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Meprin A and meprin α generate biologically functional IL-1 β from pro-IL-1 β

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Abstract

The present study demonstrates that the oligomeric metalloendopeptidase meprin A purified from kidney cortex and recombinant meprin α are capable of generating biologically active IL-1 β from its precursor pro-IL-1 β . Amino-acid sequencing analysis reveals that meprin A and meprin α cleave pro-IL-1 β at the His¹¹⁵-Asp¹¹⁶ bond, which is one amino acid N-terminal to the caspase-1 cleavage site and five amino acids C-terminal to the meprin β site. The biological activity of the pro-IL-1 β cleaved product produced by meprin A, determined by proliferative response of helper T-cells, was 3-fold higher to that of the IL-1 β product produced by meprin β or caspase-1. In a mouse model of sepsis induced by cecal ligation puncture that results in elevated levels of serum IL-1 β , meprin inhibitor actinonin significantly reduces levels of serum IL-1 β . Meprin A and meprin α may therefore play a critical role in the production of active IL-1 β during inflammation and tissue injury.

Keywords

Meprin A; meprin α ; interleukin-1 β ; caspase-1; inflammation; sepsis; cecal ligation puncture

INTRODUCTION

Meprins are zinc-dependent metalloproteinases of the ‘astacin’ family that are highly expressed as oligomeric proteins at the brush-border membranes of the kidney and intestine [1–2]. Meprin A, the heterooligomeric form comprised of α and β subunits, is the predominant form expressed abundantly in the apical membranes of renal proximal tubules [2–4]. Meprin A is anchored to the brush-border membranes through the membrane-spanning domain of the β subunit [5]. Thus, meprin A containing both α and β subunits is a membrane-associated protease anchored to the brush-border membranes through the β

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subunit. Meprins are capable of mediating proteolysis of a wide variety of proteins *in vitro*. Meprins are able to cleave extracellular matrix (ECM) proteins [4, 6–8], peptide hormones, growth factors [6, 9], and cytokines [10,11].

In addition to the abundant expression of meprin A in the kidney and small intestines, the expression of meprin α and meprin β , most likely in their homooligomeric forms, has been reported in other tissues and cells. Meprin α has been shown to be expressed in the large intestines and colon [12,13] and in cell culture systems [5] including Caco-2 colon carcinoma cells [13] and organ cultures of human fetal gut that constitutively secrete meprin α in the culture supernatants. Both meprin α and meprin β are expressed in the leukocytes of inflamed lamina propria mucosae [12] and mouse mesenteric lymph nodes [14]. In the human epidermis, each meprin subunit is expressed in distinct layers of epidermis [15].

Interleukin-1 beta, a key proinflammatory cytokine produced by multiple cells including monocytes, macrophages, neutrophils, and hepatocytes, plays important roles in many acute and chronic inflammatory diseases ranging from infections to tumor invasiveness [16–19]. Our recent studies demonstrated that meprin β can proteolytically process inactive pro-IL-1 β into a biologically active form [11]. It is generally accepted that IL-1 β is synthesized as an inactive precursor of 32 kDa (pro-IL-1 β), which is processed into a 17-kDa active form (IL-1 β) by caspase-1 [20,21]. However, many reports have suggested that caspase-1-independent proteolytic processing of pro-IL-1 β can also occur. Caspase-1-independent processing has been reported previously in macrophages treated with zymosan and turpentine [22], Fas-stimulated neutrophils [23], and leptin-induced release of IL-1 β . Some extracellular proteases have been shown to be capable of processing pro-IL-1 β [11,24]. A recent study showed that LPS-induced pro-IL-1 β processing occurred in caspase-1-deficient cells [25] caspase-1-deficient mice [26].

In view of the abundant expression of meprin A and meprin α in various organs and inflammatory sites, the present study was therefore undertaken to study whether meprin A and meprin α are capable of producing biologically active IL-1 β *in vitro* and *in vivo*. Furthermore, the studies examined the specific site of cleavage in the pro-IL-1 β molecule by meprin A and meprin α .

MATERIALS AND METHODS

Reagents

Chemicals for cell culture were purchased from Invitrogen (Carlsbad, CA) and all other chemicals from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Antibodies against human IL-1 β and against human meprin β were from Santa Cruz Biologicals (Santa Cruz, CA).

Purification of meprin A from rat kidney cortex

Meprin A was purified from the kidney cortices essentially as we described previously [4].

Expression and purification of recombinant His-tagged rat meprin α

HEK-293 cells transfected with His₆-tagged meprin α (Clone #2) were kindly provided by Judith S. Bond [27]. The cells were expanded in DMEM supplemented with 10% fetal bovine serum (FBS) and 1x antibiotic-antimycotic (Invitrogen; Carlsbad, CA). For meprin α expression, cells were grown in 150 mm dishes in serum-free DMEM/F12 until they reached 80% confluency. The medium was centrifuged to remove cells and was extensively dialyzed against 10 mM Tris/Cl pH 7.1, 0.1mM PMSF, and 0.1% Nonidet P-40, at 4° C. The dialyzed sample was loaded on an iminodiacetic acid (IDA)-Sephacrose column (GE Healthcare;

Piscataway, NJ) charged with Cu^{2+} in 10 mM Tris/Cl pH 7.1, 100 mM NaCl. Bound protein was eluted with 20 mM L-histidine in the same buffer. The enzyme activity was monitored by OCK^+ activity assay [11]. Active fractions were concentrated with a Centriprep YM-30 filtration device (Millipore), and dialyzed against 20 mM Tris/Cl pH 7.5, 100 mM NaCl and stored at 4°C.

Expression, purification, and analysis of human recombinant meprin β

The meprin β expression construct, pMep $\beta\Delta\text{TM1}$ puro3GW, was prepared and analyzed as described in our previous studies [11].

Analysis of purified meprins α , β and A

Protein concentration was measured with a Bradford dye-binding assay (BioRad; Hercules, CA) and meprin enzyme activity was determined using the fluorogenic peptide substrate H-Abz-Met-Gly-Trp-Met-Asp-Glu-Ile-Asp-Lys(Dnp)-Ser-Gly-OH (OCK^+ ; Abz, 2-amino-benzoic acid; Dnp, 2,4-dinitrophenyl) (Diver Drugs, SL; Gava, Spain) as described by Bertenshaw [6]. Briefly, pro-meprin α or β at 1.32 nM was activated by limited proteolysis with 26.3 nM trypsin at 37°C for 30 min [27]. Trypsin was then inhibited with a two-fold excess of soybean trypsin inhibitor (SBTI) at room temperature for 15 min. The enzyme assay was started by the addition of OCK^+ at a final concentration of 6.6 μM and the change in fluorescence (excitation at 320 nm/emission at 417 nm) was followed over 20 min at 30°C. All spectroscopic measurements were recorded in 96-well plates (Greiner Bio-One; San Francisco, CA) with a SpectramaxM5 plate reader (Molecular Devices; Sunnyvale, CA). To inhibit meprin activity, actinonin was added to a final concentration of 2.24 μM and incubated at room temperature for 15 min [11]. The protease inhibitor set V without EDTA (Calbiochem; San Diego, CA) was used to inhibit a broad-spectrum of serine and cysteine proteases.

Expression and purification of recombinant proIL-1 β

Recombinant proIL-1 β was expressed and purified as described in our previous studies [11].

Biological activity assay of processed proIL-1 β

Pro-IL-1 β (163 nM) was digested with 2.8 nM trypsin-activated meprin α , 2.4 nM meprin β , 1.7 nM meprin A, or 8 nM caspase-1 in a total volume of 100 μl and sterilized using 0.45 μm Millex-GV syringe filters (Millipore). Cell proliferation assays were performed in duplicate in three independent experiments using the helper T-cell line D10S as described [11].

Cecal Ligation Puncture (CLP)

CLP-induced acute kidney injury (AKI) was established as described by Wu *et al.* [28]. All animals were housed and killed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and with approval of the Institutional Animal Care and Use Committee. Following the surgery as described previously [28], 1 ml of pre-warmed normal saline was given i.p., animals were placed in individual cages, and set on a warming pad. At 7 h post CLP surgery, mice were anesthetized with isoflurane and blood collected from the vena cava. Serum IL-1 β levels were determined using a murine specific sandwich ELISA kit from R&D Systems.

RESULTS

Proteolytic cleavage of proIL-1 β with meprin α , meprin β , and meprin A and identification of cleavage sites

Meprin A was purified from kidney cortices and recombinant meprin α and meprin β (Figure 1A) were purified from HEK cells stably transfected with meprin α and meprin β , respectively, as described in “Materials and Methods.” The purified meprins were used to examine their ability to proteolytically process pro-IL-1 β . For incubation, 22 nM of meprin A or 37 nM of trypsin-activated meprin α or 31 nM of meprin β in 20 mM Tris-HCl, pH 7.5 containing 100 mM NaCl were incubated with 208 nM proIL-1 β at 37° C in a total volume of 15 μ l for 90 min. Proteolysis was terminated by heating the samples in the presence of sample loading buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% (w/v) SDS, 5% 2-ME) at 95° C for 5 min. Samples were resolved on 10% NuPAGE gels (Invitrogen), blotted to nitrocellulose membranes (BioRad), and probed with polyclonal rabbit anti-human IL-1 β antibody (Figure 1B). Meprin α produced a 17-kDa IL-1 β fragment that was slightly larger than the one produced by caspase-1 as shown in the figure. Meprin β cleaved proIL-1 β to produce a 20-kDa IL-1 β product. Meprin A produced both 17-kDa and 20-kDa products. For N-terminal sequencing, 7 μ M proIL-1 β was digested with 83 nM trypsin-activated meprin α , 71 nM meprin β , or 50 nM meprin A at 37° C in a total volume of 40 μ l for 90 min, loaded onto multiple lanes of a 10% NuPAGE gel (Invitrogen), blotted to PVDF, and visualized with Coomassie Brilliant Blue staining. The bands corresponding to the processed IL-1 β products were excised and submitted for amino acid sequencing by Edman degradation at the Harvard Microchemistry Facility. As depicted in Figure 2(A and B), the N-terminal amino acid of the 17-kDa fragment resulting from digestion with either meprin α or meprin A differs from the cleaved product produced by caspase-1 by a single additional amino acid. Meprin α or meprin A cleaved pro IL-1 β at the His¹¹⁵-Asp¹¹⁶ site whereas caspase-1 cleaved at position Asp¹¹⁶-Ala¹¹⁷. As reported previously, meprin β cleaved pro-IL-1 β at the Asn¹¹⁰-Glu¹¹¹ site.

Biological activity assay IL-1 β products produced by meprin A and meprin α

As shown in Figure 3, the biological activity of meprin α was almost 3-fold higher than that of meprin β and 1.6-fold higher for meprin A. These studies suggest that IL-1 β products produced by meprin A and meprin α have a higher biological activity than those produced by meprin β or caspase-1. We previously showed that biological activity of IL-1 β produced by caspase-1 is same to that produced by meprin β [11].

Meprin inhibitor actinonin prevents sepsis-induced generation of serum IL-1 β levels

In both experimental and clinical sepsis, bacterial compounds induce production and release of cytokines, resulting in a significant inflammatory response [18,29,30]. A sepsis-induced inflammatory response causes multi-organ failure and kidneys are often affected [29,30]. The experimental model of sepsis induced by cecal ligation puncture promotes IL-1 β and maximum induction occurs between 4 to 8 hours after infection [18, 31,32]. Actinonin, a naturally occurring hydroxamate, has proved to be a most effective inhibitor of meprin A and inhibits meprin α and meprin A at a nanomolar range (K_i =20 nM) compared to about 5 mM to other metalloproteinases [7]. Actinonin protected renal function in a mouse sepsis model of AKI [33] similar to data reported for other models of AKI injury [34,35]. Thus we examined the effect of the meprin inhibitor actinonin on the formation of mature IL-1 β from pro-IL-1 β in the sepsis model of AKI. As shown in Figure 4, 7 h after CLP serum IL-1 β levels increased 24-fold compared to Sham mice. Treatment with the meprin inhibitor actinonin significantly inhibited the generation of IL-1 β (Figure 4), suggesting that meprin A plays a functional role in the cleavage of pro-IL-1 β during sepsis.

DISCUSSION

Our studies demonstrate that meprin A purified from rat kidney cortices and purified recombinant meprin α are capable of cleaving pro-IL-1 β to produce a biologically active form of IL-1 β . Meprin A and meprin α produced a 17-kDa IL-1 β fragment that was one amino acid larger than that produced by caspase-1. Both meprin A and meprin α were found to cleave the His¹¹⁵-Asp¹¹⁶ bond whereas caspase-1 cleaves the Asp¹¹⁶-Ala¹¹⁷ site. The amino acid residues C-terminal to the cleavage in the P1' through P4' sites are DAPV in the IL-1 β fragment produced by Meprin A and meprin α . The Ala and Pro at positions P2' and P3', respectively, are in complete accordance with meprin A's preference for these residues as described previously [6]. Also, meprin A has some preference for the Asp in the P1' position [6]. We have demonstrated previously that recombinant meprin β cleaved pro-IL-1 β at the Asn¹¹⁰-Glu¹¹¹ site to result in a biologically active IL-1 β fragment [11]. Thus, the IL-1 β fragment produced by meprin A and meprin α is five amino acids shorter than that produced by meprin β . In addition to the formation of the major fragment produced by cleavage at His¹¹⁵-Asp¹¹⁶, meprin A also produced a minor fragment whose size was similar to that produced by meprin β ; however, the amount of this product was insufficient to allow its N-terminal sequence determination. IL-1 β fragments produced by meprin A and meprin α were biologically active and this activity was significantly higher than the IL-1 β produced by meprin β or caspase-1. We have reported previously that IL-1 β produced by caspase-1 and meprin β had similar biological activity [11]. These studies demonstrate that homo- and hetero-oligomeric forms of meprins are capable of cleaving pro-IL-1 β at different sites and producing biologically active fragments. A recent study has shown that pro-IL-18, another substrate of caspase-1, is also processed by meprin β to form biologically active IL-18 [10]. Meprin β cleaved pro-IL-18 at the Asn⁵¹-Asp⁵² bond, suggesting that meprin β prefers an acidic amino acid in the P1' position. This specificity of meprin β for acidic residues is consistent to our previous studies on the cleavage specificity at Asn¹¹⁰-Glu¹¹¹ bond for pro-IL-1 β [11].

Meprin A has been shown previously to be involved in urinary tract infections [1, 36], the pathogenesis of renal ischemia-reperfusion injury [34], cisplatin nephrotoxicity [35], sepsis-induced AKI [33], bladder inflammation [36], and inflammatory bowel disease [1, 10]. The high expression of meprin α in an inflammatory site suggests that meprin A or meprin α may be involved in activation of IL-1 β in inflammatory diseases. Tissue damage may be caused by inflammatory mediators and cytokines, including interleukin-1, released in response to the infection [30]. One of the most important and early pro-inflammatory cytokines released in response to sepsis is IL-1 β [18,37], which may play a critical role in host defense and tissue remodeling following injury [38]. IL-1 β is known to promote its own production and also induce the expression of many pro-inflammatory cytokines such as IL-6, IL-8, and TNF- α [39]. Sepsis is associated with a systemic inflammatory response thought to be orchestrated by pro-inflammatory cytokines. Our studies show that actinonin, a potent inhibitor of meprin A, significantly reduced sepsis-induced IL-1 β production. The results of this study, therefore, suggest that meprins may play a critical role in processing pro-IL-1 β to its active form during tissue injury and inflammation.

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Figure 1.

Purified meprins process pro-IL-1 β to mature IL-1 β **A**. Recombinant meprin α and β , and meprin A purified from kidney cortices were purified as described in Materials and Methods. The purified meprins were resolved by SDS-PAGE and visualized with Coomassie blue. **B**. Meprin α , meprin β , or meprin A were incubated with purified recombinant pro-IL-1 β and the reaction products were analyzed by Western blot using a specific antibody to IL-1 β .

A

	N-terminus of cleaved pro-IL-1 β
Partial sequence of pro-IL-1 β	... ⁷⁰ AMDKLRKMLVPCPQTFQENDLSTF FPFIFEEPIFFDTWDNEAYVHDAPVRS LNCT ¹²⁵ ...
Caspase-1	¹¹⁷ APVRSLN...
Meprin α	¹¹⁶ DAPVRSLN...
Meprin β	¹¹¹ EAYVHDAPVRSLN...
Meprin A	¹¹⁶ DAPVRSLN...

B **Figure 2.**

Identification of cleavage sites of meprin A, meprin α , meprin β , and caspase-1 in the pro-IL-1 β sequence. **A.** N-terminal amino acid sequence of the IL-1 β products produced by meprin A, meprin α , meprin β , and caspase-1. N-terminal amino acid sequences of the IL-1 β products obtained are shown. **B.** Cleavage sites of meprin A, meprin α , meprin β , and caspase-1 in the pro-IL-1 β sequence as revealed by amino acid sequencing are indicated by arrows.

Figure 3.

Meprin A and meprin α produce biologically active IL-1 β products from pro-IL-1 β . The ability of indicated treatments with purified recombinant pro-IL-1 β to induce cell proliferation of the helper T-cell line D10S was assessed as described in the text. Data are expressed relative to the activity of mature IL-1 β produced by meprin β (meprin $\beta\Delta TM$) (100%). Bars represent the mean relative expression, thin lines are SEM (n=6).

Figure 4.

Effects of actinonin on IL-1 β levels in serum from mice subjected to CLP-induced sepsis. Serum IL-1 β levels were determined using a murine-specific sandwich ELISA kit from R&D Systems. Samples were obtained from C57BL/6 mice 7h after CLP treatment or Sham surgery. Actinonin was administered 30 min before CLP treatment. Data are mean $\pm$ SEM (n = 4–6 per group). *P < 0.05 compared to Sham and CLP 7h + actinonin; **P < 0.05 compared to Sham and CLP 7h.