Review Letter

STRUCTURE, SPECIFICITY AND LOCALIZATION OF THE SERINE PROTEASES OF CONNECTIVE TISSUE

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Received 26 February 1980

1. Introduction

It is the purpose of this review to describe several well characterized proteases present in tissues and cells which appear to be related to the pancreatic serine proteases. Much of the early work concerning the nature of tissue proteases was done during the late 19th century by German physiologists who were studying tissue autolysis [1]. Hedin and Rowland [2] examined homogenates of various mammalian organs for proteolytic activity and observed that, with the exception of muscle, autolysis was greatest at acid pH. The term 'cathepsin' was introduced by Willstätter and Bamann [3] in 1929 to describe proteolytic activity of tissues in the weakly acid pH range. In recent years, however, this term has sometimes been applied to include tissue proteases in general, such as cathepsin G which has an optimum activity at pH 8 and cathepsin E which is most active at pH 2.5. The rapidly growing literature dealing with tissue and cellular proteases has been recently reviewed by Barrett [4].

Tissue and cellular serine proteases, which are most active at the physiological pH (pH 7–8), have been implicated in such processes as cellular chemotaxis [5], endocytosis, exocytosis [6], protein turnover in tissues [4,7] tumorigenesis [8] and fertilization [9], but very few of these enzymes have been purified or characterized in detail. Two notable exceptions are the human neutrophil serine proteases, cathepsin G, and elastase which have been intensely studied because of their implication in emphysema and other respiratory [10] disorders. Imbalances in the levels or regulation of tissue and cellular protease are generally thought to manifest themselves in various disease states, e.g., muscular dystrophy, arthritis, degenerative skin disorders as well as certain respiratory and gastrointestinal diseases [4]. Malignant tissues also show significant changes in proteolytic activity compared to normal, particularly that due to collagenases [11].

As important as abnormal proteolytic activity in tissues may be in disease states, intra- and extracellular proteases are necessary for maintaining normal tissue homeostasis. The steady-state concentration of proteins in cells and tissues is controlled by the rates of their synthesis and degradation [12,13] and although lysosomal proteases play a major role in intracellular protein degradation [14], there is considerable evidence that other proteases are of equal importance [15].

Several hypotheses have been advanced suggesting that specific limited proteolysis initiates the degradation of certain intracellular or extracellular proteins or groups of similar proteins [15,16]. For example, mammalian collagenases, secreted by several types of connective tissue cells exhibit specific and limited activity and so do the cytoplasmic proinsulin degrading proteases [17]. Support for the existence of intracellular degradative pathways of this type was derived from the investigations of Katunuma and coworkers [18] who have isolated several proteases exhibiting relatively restricted specificities and optimal activities at pH 8–9. It was reported that these proteases inactivated by limited proteolysis the apo-forms of pyridoxal phosphate dependent enzymes but not those requiring other cofactors [19]. These intracellular 'group-specific' proteases were shown to possess chymotrypsin-like specificity toward small synthetic substrates, were inactivated by DFP, suggesting that their catalytic sites were structurally related to those of the pancreatic serine proteases [19]. Because of the increasing awareness of the role

of limited proteolysis in many biological control mechanisms [20] and in tissue homeostasis [4,21], we have studied in detail the nature of these group-specific proteases of rat tissues. Some of our findings were unexpected while others emphasize the difficulties in attempting to interpret cellular events on the basis of observations made using tissue homogenates. Wherever appropriate we shall relate our findings about the nature of these enzymes to those which have been described for the other well characterized tissue serine proteases; i.e., neutrophil cathepsin G and elastase.

2. Purification

Katunuma and coworkers [19,22,23] describe the purification and characterization of four similar but distinct 'group-specific' proteases from rat tissue; two from the small intestine (one from mucosa and the other reported to originate in the smooth muscle layer) and one each from skeletal muscle and liver. The liver protease was thought to be a mitochondrial enzyme [19] on the basis of determinations of marker enzyme levels of fractions obtained by differential centrifugation. We decided to study the proteases from the muscle layer of small intestine and from skeletal muscle since these enyzmes could be obtained in sufficient quantity to allow detailed chemical characterization. Affinity chromatographic methods were developed in order to purify the proteases more efficiently and rapidly than previously possible.

The immobilized ligands were potato chymotrypsin inhibitor I immobilized on Sepharose 4B for the purification of the intestinal protease [24] and ovoinhibitor coupled to Sepharose 4B for the rat skeletal muscle protease [25]. The proteases isolated by these methods were chemically, physically, enzymatically and immunologically identical to those prepared by the described procedures [19,22].

3. Localization and identification

During the development of the affinity chromatographic purification method, it became apparent that the intestinal protease was not present in the muscle layer as reported [19]. Immunofluorescent and histochemical studies revealed that, in fact, the protease was contained in the so-called 'atypical' mast cells of the intestinal mucosa [26]. Cells containing the protease were observed in the bronchial and tracheal mucosa as well.

A similar chymotrypsin-like protease, known as chymase, is present in mast cells obtained from rat peritoneum [27]. The amino acid composition (table 1) of this enzyme is clearly different from that of the atypical mast cell protease, but it is remarkably similar to that of the skeletal muscle protease [28]. The amino-terminal sequences of the mast cell protease and the skeletal muscle protease are identical for the first 35 residues [28]. Immunodiffusion tests for cross-reactivity using anti-rat mast cell protease indicated that the mast cell and skeletal muscle proteases are immunologically identical [28]. Finally, immunofluorescent localization of skeletal muscle protease showed that this enzyme was in fact derived from mast cells present in the connective tissue of muscle (N. Katunuma, personal communication). Thus, both of these 'group-specific' proteases are in fact derived from mast cells.

Several other investigators have isolated chymotrypsin-like serine proteases from a variety of rat

Table 1
Comparison of the amino acid compositions of rat mast cell proteases

Amino acid	mol amino acid/mol protein	
	RMCP I[a]	RMCP II[b]
Aspartic acid	18.5	14
Threonine	16.8	13
Serine	12.0	13
Glutamic acid	20.0	17
Proline	16.2	15
Glycine	22.4	18
Alanine	15.6	16
Valine	22.1	22
Methionine	5.7	5
Isoleucine	12.4	18
Leucine	12.5	16
Tyrosine	9.9	9
Phenylalanine	8.0	6
Histidine	8.8	9
Lysine	22.7	13
Arginine	13.3	12
Half-cystine	n.d.	6
Tryptophan	n.d.	2

[a] Determined by analysis of samples after 24 h hydrolysis in 6 N HCl at 110°C [28]
[b] From sequence analysis [37]

n.d., Not determined

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