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Mastin is a gelatinolytic mast cell peptidase resembling a mini-proteasome☆

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Abstract

Mastin is a tryptic peptidase secreted by canine mast cells. This work reveals that mastin is composed of catalytic domain singlets and disulfide-linked dimers. Monomers unite non-covalently to form tryptase-like tetramers, whereas dimers aggregate with monomers into larger clusters stabilized by hydrophobic contacts. Unlike tryptases, mastin resists inactivation by leech-derived tryptase inhibitor, indicating a smaller central cavity, as confirmed by structural models. Nonetheless, mastin is strongly gelatinolytic while not cleaving native collagen or casein, suggesting a preference for denatured proteins threaded into its central cavity. Phylogenetic analysis suggests that mammalian mastins shared more recent ancestors with soluble α/β/δ tryptases than with membrane-anchored γ-tryptases, and diverged more rapidly. We hypothesize that gelatinase activity and formation of inhibitor-resistant oligomers are ancestral characteristics shared by soluble tryptases and mastins, and that secreted mastin is a mini-proteasome-like complex that breaks down partially degraded proteins without causing bystander damage to intact, native proteins.

Keywords: Mastin; Tryptase; Mast cell; Proteasome; Serine peptidase; TC30; Mast cell protease-11; Leech-derived tryptase inhibitor

Tryptases are tryptic serine peptidases secreted by mast cells and basophils and are suspected to play important roles in allergic inflammation, tissue remodeling, and host defense against bacteria [1–3]. The most extensively characterized member of this group of enzymes is human β-tryptase, which differs from most trypsin-family enzymes in key structural details [4–6], including self-compartmentalization into non-covalently associated tetramers [7], which form a central cavity containing the four enzymatically active sites [8]. Substrates and inhibitors must enter the cavity to access the active sites. The amidolytic activity of the tetramer is protected from inactivation by serpins and other large serine peptidase inhibitors in plasma by the small dimensions of the cavity, into which the inhibitors do not fit. Under some conditions, the tetramer dissociates into active but unstable monomers, which can cleave larger substrates and are more sensitive to inhibition [9,10]. Another characteristic is storage of the active enzyme with histamine and other mediators in mast cell secretory granules after excision of the zymogen propeptide. In this respect, tryptases differ from trypsinogen and other zymogens of pancreatic acinar cell granules—and from most other secreted trypsin-family peptidases, which are secreted as inactive precursors and processed to catalytically competent forms outside of the cell.

Tryptase tetramerization appears to require removal of propeptide from monomeric zymogen [11–13]. In the test tube, the β-tryptase tetramer is stabilized by high concentrations of salt, which promote hydrophobic con-

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tacts between monomers [14,15]. Under physiological conditions, the β oligomer is stabilized by co-secreted heparin, which bridges pairs of monomers via electrostatic contacts, thereby stabilizing the tetrameric configuration [8,16]. The behavior of β-tryptase's closest human relative, α, is markedly different. Rather than being stored in granules and secreted as an active tetramer, it is constitutively released from mast cells largely as an inactive pro-monomer (along with any unprocessed β) because of a mutation in its propeptide that interferes with processing [17]. Some of the human enzymes' closest relatives in rodents are oligomers that vary widely in affinity for heparin and in half-life of the active tetramer after secretion [12,18,19]. Thus, tryptases highly similar in primary structure vary markedly in behavior and tertiary structure, and can be sensitive to small differences in amino acid sequence.

Variation among tryptases complicates determining the essential or ancestral characteristics of tryptases as a group. Clues can be obtained by examining similar enzymes, but tryptases have few close relatives among well-characterized mammalian trypsin-family enzymes, hinting of origins that lie deep in vertebrate evolution. Although the placement of introns and exons in genes encoding many trypsin-family serine protease genes fall into one of just a few patterns, that of soluble mast cell tryptases (like human α, β, and δ tryptases and rodent mast cell protease-6) is unique [4]. Furthermore, tryptases have a 10–12-residue propeptide ending in a glycine at the site of detachment from the mature catalytic domain [17,20]. This is unique among trypsin-family peptidases and predicts a novel mode of activation, which appears to proceed in two stages in the case of human β-tryptase [11].

The closest known relatives of soluble mammalian mast cell tryptases are mastins [21], implantation serine proteases (ISPs)[1] [22,23], ε-tryptase [24], and membrane-anchored γ-tryptases [25,26] and prostasins [27,28]. Of these, only mastins and γ-tryptase are suspected to originate primarily from mast cells, and mastin alone forms inhibitor-resistant oligomers. Thus, in an effort to understand the origins and essential features of tryptases, we concentrate here on mastins. The existence of mastin-like enzymes was first predicted by the sequence of a cDNA in a library prepared from a canine mastocytoma [20]. Originally termed mastocytoma protease, this peptidase was later termed dog mast cell protease-3 and ultimately mastin after purification, enzymological characterization, and immunohistochemical studies, which identified similarities as well as key differences with mast cell tryptases and demonstrated expression in normal mast cells and leukocytes [21,29,30]. Recently, mastin-like enzymes were identified in pigs and mice [31,32]. The present work looks more closely at mastin to understand its relation to the tryptase family with the hope of predicting the ancestral state and essential features of this unique group of enzymes.

[1] *Abbreviations used:* ISPs, implantation serine proteases; LDTI, leech-derived tryptase inhibitor; BPTI, bovine pancreatic tryptase inhibitor.

Materials and methods

Sequencing of mastin cDNA and gene

This laboratory deduced a partial amino acid sequence of canine mastin's catalytic domain from mastocytoma cDNA [20]. To confirm the sequence with genetic material from normal tissue and to obtain cDNA corresponding to mastin's prepro-peptide, we prepared PCR primers (5′-ATGTTGTGGC TGCTG GTTCT GACC and 5-GGTTTTCCCA GCATGTTT AA TAAGAATCCA A) based on GenBank sequences AACN011015288.1 and M24665. The 5′ primer is based on open reading frames corresponding to a hydrophobic sequence compatible with a signal peptide in a fragment of canine genomic DNA also containing sequence corresponding to mastin's N-terminal catalytic domain [20]. The 3′ primer is based on a portion of previously cloned cDNA corresponding to mastin's 3′-untranslated region, including the polyadenylation signal [20]. These primers were used to amplify mastin-specific cDNA and genomic DNA extracted from mongrel dog lung. The organization of the canine mast cell tryptase gene, including intron phase and splice sites was determined by aligning fragments of publicly deposited canine genomic DNA with tryptase cDNA using BLAST algorithms. Prediction of the most probable signal peptide cleavage site was made by subjecting deduced N-terminal mastin sequences to Signal P 3.0 analysis (www.cbs.dtu.dk/services/SignalP).

Enzyme purification

Canine mastin and tryptase were isolated from mastocytoma cells and separately purified as described [29]. Canine mastin possesses a single N-terminal amino acid sequence corresponding to its predicted mature catalytic domain [20]. When subjected to denaturing gel electrophoresis under reducing conditions, purified mastin migrates as a single, broad band, which sharpens and decreases in apparent M_r after incubation with N-glycosidases [29]. Canine mastin and tryptase are readily purified from each other by exploiting differences in extraction in low-ionic strength buffers and in binding to heparin-affinity media [29]. Like mastin, canine tryptase purified from mastocytoma cells is N-glycosylated with an N-terminal amino acid sequence corresponding to its predicted mature catalytic domain [29,33].