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Apolipoprotein A-IV is a novel substrate for matrix metalloproteinases

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Screening of matrix metalloproteinase (MMP)-14 substrates in human plasma using a proteomics approach previously identified apolipoprotein A-IV (apoA-IV) as a novel substrate for MMP-14. Here, we show that among the tested MMPs, purified apoA-IV is most susceptible to cleavage by MMP-7, and that apoA-IV in plasma can be cleaved more efficiently by MMP-7 than MMP-14. Purified recombinant apoA-IV (44-kDa) was cleaved by MMP-7 into several fragments of 41, 32, 29, 27, 24, 22 and 19 kDa. N-terminal sequencing of the fragments identified two internal cleavage sites for MMP-7 in the apoA-IV sequence, between Glu^{185} and Leu^{186}, and between Glu^{262} and Leu^{263}. The cleavage of lipid-bound apoA-IV by MMP-7 was less efficient than that of lipid-free apoA-IV. Further, MMP-7-mediated cleavage of apoA-IV resulted in a rapid loss of its intrinsic anti-oxidant activity. Based on the fact that apoA-IV plays important roles in lipid metabolism and possesses anti-oxidant activity, we suggest that cleavage of lipid-free apoA-IV by MMP-7 has pathological implications in the development of hyperlipidemia and atherosclerosis.

Keywords: anti-oxidant activity/apolipoprotein A-IV (ApoA-IV)/lipid metabolism/matrix metalloproteinase (MMP)/substrate.

Abbreviations: APMA, 4-aminophenyl mercuric acetate; ApoA-IV, apolipoprotein A-IV; ApoC-II, apolipoprotein C-II; CBB, Coomassie brilliant blue; ECM, extracellular matrix; FRA, ferric-reducing ability; HDL, high-density lipoprotein; MMP, matrix metalloproteinase; PCR, polymerase chain reaction; PIC, protease inhibitor cocktail; TEV, tobacco etch virus; TIMP, tissue inhibitor of metalloproteinases.

Matrix metalloproteinases (MMPs) are a family of closely related zinc-dependent endoproteinases (*1*). Twenty-three different types of MMPs, which are either secreted or associated with the cell membrane, have been described in humans (*2*). These endoproteinases degrade virtually all extracellular matrix (ECM) components as well as many non-ECM functional proteins and play important roles in a variety of physiological and pathological processes including cell proliferation, tissue repair, tumor invasion, cancer progression and atherosclerosis (*1–3*). Most MMPs are synthesized and released into the extracellular space as pro-enzymes, and converted into active forms through the removal of a pro-domain by other proteases (*2*, *4*, *5*). The catalytic activities of MMPs are inhibited by their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) (*3*, *6*).

Apolipoprotein A-IV (apoA-IV) was first found in rat plasma lipoproteins (*7*). Human apoA-IV, a 46-kDa glycoprotein composed of 376-amino acid residues and modified by *O*-linked glycosylation, is synthesized in the enterocytes of the small intestine and secreted as a component of triglyceride-rich chylomicrons (*8–11*). Following lipolysis of these chylomicrons, apoA-IV released from the particle circulates either in a lipoprotein-free state, or bound to high-density lipoprotein (HDL) (*8*, *12*). Accumulating data suggest that apoA-IV plays many roles in lipid metabolism. In addition to its role in chylomicron assembly and secretion, apoA-IV is involved in enhancement of lecithin cholesterol acyl transferase activity (*13*), stimulation of the cholesterol ester transfer protein (*14*), and modulation of lipoprotein lipase activity (*15*). Moreover, there is evidence for the role of apoA-IV as an anti-oxidant; overexpression of apoA-IV in apoE-deficient mice has been shown to reduce the levels of oxidative markers (*16*). Inhibition of lipid peroxidation by apoA-IV has also been biochemically demonstrated (*17*). Therefore, apoA-IV seems to play a protective role against atherosclerosis by virtue of both its classical roles in lipid metabolism and its anti-oxidant properties.

We have previously screened MMP-14 substrates from human plasma using a proteomics approach (*18*) and identified apoA-IV as a novel substrate for MMP-14. In the present study, we expressed and purified human apoA-IV as a soluble protein in *Escherichia coli* and analysed its susceptibility to cleavage by various MMPs *in vitro*. We examined whether apoA-IV in plasma can be cleaved by MMP-7 and MMP-14, studied the cleavage process, and identified MMP-7 cleavage sites in recombinant apoA-IV. We then compared the MMP-7-mediated cleavage of the lipid-bound and lipid-free forms of apoA-IV. Finally, we analysed the effect of MMP-7-mediated cleavage on the anti-oxidant activity of apoA-IV.

Materials and Methods

Construction of the human apoA-IV expression plasmid

An *E. coli* expression vector for human apoA-IV fused to an affinity tag and a protease cleavage site was constructed as previously described (*19*), with minor modifications. A cDNA encoding human apoA-IV polypeptide containing the hexa-histidine (His) tag and a tobacco etch virus (TEV) protease cleavage site (TEV; Glu-Asn-Leu-Tyr-Phe-Gln↓Gly) at the N-terminus was amplified by polymerase chain reaction (PCR) using Pfu polymerase (Cat. No. CMT4001; Cosmo Genetech, Seoul, Korea) and cDNA from Caco-2 cells as a template. The sequences of primers used for the PCR were as follows: forward primer, 5′-ggaattcCATA**TG***CATCAT CATCATCATCAT**GAAAATTTATATTTCAAGGC*GAGGTCAG TGCTGACCAGGTGGC-3′ (nt. 165–187 of GenBank NM_ 000482), including an NdeI site (underlined), a start codon (bold), a hexa-histidine sequence (italicized), and a TEV cleavage site (bold italicized); reverse primer, 5′-cgGGATCC**TCA**GCTCTCCAAAGG GGCCAGCAT-3′ (nt. 1292–1272 of NM_000482), including a BamHI site (underlined) and a stop codon (bold). The PCR product and pET-22b(+) vector were cut using the restriction enzymes NdeI and BamHI and ligated. The resultant construct, pET-22b(+)-His-TEV-apoA-IV, was sequenced to confirm the absence of PCR errors and then transformed into *E. coli* BL21(DE3) cells.

Purification of human apoA-IV

E. coli BL21(DE3) cells harbouring the pET-22b(+)-His-TEV-apoA-IV construct were cultured in Luria-Bertani medium with 0.1 mg/ml ampicillin. When the absorbance of the culture at 600 nm reached 0.5, isopropyl-1-β-D-galactopyranoside was added to a final concentration of 1 mM, and the cells were further cultured for 4 h at 37°C. Cells were sedimented by centrifugation, and the cell pellet was resuspended in 20 ml of lysis buffer (20 mM Tris–HCl, pH 8.0, 300 mM NaCl, 0.1% Triton X-100) and sonicated on ice. Following centrifugation of the cell lysate, the His-TEV-apoA-IV polypeptide was affinity-purified from the supernatant using Ni^{2+}-NTA agarose resin (Cat. No. 30210, QIAGEN), according to the manufacturer's recommendations. The purified His-TEV-apoA-IV fusion protein was incubated with His-tagged TEV protease (*19*) in a 1:2 enzyme/substrate molar ratio in TEV protease reaction buffer (50 mM Tris–HCl, pH 8.0) overnight at 25°C, and the His-TEV protease and the cleaved His-TEV fragment were eliminated using Ni^{2+}-NTA resin. Purified apoA-IV was dialyzed against 20 mM Tris–HCl buffer, pH 7.4, containing 150 mM NaCl.

Preparation of MMPs and TIMPs

The catalytic domains of human MMP-3 (cMMP-3; Phe^{100}-Pro^{273}), human MMP-7 (Ala^{93}-Lys^{267}), and human MMP-14 (cMMP-14; Tyr^{112}-Ile^{318}), and the pro, catalytic and hinge domains of MMP-1 (pchMMP-1; Phe^{20}-Ala^{277}) were expressed as inclusion bodies in *E. coli* and refolded as described previously (*20–22*). Recombinant human proMMP-2 and proMMP-9 were expressed in Sf9 cells by infection with baculoviruses and purified by gelatin-agarose column chromatography as described previously (*23*). The pro-forms of MMPs were activated using 1 mM 4-aminophenyl mercuric acetate (APMA). Recombinant human TIMP-1 and TIMP-2 were expressed in Sf9 cells and purified as previously described (*24*).

Cleavage of apoA-IV by MMPs

Purified apoA-IV (1 μg) was cleaved with catalytically active MMPs in a 1:10 enzyme/substrate molar ratio in 16 μl of MMP reaction buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM $CaCl_2$, 0.5 mM $ZnCl_2$, 0.001% Brij-35) at 37°C for the indicated time intervals unless specified. To analyse cleavage of apoA-IV present in plasma proteins by MMPs, plasma proteins (75 μg) were incubated with 100 ng of MMP-7 or MMP-14 in 16 μl of MMP reaction buffer at 37°C for different times. When the effect of protease inhibitors was investigated, reactions were performed in the presence of protease inhibitor cocktail (PIC) (0.15 μl, Cat. No. 539134, Calbiochem, Torrey Pines, CA, USA), pan-MMP inhibitor GM6001 (8.75 ng, Cat. No. CC1010, Chemicon, Temecula, CA, USA), TIMP-1 (250 ng), or TIMP-2 (210 ng). Reactions were terminated by adding SDS sample buffer unless otherwise specified. Samples were subjected to 15% SDS–PAGE and stained with Coomassie Brilliant Blue (CBB) R-250 or analysed by western blotting using anti-apoA-IV antibody (Cat. No. sc-19036, Santa Cruz Biotechnology, Santa Cruz, CA, USA). A protein marker (Bio-Rad Laboratories, Hercules, CA, USA) was also run on the gel for molecular weight identification.

N-terminal sequencing of MMP-7-cleaved apoA-IV fragments

The N-termini of the MMP-7-cleaved apoA-IV fragments were sequenced by the Edman degradation method in the Tufts Core Facility (Tufts University, Boston, MA, USA) as previously described (*25*).

Formation of apoA-IV-containing proteoliposomes

ApoA-IV-containing proteoliposomes were prepared by the Tris-buffered saline (10 mM Tris–HCl, pH 8.0, 140 mM NaCl, 1 mM EDTA) dialysis method using a 95:5:1:150 initial molar ratio of palmitoyloleoyl phosphatidylcholine:cholesterol:apoA-IV:sodium cholate (*26*). Proteoliposomes were dialyzed against 20 mM Tris–HCl buffer, pH 7.4, containing 150 mM NaCl.

Ferric-reducing ability assay of MMP-7-degested apoA-IV

The ferric-reducing ability (FRA) assay was performed as described previously (*27*, *28*). The FRA reagent (500 μl) was mixed with an equal volume of MMP-7-digested apoA-IV (2 μM). Absorbance at 593 nm was measured at 25°C every 30 s over a period of 30 min using a DU 800 Spectrophotometer (Beckman Coulter, Fullerton, CA, USA) equipped with a MultiTemp III Thermocirculator (Amersham Biosciences, Uppsala, Sweden).

Results

Purification of apoA-IV and its cleavage by MMPs

Recombinant human apoA-IV containing a His_6 tag and a TEV protease cleavage site at the N-terminus (His-TEV-apoA-IV) (Fig. 1A) was expressed in *E. coli* and purified using Ni^{2+}-NTA resin (Fig. 1B). ApoA-IV protein containing an N-terminal Gly residue (Fig. 1A) was purified by incubating His-TEV-apoA-IV with His-TEV protease (*19*), followed by removal of both the His-TEV fragment and the His-TEV protease using Ni^{2+}-NTA resin (Fig. 1C). The recombinant apoA-IV was detected as a 44-kDa band in SDS gels (Figs 1C and 2), slightly lower than the 46-kDa band observed in the case of plasma apoA-IV (Fig. 3A), which contains *O*-linked glycans that constitute up to 6% of its molecular weight (*9*).

To analyse whether apoA-IV is a substrate of MMPs, the purified apoA-IV was incubated with various MMPs (MMP-1, MMP-2, MMP-3, MMP-7, MMP-9 and MMP-14) in a 1:10 enzyme/substrate molar ratio for 1 h at 37°C. Among MMPs used here, MMP-3, MMP-7 and MMP-14, of which catalytic domains were refolded, are catalytically active. However, pchMMP-1, proMMP-2 and proMMP-9, each of which contains a pro-domain, were activated to active enzymes by APMA in an optimal condition (Supplementary Figs S1 and S2). The cleavage of apoA-IV was maximal by MMP-7, followed by MMP-14, MMP-1 and MMP-3, in that order (Fig. 2).

In vitro cleavage of apoA-IV in plasma protein preparations by MMP-7 and MMP-14

To analyse the cleavage of apoA-IV in plasma proteins by MMP-7 and MMP-14, plasma proteins were incubated with each of these MMPs and the reaction mixtures were subjected to western blot analysis using the anti-apoA-IV antibody. While apoA-IV in plasma proteins was not digested by an 180-min incubation in the absence of the MMPs, the level of apoA-IV gradually

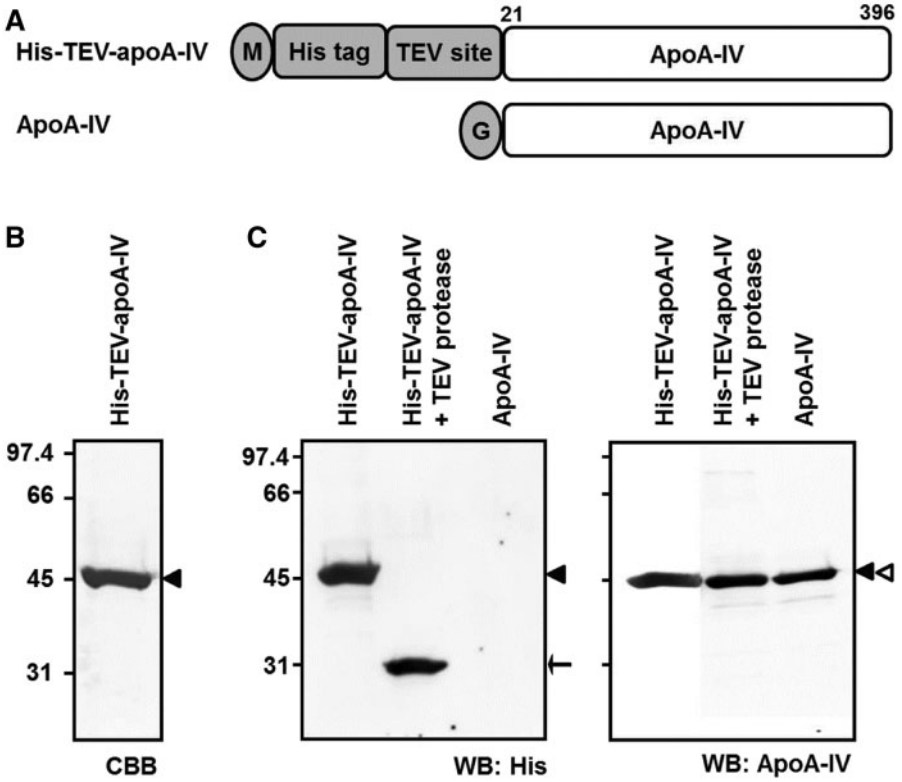

Fig. 1 Purification of recombinant apoA-IV. (**A**) Schematic diagrams of His-TEV-apoA-IV and apoA-IV. His-TEV-apoA-IV contains a translation initiation codon, a His tag, a TEV cleavage sequence and the 376-amino acid sequence of apoA-IV (Glu^{21} to Ser^{396}). The final recombinant apoA-IV consists of a Gly residue derived from the C-terminus of the TEV cleavage site, followed by the 376-amino acid sequence of apoA-IV (Glu^{21} to Ser^{396}). (**B**) Purified His-TEV-apoA-IV. The purified His-TEV-apoA-IV (45 kDa) was analysed by 10% SDS–PAGE and stained with CBB R-250. (**C**) Production of recombinant apoA-IV. His-TEV-apoA-IV was incubated overnight with His-TEV protease (29 kDa) in a 1:2 enzyme/substrate molar ratio at 25°C. The reaction mixture was applied to a Ni^{2+}-NTA affinity column to remove the His tag derived from His-TEV-apoA-IV, as well as the His-TEV protease. His-TEV-apoA-IV, the reaction mixture of His-TEV-apoA-IV and TEV protease, and purified apoA-IV after Ni^{2+} affinity chromatography were subjected to western blot analysis using anti-His antibody (left panel) and anti-His monoclonal antibody (right panel). The closed and open arrowheads indicate the 45-kDa His-TEV-apoA-IV and the 44-kDa recombinant apoA-IV, respectively. An arrow shows the position of the His-tagged TEV protease. The numbers of left side of the gels are the molecular weights (MW; kDa) of bands from the protein marker.

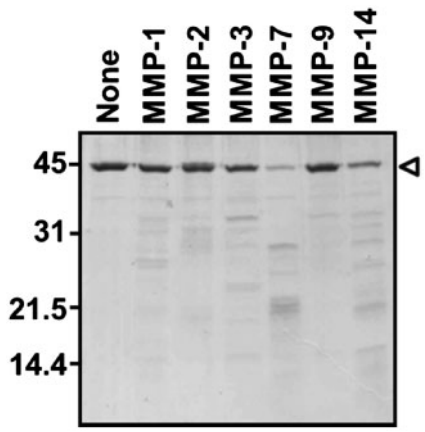

Fig. 2 Cleavage of purified recombinant apoA-IV by various MMPs. Proforms of MMPs (200 nM) were activated by incubation with 1 mM APMA at 37°C;15 min for pchMMP-1 and proMMP-2 and 30 min for proMMP-9. Purified recombinant apoA-IV was incubated with active MMPs in a 1:10 enzyme/substrate molar ratio at 37°C for 1 h. Samples were subjected to 15% SDS–PAGE and stained with CBB R-250. The open arrowhead indicates the position of intact recombinant apoA-IV containing an N-terminal Gly residue.

decreased in the presence of MMP-7 and MMP-14 in a time-dependent manner (Fig. 3A). Consistent with the results from purified apoA-IV, MMP-7 was more effective in cleavage of apoA-IV than was MMP-14.

To directly examine whether apoA-IV in plasma proteins can be cleaved by MMP-7, plasma proteins were incubated with MMP-7 in the presence of various protease inhibitors (Fig. 3B). Protease inhibitor cocktail (PIC) contains inhibitors for aspartic, cysteine and serine proteases, and amino peptidases, but not an inhibitor for MMPs. TIMP-1 inhibits many MMPs but not MMP-14, and TIMP-2 inhibits MMP-2, MMP-7 and MMP-14. As shown in Fig. 3B, apoA-IV in plasma proteins was digested by MMP-7 in the presence of the PIC, but not in the presence of the pan-metalloprotease inhibitor GM6001, TIMP-1 and TIMP-2. Taken together, these results suggest that apoA-IV in plasma can be specifically degraded by MMP-7.

Process of cleavage of apoA-IV by MMP-7

When apoA-IV was incubated with MMP-7, the 44-kDa apoA-IV protein was processed into seven

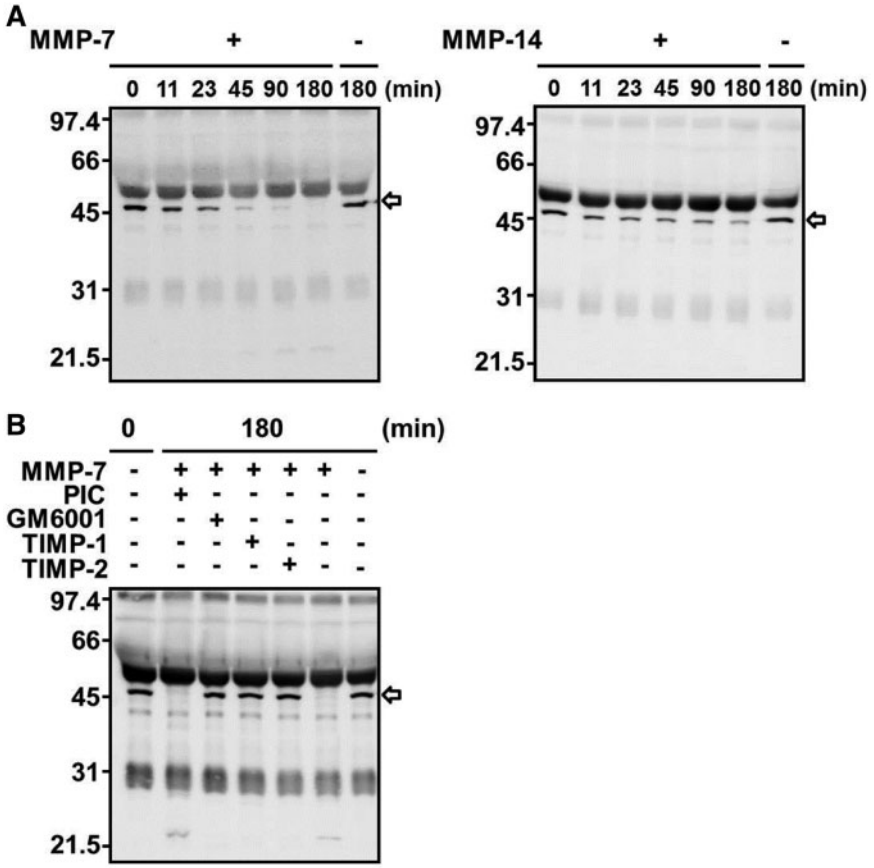

Fig. 3 Cleavage of apoA-IV in plasma by MMPs. (**A**) Cleavage of apoA-IV in plasma by MMP-7 and MMP-14. Plasma proteins were incubated with MMP-7 (left panel) or MMP-14 (right panel) at 37°C for the indicated time intervals. (**B**) Effect of protease inhibitors on the cleavage of apoA-IV in plasma by MMP-7. Plasma proteins were digested with MMP-7 for 180 min at 37°C in the presence of PIC, GM6001, TIMP-1 or TIMP-2. Samples were subjected to 15% SDS–PAGE and analysed by western blotting using anti-apoA-IV antibody. The open arrow indicates the position of intact apoA-IV present in plasma.

detectable fragments in a time-dependent manner (Fig. 4A). The apparent molecular weights of the fragments were 41 (A), 32 (B), 29 (C), 27 (D), 24 (E), 22 (F) and 19 (G) kDa. As expected, the larger bands were detected at earlier time points and the smaller bands were seen at later time points. N-terminal sequencing of each fragment showed that the A, C, D, E and F bands as well as the intact apoA-IV band start at Gly-Glu^{21}-Val-Ser-Ala (Fig. 4B). The first Gly residue was derived from TEV protease cleavage site and the following amino acid residues corresponded to the N-terminus of apoA-IV (the numbering system of the apoA-IV sequence presented here follows that of GenBank AAA51744.1). In addition, the B and G bands start at Leu^{186}-Lys-Ala-Lys-Ile and Leu^{263}-Arg-Gln-Arg-Leu, respectively, indicating that MMP-7 cleaves apoA-IV between residues Glu^{185} and Leu^{186} and between Glu^{262} and Leu^{263}.

MMP-7-mediated cleavage of lipid-free and lipid-bound apoA-IV

ApoA-IV is present in lipid-free and lipid-bound forms in human plasma (*10*). We showed that apoA-IV in plasma was cleaved by MMP-7 (Fig. 2A). To analyse whether MMP-7 is able to cleave the lipid-bound form of apoA-IV, a liposome containing apoA-IV was reconstituted with phospholipid and cholesterol (*26*). One microgram of ApoA-IV in either lipid-bound state or lipid-free state was subjected to digestion by MMP-7. Incubation of lipid-bound apoA-IV with MMP-7 resulted in accumulation of bands corresponding to fragments C and F (Fig. 5), whereas lipid-free apoA-IV was much more susceptible than lipid-bound apoA-IV to MMP-7 cleavage.

Anti-oxidant activity of recombinant apoA-IV digested by MMP-7 in vitro

It is known that recombinant apoA-IV inhibits peroxidation of low-density lipoproteins, which is mediated by Cu^{2+} and thiobarbituric acid-reactive substances (*29*, *30*). We therefore analysed whether cleavage of the lipid-free form of apoA-IV by MMP-7 affects its anti-oxidant activity. The anti-oxidant activity of apoA-IV was measured as a change in absorbance at 593 nm in the presence of FRA assay reagents. As expected, cleavage of lipid-free apoA-IV by MMP-7 resulted in a time-dependent decrease of its anti-oxidant ability (Fig. 6).

Discussion

We previously screened MMP-14 substrates using a proteomics approach, and identified apoA-IV as a novel MMP-14 substrate (*18*). Here, we have shown

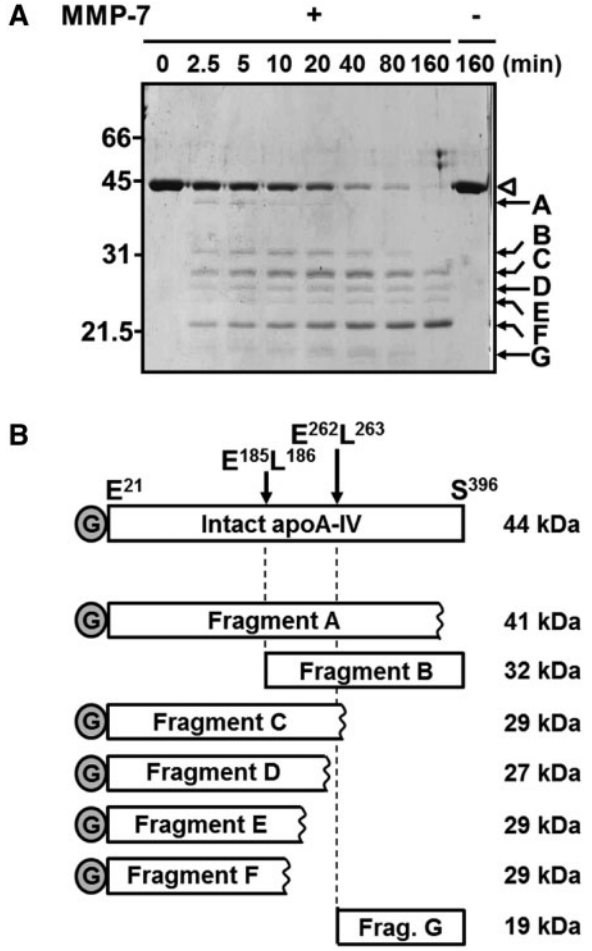

Fig. 4 Process of cleavage of apoA-IV by MMP-7. (**A**) Cleavage of apoA-IV by MMP-7 as a function of time. Purified recombinant apoA-IV was digested by MMP-7 in a 1:10 enzyme/substrate molar ratio at 37°C for the indicated time intervals. Samples were analysed by 15% SDS–PAGE and stained with CBB R-250. The open arrowhead indicates the position of intact recombinant apoA-IV. The seven major fragments (bands A–G) detected are shown. (**B**) Relative position of the major fragments of apoA-IV resulting from MMP-7 cleavage. The N-terminal sequences of the seven major fragments as well as intact apoA-IV were determined. Based on N-terminal sequence data and apparent MWs, the relative positions of the fragments were assigned. Recombinant apoA-IV polypeptide is represented by a bar for apoA-IV (Glu^{21}-Ser^{396}) in addition to a circle denoting the extra N-terminal Gly residue.

that among the tested MMPs, MMP-7 cleaved purified apoA-IV most efficiently, whereas MMP-14 exhibited lower efficiency. Consistent with these results from the cleavage of purified apoA-IV, apoA-IV present in plasma is also cleaved by MMP-7 and, to a lesser extent, by MMP-14.

MMP-7 (also known as matrilysin and PUMP-1) is secreted as a 28-kDa pro-enzyme form and activated by the removal of a 9-kDa N-terminal pro-domain (*31*). Similar to other MMPs, MMP-7 cleaves various components of the ECM, including fibronectin, laminin, collagens, gelatin and proteoglycans (*2*). MMP-7 is also involved in processing of pro-proteins into mature forms (*e.g.* MMPs, pro-α-defensin, and pro-tumor necrosis factor-α), and in the shedding of cell-surface molecules (*e.g.* heparin-binding epidermal growth factor, Fas ligand and E-cadherin) (*3*, *32–34*).

ApoA-IV has 22-amino acid tandem repeats, which are located between Ala^{60} and Asn^{352} (*35*). These repeats form amphipathic α-helices, a characteristic feature of apolipoproteins (*10*, *36*). These α-helical repeats, as well as the N-terminal 40 amino acids, facilitate the association of apoA-IV with lipids, although the lipid-binding affinity of apoA-IV is weaker than that of other apolipoproteins (*9*, *10*, *35*).

In the initial analysis of MMP-7-mediated degradation of recombinant apoA-IV, we detected seven discrete bands of apoA-IV fragments in SDS–PAGE. N-terminal sequencing revealed that the 41-kDa A, 29-kDa C, 27-kDa D, 24-kDa E and 22-kDa F fragments have the same N-terminus as the 44-kDa intact apoA-IV, whereas the 32-kDa and 19-kDa fragments start at Leu^{186} and Leu^{263}, respectively. Because most of the fragments have an intact N-terminus, it appears that apoA-IV is gradually degraded from its C-terminal end by MMP-7. This analysis also identified two internal cleavage sites in the apoA-IV sequence; Pro-His-Ala-Asp-Glu^{185}↓Leu^{186}-Lys-Ala and Ala-Ser-Ala-Glu-Glu^{262}↓Leu^{263}-Arg-Gln. A comparison of these sites with the consensus MMP-7 cleavage site motif, (Pro or Ile) (Val, Ile or Arg) (Pro, Val or Ile) (Leu, Met or Tyr) (Ser, Glu, Asn or Ala) ↓ (Leu, Ile or Met) (Val, Thr, Ile, Met, Lys or Arg) (Met, Tyr or Gln), determined by MMP-7 cleavage of oligopeptide libraries (*37*), revealed that, interestingly, both cleavage sites were matched at only 4 of the 8 positions in the MMP-7 cleavage site consensus motif. This result indicates that screening of MMP substrates by digestion of protein mixtures or proteomes is more practical than *in silico* screening of MMP substrates based on cleavage site motifs.

ApoA-IV is mainly secreted from the enterocytes of the intestine as a component of triglyceride-rich chylomicrons (*8*). Lipolysis of these chylomicrons leads to the dissociation of apoA-IV, and the released apoA-IV circulates either in a lipid-free form or bound to HDL particles (*8*, *36*). We therefore analysed the cleavage of lipid-bound and lipid-free forms of apoA-IV by MMP-7. Our results show that the half-life of the intact apoA-IV band was 13.8 min while that of the lipid-bound apoA-IV was about 138 min, under our reaction conditions. This suggests that lipid-free apoA-IV is much more susceptible to cleavage by MMP-7 than apoA-IV located within chylomicrons or bound to HDL particles in physiological conditions.

Although human apoA-IV deficiency has not been reported, apoA-IV is likely involved in lipid metabolism; apoA-IV has been shown to increase the activity of the lecithin cholesterol acyltransferase (*13*) and of the cholesterol ester transfer protein (*14*), and to play a role in the activation of lipoprotein lipase (*15*). We have previously showed that apolipoprotein C-II (apoC-II), a cofactor of lipoprotein lipase, is readily cleaved by MMP-14 and MMP-7 (*21*). Thus, the degradation of both apoA-IV and apoC-II by these MMPs accelerates the disruption of normal lipid physiology. In addition, apoA-IV exhibits anti-oxidant and anti-atherogenic activity *in vitro* and in animal models (*29*). The anti-oxidant activity of apoA-IV is significant because apoA-IV is present in both

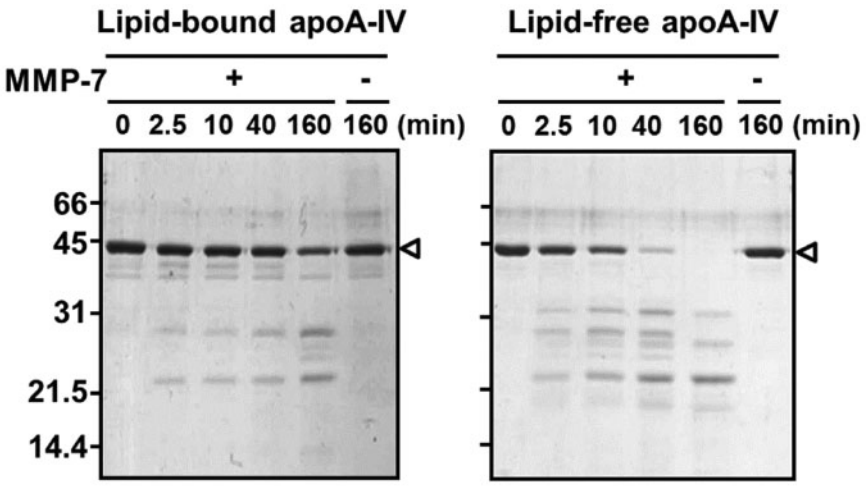

Fig. 5 Comparison of MMP-7 cleavage of lipid-bound and lipid-free apoA-IV. Recombinant apoA-IV (1 μg) in lipid-bound (lipid:protein = 95:1) or lipid-free state was digested by MMP-7 in a 1:10 enzyme/substrate molar ratio at 37°C for the indicated time intervals. Samples were analysed by 15% SDS–PAGE and stained with CBB R-250.

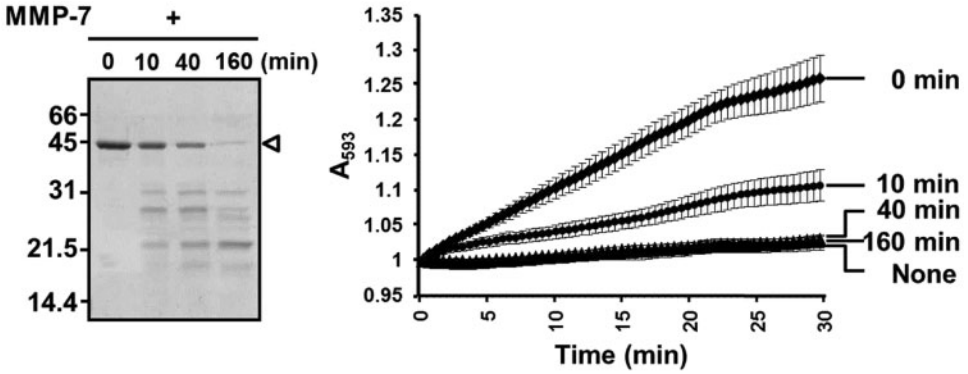

Fig. 6 Effect of apoA-IV degradation by MMP-7 on its antioxidant activity. Recombinant lipid-free apoA-IV (50 μg) was digested by MMP-7 in a 1:10 enzyme/substrate molar ratio in 50 μl of MMP reaction buffer at 37°C for the indicated time intervals. Samples were dialyzed into phosphate-buffered saline after the reaction was stopped by adding EDTA to a final concentration of 10 mM. Aliquots of the samples were analysed by 15% SDS–PAGE and stained with CBB R-250 (left panel). The MMP-7-cleaved apoA-IV samples (44 μg) were analysed for anti-oxidant activity by the FRA assay using a DU 800 Spectrophotometer equipped with a MultiTemp III Thermocirculator. The plotted absorbance at 593 nm at each time point is the mean ± SD of three independent determinations.

lipoprotein-bound and lipoprotein-free forms in circulation due to its amphipathic property, unlike most other anti-oxidants (*38*). Our results demonstrate that the cleavage of apoA-IV by MMP-7 results in loss of its anti-oxidant activity.

To our knowledge, this is the first demonstration of apoA-IV as a novel substrate for MMP-7. The classical roles of apoA-IV in lipid metabolism, as well as its anti-oxidant activity, appear to be important to protect against the development of hyperlipidemia and atherosclerosis. MMP-7 is known to be expressed primarily at the border between the fibrous cap and lipid core in atherosclerotic lesions (*39*, *40*). In light of these observations, we suggest that the cleavage of apoA-IV by MMP-7 might be an important step leading to the disruption of normal lipid metabolism and the induction of atherosclerosis.

Supplementary Data

Supplementary Data are available at *JB* Online.

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Conflict of interest

None declared.

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