Isolation of an aspartic proteinase precursor from the egg of a hard tick, *Boophilus microplus*

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SUMMARY

An aspartic proteinase precursor, herein named BYC (*Boophilus* Yolk pro-Cathepsin) was isolated from eggs of the hard tick, *Boophilus microplus*. As judged by electrophoresis on sodium dodecyl sulfate polyacrylamide slab gel (SDS–PAGE), purified BYC presented 2 bands of 54 and 49 kDa, bearing the same NH_2-terminal amino acid sequence. By Western blot analysis, BYC was also found in the haemolymph, indicating an extraovarian site of synthesis. Several organs were incubated in culture medium with [^{35}S]methionine, and only the gut and fat body showed synthesis of BYC polypeptides. Protein sequencing of both the NH_2-terminal and an internal sequence obtained after cyanogen bromide (CNBr) cleavage of BYC revealed homology with several aspartic proteinase precursors. Incubation at pH 3·5 resulted in autoproteolysis of BYC, which produced the mature form of the enzyme, that displayed pepstatin-sensitive hydrolytic activity against haemoglobin. Western blot analysis using anti-BYC monoclonal antibodies showed proteolytic processing of BYC during embryogenesis and suggested activation of the enzyme during development. A role of BYC in degradation of vitellin, the major yolk protein of tick eggs, is discussed.

Key words: tick, *Boophilus microplus*, embryogenesis, aspartic proteinase, yolk proteins.

INTRODUCTION

Eggs of arthropods require large amounts of yolk proteins to develop, most of which are synthesized outside the oocyte, mainly in the fat body (Bianchi *et al*. 1985; Chinzei & Yano, 1985; Kunkel & Nordin, 1985), but also by follicle cells (Sato & Yamashita, 1991) or the midgut (Taylor *et al*. 1991). Yolk proteins are localised in structures called yolk granules or yolk spheres (Hagedorn & Kunkel, 1979; Kunkel & Nordin, 1985; Raikhel *et al*. 1990). These proteins are considered to be amino acid reserves designed to support embryo growth, their utilization being accomplished by the action of proteases (Hagedorn & Kunkel, 1979; Takahashi *et al*. 1993; Izumi *et al*. 1994). Different classes of enzymes have been implicated in yolk degradation, ranging from neutral serine proteinases (Ikeda, Sasaki & Yamashita, 1990) to acidic cysteine proteinases such as cathepsins B and L (Medina, León & Vallejo, 1988; Fagotto, 1990, 1991; Takahashi *et al*. 1993). In a single report, an aspartic proteinase has been suggested to play a role in yolk proteolysis (Nussenzveig, Oliveira & Masuda, 1992). During attempts to characterize the major protein components of the egg of the hard tick, *Boophilus microplus*, we purified a precursor of an aspartic proteinase, herein named BYC (Boophilus Yolk pro-Cathepsin).

Aspartic proteinases are synthesized as prepro-enzymes containing a signal sequence for targeting the nascent polypeptide to the endoplasmic reticulum (Marquardt *et al*. 1987) and a propeptide involved in the control of enzyme activity (Erickson, Conner & Blobel, 1981). Proteolytic removal of the propeptide by self-hydrolysis results in the activation of the enzyme (Richo & Conner, 1994).

In this article we describe the isolation and partial characterization of BYC, and provide evidence suggesting that this enzyme plays a role in tick embryo development.

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MATERIALS AND METHODS

Ticks

Ticks were obtained from a colony maintained at the Faculdade de Veterinária at the Universidade Federal do Rio Grande do Sul, Brazil. *Boophilus microplus* of the Porto Alegre strain, free of *Babesia* spp., were reared on calves obtained from a tick-free area. Engorged adult females were kept in Petri dishes at 28 °C and 80 % relative humidity until completion of oviposition.

Eggs and haemolymph

Eggs laid less than 24 h beforehand were collected and homogenized in a Potter–Elvehjem tissue grinder in 20 mM Tris–HCl buffer, pH 7·4 with 0·05 mg/ml soybean trypsin inhibitor, leupeptin and antipain, and 1 mM benzamidine (approximately 1 g of eggs/10 ml). Egg homogenate was centrifuged at 11 000 ***g*** for 10 min at 4 °C. The floating lipids and the pellet were discarded, and the supernatant was used for protein isolation.

Haemolymph (100–500 μl) was collected with a glass microcapillary pipette by cutting the cuticle and applying a gentle pressure to the tick abdomen. Haemolymph was mixed with an equal volume of the same buffer used for the eggs, containing the protease inhibitors, and centrifuged at 11 000 ***g*** for 5 min at room temperature. The cell pellet was discarded and the haemolymph was stored under liquid nitrogen until use.

Protein purification

Egg homogenate was applied to a column (10 × 3 cm) of DEAE–Toyopearl 650M equilibrated with 20 mM Tris–HCl, pH 8·4, and eluted with a NaCl gradient. One fraction containing BYC (identified by SDS–PAGE), concentrated in a Speed-Vac system (Savant SVC 100) was applied to a Sephacryl S-200 column (100 × 1·5 cm) equilibrated with 0·15 M NaCl, 10 mM Tris–HCl, pH 7·2.

Protein purity was monitored by SDS–PAGE (Laemmli, 1970) and Coomassie Blue staining of the gels. Molecular masses of polypeptides were determined using the following protein standards: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine albumin (67 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12·5 kDa). For blotting experiments a pre-stained mix of standards (Sigma Chemical Co., St Louis, MO) was used, containing α_2-macroglobulin (190 kDa), β-galactosidase (108 kDa), fructose-6-phosphate kinase (84 kDa), pyruvate kinase (67 kDa), fumarase (55 kDa), lactic dehydrogenase (39·5 kDa) and triosephosphate isomerase (35 kDa).

Amino acid sequences

BYC (200 pmol) was subjected twice to automatic Edman degradation using a liquid-phase sequencer (Porton PI 2020/2090). Phenylthiohydantoin amino acids were identified in a Hewlett Packard HPLC system (model 1090) with an amino-quant column (200 mm × 2·1 mm). Purified BYC (1 mg) was incubated with CNBr (1·5 mg in 600 μl of 70 % formic acid) for 18 h at room temperature. CNBr-treated samples were Speed-Vac dried, solubilized in 200 μl of sample buffer, subjected to SDS–PAGE (10–20 %; 40 μl/lane) and transferred to a polyvinylidene difluoride (PVDF) membrane (Matsudaira, 1987). After staining with Coomassie Blue for 5 min the chosen polypeptide bands were cut and applied to the protein sequencer.

Sequence analysis was carried out with programs from the GCG Software Package, Version 8.0, Wisconsin University. The softwares used were FASTA for homology searches, PILEUP for sequence alignments and BESTFIT for determination of similarity percentages.

Polyclonal antibodies

Rabbits were inoculated subcutaneously with BYC (0·25 mg in 0·25 ml of NaCl 0·15 M) emulsified with an equal volume of Freund's complete adjuvant. After 1 month, animals were boosted with 0·25 mg of BYC in 0·15 M NaCl. Sera were prepared from blood collected at least 1 month after the second injection.

Monoclonal antibody (*mAb*)

BALB/c mice were inoculated 3 times at 10-day intervals by the intraperitoneal route with 100 μg of BYC in 0·2 ml of 10 mM sodium phosphate buffer, pH 7·4, with 0·15 M sodium chloride (PBS). In the first inoculum the antigen was emulsified in 0·2 ml of Freund's complete adjuvant and in the 2 boosters, immunizations were performed using BYC emulsified in 0·2 ml of incomplete Freund's adjuvant. Three days before fusion mice received an intrasplenic booster with 50 μg of BYC in PBS.

Spleen cells were fused to SP2/o myeloma cells with polyethylene glycol (Köhler & Milstein, 1975). Hybridoma culture supernatants were screened by ELISA for antibodies against BYC (Harlow & Lane, 1988). Cloned hybridoma cells secreting mAbs were inoculated into BALB/c mice previously injected with Pristane to induce ascites formation (Harlow & Lane, 1988). The ascites were dialysed with 20 mM sodium phosphate buffer, pH 8·4, and applied to a protein G-Sepharose column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with the same buffer. All subsequent experiments were performed with the mAbs eluted from this column.

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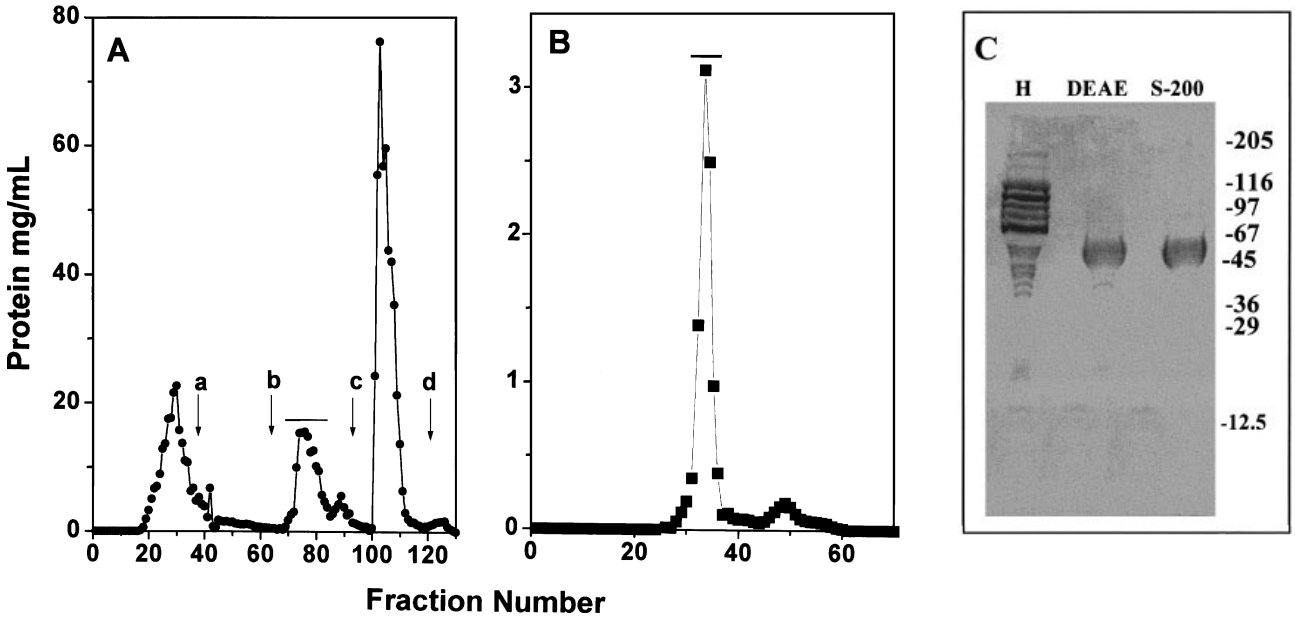

Fig. 1. Isolation of BYC from crude egg homogenate. (A) DEAE–Toyopearl – the sample was applied to the column equilibrated with 10 mM Tris–HCl buffer, pH 8·4. A NaCl stepwise gradient was applied as indicated: (a) 0–75 mM; (b) 75–150 mM; (c) 150–400 mM; (d) 1 M. (B) Sephacryl S-200 – the column was equilibrated with NaCl 0·15 M; 10 mM Tris–HCl, pH 7·4. Fractions containing BYC are indicated with a bar. (C) Summary of BYC isolation by SDS–PAGE (10 %). H, egg homogenate; DEAE, after DEAE–Toyopearl; S-200, after Sephacryl S-200 column. Numbers at right are molecular masses of protein standards.

Western blot

For Western blot analysis, samples were first separated by SDS–PAGE (10 %) and then transferred to nitrocellulose (Towbin, Staehelin & Gordon, 1979). Nitrocellulose sheets were then blocked by incubation with 5 % (w/v) non-fat dry milk, 0·5 % (v/v) Tween 20 in PBS, and incubated with mAbs (1 mg/ml) and 5 % non-fat dry milk in PBS. Goat anti-mouse IgG conjugated to alkaline phosphatase diluted 1:10000 (v/v) was used as secondary antibody and developed with nitroblue tetrazolium (NBT) 0·033 % (w/v) and bromochloroindolyl phosphate (BCIP) 0·016 % (w/v).

Proteolytic activity

BYC (1·5 mg/ml) proteolytic activity was assayed in 0·1 M glycine–HCl buffer, pH 3·5, at 37 °C for 6 h. Bovine haemoglobin (to 1·5 mg/ml) and pepstatin (to 10^{-4} M) were added when indicated. Reactions were stopped by addition of 200 μl of SDS–PAGE sample buffer to 400 μl of the reaction mixtures and heating at 100 °C for 3 min. Aliquots of 30 μl were analysed by SDS–PAGE (20 %).

In vitro *protein synthesis*

Gut, fat, body, ovary and malpighian tubules were dissected and incubated in 100 μl of Dulbecco's modified medium containing [^{35}S]methionine (0·1 μCi/μl) for 2 h at 28 °C and ambient atmosphere. Tissues were homogenized in a tissue grinder, centrifuged at 11000 ***g*** for 10 min, and the supernatant fractions were added to 50 μl of *Staphylococcus aureus* protein A (Sigma Chemical Co., St Louis, MO), incubated at room temperature for 15 min and centrifuged again in a microcentrifuge (2 min). The pellets were discarded and 5 μl of rabbit polyclonal antiserum against BYC were added to the supernatants. After 1 h (37 °C), 50 μl of the protein A suspension were added, mixtures were kept for 15 min at room temperature and centrifuged for 2 min (Faria, Garcia & Goldenberg, 1993). Immunoprecipitates were washed 4 times with 800 μl of 10 mM Tris–HCl, pH 8·0, 0·15 M NaCl, 0·1 % (v/v) NP-40 and resuspended in 40 μl of SDS–PAGE sample buffer (Contreras, Morel & Goldenberg, 1985). The immunoprecipitates and total crude organ extracts were analysed by SDS–PAGE (10 %) and fluorography (Laskey & Mills, 1975).

RESULTS

BYC purification was achieved by anion-exchange chromatography on DEAE–Toyopearl (Fig. 1A), followed by gel filtration in a Sephacryl S-200 column (Fig. 1B). In this last column the protein was obtained in an elution volume that indicated an apparent molecular mass of 54 kDa (not shown). In SDS–PAGE using heavily loaded gels (30 μg protein/lane) BYC appeared as a single band (Fig. 1C). However, in gels with smaller amounts of protein, 2

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                    5         10        15
NH2-terminal:  K I R I P L R K D R I I M X N L F H

                5         10        15        20        25
Peptide 1: F I K M L I P E P V F A F Y F M P T V V G S D D E

      30
L I L G G I E K G

                5         10        15        20        25
Peptide 2: R W L Q T E Y I Y A K P V S T L P Y I Y G P Q S Q I D
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Fig. 2. BYC protein sequences. The NH_2-terminal and internal sequences of BYC were determined by automated Edman degradation. Peptides 1 (36 kDa) and 2 (23 kDa) were obtained after cleavage with cyanogen bromide, followed by SDS–PAGE (10–20 %) and blotting to a PVDF membrane.

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                                                PS   PI
(PEPSIN F)      LVTIPIMKVKSMRENLRE              60   33
(RENINMOUSE)    FERIPIKKMPSVREILEE              57   33
(CATDRAT)       LIRIPLRKFTSIRRTMTE              53   57
(CATE)          LHRVPISRRESLRKKLRA              50   29
(N-TERMINAL)    KIRIPIRKDRIIMXNLFH
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                                                                PS   PI
(PEPSIN F)      WAQGLISQNLFAFYLS.SKEERGSNMLMLGGVDPS             59   26
(RENINMOUSE)    LSQGVLKEEVFSVYYNRGSH.LLGGEVVLGGSDQP             50   26
(CATDRAT)       MKQKLVEKNIFSFYLNRDPTGQPGGELMLGGTDSR             55   29
(CATE)          MAQNLVALPMFSVYMSSNPGG.SGSELTFGGYDPS             56   29
(peptide 1)     FIKMLLPEPVFAFYFMPTVVG.SDDELILGGIEKG
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Fig. 3. Homology of NH_2-terminal and polypeptide 1 amino acid sequences to aspartic proteases precursors. The sequences of NH_2-terminal portion of BYC and peptide 1 were compared to rabbit pro-pepsin F (PEPSIN F; Kageyama, Tanabe & Koiwai, 1990), mouse pro-renin (RENINMOUSE; Panthier *et al.* 1982), rat pro-cathepsin D (CATDRAT; Yonezaga *et al.* 1988) and guinea-pig pro-cathepsin E (CATE, Kageyama *et al.* 1992) using a GCG software package version 8.0, Wisconsin University. Boxes indicate a conserved site of proteolytic cleavage related to aspartic proteinase maturation. PS, similarity percentage; PI, identity percentage.

bands of 54 and 49 kDa could be distinguished. Using higher resolution SDS–PAGE conditions (8 % polyacrylamide, 10 μg of protein/lane and overrunning the gel until BYC was close to the end of the gel) these polypeptides were clearly separated and blotted to a PVDF membrane. The same NH_2-terminal sequence was obtained for the first 10 amino acid residues for both polypeptides (not shown), indicating that they are isoforms of the same protein.

BYC NH_2-terminal sequence as determined by Edman degradation is shown in Fig. 2. Sequences of 2 internal fragments obtained by cleavage with cyanogen bromide are also shown. The NH_2-

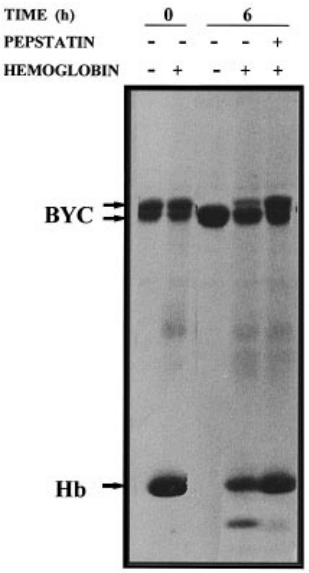

Fig. 4. BYC proteolytic activity. BYC (1·5 mg/ml) was incubated in 0·1 M glycine–HCl buffer, pH 3·5, at 37 °C for 6 h in the presence of haemoglobin (1·5 mg/ml) or haemoglobin (1·5 mg/ml) + pepstatin (10^{-4} M). BYC and haemoglobin polypeptides are indicated at the side of the figure.

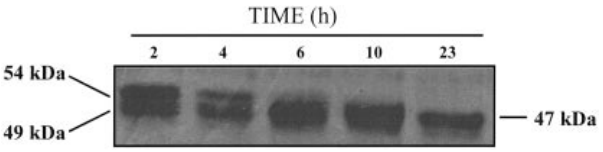

Fig. 5. BYC autoproteolysis. Purified BYC was incubated in 0·1 M glycine–HCl buffer, pH 3·5, at 37 °C for the times indicated in the figure. The molecular masses of the polypeptides are indicated.

terminal portion and polypeptide 1 showed significant homology with aspartic proteinase precursors (60–50 % and 59–50 % of similarity for the terminal portion and peptide 1, respectively) (Fig. 3). In polypeptide 1 a sequence for a conserved site for proteolytic cleavage in this class of enzymes was identified. In the NH_2-terminal sequence a conserved site for signal peptide cleavage was found. No significant homologies were found for peptide 2. However, definitive identification of BYC as a proteinase precursor was obtained by observation of its proteolytic activity. Purified BYC displayed hydrolytic activity against bovine haemoglobin at pH 3·5 (Fig. 4). Following incubation at this pH, BYC polypeptides were converted to a slightly lower molecular mass form of 47 kDa (Fig. 5). BYC self-hydrolysis was slower in the presence of haemoglobin, probably due to competition between BYC and haemoglobin for binding to the active site of newly matured enzyme molecules. When pepstatin A, a specific inhibitor of aspartic proteases, was added to the incubation mixture, neither BYC activation nor haemoglobin hydrolysis were observed

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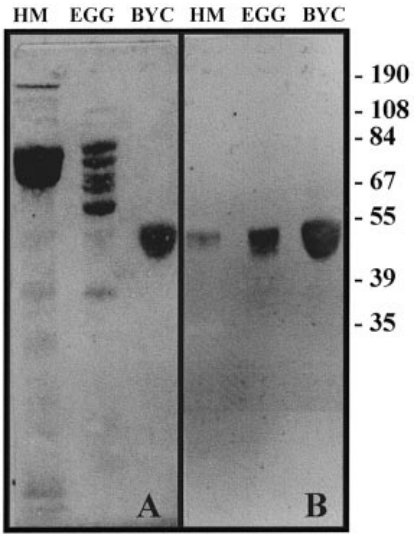

Fig. 6. Western blot of haemolymph and eggs. Samples were run on a 10 % SDS–PAGE gel and transferred to a nitrocellulose membrane. Anti-BYC monoclonal antibody was used for Western blot analysis. (A) Membrane stained with Ponceau Red; (B) membrane probed with anti-BYC mAb. HM, haemolymph; EGG, egg homogenate; BYC, purified BYC. Numbers at right are molecular masses of protein standards.

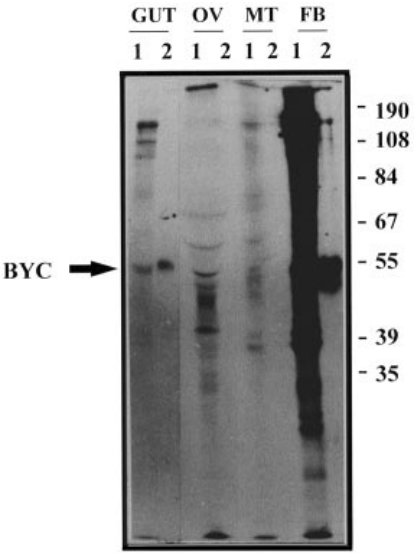

Fig. 7. Synthesis of BYC by the gut and fat body. Tissues from fully engorged females that had fallen from cattle 4 days beforehand were dissected and incubated in culture media containing [^{35}S]methionine for 2 h. Shown are GUT (gut), OV (ovary), MT (malphigian tubules), and FB (fat body). Total organ homogenates (1) and immunoprecipitates (2) were analysed by SDS–PAGE (10 %) and fluorography. Numbers at right are molecular masses of protein standards.

(Fig. 4), confirming that active BYC is an enzyme of this type.

Western blot analysis using monoclonal antibodies against BYC showed that BYC is present not only in the eggs, but also in the haemolymph of engorged

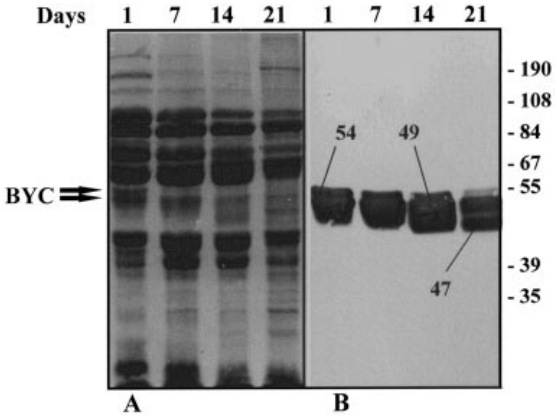

Fig. 8. BYC proteolytic processing during embryogenesis. Newly laid *Boophilus microplus* eggs were maintained at 80 % relative humidity at 27 °C. After oviposition, at the days indicated in the figure, eggs (days 1, 7 and 14) or larvae (day 21) were homogenized in the same mixture of protease inhibitors used in the other experiments (with pepstatin 10^{-4} M added). Samples were analysed by SDS–PAGE (10 %) and Western blot. (A) Coomassie Blue-stained gel; (B) blotted membrane probed with anti-BYC mAb. Numbers at right are molecular masses of protein standards.

females (Fig. 6). In order to identify the site of BYC synthesis, tick organs were dissected and incubated in [^{35}S]methionine culture medium, and the fat body and gut showed radioactivity incorporation into BYC polypeptides (Fig. 7).

BYC activation *in vitro* proceeded through the formation of a 47 kDa polypeptide (Fig. 5). To test whether the same phenomenon occurred *in vivo*, the time-course of BYC cleavage was followed during embryonic development by means of Western blot analysis with anti-BYC monoclonal antibody. Egg development took about 21 days, and limited proteolysis of BYC was already observed in the 7th day after oviposition (Fig. 8). BYC was continuously processed by partial hydrolysis during embryogenesis and when the first instar larvae ecloded at the 21st day, the 54 kDa polypeptide was barely visible (Fig. 8B). When a gel containing the same samples was stained with Coomassie Blue, the total protein profile showed that some major bands continuously decreased during embryogenesis (Fig. 8A). In order to investigate BYC participation in yolk degradation, newly laid eggs were homogenized and incubated at pH 3·5. As can be seen in Fig. 9, several of the major polypeptides present in the tick egg homogenate were totally hydrolysed after a 24 h incubation period and degradation was completely inhibited by addition of pepstatin A.

DISCUSSION

In ticks, VT, the main yolk protein and its precursor vitellogenin (VTG) from different species have been

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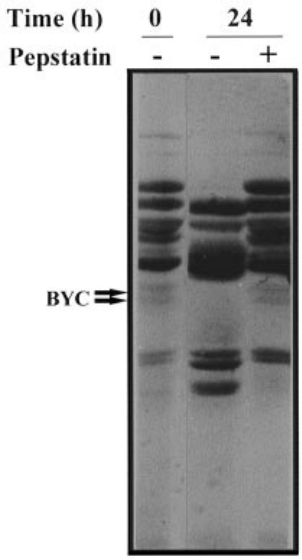

Fig. 9. Proteolytic activity in egg homogenate. Egg homogenate (1 mg/ml) was incubated for 24 h in 0·2 M glycine–HCl, pH 3·5, with or without pepstatin A (10^{-4} M). Samples (30 μg protein) were analysed by SDS–PAGE (10 %).

isolated and characterized (Boctor & Kamel, 1976; Chinzei, Chino & Takahashi, 1983; Rosell & Coons, 1991). Yolk protein degradation during embryogenesis in this group of animals has been attributed to a cathepsin L-like proteinase which is supposed to be stored in yolk platelets as a proenzyme which is proteolytically activated after lowering of platelet pH during development (Fagotto, 1990). A very similar pattern has been found in insects, a closely related group. Both in *Bombyx mori* and *Musca domestica*, cystine proteinase precursors from eggs are converted to active enzymes by autoproteolysis at acid pH (Takahashi *et al*. 1993; Ribolla & Bianchi, 1995). Two trypsin-like serine proteinases, named egg-specific protein protease (Indrasith, Sasaki & Yamashita, 1988) and VT-specific protease (Ikeda *et al*. 1990), have also been implicated in the digestion of yolk proteins in *B. mori*. In the case of the yellow fever mosquito, *Aedes aegypti* (Cho, Deitsch & Raikhel, 1991), a serine carboxypeptidase is activated in eggs at the onset of embryo development in parallel with the conversion of a 53 kDa precursor to a 48 kDa active enzyme. Here we report the isolation and characterization of BYC, an aspartic proteinase precursor from eggs of the cattle tick, *Boophilus microplus*. To our knowledge, this is the first characterization of an aspartic proteinase involved in yolk degradation in arthropods.

Two out of three BYC amino acid sequences were homologous to other aspartic proteinase precursors (60 % for the NH_2-terminal portion and 59 % for peptide 1). The amino acid composition of BYC was also very similar to other pro-enzymes of this class (data not shown). These observations, together with BYC self-hydrolysis suggested that BYC is a latent proteinase precursor, the maturation of the active enzyme being accomplished by autoproteolytic activation, a pattern that is common to other aspartic proteinases (Hasilik, 1992). As already mentioned in the Results section, the 2 bands (54 and 49 kDa) found in purified BYC are probably isoforms of the same enzyme precursor, similar to the structural microheterogeneity described for porcine cathepsin D (Takayuki & Tang, 1981) and for bovine pepsinogen (Meitner & Kassel, 1971), where the presence of several isoforms has been attributed to small differences in amino acid sequence or in protein phosphorylation.

A particularly remarkable degree of homology between BYC and mammalian pro-enzymes was observed in 2 sequences of possible functional significance. This was the case for the signal peptide cleavage sequence found in the NH_2-terminal portion (R-I-P-L) (Hasilik, 1992) and for 1 sequence (L-G-G) found in peptide 1 enclosing a proteolytic cleavage site involved in maturation of other aspartic proteinases. This site is responsible for the generation of the predominant form of the mammalian mature cathepsin D that presents 2 polypeptide chains (35 and 15 kDa) (Huang, Huang & Tang, 1979). Despite the presence of this sequence in BYC peptide 1, a 30 kDa polypeptide was not observed either *in vivo* during embryogenesis or *in vitro* after incubations at pH 3·5. On the other hand, the generation of a 47 kDa polypeptide is very close the pattern expected after removal of a propeptide sequence by autocatalysis, as has been found in other aspartic proteases (Hasilik, 1992). This 47 kDa form is enzymatically active *in vitro*, and its presence *in vivo* indicates that BYC activation is likely to occur during embryogenesis as part of the developmentally programmed degradation of yolk proteins. Incubation of egg homogenate at pH 3·5 for 24 h also resulted in selective degradation of several major polypeptides of the egg, which are also degraded during embryogenesis *in vivo*. Most of these polypeptides in fact belong to the *Boophilus* main yolk protein, VT, which has been isolated and characterized in our laboratory (data not shown). VTs comprise between 80 and 90 % of the total protein content of eggs of ticks and most insects (Boctor & Kamel, 1976; Hagedorn & Kunkel, 1979; Chinzei *et al*. 1983; Rosell & Coons, 1991). Tick VTs that have been described are very similar to each other, with 6–9 apoproteins ranging from 35 to 160 kDa (Rosell & Coons, 1991). Furthermore, cattle vaccinated with BYC showed an immune response that was partially protective against tick infestation (manuscript in preparation). Consistent with the role for BYC in yolk degradation proposed here, the protective effect of cattle immunization was mostly due to an increase in the number of sterile eggs.

A great diversity concerning the site of synthesis of proteinases that degrade yolk is found when

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different enzymes and animal species are compared. The serine (trypsin-like) proteinases from *B. mori* (ESP-protease and VT-specific protease) are synthesized by the eggs during the course of embryo growth (Indrasith *et al*. 1988). Cysteine proteinases from *B. mori* are synthesized during vitellogenesis by the ovarian follicle cells and accumulated by the oocytes (Yamamoto *et al*. 1994). The *A. aegypti* carboxypeptidase is synthesized by the fat body, secreted to the haemolymph and also specifically accumulated by the oocytes (Cho *et al*. 1991). The presence of BYC in the haemolymph could be explained by synthesis in the ovary, followed by secretion to the haemolymph or, alternatively, by an extraovarian site of synthesis and incorporation by vitellogenic oocytes. Incubation of several insect organs in culture medium with [^{35}S]methionine showed BYC synthesis in the gut and fat body. Interestingly, VT synthesis has also been ascribed to these organs in other ticks (Chinzei & Yano, 1985; Rosell & Coons, 1992). Taken together, these data suggest that BYC may be specifically taken up by the growing oocytes, by means of an endocytic pathway that may be shared with VT. Investigation of this possibility by immunocytochemical techniques is under way in our laboratory.

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