STRUCTURE AND FUNCTION OF MAST CELL PROTEASES

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The properties of the rat mast cell proteases, RMCP I and RMCP II are compared. How the structures of these enzymes may relate to substrate specificity and function is discussed. Additionally, it is proposed that the mast cell proteases represent the best characterized members of a distinct subclass of serine proteases that also include proteolytic enzymes found in cytotoxic lymphocytes and granulocytes.

Although the mast cell has been known for nearly one hundred years, its origin, functions and relationship to other, better characterized leukocytes remain poorly understood. In the past, much of the work on mast cells focused on attempts to understand how these cells participate in hypersensitivity and anaphylactic reactions and how to overcome the effects of substances, such as histamine, that are secreted by mast cells.

The role of the mast cell in disease still is an important area of investigation. Recently, however, several hypotheses have emerged suggesting mast cells perform important functions in homeostasis and tissue repair under normal conditions and that they serve as "sentinel" cells in defense against invading organisms (1).

Our work on mast cells during the past 10 years has centered on the identification, purification and characterization of the major protein products of these cells; namely, proteolytic enzymes. This paper reviews the structure and enzymology of mast cell proteases and discusses these properties as they may relate to function.

The Types and Distribution of Mast Cells

Mast cells are tissue granulocytes that are similar in several properties to blood basophils. In some laboratory animals there is an inverse relationship between blood basophil and tissue mast cell numbers. For example, in rats the occurrence of blood basophils is extremely low whereas tissue mast cells are abundant. In contrast just the opposite distribution of these cells is observed in rabbits (4).

Although mast cells can be observed anywhere in loose connective tissue of vertebrates they are located most frequently around blood vessels and in the vicinity of nerve endings (18). This observation is consistent with the hypothesis that mast

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Abbreviations: CTMC: connective tissue mast cell, MMC: mucosal mast cell, RMCP I: rat mast cell protease I, RMCP II: rat mast cell protease II.

cells serve as sentinel cells and function as "biological filters" that cleanse environmentally sensitive areas of potentially toxic substances.

In rats (17) and in humans (29) there are two types of mast cells. They differ from each other in distribution, morphology, histology and cellular contents among other properties. The type of mast cell that has received the most attention in the past is the connective tissue mast cell (CTMC), which, in the rat, can be isolated as a free cell from the peritoneal cavity. The other cell type is known as the mucosal mast cell (MMC) because it is located primarily in the lamina propria of mucosal tissue in healthy rats.

The relationship between the two types of mast cells is not understood. One possibility is that the cells represent differentiated subsets of the same cell and that its cellular properties are influenced by factors in the local environment (14). Another possibility is that, despite similarities, the two types of mast cells are not directly related to each other. Evidence exists in support of each possibility. It is known, however, that the MMC is under immuno-regulation and it can proliferate rapidly under certain conditions such as during parasite infections (32) in response to soluble T-cell factors (8). Such regulation, rapid proliferation and T-cell dependency has not been observed for the CTMC.

Mast Cell Contents and Properties

A prominent feature of mast cells is the presence of numerous basophilic secretory granules containing high molecular weight proteoglycans (38), proteolytic and other types of enzymes (12), chemotactic molecules (19), and various substances such as histamine, serotonin and leukotrienes (21) that exert powerful vasoactive and neuroactive effects when released from the cells.

Mast cells can be stimulated to secrete their granules in the presence of an appropriate antigen by a process mediated by specific IgE molecules that bind to receptors on the cell surface (9). Also, secretion can be initiated through non-immunological processes by chemical agents such as compound 48/80 (28), peptides such as bradykinin and those present in insect venom (7), and calcium ionophores (6).

Exocytosis is a non-cytotoxic process involving fusion of perigranular membranes of adjoining granules and finally to the plasma membrane (18). When the granules come into contact with the extracellular fluid, substances such as histamine that are loosely associated with granules are released and can be transported away from the perturbed cell by diffusion. Other, more tightly-associated substances remain bound to the granule matrix. As the cell returns to a normal state it may recover many of the spent granules (18). Alternatively, other granules that have drifted away from the mast cell and cannot be reclaimed, may be phagocytosed by macrophages (18). Despite some apparent similarities in morphology and histology, the CTMC and MMC of rats differ significantly by nearly every criterion of comparison observed to date. For example, although secretory granules of each type of mast cell contain sulfated proteoglycans, CTMC granules contain heparin and those of MMC contain chondroitin sulfate (22). The histamine and serotonin levels in CTMC are high, whereas the concentrations of these mediators in MMC are much lower. In contrast, the leukotriene concentration is higher in MMC than it is in CTMC (21). Table 1 provides a comparison of some of the known properties of rat mast cells.

TABLE 1. *Comparison of some properties of rat mast cells*

Property	CTMC	MMC
Distribution	Connective tissue peritoneum	Mucosal tissue
Origin	Connective tissue (bone marrow?)	Bone marrow
Immunoregulation	None observed	T-cell dependency (IL-3)
Response to IgE-antigen complexes	Degranulation	Degranulation
Response to compound 48/80 or basic peptides	Degranulation	No effect
Solubility of secretory granules	Insoluble in physiological buffers	Soluble in physiological buffers
Granule contents	Heparin	Chondroitin sulfate B
	Histamine	Histamine
	Serotonin	Serotonin (?)
	RMCP I	RMCP II
	Carboxypeptidase A	

Mast Cell Proteases

Rat CTMC are known to contain at least three different proteolytic enzymes: a carboxypeptidase A-like enzyme (30), a trypsin-like enzyme (11), and a chymotrypsin-like enzyme (30). Only a chymotrypsin-like protease and none of the others has been found to date in MMC (30). The chymotrypsin-like enzyme are the best characterized of the mast cell proteases. Work in our laboratory and by others has provided information concerning the physical, chemical, enzymological, immunological and structural properties of the chymotrypsin-like proteases (30, 37). This enzyme in CTMC was first discovered by Benditt and Arase in 1959 (2). The analogous enzyme from MMC was first isolated from rat intestine as the so-called "group-specific protease" (10). When the actual source of the enzyme was discovered (31), the protease was more properly referred to as rat mast cell protease II

TABLE 2. *Comparison of some properties of RMCP I and RMCP II*

Property	RMCP I	RMCP II
Substrate specificity	Chymotrypsin-like	Chymotrypsin-like
Optimum pH	8-9	8-9
Molecular mass (daltons)	26,000	24,655
Amino acid sequence	>95% determined (75% identity to RMCP II)	determined
	Homologous relationship to lymphocyte proteases	
Storage form in granule	Active	Active
Extraction conditions	Buffers of high ionic strength (e.g. >1M KCl)	Physiological buffers
Location following exocytosis	Remains associated with insoluble granules	Released from granule
		significant levels observed in blood and gut lumen

(RMCP II) to distinguish it from chymase of CTMC which was designated rat mast cell protease I (RMCP I) (35).

Although RMCP I and RMCP II share several similar properties, there are a number of significant differences between these proteases (30). A comparison of properties of these enzymes is presented in Table 2.

The functions of RMCP I and RMCP II remain unknown. However, based on available information, it is likely that the proteases perform different functions. For example, there are significant differences in the activities, substrate specificities and chemical properties of the proteases (30, 37). Additionally, a potentially important difference is that RMCP I remains bound to its insoluble granule after exocytosis (25) whereas RMCP II diffuses away from its highly soluble granule in the presence of extracellular fluids (16). One interpretation of these observations is that RMCP I activity may be restricted to the vicinity of the perturbed mast cell, but the activity of RMCP II could be expressed at some distance from the mast cell.

The carboxypeptidase A-like (5) and trypsin-like (11) enzymes of the rat CTMC have been isolated and partially characterized, but no functions have yet been ascribed to them.

The complete covalent structure of RMCP II is known (34) and over 95% of the amino acid sequence of RMCP I has now been obtained in our laboratory (Le Trong, Neurath and Woodbury, unpublished). The structures of the proteases reveal a clear homologous relationship since approximately 75% of the amino acid

	174	*	**	180	***	186
RMCP I	Ile - Arg - Ser - Ala - Tyr - Lys - Gly - Asp - Ser - Gly - Gly - Pro - Leu					
RMCP II	Leu - Arg - Ala - Ala - Phe - Met - Gly - Asp - Ser - Gly - Gly - Pro - Leu					
CCPI	Lys - Arg - Ala - Ser - Phe - Arg - Gly - Asp - Ser - Gly - Gly - Pro - Leu					
CCP II	Lys - Gly - Ala - Ser - Phe - Glu - Gly - Asp - Ser - Gly - Gly - Pro - Leu					
HLP	Lys - Lys - Thr - Ser - Phe - Lys - Gly - Asp - Ser - Gly - Gly - Pro - Leu					
CA	Gly - Val - Ser - Ser - Cys - Met - Gly - Asp - Ser - Gly - Gly - Pro - Leu					
Tr	Gly - Lys - Asp - Ser - Cys - Gln - Gly - Asp - Ser - Gly - Gly - Pro - Val					

Fig. 1. Comparison of the partial amino acid sequences near the active site of related serine proteases. RMCP I: rat mast cell protease I, RMCP II: rat mast cell protease II, CCP I: mouse cytotoxic T-cell protease I, CCP II: mouse cytotoxic T-cell protease II, HLP: human lymphocyte protease (J. Schmid and C. Weissmann, Zürich, personal communication), CA: bovine chymotrypsin, Tr: bovine trypsin, *: residue participating in substrate binding, **: position of a half-cystinyl residue that, in most serine proteases, contributes to a disulfide bond (the absence of this bond appears to be characteristic of the mast cell and lymphocyte serine proteases), ***: active site serine.

preference for substrates containing hydrophobic residues at P_2 and P_3 as well as P_1 (37). Further, the results of specificity studies on the activity of RMCP I toward where. However, for the purposes of the present paper, the amino acid sequence around the substrate binding site and the active site seryl residue of RMCP I is shown in Figure 1. The structures in this region of RMCP II and other related serine proteases are included for comparison.

During the past year, several cDNA clones have been described that appear to be derived from mRNA specific to activated T-cells or natural killer cells and which encode serine proteases (15) displaying a strong homologous relationship to RMCP II (Fig. 1). Overall the amino acid sequences of these "lymphocyte" proteases are about 55% identical to that of RMCP II. Additionally, the mast cell proteases RMCP I and RMCP II and the putative lymphocyte proteases have other structural features in common that have been discussed in detail for RMCP II (34). For example, the mast cell and lymphocyte proteases lack a disulfide bond near the active site seryl residue which is present in all other known serine proteases (34).

Recently, the cDNA structure of human Cathepsin G has been determined and this protease appears closely related in structure to both the mast cell and the lymphocyte proteases (Dr. James Travis, University of Georgia, personal communication). Interestingly, cathepsin G also lacks the disulfide bond near the active site seryl residue found in most serine proteases.

The identification of what appears to be a family of similar serine proteases in mast cells, lymphocytes and leukocytes provides evidence that is consistent with the hypothesis that RMCP II (and RMCP I) is representative of a distinct class of serine proteases that is different in an evolutionary sense from other types of serine proteases (34, 36). Thus, in addition to the families of pancreatic serine proteases, the blood coagulation and fibrinolytic factors, and the proteases of complement it appears that the serine proteases of mast cells, granulocytes and lymphocytes form a distinct family of proteases. It is unknown whether or not these apparently related proteases also have similar functions.

The substrate specificities of RMCP I and RMCP II have been investigated using peptide-p-nitroanilide substrates (37). Recently, the specificity of RMCP I (both free, and bound to isolated mast cell granules) toward many different peptide and proteins has been examined (13). The results of these studies indicate that the specificities of RMCP I and II are quite different from those of chymotrypsin and cathepsin G. In particular, the mast cell proteases demonstrate a greater preference than chymotrypsin for substrates that have extended hydrophobic structures on both sides of the susceptible peptide bond (13, 37).

Using the nomenclature of Schechter and Berger (24) a peptide substrate can be designated as

whereby the peptide bond P_1-P_1' represents the susceptible peptide bond. Chymotrypsin-like proteases will hydrolyze peptides at this bond if P_1 is a residue with an aromatic or hydrophobic side chain. For chymotrypsin, the nature of the P_2 residue has a significant effect on the rate of hydrolysis of the P_1-P_1' bond (26). The residues at the other positions have little effect on the rate of hydrolysis (26). In contrast, the mast cell proteases RMCP I and RMCP II show a significant

sequences are identical. A detailed discussion of the amino acid sequence of RMCP I and how it relates to RMCP II and other serine proteases will be published elsewhere. Peptides and proteins indicate that, unlike chymotrypsin, this protease prefers a hydrophobic residue at P_1' as well (13).

The 3-dimensional structure of RMCP II has been determined by x-ray diffraction studies (23). A comparison of this structure to that of bovine chymotrypsin (3) reveals a high degree of overall similarity between the proteases. However, there appears to be a major difference between the 3-dimensional structure of RMCP II and that of chymotrypsin in the vicinity of the active sites of the proteases (23) that may affect substrate specificity.

Since the amino acid sequence in this region of RMCP I is more closely related to that of RMCP II than to chymotrypsin (Le Trong, Neurath, and Woodbury, unpublished) the 3-dimensional structures of RMCP I and RMCP II may be similar in this region as well. If so, it would appear that the P_1' residue of peptide substrates can interact with regions of the mast cell proteases, but not with the same regions of chymotrypsin.

Until recently, the activity of RMCP I has been studied only with purified protease. Under physiological conditions of pH and ionic strength, however, it appears that the protease remains associated with the extruded granule after exocytosis (12, 25). Thus, we felt it relevant to study the substrate specificity of RMCP I while it was bound to isolated insoluble mast cell granules that lack perigranular membranes (12). The results indicate that peptide/protein substrates as large as approximately 15,000 daltons are readily hydrolyzed by granule-bound RMCP I (13). Proteins larger than this size, however, are not hydrolyzed by granule-bound protease, but are degraded by the free enzyme. Further, neither normal rat serum nor α -1-anti-trypsin, both of which inhibit purified RMCP I, could inhibit the activity of granule-bound RMCP I (13). These results strongly suggest that under physiological conditions the specificity and regulation of activity of RMCP I may be significantly restricted compared to the same properties of purified RMCP I.

Although the function of the mast cell proteases are not known, the study of these enzymes has provided considerable information about mast cells in general. For example, mast cells can be cultured from bone marrow cells in the presence of a T-cell factor which in the mouse is Interleukin-3 (20). The observation that these cultured cells produced RMCP II, but not RMCP I, in addition to other considerations, established that the cultured rat bone marrow cell was the MMC (8). Also, similar observations have established that the rat basophilic leukemia (RBL) cell line, which has long served as a model in the study of basophilic granulocytes, is closely related, if not identical to the MMC (27). Additionally, the detection of RMCP II in the blood and intestinal lumen of rats during parasite expulsion and during systemic anaphylaxis (16, 33) has implicated a direct participation of the MMC in these processes.

Clearly, the measurement of expression and distribution of the mast cell proteases has been, and should continue to be, a significant biochemical marker in studying mast cell heterogeneity and function.

Summary

Although there now exists a considerable amount of information about the primary and tertiary structures and the substrate specificities of the rat mast cell

chymotrypsin-like proteases, little is known about how they function in hypersensitivity and inflammatory reactions. It is known that MMC proliferation and secretory activity are greatly increased during the course of intestinal parasite infection (32). There is also a dose-response related release of RMCP II from MMC of rats undergoing systemic anaphylactic shock (16). Additionally, RMCP II is secreted from MMC at the same time rats harboring intestinal parasites damage and expel the parasites by an immunologically-mediated process (33). Thus, the MMC and its principal protein product, RMCP II, probably play fundamental roles in the immunological defense mechanisms of the gastrointestinal and respiratory tracts.

The recent observation that it may be more relevant to study the properties of RMCP I as it is associated with isolated secretory granules than those of the free enzyme (13) offers a new experimental approach to understanding how this protease functions.

There seems to be little doubt that several proteases comprise a distinct family of serine proteases that have closely related properties in common. The mast cell proteases RMCP I and RMCP II represent the best characterized examples of this family of granulocyte proteases. It is our opinion, that much more work is required in this area and that the mast cell proteases, which can be isolated in relatively large amounts, provide excellent models to investigate the functions of proteases in hypersensitivity, anaphylactic and inflammatory reactions.

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