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Short Report

An aspartic proteinase in *Drosophila:* maternal origin and yolk localization

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ABSTRACT. An aspartic proteinase activity has been found in *Drosophila* oocytes and embryos. The proteinase is maximally active at pH 3.5 and has been characterized by its sensitivity to specific inhibitors and by the specificity of cleavage. The activity is very low and has been localized in the yolk granules. The proteinase is detected in mature oocytes (i.e., it is of maternal origin) and remains essentially constant during embryogenesis. This suggests that the *Drosophila* aspartic proteinase functions mainly before embryogenesis.

KEY WORDS: Drosophila *aspartic proteinase, yolk granules associated proteinase, maternal origin*

Although it is thought that proteolytic mechanisms must be involved in the control of early development, the data available on the regulation of proteolytic activities in different developmental systems is scarce. In *Drosophila*, we have described a cathepsin B-like proteinase which is developmentally regulated and we have linked it with yolk degradation (Medina *et al.*, 1988; Medina and Vallejo, 1989a). We have studied a serine proteinase (Medina and Vallejo, 1989b) that is also regulated during development and we suggested its involvement in the initial activation of the cathepsin B-like proteinase. In this report, we shall discuss a cathepsin D-like proteinase activity that is present in the mature oocyte and which appears to remain constant during embryogenesis.

The proteinase activity we have found in *Drosophila* embryos has optimum activity at pH 3.5 (Fig. 1). Different characteristics of the proteinase are outlined below.

Developmental regulation

The assay of the proteinase in mature oocytes and after fertilization indicated that the activity remained essentially constant during embryogenesis (Table I). The fact that the activity was detected in the mature oocytes indicates that the enzyme is of maternal origin. The activity was very low and represented about 1% of the activity of the other acidic proteinase — the cathepsin B-like proteinase (Medina *et al.*, 1988), with both proteinases measured in optimal conditions. The sensitivity of the method (Garesse *et al.*, 1979) made it possible to carry out the measurements with precision.

Subcellular distribution

The study of the subcellular distribution of the proteinase activity in oocytes and embryos indicated that the activity was mainly associated with the 500xg fraction (about 90%). In early embryos, this fraction contains yolk granules and nuclei revealed by microscopic observations (not shown). The fact that the oocyte has only one nucleus but is loaded with yolk granules (Campos-Ortega and Hartenstein, 1985) suggests that the proteinase is associated with these embryonic structures. In addition, the subcellular distribution observed is very similar to that found for acid phosphatase in early embryos (Medina and Vallejo, 1989a), which we have shown to be associated with yolk granules. Acid phosphatase has also been used as a marker for yolk granules in *Artemia* (Perona *et al.*, 1987, 1988) and sea urchin (Armant *et al.*, 1986; Yokota and Kato, 1988).

Characterization of the proteinase activity

a) By specific inhibitors. The characterization by specific inhibitors indicated that the proteinase was acidic, of the aspartic type (Table 2). The activity was not inhibited by soybean trypsin inhibitor, leupeptin and p-hydroximercuribenzoate (OH-HgBzOH), indicating that the enzyme was neither a serine proteinase nor a cysteine proteinase. Pepstatin, the inhibitor of aspartic proteinases, inhibited the proteolytic activity indicating that it was an aspartic proteinase. Diazo-acetyl-DL-norleucine (DANME), which is another inhibitor of this type of proteinases (Barret and McDonald, 1980), also inhibited significantly.

b) Specificity of cleavage. Among the protein substrates tested, casein was preferred over albumin and protamine (Table 2). Protamine, which is 66% arginine (Devereux *et al.*, 1984), is not recognized by this proteinase. Protamine, however, is a very good substrate for the acidic cathepsin B-like proteinase, which we have already described in *Drosophila* (Medina *et al.*, 1988) and for the serine protein-

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TABLE 1

THE ACTIVITY OF THE ASPARTIC PROTEINASE OF *DROSOPHILA* DURING EMBRYOGENESIS

	0 h	1 h	3 h	6 h	9 h	12 h	18 h	21 h
Aspartic proteinase (Units/10^6 embryos)	178	311	498	338	284	222	293	302

The activity was assayed in the homogenates of embryos of the times indicated. The value detected in oocytes is probably low, due to the difficulty in assaying proteolytic activity in the yolk granule before entering degradation (Medina *et al.*, 1988; Medina and Vallejo, 1989b).

ase also found in *Drosophila* (Medina and Vallejo, 1989b). These two proteinases cleave by arginine. Aspartic proteinases cleave by hydrophobic residues (Barrett and McDonald, 1980). Protamine is very poor in hydrophobic residues (only one residue of the 32 aminoacids of the molecule). Of the two substrates cleaved, casein is richer in hydrophobic residues (about 30%) than albumin (23%) (Devereux *et al.*, 1984). Therefore, the specificity of cleavage supports the specificity of inhibition.

The aim of this report is to communicate the presence of an aspartic proteinase in *Drosophila*. The activity is very low and this has precluded a more extensive study of the enzyme. An aspartic proteinase has been involved in the accumulation of yolk protein in *Xenopus* (Opresko and Karpf, 1986). If the same were true for *Drosophila*, it would explain why the activity is not regulated during embryogenesis. This could also explain its localization in the yolk and its maternal origin. It is therefore likely that the aspartic proteinase functions mainly before embryogenesis. This report completes the description of the proteinase activities we have detected during embryogenesis of *Drosophila*.

TABLE 2

THE CHARACTERIZATION OF THE ASPARTIC PROTEINASE OF *DROSOPHILA*

BY INHIBITORS		BY SUBSTRATES	
	Activity		Activity
Leupeptin 2.5 µg/ml +	100	Casein	100
STI 0.1 mg/ml + Pepstatin 2.5 µg/ml	0	Albumin	54
DANME 2 mM	19		
OH-Hg-BzOH 2.5 mM	106	Protamine	0

The characterization was carried out with the enzyme from embryos of 3.5 h of development. The characterization by inhibitors was determined using 0.5 mg/ml casein as substrate in the presence of leupeptine (2.5 µg/ml) to avoid the activity of the cysteine proteinase (Medina *et al.*, 1988) and soybean trypsin inhibitor (STI, 0.1 mg/ml) to avoid the activity of the serine proteinase (Medina and Vallejo, 1989b).

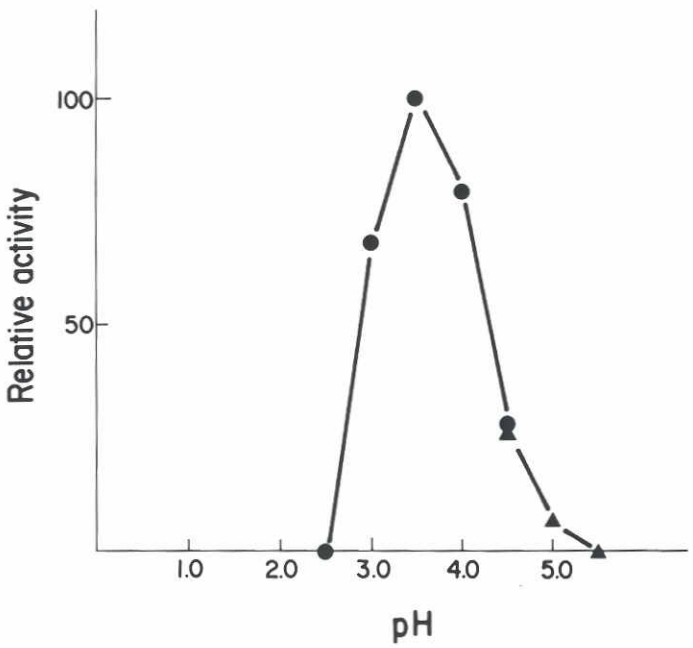

Fig. 1. pH-dependent activity of the aspartic proteinase of *Drosophila*. *The proteinase was assayed in 50 mM buffer citrate (● - ●) and acetate (▲ - ▲) at the pH indicated. The homogenate of 3.5 h old embryos was used as source of the enzyme.*

Experimental Procedures

Drosophila melanogaster (Oregon-R strain) was cultured at 25° C as usual (Elgin and Miller, 1977). Mature oocytes were obtained (Petri *et al.*, 1977) taking advantage of the fact that mature oocytes adhere to glass while the immature ones do not. Embryos were collected routinely for 2 h, with these embryos considered on average to be 1 h old. The collected embryos were allowed to develop the additional time required (for example, 5 h to obtain 6 h old embryos).

Homogenization and fractionation. Embryos and oocytes were dechorionated, unless otherwise indicated, prior to hand homogenization in 2-4 vols of a medium (Vallejo *et al.*, 1981) composed of 0.3 M sucrose, 15% Ficoll 400, 25 mM Hepes buffer, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM sodium borate, adjusted to pH 7.5. This medium has been proven to preserve the embryonic subcellular structures (Vallejo *et al.*, 1981; Roggen and Slegers, 1985). Subcellular fractionation was accomplished by centrifuging the homogenates at 2500 rpm 20 min (500xg fraction). The supernatant was further centrifuged at 15000 rpm 30 min (27000xg fraction) and the resulting supernatant was spinned at 38000 rpm 60 min giving a pellet (100000xg fraction) and a supernatant (soluble fraction).

Proteinase assay. The proteinase activity in the resuspended particulate fractions was assayed after disruption by vortexing followed by 4-5 cycles of freezing and thawing. The assay was carried out routinely at 37°C in a final volume of 1 ml, with α-casein (Sigma, 0.5 mg/ml) and 50 mM potassium citrate buffer, pH 3.5 in the presence of leupeptine (2.5 μg/ml) to block the activity of a cysteine proteinase (Medina *et al.*, 1988) and of soybean trypsin inhibitor (0.1 mg/ml) to block the activity of a serine proteinase (Medina and Vallejo, 1989b), according to the fluorescamine-based method of Garesse *et al.*, (1979). One unit is, as defined previously (Garesse *et al.*, 1979), the amount of proteolysis equivalent to the increase in fluorescence observed after labeling with fluorescamine the new-α-amino-groups produced during the hydrolysis of protamine by 7 ng of trypsin (IX Sigma) at 37°C for 10 min. The activity on albumin and protamine (Sigma) was also determined with fluorescamine. The effect of inhibitors was determined after 20 min preincubation with the enzyme at 4°C, using casein as substrate.

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