Persian J. Acarol., 2025, Vol. 14, No.2, pp. 209–223.
https://doi.org/10.22073/pja.v14i2.85470
Journal homepage: http://www.biotaxa.org/pja

Article

Cathepsin D-like enzyme from embryo of camel tick *Hyalomma dromedarii* (Acari: Ixodida)

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ABSTRACT
It is well known that cathepsin D (CD) has a fundamental role in the degradation of yolk proteins during embryo development as it has a dual function in embryo feeding and defending against pathogens. Cathepsin D-like activity is revealed during embryogenesis of *Hyalomma dromedarii* (Acari: Ixodida) tick and attained the highest activity in 3–4 days-old embryos during the cleavage stage and nominated camel tick embryo cathepsin D (CTECD). After purification by cationic CM-cellulose gel filtration Sephacryl S-300 columns, CTECD turned out to be homogeneous with specific activity of 40 U mg^{-1} proteins, 7.4 purification factor and 55.3% yield. CTECD molecular mass was deduced from the size exclusion column to be almost 40 kDa. CTECD hydrolyzed the specific synthetic substrate Bz-Arg-Gly-Phe-Phe-Pro-4-methoxy-β-naphthylamide confirming that the enzyme is cathepsin D. Both Zn^{2+} and Pepstatin A were potent inhibitors of CTECD activity. Also, CTECD displayed its maximum activity at pH 2.5 with a pH profile typical for cathepsin D. This study suggests the involvement of CTECD in yolk proteins utilization by the developing embryos as it attained its highest activity in cell cleavage stage. The study of biochemical changes during tick embryogenesis will be helpful in understanding the tick cells adaptability for different pathogens.

KEYWORDS: Characterization, embryogenesis, CTECD, purification, yolk proteins.

PAPER INFO.: Received: 09.04.2024, Accepted by: *A. Hossini-Chegeni*, 15.12.2024, Published: 15.04.2025

INTRODUCTION

Ticks are hematophagous species that are distributed everywhere in the world acting as particular vectors of numerous pathogens and are usually treated as the most important transmitter of diseases to animals (Mencke 2013). Moreover, ticks have great burdens on livestock that cause huge economic losses on milk, leather and meat production. The use of acaricides is the most widespread method for tick control which has many disadvantages such as tick resistance to chemicals, remnants in livestock industries and environmental pollution. Therefore, alternative tick control methods have evolved such as tick vaccines (Saidi *et al*. 2016). Vaccines usage is a promising procedure for tick control (Ali *et*

How to cite: Helmy, M., Masoud, H.M.M., Darwish, D.A., Abdel-Monsef, M.M. & Ibrahim, M.A. (2025) Cathepsin D-like enzyme from embryo of camel tick *Hyalomma dromedarii* (Acari: Ixodida). *Persian Journal of Acarology*, 14(2): 209–223.

al. 2020). Yolk cathepsin of *Boophilus microplus* (Canestrini) is an aspartic proteinase present in eggs that participates in *B. microplus* embryogenesis and was suggested as a significant antigen in developing anti-tick vaccines (Leal *et al*. 2006). *Hyalomma dromedarii* Koch (Acari: Ixodidae) is the species infecting camels in Egypt (El Kammah *et al*. 2001; Alsarraf *et al*. 2017). Ticks overcome blood coagulation of the host through improvement of their haemostatic mechanisms to prolong the feeding periods during which the pathogen transmission occurs. Ticks digest blood slowly by intracellular processes compared to protein digestion in blood sucking arthropods (Mulenga and Erikson 2011). Many tick species like Argasidae and Ixodidae digest blood in their midgut epithelial cells (Reyes *et al*. 2020). Oviparous animal's embryos need to overcome the early development stages in absence of external nutrition. In this state, storage of maternal nutrients is carried out in the eggs in the form of yolk granules (Martins *et al*. 2018). The large lysosomal-like organelles are filled with yolk phospholipo-glycoproteins that are originated from larger precursor, named Vitellogenin (VTG). Activation of lysosomal-like hydrolases that exist in yolk granules of invertebrate eggs happens at start of embryogenesis, leading to restricted proteolytic cleavage of yolk proteins (Logullo *et al*. 2002). In oviparous species, the yolk degradation procedure is always accompanied with cysteine and aspartic endopeptidases (Bergamo *et al*. 2010; Bencosme-Cuevas *et al*. 2023). The formation of yolk in the ovary needs uptaking of the precursor proteins from the hemolymph such as Vitellogenins. VTG is produced in the fat body (Seixas *et al*. 2010), which then in the egg turns into vitellins (VTs); large molecular weight proteins consist of various subunits (Canal *et al*. 1995). Tick proteinases maximum activity is around pH 3.0 (Reich and Zorzópulos 1978). Cathepsin D is an intracellular aspartic protease formed in rough endoplasmic reticulum with a signal peptide, a pro-peptide and a mature peptide (Zaidi *et al*. 2008). After signal peptide removal, cathepsin D with the mature peptide reaches its intracellular vesicular structures (endosomes, lysosomes, and phagosomes), where endopeptidases activate it (Laurent-Matha *et al*. 2006; Calvo *et al*. 2008; Horn *et al*. 2009). Various researches have studied cathepsin D of blood sucking parasites which prove its relation to the blood digestion procedure because of its capability of haemoglobin degradation (Brinkworth *et al*. 2001). Cathepsin D-like peptidases of parasites are engaged in several adaptive missions implicate larval migration, tissue penetration, molting, immune evasion, coagulation, host blood proteins digestion, and cellular matrix degradation (Sajid and McKerrow 2002). Cathepsin D was identified in the gut of *Ixodes ricinus* (L.) ticks (Sojka *et al*. 2012) and the small intestine of *Triatoma infestans* Klug bug (Balczun *et al*. 2012). During pathogenic microorganisms infection, cathepsin D expression level was elevated in different tissues like grass carp, half-smooth tongue sole, channel catfish, hard tick and triatomine (Boldbaatar *et al*. 2006; Borges *et al*. 2006; Jeffers and Roe 2008; Horn *et al*. 2009; Franta *et al*. 2010; Chen *et al*. 2011; Feng *et al*. 2011; Dong *et al*. 2012; Mu *et al*. 2013). Cathepsin D participates also in embryo development and tissue invasion mechanisms (Follo *et al*. 2013). Various cathepsin D homologues have been isolated and characterized in bugs, hook worms, fishes, mites, schistosomes, nematodes and ticks' tissues (Verity *et al*. 1999; Williamson *et al*. 2002, 2003; Fragoso *et al*. 2009; Chen *et al*. 2011; Feng *et al*. 2011; Balczun *et al*. 2012; Bartley *et al*. 2012; Dong *et al*. 2012; Sojka *et al*. 2012; Mu *et al*. 2013). The proteolytic activity should be involved for yolk proteins degradations to provide the tick embryos with the necessary nutrients and for the synthesis of new proteins required for organogenesis. Therefore, the purpose of this study is to purify and characterize cathepsin D-like activity from *H. dromedarii* embryo for investigating its role during early steps of development.

MATERIALS AND METHODS

Tick embryos

Engorged *H. dromedarii* females were obtained from a market for camels near Giza and preserved at 85% relative humidity and 28 ℃. Collection of embryos were done daily from fertilized ovipositing ticks and either frozen directly (zero-day age) or incubated under the same condition until

a particular age and stored at (−20 ℃) at three days intervals (3, 6, 9, 12, 15, 18, 21, 24) until hatching of larvae at 27th day.

Chemicals

Haemoglobin, Casein, albumin, phenyl methyl sulphonyl fluoride (PMSF), carboxy-methyl-cellulose (CM-Cellulose), Sephacryl S-300, bestatin, soyabean trypsin inhibitor, Pepstatin A, gel filtration calibration Kit and molecular weight standard markers were purchased from Sigma Chemical Co. All other used chemicals were of analytical grade.

Protein determination

Protein was detected by Bradford method using (BSA) as a standard protein (Bradford 1976).

Assay of cathepsin D

Using hemoglobin as substrate

The acid denatured hemoglobin solution (2% w/v) was prepared by dissolving 2 g bovine hemoglobin in 100 ml 0.06 N HCl then centrifuged at 5000× g for 10 min at 4 ℃ and supernatant was kept frozen until use (Herriott 1955). Assay reaction mixture contained 1 ml volume; 0.1 M citrate phosphate pH 3.0 containing 5 mg hemoglobin and suitable dilution of enzyme then incubated at 37 ℃ for four hours and stopped by adding of 1 ml 10% trichloroacetic acid (TCA) then centrifuged at 5000× g for 15 min to remove the precipitate and supernatant was read spectrophotometrically at 280 nm. Control reaction mixture without enzyme was incubated under the same conditions. One cathepsin D-like activity unit is defined as the enzyme quantity needed to liberate 1 µg of small peptides from the substrate in 1 h (Barrett 1977).

Using Bz-Arg-Gly-Phe-Phe-Pro-4-methoxy-β-naphthylamine as substrate

Two ml reaction mixture of 75 mM citrate phosphate buffer pH 3, containing 1 mM EDTA, 1 mM DTE, 50 µl of 20 mM substrate stock solution and 500 µl of enzyme solution were incubated at 37 ℃ for 1 h. The liberated 4-methoxy-β-naphthylamine was conjugated with 500 µl Fast Garnet GBC (1 mg/ml in 0.1 M acetate buffer pH 4.2 containing 10% Tween 20). After 10 min., the coupled product was extracted in 4 ml n-butanol and color absorbance was recorded at 520 nm. One unit of enzyme activity was defined as the amount of enzyme that liberates 1 µmol 4-methoxy-β-naphthyl-amine per min at 37 ℃ (Barrett 1977).

Hyalomma dromedarii *cathepsin D-like activity purification*

All purification procedures were carried out at 4 ℃. Three grams of *H. dromedarii* eggs were homogenized in 15 ml 0.02 M citrate-phosphate buffer, pH 3.0 by a Teflon-pestle homogenizer then centrifuged using Sigma 1–16 K high speed refrigerated centrifuge for 20 min. at 10000× g to eliminate cell debris and insoluble materials. Clear supernatant was kept at −20 ℃ as crude extract.

CM-cellulose chromatography

Hyalomma dromedarii eggs crude extract (3–4 days-old) was applied on a CM-cellulose column (24 cm × 2.5 cm) previously equilibrated with 0.02 M citrate-phosphate pH 3.0. Proteins were resolved utilizing NaCl stepwise gradient (0.0 to 1 M) dissolved in the equilibration buffer. The column flowing rate was set at 60 ml/h and 5 ml fractions were collected and those containing cathepsin D activity were combined, freeze dried using Christ 1–4 LSC Plus instrument under 0.1 mbar vacuum and at (−55 ℃) for 12 h then dissolved in 3 ml of equilibration buffer.

Gel filtration chromatography

The lyophilized fraction holding the cathepsin D activity was fractionated through a Sephacryl

S-300 column (150 cm × 1.75 cm) previously equilibrated and eluted with 0.02 M citrate-phosphate pH 3.0 at 30 ml/h flowing rate. This size exclusion column was standardized with ferritin, catalase, alcohol dehydrogenase, bovine serum albumin and myoglobin as standard calibration markers for molecular mass locating of *H. dromedarii* embryo cathepsin D (Andrews 1964).

Electrophoretic analysis

7% Native PAGE was carried out at 150 V for 2 h and 0.25% Coomassie Brilliant Blue R-250 was used for staining of proteins (Smith 1969).

Optimum pH of cathepsin D activity

The effect of pH on the purified CTECD enzyme was tested on hemoglobin as a substrate using 0.1 M citrate-phosphate buffer, pH (1.0–6.0) at standard assay conditions.

Effect of metal ions and inhibitor on cathepsin D activity

Effect of ions and inhibitors on purified CTECD activity was achieved by incubating the purified enzyme with 5 mM with each of the (Co^{2+}, Zn^{2}+, Cu^{2+}, Mg^{2+}, Ni^{2+}, Ca^{2+}, Mn^{2+}, Bestatin HCI, β-Mercaptoethanol and Iodoacetic acid), 2 mM Phenylmethylsulfonylfluoride (PMSF), 2 µM Pepstatin A and 5 µg Soya bean trypsin inhibitor at 37 ℃ for 15 min before activity assay. Then, CTECD activity test was evaluated in comparison with a control test without any compound.

Statistical analysis

The commonly used index of the variation is the standard error of the mean (S.E.) (Bailey 1997). The standard deviation (SD) is the geometric mean of the deviation from the mean and n is the number of individuals.

RESULTS

Influence of pH on proteolytic activity of crude H. dromedarii *using acid denatured haemoglobin as substrate*

Effect of pH on proteolytic activity of crude *H. dromedarii* embryos homogenate (6 and 18 days after oviposition) was examined between pH 1.9 and 6.5 using 0.1 M citrate-phosphate buffer. The pH profile for proteolytic activity of *H. dromedarii* embryo homogenate of age 6 fell sharply in acid side with maximum activity at pH 2.5 (Fig. 1a) while age 18 embryo homogenate exhibited optimum pH at 3.5 (Fig. 1b).

Cathepsin D specific activity developmental profile during H. dromedarii *embryonic development*

The cathepsin D activity wasestimated during embryogenesis of *H. dromedarii* arising a developmental profile utilizing 0.1 M citrate-phosphate buffer, pH 2.5 (Fig. 2). The developmental profile revealed that cathepsin D displayed the highest specific activity (7.3 ± 0.184 U/mg protein) at the 3–4 days-old embryo.

Purification of cathepsin D-like activity from H. dromedarii *3–4 days-old embryos*

The cathepsin D purification process is shown in Table 1 and composed of two main chromatographic steps using cation exchange on CM-cellulose (Fig. 3a) and gel filtration on Sephacryl S-300 (Fig. 3b). The CD starting specific activity of the 3–4 days-old embryo crude extract was 5.4 U/mg protein. One major CD activity peak was obtained from the CM-cellulose column using 0.02 M citrate phosphate buffer with specific activity 13.8 U/mg proteins. For more purification, the major CD activity exhibited fractions were collected, lyophilized and applied on a Sephacryl S-300 resin. The elution profile from this column displayed one CD activity peak that designated camel tick embryo cathepsin D (CTECD) and its apparent native molecular weight is obtained to be 40 kDa.

The gel filtration chromatography elevated the specific activity of CTECD to 40 U/mg proteins which represent 7.4 folds over the crude extract with 55.3% recovery.

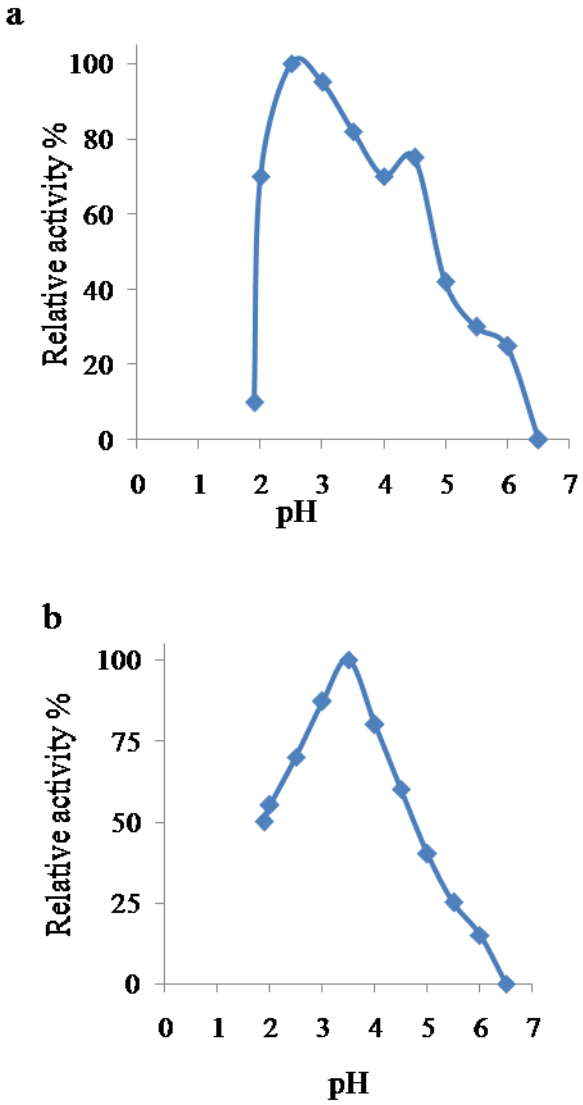

Figure 1. **a.** Effect of pH on proteolytic activity of 6–7 days-old embryo crude extract using acid denatured hemoglobin as substrate using 0.1 M citrate phosphate buffer, pH (1.0–6.0); **b.** Effect of pH on proteolytic activity of 18–19 days-old embryo crude extract using acid denatured hemoglobin as substrate using 0.1 M citrate phosphate buffer, pH (1.0–6.0).

Electrophoretic analyses

Protein aliquots from all CTECD purification steps were analyzed electrophoretically, for protein patterns on 7% native PAGE, the purity of CTECD enzyme was examined and appeared as one major protein band demonstrating its homogeneity (Fig. 4a).

Substrate specificity

Substrate specificity of CTECD purified from *H. dromedarii* 3–4 days-old embryo was tested utilizing Bz-Arg-Gly-Phe-Phe-Pro-4-methoxy-β-naphthylamine, hemoglobin, casein and albumin as

substrates (Table 2).

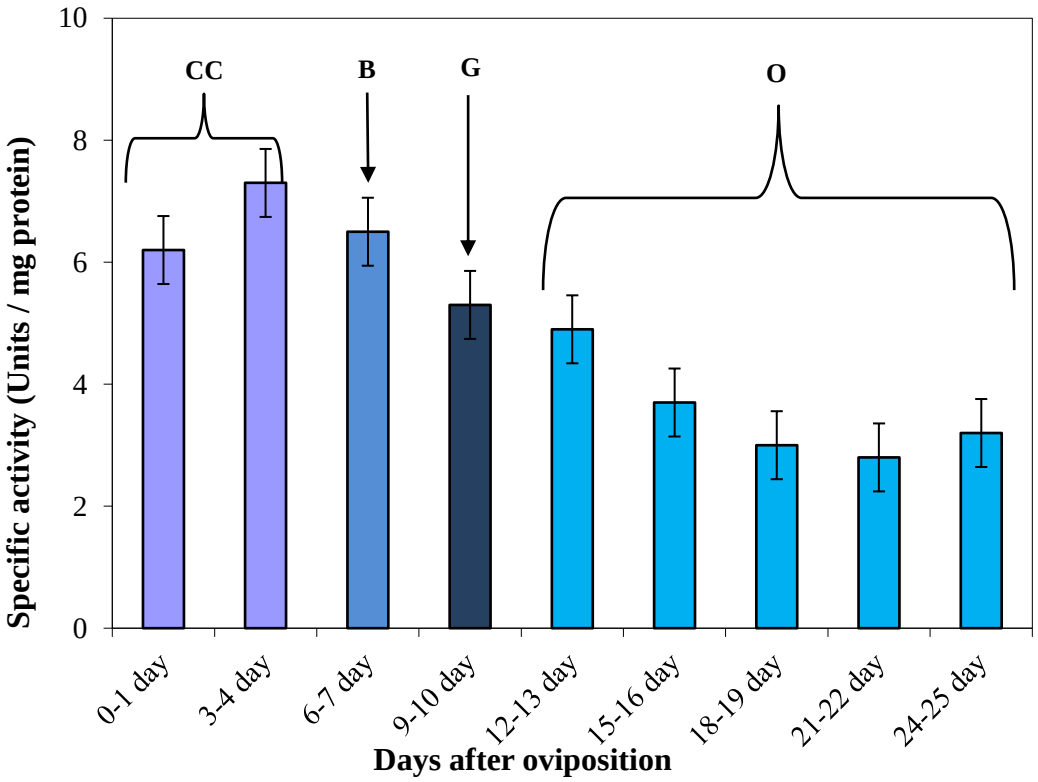

Figure 2. Developmental profile of cathepsin D specific activity (Units/mg protein) during embryogenesis of camel tick *H. dromedarii*. Each point represents the mean of at least 4 runs ± S.E. CC: Cell Cleavage; B: Blastula formation; G: Gastrulation; O: Organogenesis.

Table 1. Purification scheme for *H. dromedarii* 3–4 days-old embryo CD.

Purification steps	Total protein (mg)	Total units	Recovery (%)	Specific activity	Fold purification
Crude extract	40	217	100.0	5.4	1.0
CM-cellulose fractions					
0.0 M NaCl	13	180	82.95	13.8	2.5
Sephacryl S-300					
0.0 M NaCl	3	120	55.3	40	7.4

Optimum pH for cathepsin D activity

The purified CTECD activity was tested on hemoglobin using 0.1 M citrate-phosphate buffer, pH (ranges from 1 to 6) that reached its optimum activity at pH 2.5 (Fig. 4b).

Effect of metal ions and inhibitor on cathepsin D activity

The activity of purified CTECD in the presence of 5 mM of different cations is summarized in Table 3. A control without any cation was considered as 100% relative activity. The CTECD activity was completely inhibited by the metal ion Zn^{2+}. Effect of inhibitors on purified CTECD exists in Table 4, which showed that the most potent inhibitor of CTECD was found to be Pepstatin A as concentration of 2 µM resulted in 97% inhibition.

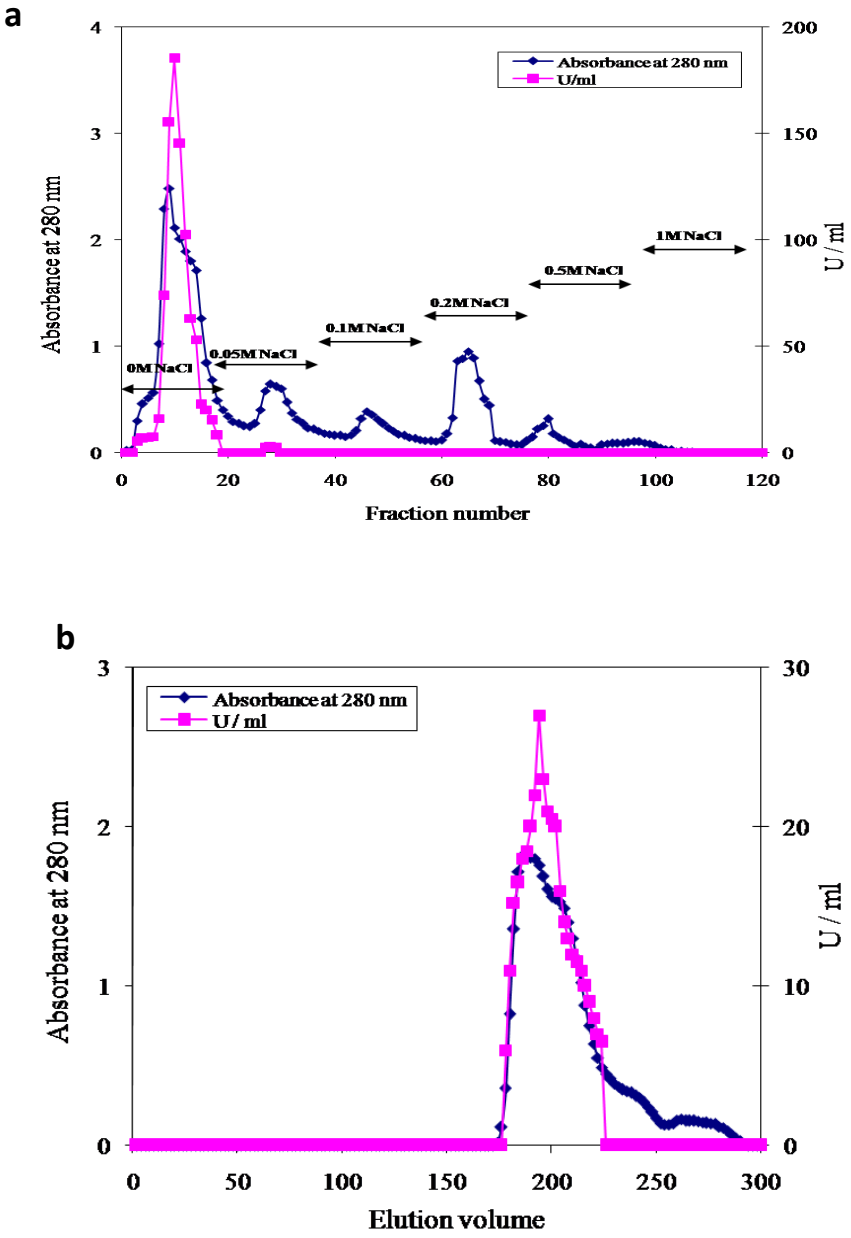

Figure 3. **a.** The chromatographic pattern of *H. dromedarii* 3–4 days-old embryo crude extract on CM-cellulose column (6 × 2.4 cm) previously equilibrated and washed with 0.02 M citrate phosphate pH 3.0. **b.** The chromatography of CTECD on Sephacryl S-300 column (142 × 2.4 cm) equilibrated and run with 0.02 M citrate phosphate pH 3.0.

Table 2. Substrate specificity of *H. dromedarii* 3–4 days-old embryo CD.

Substrate	Final Conc	Residual activity %
Bz-Arg-Gly-Phe-Phe-Pro-4-methoxy-β-naphthylamine	0.5 mM	100
Hemoglobin	0.077 mM	100
Albumin	0.075 mM	0
Casein	0.048 mM	0

Table 3. Effect of divalent ions on *H. dromedarii* 3–4 days-old embryo CD.

Reagent	Final concentration (mM)	Residual activity (%)	Inhibition (%)
Control	-	100.0	0
$CoCl_2$	5	11.0	89
$MnCl_2$	5	10.0	90
$ZnCl_2$	5	2.0	98
$CuCl_2$	5	34.0	66
$NiCl_2$	5	27.0	73
$MgCl_2$	5	51.0	49
$CaCl_2$	5	8.0	92

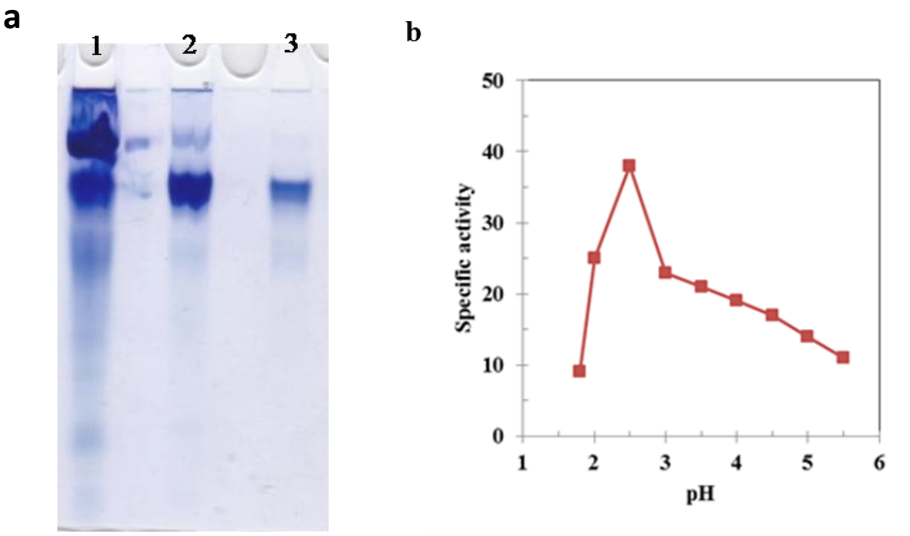

Figure 4. a. 7 % native gel of CTECD protein pattern: (1) crude extract, (2) CM cellulose fraction and (3) Sephacryl S-300 purified CTECD fraction. **b.** Effect of pH on the activity of purified CTECD using 0.1 M citrate phosphate buffer, pH (1.0–6.0).

Table 4. Effect of inhibitors on *H. dromedarii* 3–4 days-old embryo CD.

Inhibitor	Final	Inhibition %	Residual activity %
Control	-	0.0	100.0
Bestatin HCI	5 mM	72	28
Soya bean trypsin inhibitor	5 *µg*	5	95
β-Mercaptoethanol	5 mM	33	67
Pepstatin A	2 µM	97	3
Iodoacetic acid	5 mM	10	90
Phenylmethylsulfonylfluoride	2 mM	76	24

DISCUSSION

Inhibition of cathepsin D like proteases was discussed as an anti-parasitic interventional strategy since it has been explained to have significant functions in protein trafficking techniques of parasites. It initiates blood digestion as a component of multi-proteolytic enzymes complex in Platyhelminthes, nematodes, malaria and ticks (Sojka *et al*. 2016). Cathepsin D is a lysosomal soluble aspartate endopeptidase implicated in degrading and activating hormones, enzymes and proteins (Benes *et al*. 2008). In many organisms the involvement of various acid proteases classes has already been

confirmed during embryogenesis (Liu and Nordin 1998). It is involved in yolk proteins degradation of *R. prolixus* embryos (Fialho *et al*. 2005; Gomes *et al*. 2010), digesting proteins of ingested blood in *T. infestans* (Balczun *et al*. 2012) and promoting the early degradation of vitellin (VT) (Aguirre *et al*. 2011; Leyria *et al*. 2015). The degradation of VT is a basic biochemical process during embryogenesis for nutrition of tick embryos, shared by different groups of animals (Abreu *et al*. 2004; Zhang *et al*. 2019). Two aspartic and one cysteine proteinases were characterized in *B. microplus* eggs, suggesting their key role in embryogenesis (Renard *et al*. 2000; Sorgine *et al*. 2000). The role of cathepsin L-like proteases in embryos of *B. microplus* and *Ornithodorus moubata* ticks was described and suggested that these enzymes may be related to proteolysis of VT (Abreu *et al*. 2004). Yolk platelets acidification during embryogenesis of *B. microplus* increases during the primary development, being more intensive in the egg cortex region (Renard *et al*. 2000). In this investigation, *H. dromedarii* embryo homogenate at early stage of embryogenesis (6 days-old embryo) cleaved the acid-denatured hemoglobin with maximum activity at pH 2.5 (Fig. 1a). However, it exhibited a distinct pH optimum at pH 3.5 (Fig. 1b) at the late stage of embryogenesis (18 days-old embryo). This behavior suggested the existence of more than one enzyme involved in the degradation of egg yolk proteins during early and late stages of tick embryogenesis. The activity of cathepsin D was estimated during successive intervals of *H. dromedarii* embryogenesis. This profile showed that the highest cathepsin D specific activity level was acquired at 3–4 days-old embryo which represents the cell cleavage phase (Fig. 2). We purified cathepsin D from *H. dromedarii* tick 3–4 days-old embryo via cation exchange chromatography and size exclusion chromatography through a standard scheme specific for cathepsin D and various peptidases (Defferrari *et al*. 2011). The CM cellulose chromatographic pattern of *H. dromedarii* 3–4 days-old embryo showed one major cathepsin D activity peak nominated CTECD (Fig. 3a). Further chromatography of CTECD on Sephacryl S-300 column (Fig. 3b) also showed one CTECD activity peak. After size exclusion chromatography, CTECD specific activity was raised to 40 units/mg protein representing 7.4 folds and 55.3% yield (Table 1). The purity of purified CTECD enzyme was examined by analysis on 7% native PAGE that appeared as one major protein band (Fig. 4a) demonstrating the homogeneity of the preparation. CTECD exhibited an optimum pH at 2.5 (Fig. 4b) while cathepsin D from the mosquito *Aedes aegypti* (L.) obtained at pH 3.0 (Cho and Raikhel 1992), from pupae of the blowfly *Aldrichina grahami* (Aldrich) obtained at pH 3.5 (Kawamura *et al*. 1987), from *Tribolium castaneum* (Herbst) larvae gut obtained at pH 3 (Blanco-Labra *et al*. 1996) and the gut of *Ixodes ricinus* ticks obtained at pH 4 (Sojka *et al*. 2012). Optimal hydrolysis of hemoglobin by midgut cathepsin D of insects occurred at an acidic pH value of 2.5 for Notonectidae (*Notonecta* sp.) and Belostomatidae (*Belostoma* sp.) or 3.0 for Reduviidae (*Sinea* sp.), Phymatidae (*Phymata* sp.), Pentatomidae (*Phymata* sp.), and Lygaeidae *Oncopeltus fasciatus* (Houseman *et al*. 1983). The purified CTECD was highly specific to Bz-Arg-Gly-Phe-Phe-Pro-4-methoxy-β-naphthylamine colorimetric cathepsin D substrate ensuring that the purified enzyme is cathepsin D since it hydrolyzed it preferentially (Table 2). All of Co^{+2}, Zn^{+2}, Mn^{+2}, Cu^{+2}, Mg^{+2}, Ni^{+2} and Ca^{+2} ions strongly inhibited CTECD activity (Table 3). Bestatin HCI, β-Mercaptoethanol, PMSF and Pepstatin A highly inhibited the purified CTECD activity with the most potent of which being Pepstatin A (Table 4) in agreement with cathepsin D from pupae of the blowfly *A. grahami* (Kawamura *et al*. 1987), from the mosquito *Aedes aegypti* (Cho and Raikhel 1992), from midgut of Notonectidae (*Notonecta* sp.), Belostomatidae (*Belostoma* sp.), Reduviidae (*Sinea* sp.), Phymatidae (*Phymata* sp.), Pentatomidae (*Phymata* sp.), and Lygaeidae *Oncopeltus fasciatus* (Dallas) (Houseman *et al*. 1983), from midgut of *Tribolium castaneum* larvae (Blanco-Labra *et al*. 1996). The potent inhibition of CTECD enzyme by pepstatin A indicates that *H. dromedarii* embryo CTECD is an aspartic peptidase. Peptidases are considered to be prospective targets for vaccines and drugs in parasites control mechanisms owing to their critical roles during the initial embryonic periods or to the parasite pathogenicity (Sajid and McKerrow 2002; Loukas *et al*. 2004).

CONCLUSION

This is the first study of CTECD purification from 3–4 days-old embryo of the camel tick *H. dromedarii*. This CTECD might have a basic part in yolk protein degradation during the early embryonic development or putatively have double function acting in embryo feeding and defense against pathogens. As our research project aims at understanding the biochemical changes during tick embryogenesis, such a study will be helpful for future target of camel tick *H. dromedarii* biocontrol by using prepared CTECD antibodies as a substantial source of anti-tick vaccines.

ACKNOWLEDGMENT

This study was supported and funded by National Research Centre, Egypt which is greatly appreciated.

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آنزیم شبه کاتپسین D از جنین کنهٔ شتر *Hyalomma dromedarii* (Acari: Ixodida)

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چکیده

به خوبی شناخته شده است که کاتپسین D (CD) نقش اساسی در تخریب پروتئین‌های زرده در طول رشد جنین دارد زیرا عملکردی دوگانه در تغذیهٔ جنین و دفاع در برابر عوامل بیماریزا دارد. فعالیت شبه کاتپسین D در طول جنین‌زایی تین *Hyalomma dromedarii* (Acari: Ixodida) اثبات و بیشترین فعالیت را در جنین‌های ۳ تا ٤ روزه در طول مرحله تقسیم و کاتپسین D جنین کنهٔ شتر (CTECD) به دست آورد. پس از خالص‌سازی توسط ستون‌های سفاکریل S-300 با ژل CM-سلولز کاتیونی، CTECD با فعالیت ویژه ٤٠ U/mg پروتئین، فاکتور خالص‌سازی ۷/٤ و بازده ۵۵/۳ درصد همگن بود. جرم مولکولی CTECD از ستون حذف اندازه تقریبا ٤٠ کیلو دالتون استنباط شد. CTECD سوبسترای مصنوعی خاص Bz-Arg-Gly-Phe-Phe-Pro-4-methoxy-β-naphthylamide را هیدرولیز کرد و تایید کرد که آنزیم کاتپسین D است. هر دو Zn2+ و Pepstatin A مهارکننده‌های قوی فعالیت CTECD بودند. همچنین، CTECD حداکثر فعالیت خود را در اسیدیته ۲/۵ با مشخصات اسیدیته تیپ برای کاتپسین D نشان داد. این مطالعه نشان می‌دهد که CTECD در استفاده از پروتئین‌های زرده توسط جنین‌های در حال رشد نقش دارد زیرا به بیشترین فعالیت خود در مرحله تقسیم سلولی دست یافت. مطالعهٔ تغییرات بیوشیمیایی در طول جنین‌زایی کنه در درک سازگاری سلول‌های کنه برای پاتوژن‌های مختلف مفید خواهد بود.

کلمات کلیدی: بروز ویژگی‌ها، جنین‌زایی، کاتپسین D جنین کنهٔ شتر، خالص‌سازی، پروتئین‌های زرده.

اطلاعات مقاله: تاریخ دریافت: ۱٤۰۳/۱/۲۱، پذیرش توسط: /. حسینی چگنی، ۱٤۰۳/۹/۲۵، تاریخ چاپ: ۱٤۰٤/۱/۲٦