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Biochemistry

A major serine protease in rat skeletal muscle: Evidence for its mast cell origin

(chymase/amino-terminal sequences/atypical mast cells)

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ABSTRACT **The physical, chemical, and immunologic properties of a protease from rat skeletal muscle, proposed to function in the degradation of certain intracellular enzymes, are identical to those of a chymotrypsin-like serine protease isolated from peritoneal mast cells. The results of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 8 M urea indicate that the two rat proteases have identical mobilities corresponding to a molecular weight of 26,000. The relative amino acid compositions of the proteases are nearly identical. Immunodiffusion tests for crossreaction between the muscle protease and antisera directed toward mast cell protease indicate that the former is immunologically identical to mast cell protease. The first 35 amino-terminal residues of the two enzymes are identical and indicate homology of these proteins to other mammalian serine proteases. The sequence analysis of the protease from muscle was extended for an additional 16 positions, and comparison of this amino-terminal sequence with that of a similar enzyme from small intestine showed approximately 75% sequence identity. In contrast, only 40% of the residues in this region of bovine chymotrypsin A were found at corresponding loci in rat muscle protease. It is concluded that the protease from muscle or mast cells is closely related to the enzyme from small intestine which recently was localized in the "atypical" mast cells of gut mucosa [Woodbury, R. G., Gruzenski, G. M. & Lagunoff, D. (1978) *Proc. Natl. Acad. Sci. USA* 75, 2785–2789].**

Katunuma and coworkers (1, 2) have isolated several chymotrypsin-like serine proteases from various rat tissues and proposed that these enzymes initiate the degradation of several intracellular pyridoxal phosphate-dependent enzymes such as ornithine aminotransferase. The apo forms of these enzymes were inactivated by the proteases, but not the holo enzymes nor the apo forms of enzymes requiring other cofactors.

Recently, an improved method was developed to purify a serine protease from rat skeletal muscle (unpublished data). During that study it was observed that the relative amino acid composition of this enzyme and that of the chymotrypsin-like protease (chymase) of peritoneal mast cells (3) were similar. The present study shows that the amino-terminal sequences and the immunologic properties of these two proteases are identical.

EXPERIMENTAL

Materials. For the purpose of developing specific antisera, a small amount of the chymotrypsin-like protease of rat peritoneal mast cells was purified as described by Lagunoff and Pritzl (4). Larger quantities of enzymes were prepared by a method which includes affinity adsorption chromatography on ovoinhibitor immobilized on Sepharose 4B (unpublished data). The protease from skeletal muscle was purified by the method of Sanada *et al.* (5) or by the affinity adsorption method described above. Rabbit antisera directed toward mast cell protease or the "atypical" mast cell protease of small intestine were prepared and their specificities were determined as described (3).

Methods. Proteins were reduced and pyridylethylated (6) before sequence analyses were carried out on a Beckman Sequencer model 980B by the method of Edman and Begg (7) as modified by Hermodson *et al.* (8). Phenylthiohydantoin-amino acids were identified by gas chromatography and by high-performance liquid chromatography (9). Amino acid analyses were performed on a Durrum model D-500 amino acid analyzer. Duplicate samples were hydrolyzed in 6 M HCl at 110°C for 24 hr. Gel electrophoresis in the presence of sodium dodecyl sulfate and dithiothreitol was carried out by the method of Weber and Osborn (10), and also in the presence of 8 M urea.

Double immunodiffusion was performed to determine if the protease isolated from skeletal muscle crossreacted with antiserum directed toward mast cell protease.

RESULTS

The relative amino acid composition (after 24 hr of hydrolysis in 6 M HCl) of mast cell protease was found to be similar to that of the enzyme from skeletal muscle (Table 1). These compositions are similar to that determined from time-course analysis by Sanada *et al.* (5).

Electrophoresis of the two proteases on polyacrylamide gels in the presence of sodium dodecyl sulfate and dithiothreitol indicated that their apparent molecular weights were approximately 29,000 (Fig. 1). When urea (8 M) also was included in the gels the electrophoretic mobilities of the proteins corresonded to apparent molecular weights of approximately 26,000. The latter value is more consistent with that (22,000–24,000) recently determined by sedimentation equilibrium analysis (5).

Double-immunodiffusion tests showed that antisera directed toward the chymotrypsin-like protease of mast cells crossreacted with protease obtained from muscle (Fig. 2). The precipitin lines formed when the two proteases were placed in adjacent sample wells were completely fused and lacked spur lines, indicating that the two proteases are immunologically identical. The protease isolated from skeletal muscle (or peritoneal mast cells) did not crossreact with antisera directed toward the intestinal protease (Fig. 2) and vice versa; the intestinal protease did not crossreact with antisera directed toward peritoneal mast cell protease.

The amino-terminal sequences of the mast cell protease and the enzyme from skeletal muscle are identical for the first 35 residues (Fig. 3). The sequence analysis of the protease from muscle was extended to an additional 16 positions and com-

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Table 1. Comparison of relative amino acid compositions* of rat skeletal muscle and mast cell proteases

Amino acid	mol of amino acid/mol of protein		
	Mast cell protease†	Muscle protease‡	Muscle protease§
Aspartic acid	18.5	20.2	16
Threonine	16.8	16.5	16
Serine	12.0	12.5	10
Glutamic acid	20.0	21.0	18
Proline	16.2	16.5	16
Glycine	22.4	21.8	22
Alanine	15.6	16.0	16
Valine	22.1	21.4	20
Methionine	5.7	5.5	8
Isoleucine	12.4	12.7	10
Leucine	12.5	13.9	14
Tyrosine	9.9	9.1	10
Phenylalanine	8.0	8.0	8
Histidine	8.8	8.5	6
Lysine	22.7	23.4	22
Arginine	13.3	12.7	10
Half-cystine	ND	ND	8
Tryptophan	ND	ND	2

* Determined for duplicate samples after proteins had hydrolyzed for 24 hr in 6 M HCl at 110°C. ND, not determined.
† Purified from isolated peritoneal mast cells.
‡ Prepared by affinity adsorption on ovoinhibitor immobilized on Sepharose. Compositions of both proteases were referred to molecular weights of 26,000.
§ Calculated from Sanada *et al.* (5) assuming a molecular weight of 23,000.

pared (Fig. 3) to the first 51 amino-terminal residues of the "atypical" mast cell protease from rat small intestine (11) and of bovine α-chymotrypsin A (12). In this region, approximately 75% of the residues of the protease from skeletal muscle were found at corresponding loci in the enzyme from small intestine. Further, most of the amino acid residue substitutions were chemically conservative. In contrast, only 40% of the residues in this portion of the muscle protease (37% of the intestinal protease) were found to be identical in bovine chymotrypsin.

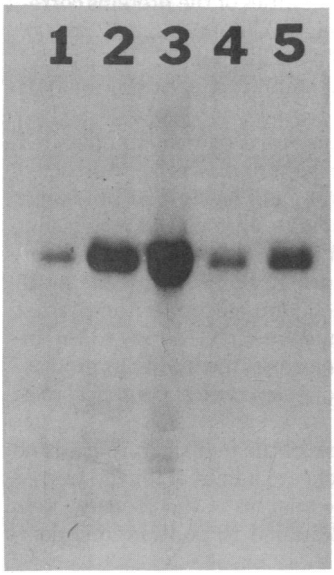

FIG. 1. Gel electrophoresis in the presence of sodium dodecyl sulfate and dithiothreitol of: gel 1, 10 μg of protease from rat skeletal muscle isolated by affinity adsorption chromatography on ovoinhibitor immobilized on Sepharose; gel 2, 20 μg of protease from rat peritoneal mast cells; gel 3, 40 μg of mast cell protease; gel 4, 10 μg of skeletal muscle protease; gel 5, 10 μg each of skeletal muscle and mast cell proteases.

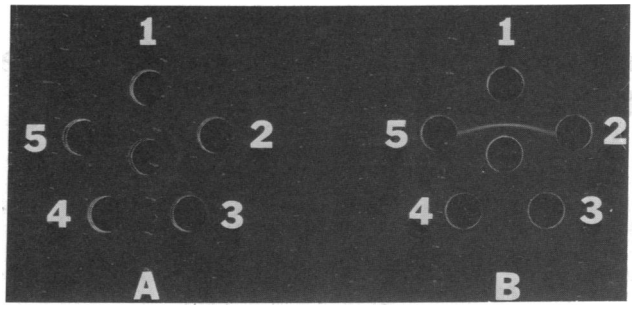

FIG. 2. Immunodiffusion test for crossreaction. (*A*) Center well, antiserum directed toward mast cell protease; 1 and 3, mast cell protease (16 μg/ml); 2 and 4, muscle protease (16 μg/ml); 5, protease from small intestine (20 μg/ml). (*B*) Center well, antiserum directed toward protease from small intestine; 1, intestinal protease (250 μg/ml); 2, muscle protease (220 μg/ml); 3, intestinal protease (50 μg/ml), resulting in a weak precipitin line; 4, mast cell protease (66 μg/ml); 5, muscle protease (66 μg/ml). Each well contained 5 μl of sample.

DISCUSSION

The physical, chemical, immunological, and structural data obtained to date indicate that the protease from rat skeletal muscle is identical with the chymotrypsin-like serine protease isolated from peritoneal mast cell granules. In addition, the two proteases possess nearly identical values of k_{cat} and K_m toward benzoyl-tyrosine ethyl ester (unpublished data). The results of preliminary immunofluorescent localization studies with antisera directed toward the skeletal muscle protease confirmed the mast cell origin of this enzyme (unpublished observation).

In recent years a number of chymotrypsin-like serine proteases have been described, and some of these have been isolated from various rat tissues such as skin, thymus, and skeletal muscle (13, 14). In addition to their similar specificities toward ester substrates, these enzymes usually require highly concentrated salt solutions to maintain their solubility. In view of the ubiquitous distribution of protease-containing mast cells in rat tissues (3), it would not be unreasonable to suggest that some of these proteases, in fact, are derived from these cells.

The protease from small intestine (1) also appears to be of mast cell origin since it is localized in the so-called "atypical" mast cells of mucosa (3). It is unclear, however, whether this cell is related to normal mast cells or represents a distinct cell line (15).

Recently, immunofluorescent evidence was obtained that each type of mast cell contained both proteases (3). Although no crossreaction was evident between the proteases and their respective antisera by immunodiffusion tests, the possibility cannot yet be excluded that the proteases share a single antigenic site. In such a case, crossreaction studies should lead to soluble complexes which would be undetected by immunodiffusion tests but could give positive immunofluorescent staining in fixed tissue. In any event, it is clear from the amino-terminal sequences determined in the current study that the proteases are not derived from one another and that each must be a distinct gene product.

In view of the close similarity of the two proteases in amino-terminal structure, cellular origin, and chymotrypsin-like specificity toward ester substrates (1), one is tempted to suggest that they perform similar physiological functions. However, the recent examinations by Kobayashi and colleagues (16, 17) of the substrate specificities of each enzyme toward several polypeptide hormones clearly indicate significant differences. The protease from "atypical" mast cells of small intestine showed a much lower catalytic rate and greater selec-

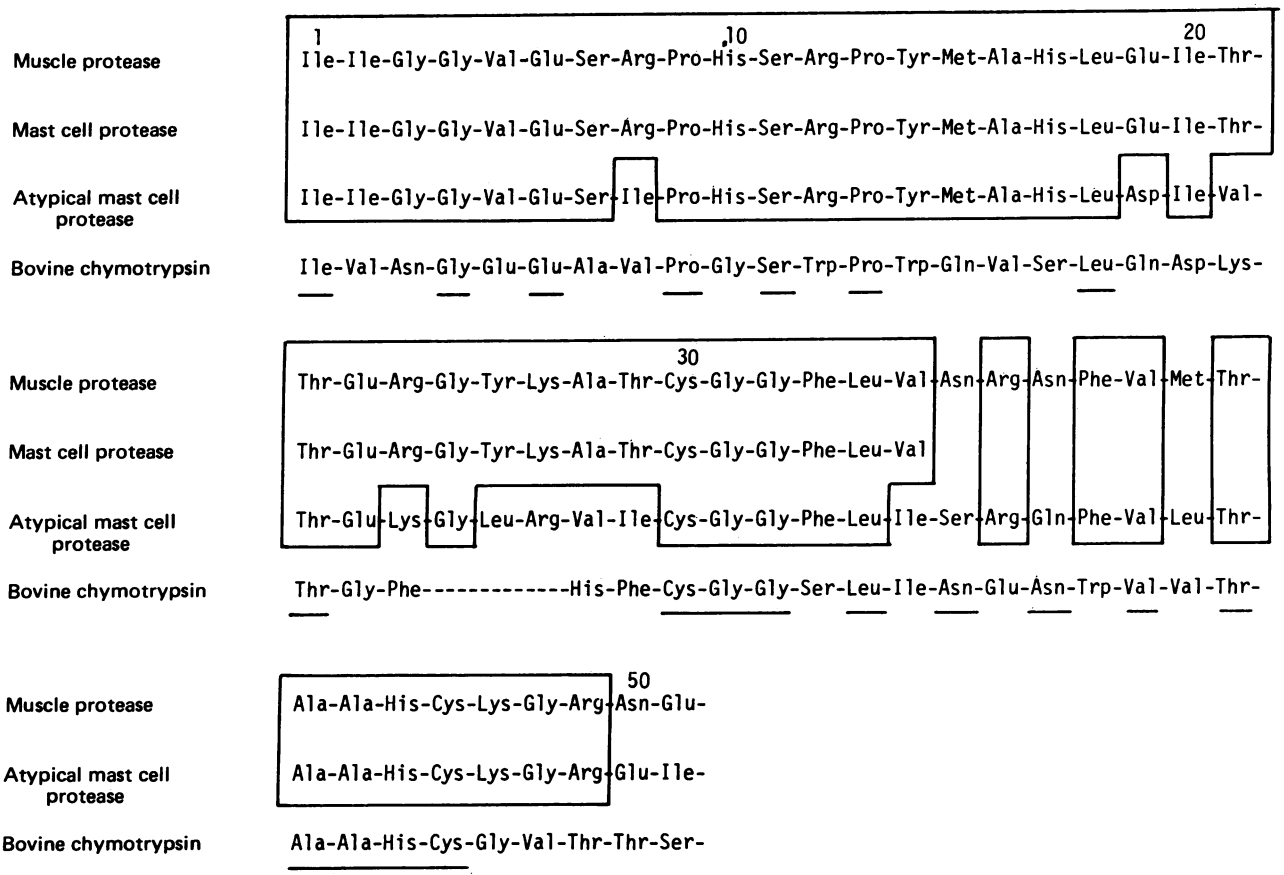

FIG. 3. Comparison of the amino-terminal sequences of rat mast cell protease (chymase) and skeletal muscle protease. The amino-terminal structures of a protease (atypical mast cell protease) from small intestine (11) and bovine chymotrypsin A (12) are included for comparison. Half-cystine was determined as *S*-pyridylethylcysteine. Dashed line (- - -) indicates a gap placed in the sequence of chymotrypsin in order to obtain an optimum alignment of this structure with the others. Residues of chymotrypsin that are underlined are identical to those in mast cell protease.

tivity in its action on peptide bonds between adjacent hydrophobic amino acid residues compared to the enzyme from normal mast cells, which resembles bovine chymotrypsin in specificity and catalytic rate. This difference suggests that perhaps these two mast cell-derived proteases may not act on the same physiologic substrates, and that there exists a degree of tissue specificity or discrimination in the effects exerted by each enzyme. The fact that "atypical" mast cells are found only in mucosal tissue, such as the digestive and respiratory tracts, whereas normal mast cells are found in the connective tissue at most other sites, is consistent with the premise that the actions of these proteases are tissue specific.

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