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Scientific Research

Arg-Ser-^{775}, ^{792} and ^{823} in Spacer Region of ADAMTS-18 Is Critical for Thrombin Cleavage

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Abstract

Cleavage of ADAMTS-18 by thrombin represents a new mechanism of platelet thrombus clearance via the release of active ~45-kDa C-terminal fragments that induces oxidative platelet fragmentation. The exact cleavage sites remain unclear, but Arg (R)^{775}/Ser (S)^{776} in spacer region of ADAMTS-18 has been shown to be one of the cleavage sites of thrombin. Here, we demonstrate that R^{792}/S^{793} and R^{823}/S^{824} are also thrombin cleavage sites by sequence analysis, amino acid mutation and mass spectrometry assay. All these cleavage sites are thrombin-specific and insensitive to other enzymes tested (e.g. cathepsin D or trypsin). Simultaneous mutation of R^{775}, ^{792}, ^{823} to S^{775}, ^{792}, ^{823} in ADAMTS-18 completely abrogated the cleavage by thrombin and the generation of active C-terminal 45-kDa fragments. Together with previous study, a total of three thrombin-specific cleavage sites have been identified in spacer region of ADAMTS-18.

Keywords

ADAMTS-18, Thrombin, Cleavage, Mass Spectrum

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1. Introduction

ADAMTS (a disintegrin and metalloproteinase domain, with thrombo spondin type-1 modules) is a family of 19 secreted Zn-metalloproteinases, which have multidomain structural components in common [1]. These include an N-terminal signal peptide, followed by a pro-domain, a metalloproteinase catalytic domain with a zinc binding motif, a disintegrin-like domain, a central thrombospondin type-1-like repeat (TSR), a cysteine rich domain (high sequence homology), a spacer region, and a variable number of C terminal TSR repeats. This family plays important roles in several pathophysiological conditions mainly including arthritis [2], spermatogenesis [3], angiogenesis [4] [5], and thrombosis-related disease [6]. Noteworthily, most of these activities are related to proteolytic processing within their C-terminal regions [6]-[9].

ADAMTS-18 has the similar domain organization as other family members. ADAMTS-18 has been shown to be epigenetically silenced in multiple carcinomas and has tumor suppressor activity [10]. Mutation of ADAMTS-18 is strongly associated with colorectal cancer [11]. The data from National Center for Biotechnology Information (NCBI) subject's gene expression omnibus (GEO) also showed that ADAMTS-18 gene was differentially expressed in subjects with normal skeletal fracture versus subjects with nonunion skeletal fracture [12]. Therefore, it is also associated with bone mineral density (BMD) determination in the major human ethnic groups. Recently, some studies indicate that the ADAMTS-18 gene is also play a crucial role in early eye development [13].

Platelet integrin αIIbβ3 (GPIIb/IIIa) is a heterodimeric receptor of the integrin family expressed at high density (50,000 - 80,000 copies/cell) on the platelet plasma membrane [14]. GPIIIa49-66 (CAPESIEFPVSEAREVLED) is a linear epitope of integrin subunit β3 (GPIIIa) in its extracellular domain. We previously reported that the unique feature of the antibodies (Abs) against GPIIIa49-66 was their ability to induce reactive oxygen species (ROS) through the activation of 12-lipoxygenase and nicotinamide adenine dinucleotide phosphate oxidase (NADPH), leading to complement-independent platelet fragmentation [15] [16]. Recently, we revealed that ADAMTS-18 was the physiologic ligand of platelet GPIIIa49-66 [17]. Thrombin generated from the endothelium of vessel injury is able to cleave ADAMTS-18. The generated ~45-kDa C-terminal cleavage product of ADAMTS-18 becomes activated. It clusters the β3 integrins and induces oxidative platelet fragmentation as we previously described anti-GPIIIa 49 - 66 Ab [17]. We have identified that R^{775}/S^{776} in spacer region of ADAMTS-18 is one of the potential cleavage sites of thrombin [18]. However, sequence analysis indicates that there still exist two same sites in spacer region neighboring R^{775}/S^{776} named R^{792}/S^{793} and R^{823}/S^{824}, which also generate similar ~45-kDa C-terminal products in theory when cleaved by thrombin. In this study, we have investigated other thrombin cleavage sites in spacer region of ADAMTS-18 through amino acid mutation and mass spectrometry assay.

2. Materials and Methods

2.1. Reagents

All reagents were obtained from Sigma (St. Louis, MO) unless otherwise designated. Full-length ADAMTS-18 cDNA coding sequence was purchased from ATCC and cloned into mammalian expression vector pBudCE4.1 from Invitrogen (Carlsbad, CA) [17] [18]. ADAMTS-18 peptides were synthesized by Sangon Biotech (Shanghai, China). The *in vitro* Transcend™ Biotinylated Translation Detection Systems was purchase from Promega (Madison, WI, USA).

2.2. *In Vitro* DNA Translation and Thrombin Cleavage Assay

Biotinylated-methionine-labeled ADAMTS-18 or its mutant was translated using an *in vitro* Transcend™ Biotinylated Translation Detection Systems following the protocol provided by the manufacturer. All the peptides or translated proteins were then digested by thrombin or cathepsin D or trypsin according to the protocol provided by the manufacture.

2.3. Immunoblotting

In vitro translation products were separated by 12% SDS/PAGE gels, transferred to a nitrocellulose membrane, and immunoblotted with horseradish peroxide (HRP) conjugated avidin for 1 hour followed by washing with

PBST (0.1% Tween 20). The signal band was detected by chemiluminescence substrate [18].

2.4. Mass Spectrometry

Mass spectrometry was performed as previously described [18]. Briefly, for analysis of ADAMTS-18 cleavage products, a fresh mixture of enzyme and ADAMTS-18 peptide was submitted to molecular weight determination by Matrix assisted laser desorption ionization quadrupole time of flight (MALDI-QTOF) mass spectrometry (MS) (Applied Biosystems 4700 Proteomics Analyzer). To determine the amino acid sequences of newly observed peaks, MS/MS peptide *de novo* sequencing using a specific software program (Applied Biosystems DeNovo Explorer) was performed.

3. Results

3.1. Thrombin Cleavage of C-Terminal ADAMTS-18 on Several Sites

Previous study has shown that the full-length ADAMTS-18 is proteolyzed by thrombin and results in ~45-kDa C-terminal fragments releasing [17]. The optimal cleavage site for thrombin is R/X [X refers to nonacidic amino acid mainly including R, lys (K), His (H), and Ser (S)] (**Figure 1(a)**). We have demonstrated that R^{775}/S^{776} in spacer region of ADAMTS-18 is the potential cleavage site of thrombin [18]. However, analysis of the primary amino acid sequence of ADAMTS18 revealed that there exist three similar thrombin cleavage sites in ADAMTS-18 spacer region named R^{775}/S^{776}, R^{792}/S^{793} and R^{823}/S^{824} (**Figure 1(b)**). The possible molecular weight from these predicted sites to C terminal is ~49-, 47- and 43-kDa, respectively (**Figure 1(c)**). Therefore, it remained uncertain whether R^{792}/S^{793} and R^{823}/S^{824} were also the actual sites of proteolysis.

3.2. Susceptibility of Thrombin for R^{792}/S^{793} and R^{823}/S^{824} of C-Terminal ADAMTS-18

To explore these predictions, we synthesized peptides covering the other two putative cleavage sites. The P06594 (PGEFPFAGTTFEYQ**RS**FNRPERLYAPG) covers R^{823}/S^{824}. The initial molecular weight (MW) of

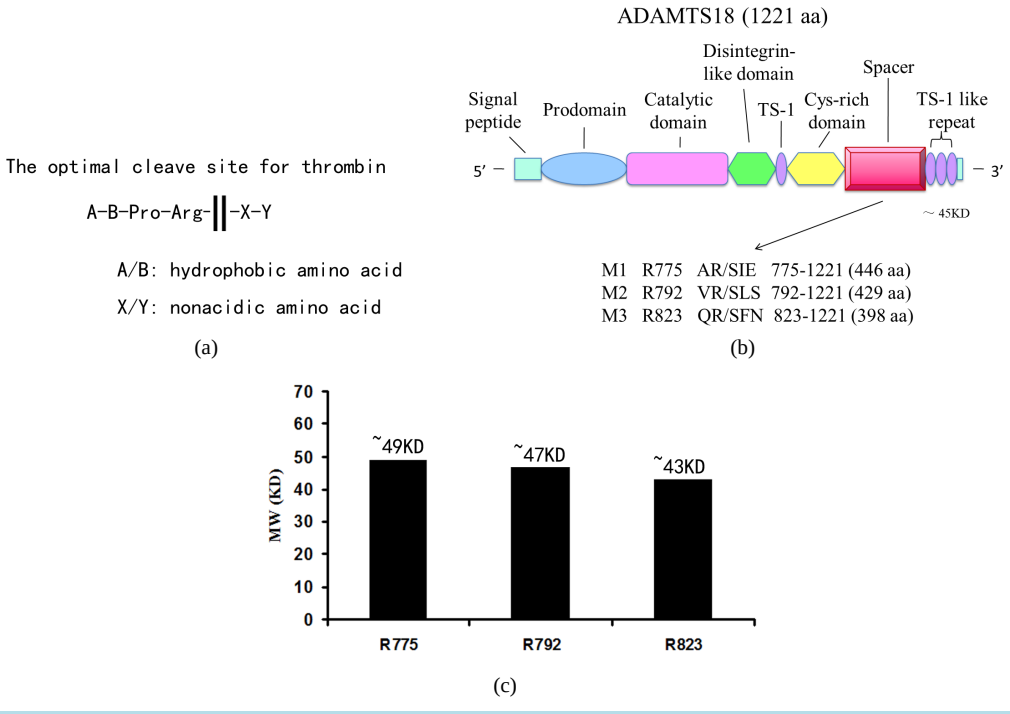
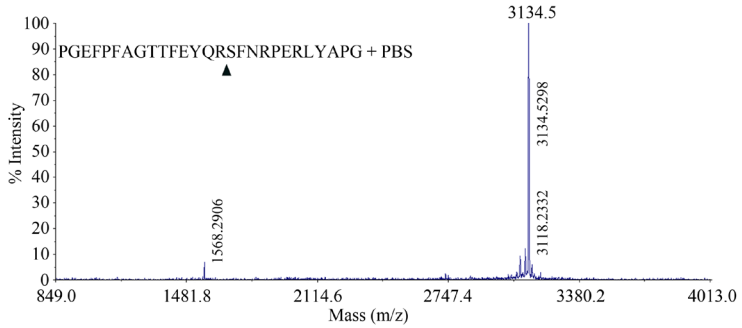


Figure 1. Putative thrombin cleavage sites in ADAMTS-18 spacer region. (a) The optimal cleave site for thrombin. (b) Diagram of ADAMTS-18 domain structure. Location of predicted thrombin cleavage sites after Arg $(R)^{775}$, R^{792} and R^{823} are shown above. (c) The possible molecular weight from these predicted cleavage sites to C-terminal of ADAMTS-18.

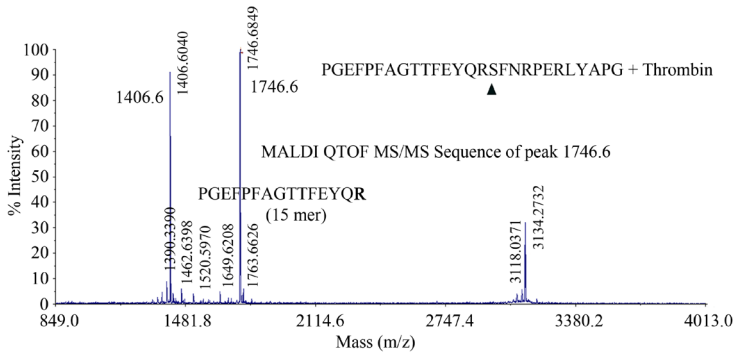
P06594 is ~3134.5 Da when incubated with PBS buffer (**Figure 2(a)**). However, thrombin cleaved 27-mer P06594 at R/S site, producing 15-mer N terminal peptide PGEFPFAGTTFEYQR (~1746.6 Da) and 12-mer C terminal peptide SFNRPERLYAPG (~1406.6 Da), respectively (**Figure 2(b)**). The generation of 15-mer peptide becomes obvious when the concentration of thrombin beyond 5 U/ml (**Figure 2(c)**). Consistently both PBS and thrombin had no effect on P06595 (PGEFPFAGTTFEYQ**SS**FNRPERLYAPG), in which R^{823} was mutated to S^{823} (**Figure 2(d)** and **Figure 2(e)**). The generation of 15-mer peptide completely abrogated when cleaved by various concentrations of thrombin (**Figure 2(f)**). Hirudin completely inhibited the generation of MW1746.6 and MW1406.6 peak suggesting the specificity of thrombin cleavage (**Figure 3**) Similar results were obtained with P06728 (ELQVSS SYLAV**RS**LSQKYYLTGGWSID), which covers R^{792}/S^{793} producing two peptide peaks (~1717.9 Da and 1351.52) at R^{792}/S^{793} site (**Table 1**). We also incubated these peptides with other enzyme cathepsin D or trypsin, and assayed by mass spectrometry. It demonstrated that these cleavage sites are thrombin-specific, and insensitive to cathepsin D or trypsin (**Table 1**).

3.3. Specificity of Thrombin for Site-Mutated ADAMTS-18 Full-Length Protein

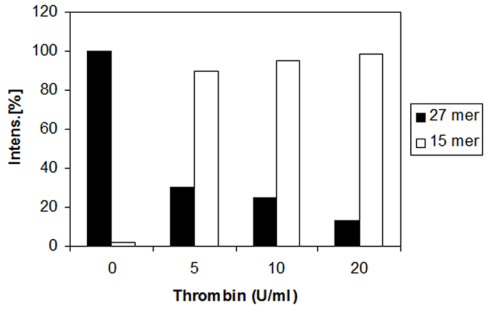
Since R/S^{775}, ^{792} and ^{823} in spacer region of ADAMTS-18 are critical for thrombin cleavage, we further constructed mammalian expression vector in which all these susceptible sites were mutated to $S/S-^{775}$, ^{792} and ^{823} (**Figure 4(a)**).



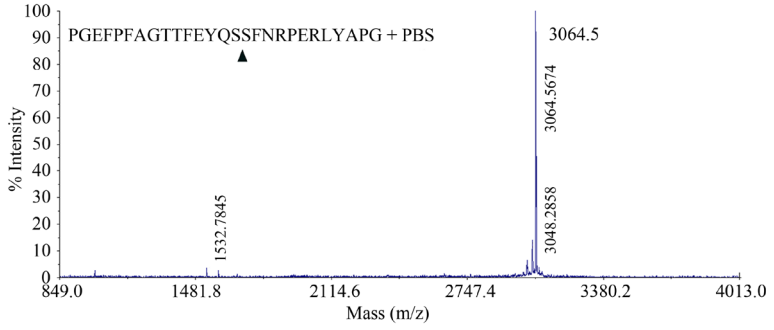
(a)



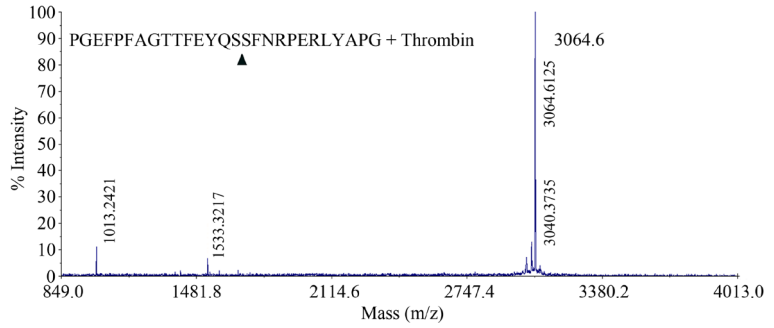
(b)



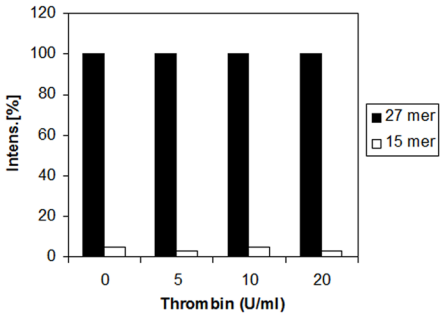
(c)



(d)



(e)



(f)

Figure 2. Susceptibility of thrombin for R^{823}/S^{824} site of ADAMTS18. (a) (b) Synthesized 27-mer ADAMTS-18 peptide P06594 containing R^{823}/S^{824} (~3134.5 Da) was incubated with PBS (a) or 5 U/ml thrombin (b) for 1 h. The putative thrombin cleavage site was confirmed by MALDI QTOF mass spectrometry. (c) The releasing of 15-mer N terminal peptide (MW 1746.6) when P06594 was cleaved by different concentration of thrombin at R^{823}/S^{824} site. (d) (e) Mutated 27-mer ADAMTS-18 peptide P06595 (~3064.5 Da, R^{823} switches to S^{823}) was incubated with PBS (d) or thrombin (e) in the same condition as P06594 and analyzed by mass spectrometry. Proteolysis after R^{823} was prevented by substitution of the arginine (Arg, R) to serine (Ser, S). (f) No 15-mer N terminal peptide (MW 1746.6) was released when P06595 was cleaved by different concentrations of thrombin at S^{823}/S^{824} site.

Bio-methionine-labeled ADAMTS18 and site-mutant ADAMTS-18 were synthesized with *in vitro* translation system using the expression vector of pBudCe 4.1/ADAMTS-18. Both pBudCe 4.1/ADAMTS-18 (lane 2) and its mutant (lane 4) demonstrated two dominant bands of ~135 kDa and ~75 kDa. The ~135 band represented intact ADAMTS-18 (1221 amino acids), and ~75 kDa band does represent the short form of ADAMTS-18 since luciferase control (lane 1) and the empty vector (lane 3) did not transcribe these two bands (**Figure 4(b)**). The expression of ~75 kDa short form of ADAMTS-18 is consistent with our previous report [18]. **Figure 4(c)** demonstrates wide type ADAMTS-18 is proteolyzed by thrombin, and the cleavage fragment is about ~45-kDa. However, thrombin had no effect on mutated ADAMTS-18 in various concentrations (**Figure 4(d)**).

Table 1. Analysis of thrombin cleavage sites in ADAMTS-18 protein.

Number	Peptide location in ADAMTS-18	Sequence	Molecular weight	Molecular weight after thrombin digestion (R/S)	Molecular weight after cathepsin D or trypsin digestion
331941	762-791	NEYYPVVIIPAGA**RS**SIEIQELQVSSSYLAV	3308.41	1447.52 + 1559.3	ND
331942	762-791	NEYYPVVIIPAGA**SS**SIEIQELQVSSSYLAV	2982.29	ND	ND
P06728	780-806	ELQVSSSYLAV**RS**LSQKYYLTGGWSID	3051.4	1351.52 + 1717.9	ND
P06729	780-806	ELQVSSSYLAV**SS**LSQKYYLTGGWSID	2982.29	ND	ND
P06594	808-834	PGEFPFAGTTFEYQ**RS**FNRPERLYAPG	3134.5	1746.6 + 1406.6	ND
P06595	808-834	PGEFPFAGTTFEYQ**SS**FNRPERLYAPG	3066.34	ND	ND

ND, no digestion.

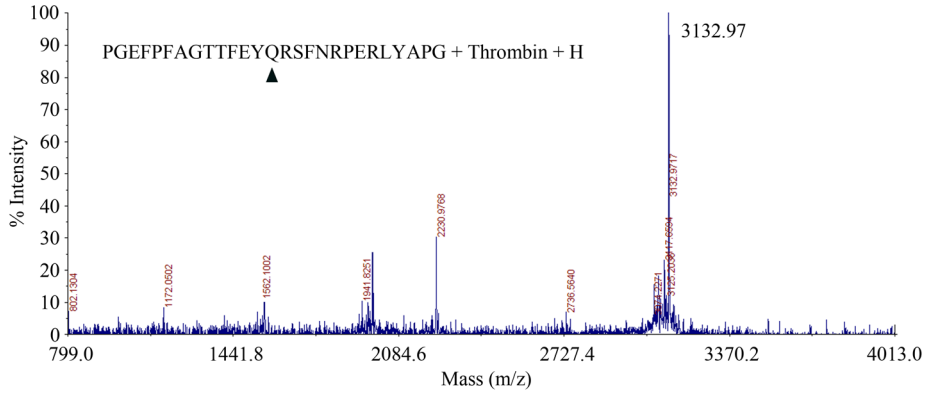


Figure 3. Inhibition effect of hirudin on thrombin cleavage. Synthesized 27-mer ADAMTS18 peptide P06594 was incubated with 5 U/ml thrombin and equal amount of hirudin for 1 h and analyzed by mass spectrometry. Representative mass spectrometry map showed proteolysis after R^{823} was completely inhibited by the addition of hirudin.

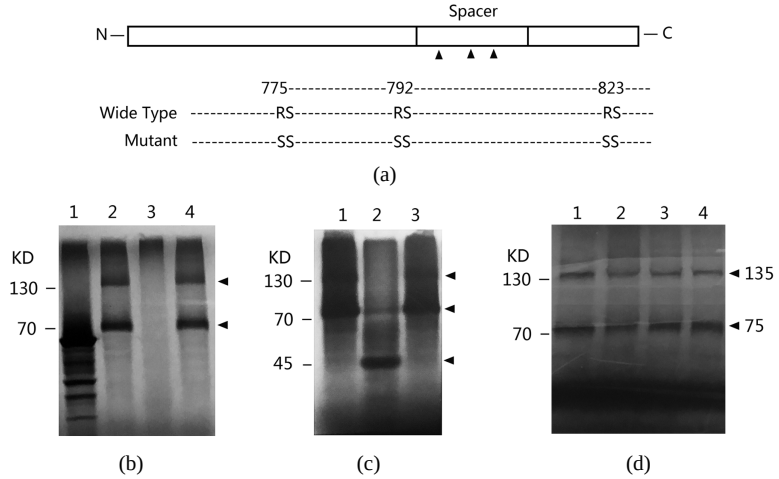


Figure 4. Effect of thrombin on ADAMTS-18 mutant. (a) Diagram of ADAMTS-18 mutation sites in which R^{775}, R^{792} and R^{823} were simultaneously mutated to S^{775}, S^{792} and S^{823}. (b) *In vitro* translation. Lane 1, luciferase (~62 kDa); lane 2, pBudCE4.1/ADAMTS-18 (~135 kDa); lane 3, pBudCE4.1; lane 4, pBudCE4.1/ADAMTS-18 mutant (~135 kDa). (c) Wide-type ADAMTS-18 was incubated with thrombin and analyzed by immunoblotting. Lane 1, Bio-ADAMTS-18 alone; lane 2, Bio-ADAMTS-18 + 5 U/ml thrombin; lane 3, Bio-ADAMTS-18 + 5 U/ml thrombin + hirudin. (d) Bio-ADAMTS18 mutant was incubated with various concentrations of thrombin and analyzed by immunoblotting. Lane 1-4 refers to 5, 10, 20, 30 U/ml thrombin, respectively.

4. Discussion

Despite the similarity shared by ADAMTS family members, most differences among them are found in the C-terminal domains of the protein, suggesting that the C-terminal domains of ADAMTS may determine their *in vivo* location and substrate specificity [19]-[22]. C-terminal processing has been shown in ADAMTS-1 [19] [21], ADAMTS-4 [20] [22], ADAMTS-8 [4], ADAMTS-9 [5], and ADAMTS-13 [6]. This splicing will shed light on the biological function of these important proteins. Noteworthily, most of cleavage events occur within the spacer region [19]-[22].

We previously reported that cleavage of ADAMTS-18 by thrombin represent a novel mechanism for platelet thrombus clearance [17]. The release of the active 45-kDa C-terminal fragment could regulate thrombus size by inducing oxidative platelet fragmentation. In this study, we first revealed that the R^{775}/S^{776}, R^{792}/S^{793} and R^{823}/S^{824} in spacer region of ADAMTS-18 are critical for thrombin cleavage. This cleavage region is similar to those of the ADAMTS family members reported previously [19]-[22]. Physiologically, thrombin is generated rapidly, and at high local concentrations during the normal hemostatic response. We found that ADAMTS-18 was proteolyzed by thrombin at a high thrombin concentration, whereas low thrombin concentration had undetectable cleavage effect on ADAMTS-18 which mimics some physiological conditions, especially platelet thrombus formation. It is of interest in this regard that ADAMTS-13 has recently been shown to be inactivated by thrombin contributing to the loss of ADAMTS-13 VWF cleavage function [6]. Furthermore, ADAMTS-13 has been reported to limit platelet thrombus formation in a shear rate dependent platelet thrombus model on collagen, by its cleavage of ultra large VWF [23].

In present study, we also find ~75-kDa band which is from ADAMTS-18 cDNA in *in vitro* translation assays. Thrombin or other enzyme (cathepsin D or trypsin) had no effect on the generation of this band. Thus, it is likely other mechanism has been involved in the ADAMTS-18 processing.

5. Conclusion

In summary, this report provides a direct proof that the existence of C-terminal proteolytic cleavage sites of ADAMTS-18 by thrombin, has potential drug application in dissolution of arterial thrombi.

Acknowledgements

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References

[1] Apte, S.S. (2009) A Disintegrin-Like and Metalloprotease (Reprolysin-Type) with Thrombospondin Type 1 Motif (ADAMTS) Superfamily: Functions and Mechanisms. *The Journal of Biological Chemistry*, **284**, 31493-31497. http://www.jbc.org/content/284/46/31493.long http://dx.doi.org/10.1074/jbc.R109.052340

[2] Guo, F., Lai, Y., Tian, Q., Lin, E.A., Kong, L. and Liu, C. (2010) Granulin-Epithelin Precursor Binds Directly to ADAMTS-7 and ADAMTS-12 and Inhibits Their Degradation of Cartilage Oligomeric Matrix Protein. *Arthritis & Rheumatology*, **62**, 2023-2036.

[3] Li, S.W., Arita, M., Fertala, A., Bao, Y., Kopen, G.C., Långsjö, T.K., Hyttinen, M.M., Helminen, H.J. and Prockop, D.J. (2001) Transgenic Mice with Inactive Alleles for Procollagen N-Proteinase (ADAMTS-2) Develop Fragile Skin and Male Sterility. *Biochemical Journal*, **355**, 271-278. http://www.biochemj.org/bj/355/0271/bj3550271.htm http://dx.doi.org/10.1042/0264-6021:3550271

[4] Georgiadis, K.E., Hirohata, S., Seldin, M.F. and Apte, S.S. (1999) ADAM-TS8, a Novel Metalloprotease of the ADAM-TS Family Located on Mouse Chromosome 9 and Human Chromosome 11. *Genomics*, **62**, 312-315. http://dx.doi.org/10.1006/geno.1999.6014

[5] Clark, M.E., Kelner, G.S., Turbeville, L.A., Boyer, A., Arden, K.C. and Maki, R.A. (2000) ADAMTS9, a Novel Member of the ADAM-TS/Metallospondin Gene Family. *Genomics*, **67**, 343-350. http://dx.doi.org/10.1006/geno.2000.6246

[6] Lam, J.K., Chion, C.K., Zanardelli, S., Lane, D.A. and Crawley, J.T. (2007) Further Characterization of ADAMTS-13

Inactivation by Thrombin. *Journal of Thrombosis and Haemostasis*, **5**, 1010-1018.
http://dx.doi.org/10.1111/j.1538-7836.2007.02514.x

[7] Cal, S., Arguelles, J.M., Fernandez, P.L. and López-Otín, C. (2001) Identification, Characterization, and Intracellular Processing of ADAM-TS12, a Novel Human Disintegrin with a Complex Structural Organization Involving Multiple Thrombospondin-1 Repeats. *The Journal of Biological Chemistry*, **276**, 17932-17940.
http://www.jbc.org/content/276/21/17932.long
http://dx.doi.org/10.1074/jbc.M100534200

[8] Liu, Y.J., Xu, Y. and Yu, Q. (2006) Full-Length ADAMTS-1 and the ADAMTS-1 Fragments Display Pro- and Anti-metastatic Activity, Respectively. *Oncogene*, **25**, 2452-2467.
http://www.nature.com/onc/journal/v25/n17/full/1209287a.html
http://dx.doi.org/10.1038/sj.onc.1209287

[9] Gao, G., Plaas, A., Thompson, V.P., Jin, S., Zuo, F. and Sandy, J.D. (2004) ADAMTS4 (Aggrecanase-1) Activation on the Cell Surface Involves C-Terminal Cleavage by Glycosylphosphatidyl Inositol-Anchored Membrane Type 4-Matrix Metalloproteinase and Binding of the Activated Proteinase to Chondroitin Sulfate and Heparan Sulfate on Syndecan-1. *The Journal of Biological Chemistry*, **279**, 10042-10051. http://www.jbc.org/content/279/11/10042.long
http://dx.doi.org/10.1074/jbc.M312100200

[10] Jin, H., Wang, X., Ying, J., Wong, A.H., Li, H., Lee, K.Y., Srivastava, G., Chan, A.T., Yeo, W., Ma, B.B., Putti, T.C., Lung, M.L., Shen, Z.Y., Xu, L.Y., Langford, C. and Tao, Q. (2007) Epigenetic Identification of ADAMTS18 as a Novel 16q23.1 Tumor Suppressor Frequently Silenced in Esophageal, Nasopharyngeal and Multiple Other Carcinomas. *Oncogene*, **26**, 7490-7498. http://www.nature.com/onc/journal/v26/n53/full/1210559a.html
http://dx.doi.org/10.1038/sj.onc.1210559

[11] Sjöblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N., Szabo, S., Buckhaults, P., Farrell, C., Meeh, P., Markowitz, S.D., Willis, J., Dawson, D., Willson, J.K., Gazdar, A.F., Hartigan, J., Wu, L., Liu, C., Parmigiani, G., Park, B.H., Bachman, K.E., Papadopoulos, N., Vogelstein, B., Kinzler, K.W. and Velculescu, V.E. (2006) The Consensus Coding Sequences of Human Breast and Colorectal Cancers. *Science*, **314**, 268-274. http://www.sciencemag.org/content/314/5797/268.long
http://dx.doi.org/10.1126/science.1133427

[12] Xiong, D.H., Liu, X.G., Guo, Y.F., Tan, L.J., Wang, L., Sha, B.Y., Tang, Z.H., Pan, F., Yang, T.L., Chen, X.D., Lei, S.F., Yerges, L.M., Zhu, X.Z., Wheeler, V.W., Patrick, A.L., Bunker, C.H., Guo, Y., Yan, H., Pei, Y.F., Zhang, Y.P., Levy, S., Papasian, C.J., Xiao, P., Lundberg, Y.W., Recker, R.R., Liu, Y.Z., Liu, Y.J., Zmuda, J.M. and Deng, H.W. (2009) Genome-Wide Association and Follow-Up Replication Studies Identified ADAMTS18 and TGFBR3 as Bone Mass Candidate Genes in Different Ethnic Groups. *The American Journal of Human Genetics*, **84**, 388-398.
http://dx.doi.org/10.1016/j.ajhg.2009.01.025

[13] Aldahmesh, M.A., Alshammari, M.J., Khan, A.O., Mohamed, J.Y., Alhabib, F.A. and Alkuraya, F.S. (2013) The Syndrome of Microcornea, Myopic Chorioretinal Atrophy, and Telecanthus (MMCAT) Is Caused by Mutations in ADAMTS18. *Human Mutation*, **34**, 1195-1199. http://dx.doi.org/10.1002/humu.22374

[14] Shattil, S.J. and Ginsberg, M.H. (1997) Perspectives Series: Cell Adhesion in Vascular Biology. Integrin Signaling in Vascular Biology. *The Journal of Clinical Investigation*, **100**, 1-5. http://www.jci.org/articles/view/119500
http://dx.doi.org/10.1172/JCI119500

[15] Nardi, M., Tomlinson, S., Greco, M.A. and Karpatkin, S. (2001) Complement-Independent, Peroxide-Induced Antibody Lysis of Platelets in HIV-1-Related Immune Thrombocytopenia. *Cell*, **106**, 551-561.
http://dx.doi.org/10.1016/S0092-8674(01)00477-9

[16] Nardi, M., Feinmark, S.J., Hu, L., Li, Z. and Karpatkin, S. (2004) Complement-Independent Ab-Induced Peroxide Lysis of Platelets Requires 12-Lipoxygenase and a Platelet NADPH Oxidase Pathway. *The Journal of Clinical Investigation*, **113**, 973-980. http://www.jci.org/articles/view/20726
http://dx.doi.org/10.1172/JCI20726

[17] Li, Z., Nardi, M.A., Li, Y.S., Zhang, W., Pan, R., Dang, S., Yee, H., Quartermain, D., Jonas, S. and Karpatkin, S. (2009) C-Terminal ADAMTS-18 Fragment Induces Oxidative Platelet Fragmentation, Dissolves Platelet Aggregates, and Protects against Carotid Artery Occlusion and Cerebral Stroke. *Blood*, **113**, 6051-6060.
http://www.bloodjournal.org/content/113/24/6051.long?sso-checked=true
http://dx.doi.org/10.1182/blood-2008-07-170571

[18] Wang, J., Zhang, W., Yi, Z., Wang, S. and Li, Z. (2012) Identification of a Thrombin Cleavage Site and a Short Form of ADAMTS-18. *Biochemical and Biophysical Research Communications*, **419**, 692-697.
http://dx.doi.org/10.1016/j.bbrc.2012.02.081

[19] Vázquez, F., Hastings, G., Ortega, M.A., Lane, T.F., Oikemus, S., Lombardo, M. and Iruela-Arispe, M.L. (1999) METH-1, a Human Ortholog of ADAMTS-1, and METH-2 Are Members of a New Family of Proteins with Angio-Inhibitory Activity. *The Journal of Biological Chemistry*, **274**, 23349-23357.

http://www.jbc.org/content/274/33/23349.long
http://dx.doi.org/10.1074/jbc.274.33.23349

[20] Gao, G., Westling, J., Thompson, V.P., Howell, T.D., Gottschall, P.E. and Sandy, J.D. (2002) Activation of the Proteolytic Activity of ADAMTS4 (Aggrecanase-1) by C-Terminal Truncation. *The Journal of Biological Chemistry*, **277**, 11034-11041. http://www.jbc.org/content/277/13/11034.long
http://dx.doi.org/10.1074/jbc.M107443200

[21] Rodriguez-Manzaneque, J.C., Milchanowski, A.B., Dufour, E.K., Leduc, R. and Iruela-Arispe, M.L. (2000) Characterization of METH-1/ADAMTS1 Processing Reveals Two Distinct Active Forms. *The Journal of Biological Chemistry*, **275**, 33471-33479. http://www.jbc.org/content/275/43/33471.long
http://dx.doi.org/10.1074/jbc.M002599200

[22] Flannery, C.R., Zeng, W., Corcoran, C., Collins-Racie, L.A., Chockalingam, P.S., Hebert, T., Mackie, S.A., McDonagh, T., Crawford, T.K., Tomkinson, K.N., LaVallie, E.R. and Morris, E.A. (2002) Autocatalytic Cleavage of ADAMTS-4 (Aggrecanase-1) Reveals Multiple Glycosaminoglycan-Binding Sites. *The Journal of Biological Chemistry*, **277**, 42775-42780. http://www.jbc.org/content/277/45/42775.long
http://dx.doi.org/10.1074/jbc.M205309200

[23] Muroi, C., Fujioka, M., Mishima, K., Irie, K., Fujimura, Y., Nakano, T., Fandino, J., Keller, E., Iwasaki, K. and Fujiwara, M. (2014) Effect of ADAMTS-13 on Cerebrovascular Microthrombosis and Neuronal Injury after Experimental Subarachnoid Hemorrhage. *Journal of Thrombosis and Haemostasis*, **12**, 505-514. http://dx.doi.org/10.1111/jth.12511

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