with a molecular weight of about 130 kDa, seems to represent the dimeric form of G1-NITEGE^{373} fragment (Fig. 1A, lane 6). This fragment is not a disulfide-bonded dimer, since electrophoresis was performed under reducing conditions and this species does not occur in digests of rat aggrecan prepared under the same conditions (Fig. 2, lane 4). It also does not appear to be generated specifically by ADAMTS1 digestion, since the same human samples digested with ADAMTS4 also contain this product and digestion with MMP3 generates the equivalent VDIPEN-reactive species at about 100 kDa [33]. These results suggest that in mature cartilage, a small portion of human aggrecan monomers form in situ non-reducible cross-links with each other or with other matrix proteins of a similar size to the G1 domain, such as link protein [34]. A second novel species described here (Fig. 2, arrow in lane 6) appears to represent the product of the ADAMTS1-dependent cleavage of the G1-TASELE fragment at an unknown site between the G2 and keratan-sulfate domains. Such activity may be responsible for the high content of the aggrecan double-globe species found in aggrecan prepared from human (Fig. 1A, white arrowhead above 148 kDa marker in lane 1), bovine, and pig cartilage [21,35]. The third novel species is the G1-VDIPEN product which were detected in incubations of ADAMTS1 with both human and rat aggrecans. This product was previously identified in incubations of aggrecan with a range of MMPs [36], cathepsin B [37] and also recombinant human ADAMTS4 [33].

Finally, versican was also found to be cleaved by ADAMTS1 and ADAMTS4 [7], and brevican by ADAMTS4 [8], at sequences very conserved in comparison with cleavage sites of aggrecan. Although the activity of ADAMTS1 on brevican remains to be tested, it is likely that it will also recognize and cleave this proteoglycan. All these substrates belong to the hyalectans subfamily of matrix proteoglycans [38] that, together with collagens, provide strength and resistance to the ECM. A common feature of these proteoglycans is their multidomain structure in which major functional roles include binding to complex carbohydrates (hyaluronan and lectins) and addition of glycosaminoglycan chains in extracellular matrices. The processing and remodeling of this group of matrix proteoglycans appear to occur by the combined action of MMP and ADAMTS proteases.

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