PROTEOLYTIC ENZYMES OF RAT LIVER MITOCHONDRIA. EVIDENCE FOR A MAST CELL ORIGIN

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1. Introduction

When rat liver mitochondria were subjected to step gradient centrifugation, a sediment of high density was obtained [1]. This sediment contained an insoluble proteinase and a carboxypeptidase. Both enzymes have been purified and their physicochemical properties were studied [2–4]. After subcellular fractionation, digitonin treatment and step gradient centrifugation both enzymes were localized in mitochondria [1,3]. After submitochondrial fractionation both proteolytic enzymes were found in the inner mitochondrial membrane [1,3]. From detailed studies on the properties of the proteinase [2,4] it became likely that the histone degrading enzyme isolated in our laboratory is identical with the 'group-specific' proteinase described [5]. The proteinase in the inner membrane of mitochondria has also been localized [6,7]. Recent experiments [8,9] have shown that the group specific proteinases isolated from small intestine [8] and from skeletal muscle [9] are of mast cell origin. In the light of these findings it became important to examine this possibility, although mast cells have not been described as an integral part of the liver [10]. It will be shown here that indeed the proteinase as well as the carboxypeptidase isolated from the mitochondrial fraction originate from mast cells.

2. Methods

The mitochondrial fractions from rat liver, heart and kidney were prepared according to [11]. The brain mitochondrial fraction was isolated by step gradient centrifugation as in [12] to achieve removal of synaptic vesicles which are abundant in the rough mitochondrial fraction. Mast cells were obtained from a peritoneal exudate by the method in [13]. The mitochondrial fraction was obtained by centrifugation at 18 000 × *g* for 5 min after homogenizing the cell suspension and removing whole cells and nuclei. The mitochondrial fractions from different origins were treated with digitonin [11]. The details of the sucrose step gradient centrifugation used to isolate the high density sediments which contained the proteinase and the carboxypeptidase have been reported [1]. The assay conditions for the measurement of both enzyme activities have been described [2,3]. For the histochemical detection of mast cells small tissue blocks of rat liver, heart, kidney and brain were frozen in isopentane cooled with liquid nitrogen. Thereafter for freeze-substitution the blocks were transferred to absolute acetone (–62°C) and brought to room temperature by programmed rewarming within 48 h. After paraffine embedding 10 μm sections were made and incubated for 2 h in a 1% diastase solution, then treated with the PAS–Alcian blue technique [14].

3. Results

When digitonin-treated mitochondria from rat liver were subjected to step gradient centrifugation, a sediment of high density containing proteinase and carboxypeptidase activity was isolated (table 1). Similarly, insoluble sediments exhibiting proteinase and carboxypeptidase activities were obtained after

Table 1
Comparison of proteinase and carboxypeptidase activities of the high density fractions from different cell types and organs

High density fraction	Proteinase (mU/mg protein)	Carboxy-peptidase	Proteinase (mU/g tissue (wet wt))	Carboxy-peptidase	Proteinase (mU/mg mitochondrial prot.)	Carboxy-peptidase
Liver	500	76	70	10	5.5	0.8
Heart	435	74	376	64	23.8	4.1
Kidney	90	12	22	3	2.3	0.3
Brain	7	0.4	0.7	< 0.1	0.03	< 0.1
Mast cells	32 000	2150	7600	504	1380	92

The preparation of the high density sediments is described in section 2. The proteinase activity was measured with *N*-acetyl-tyrosine-ethylester [2], the carboxypeptidase was tested with Cbz–Ala–Phe [3]

sucrose step gradient centrifugation of mitochondrial fractions from rat heart and rat kidney. No high density sediment could be isolated from the mitochondrial fraction of rat brain. When a digitonin-treated mast cell mitochondrial fraction was subjected to centrifugation under identical conditions, the pellet contained proteinase and carboxypeptidase activities of ~2 orders of magnitude higher than those obtained for heart and liver (table 1).

In the PAS–Alcian blue-stained liver sections the presence of mast cells could be demonstrated. They occurred exclusively in the connective tissue, sporadically around the greater afferent vessels and in large numbers under the liver capsule and around the hilum (fig.1).

In agreement with the proteinase and carboxypeptidase activities the number of mast cells found in sections of rat heart was higher, than in liver whereas essentially no mast cells could be detected in kidney and brain sections. When the high density fractions obtained from liver and peritoneal mast cell mitochondria were treated with 2 M LiCl in order to

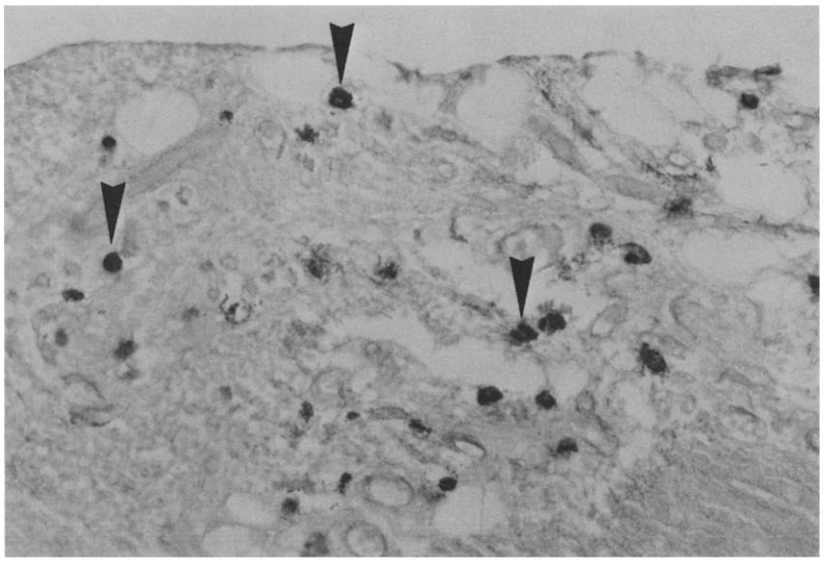

Fig.1. Occurrence of mast cells in the subcapsular portion of rat liver. A 10 μm section was pretreated with a 1% diastase solution for 2 h then stained by the PAS–Alcian blue technique as described in section 2. Magnification 250 : 1, mast cells are marked by arrows.

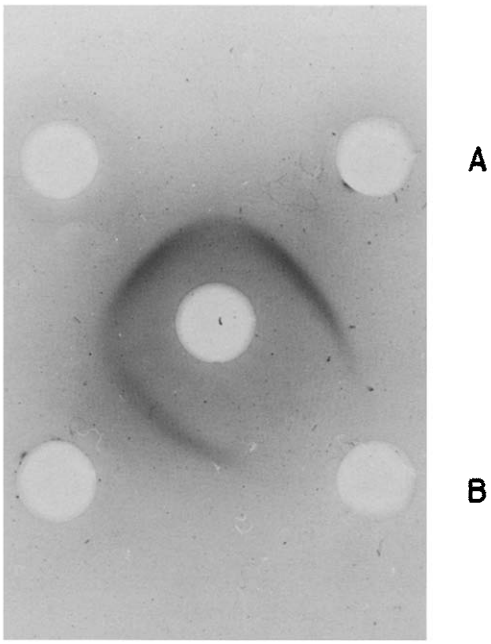

Fig.2. Immunodiffusion of mast cell antiserum against solubilized proteinases isolated from various sources. The double diffusion was carried out with 0.7% agarose gels containing 10 mM Tris–HCl (pH 8.0), 0.1% Triton X-100 and 1.5 M LiCl. Precipitates developed within 30 h at 4°C. The center well contained 8 μl antiserum against the chymotrypsin-like proteinase from peritoneal mast cells [15]. The peripheral wells containing 11 μg liver proteinase (spec. act. 2.4 U/mg) (A), 8 μg bovine α-chymotrypsin (B), 10 μg heart proteinase (spec. act. 2.2 U/mg) (C) and 3 μg mast cell proteinase (spec. act. 9.4 U/mg) (D), each solubilized in 2 M LiCl.

solubilize the proteinase and carboxypeptidase and subjected to SDS–polyacrylamide gel electrophoresis; identical protein patterns were observed (not shown).

In immunodiffusion tests crossreactions occurred between anti-mast cell proteinase and solubilized proteinases from liver and heart (fig.2).

4. Discussion

A histone degrading serine-type proteinase and a carboxypeptidase have been localized within the inner mitochondrial membrane. This conclusion has been reached after step gradient centrifugation of digitonin-treated mitochondria and a subsequent submitochondrial fractionation. Furthermore, the presence of cytochrome *c* oxidase in the high density fraction containing the proteinase–carboxypeptidase activities strongly suggested an inner mitochondrial membrane location. It was difficult, however, to explain the high density of the material (ρ = 1.25 g/cm^3) which sedimented through the layer of 1.7 M sucrose compared to an average density of 1.20 g/cm^3 measured for mitochondria [16]. We have speculated that the high density proteinase represents the 'tight junction' within mitochondria, sites, where inner and outer membrane are in close proximity [1].

A re-evaluation of the localization of the serine proteinase and carboxypeptidase was initiated by the demonstration [8,9] that the 'group-specific enzymes' [5] isolated from rat small intestine and skeletal muscle were of mast cell origin. Since it is not generally known that rat liver contains mast cells, it was necessary to demonstrate their presence in liver (fig.1). It was roughly estimated that < 0.1% of the total liver mass are mast cells. Evidence for the mast cell origin of the serine proteinase and the carboxypeptidase associated with the high density sediment obtained after step gradient centrifugation of digitonin-treated mitochondria came from the following findings:

(i) A > 100-fold higher proteinase activity/g wet wt was measured in the high density fraction obtained from a digitonin-treated mast cell mitochondrial fraction compared to liver (table 1).
(ii) In addition to the proteinase the carboxypeptidase which was only recently discovered in liver [3] is also found in the high density fraction isolated from mast cells.
(iii) The protein patterns after SDS–polyacrylamide gel electrophoresis are the same for solubilized high density fractions from liver and mast cells.
(iv) A crossreaction occurred between the liver proteinase and the mast cell antiserum (fig.2).
(v) The physicochemical properties of the liver proteinase such as molecular weight, pH optimum, solubility in KCl at high concentrations agree with the properties described for the serine proteinase isolated from mast cell granules [13,15,17].
(vi) When compound 48/80, known to cause destruction of mast cells, was injected intraperitoneally, the yield in proteinase and carboxypeptidase found in the sediment obtained after step gradient centrifugation was decreased to 30–40% (R.H., unpublished results).

In conclusion, it should be emphasized that mast cells are widely distributed in connective tissue. Thus, it is highly probable that both proteolytic enzymes may be found in various other tissues. Very likely both enzymes are localized within the granules of mast cells. The high density of these granules explains why the proteinase and carboxypeptidase contaminate various subcellular fractions. When chromatin is prepared from a total rat liver homogenate [18] by centrifugation through a 1.7 M sucrose layer, the cosedimentation of granules and chromatin occurs [19]. In order to prepare chromatin devoid of these proteolytic enzymes, it is necessary to isolate nuclei by centrifugation through 2.3 M sucrose [20]. Similarly the mast cell granules are found in the mitochondrial fraction obtained by sedimentation of a postnuclear supernatant. A separation from the mitochondria may be achieved by centrifugation on a continuous sucrose gradient.

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