Comparative Biochemistry and Physiology, Part B 215 (2018) 31–38

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

Is digestive cathepsin D the rule in decapod crustaceans?

Diana Martínez-Alarcón[a,b], Reinhard Saborowski[b], Liliana Rojo-Arreola[c], Fernando García-Carreño[a,*]

[a] Centro de Investigaciones Biológicas del Noroeste (CIBNOR), IPN 195, Col. Playa Palo de Santa Rita, La Paz BCS 23096, Mexico
[b] Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research (AWI), Am Handelshafen 12, 27570 Bremerhaven, Germany
[c] CONACYT- Centro de Investigaciones Biológicas del Noroeste (CIBNOR), IPN 195, Col. Playa Palo de Santa Rita, La Paz BCS 23096, Mexico

ARTICLE INFO

Keywords:
Cathepsin D
Crustacea
Decapoda
Digestive enzyme
Extracellular function
Lysosomal protease

ABSTRACT

Cathepsin D is an aspartic endopetidase with typical characteristics of lysosomal enzymes. Cathepsin D activity has been reported in the gastric fluid of clawed lobsters where it acts as an extracellular digestive enzyme. Here we investigate whether cathepsin D is unique in clawed lobsters or, instead, common in decapod crustaceans. Eleven species of decapods belonging to six infraorders were tested for cathepsin D activity in the midgut gland, the muscle tissue, the gills, and when technically possible, in the gastric fluid. Cathepsin D activity was present in the midgut gland of all 11 species and in the gastric fluid from the seven species from which samples could be taken. All sampled species showed higher activities in the midgut glands than in non-digestive organs and the activity was highest in the clawed lobster. Cathepsin D mRNA was obtained from tissue samples of midgut gland, muscle, and gills. Analyses of deduced amino acid sequence confirmed molecular features of lysosomal cathepsin D and revealed high similarity between the enzymes from Astacidea and Caridea on one side, and the enzymes from Penaeoidea, Anomura, and Brachyura on the other side. Our results support the presence of cathepsin D activity in the midgut glands and in the gastric fluids of several decapod species suggesting an extracellular function of this lysosomal enzyme. We discuss whether cathepsin D may derive from the lysosomal-like vacuoles of the midgut gland B-cells and is released into the gastric lumen upon secretion by these cells.

1. Introduction

Protein is the main component in the food of heterotrophic organisms. Therefore, the importance of proteolytic enzymes in digestion. Neurath (1984) stated that proteases are presumed to have arisen in the earliest phases of biological evolution since even the most primitive organisms must have required them for digestion and for the metabolism of their own proteins. In crustaceans, digestive enzymes are synthesized in the midgut gland, likewise called hepatopancreas. This organ also plays a fundamental role in the absorption and storage of nutrients (Loizzi, 1971; McGaw and Curtis, 2013; Saborowski, 2015; Vogt, 1993). Mechanisms of production and storage of digestive enzymes in crustaceans differ significantly from those in vertebrates (Vogt et al., 1989). In crustaceans, digestive enzymes are synthesized in the F-cells of the midgut gland and secreted into the midgut gland lumen (Caceci et al., 1988; Ceccaldi, 1998; Vogt et al., 1989). Then, the enzymes accumulate in the gastric chamber and contribute to the extracellular digestion of food items (Saborowski, 2015; Vogt et al., 1989).

Animals possess many types of proteases, distinguished by the amino acid at the active site and the location in which it functions. Serine proteases, including trypsin and chymotrypsin, are the most abundant and, hence, the best described endopeptidases in the digestive system of decapods. Their functions are well established as well as the mechanisms of synthesis, storage, activation, and secretion (Celis-Guerrero et al., 2004; Díaz-Tenorio et al., 2006; Garcia-Carreño et al., 1994; Hernández-Cortés et al., 1997; Muhlia-Almazán et al., 2008). Klein et al. (1996) and Sainz et al. (2004) showed that trypsin isoforms are synthesized as inactive precursors but are rapidly activated.

Digestive endopeptidases belonging to the classes of metallo-, cysteine, and aspartate-proteases have been reported and characterized as well although for a limited number of decapod species (Laycock et al., 1989; Navarrete del Toro et al., 2006; Rojo et al., 2010b; Stöcker et al., 1988; Teschke and Saborowski, 2005). Additionally, the aspartic endopeptidase, cathepsin D, was found in the gastric fluid of the clawed lobsters *Homarus gammarus* and *H. americanus* and was identified as a fully operational extracellular digestive enzyme at the acid pH of gastric fluid (Navarrete del Toro et al., 2006; Rojo et al., 2010a). The presence of cathepsin D in the gastric fluid seems unusual as this enzyme is known and well characterized as typical lysosomal and, thus, intracellular endopeptidase.

* Corresponding author.
E-mail address: fgarcia@cibnor.mx (F. García-Carreño).

http://dx.doi.org/10.1016/j.cbpb.2017.09.006
Received 6 June 2017; Received in revised form 28 September 2017; Accepted 28 September 2017
Available online 12 October 2017
1096-4959/ © 2017 Elsevier Inc. All rights reserved.

Cathepsin D (EC 3.4.23.5) plays a crucial role within the cells. By hydrolyzing intracellular proteins it participates in various physiological processes involved in maintaining tissue homeostasis, regulation of apoptosis, activation of hormones and growth factors (Benes et al., 2008), to mention a few. In insects, cathepsin D-like peptidases showed specialized functions like cellular remodelling during metamorphosis (Gui et al., 2006). In humans, cathepsin D is synthesized in the rough endoplasmic reticulum as a pre-pro-enzyme but rapidly loses the signal peptide. The enzymatically inactive pro-cathepsin D, now called precursor of cathepsin D or zymogen, is glycosylated in the endoplasmic reticulum and directed into the Golgi complex. Once in the Golgi complex the precursor is tagged with mannose-6-phosphate (M6P) and directed into the endosomes due to specific M6P receptors. The endosomes will eventually transport the precursor to lysosomes (Nicotra et al., 2010) where they will be activated by the action of cysteine lysosomal proteases. Finally the mature enzyme consists of two chains, one of 14 kDa in the amino-terminal domain and the other of 34 kDa in the carboxyl-terminal domain (Laurent-Matha et al., 2006). Once activated, the cathepsin D will participate in the hydrolysis of proteins within the lysosomes.

In contrast to serine or cysteine peptidases that form a covalent intermediate in the hydrolysis of the peptide bond, aspartyl peptidases catalyze the hydrolysis of the peptide bond by means of an acid-base mechanism that involves the coordination of a water molecule. One of the two aspartic acid residues forming the catalytic dyad activates the water molecule by taking a proton. This makes the water nucleophilic and allows it to attack on the carbonyl carbon of the substrate scissile bond, creating a tetrahedral oxyanion intermediate. Upon electron rearrangement the scissile amide is protonated which results in the splitting of the substrate peptide into two peptides (Brik and Wong, 2003; Conner, 2004).

Cathepsin D evolved to an enzyme with digestive function in invertebrates like insects (Srp et al., 2016), mites (Wajahat Mahmood et al., 2013) and bloodsucking parasites as part of a multienzyme proteolytic complex (Sojka et al., 2016) and in vertebrates like bloodsucking fish (Xiao et al., 2015) and now we found it in crustaceans (Navarrete del Toro et al., 2006; Rojo et al., 2010a). The presence of cathepsin D in the gastric fluid of clawed lobsters that contributes to the extracellular digestion (Rojo et al., 2010b) raises the question whether this situation is unique within the genus of clawed lobsters (*Homarus* sp.) or spread among other decapod taxa. Therefore, in this study we assessed the presence of cathepsin D in the gastric fluid and other tissues of 11 decapod species from six infraorders by means of catalytic activity as well as the expression of cathepsin D mRNA. The data presented here may contribute to a better understanding of the function and evolution of the digestive processes in the midgut gland of decapod crustaceans.

2. Materials and methods

2.1. Studied species

Adults of 11 species of decapod crustaceans belonging to six different infraorders (Table 1) were analysed for the presence of cathepsin D activity and mRNA. Species from the North Sea were caught during two sampling campaigns, one in September 2013 and the other in April 2016, near the island of Helgoland (54°11′N, 7°55′E), by bottom trawling. American lobster, *H. americanus*, were purchased from a local seafood merchant (Edelfisch Kontor, Bremerhaven, Germany) and the European crayfish *Astacus astacus* from a crayfish rearing facility (Edelkrebszucht Göckemeyer, Neustadt-Poggenhagen, Germany). Specimens were processed in the laboratories of the Alfred Wegener Institute in Bremerhaven, Germany. Red or California spiny lobster *Panulirus interruptus* were purchased from a local seafood merchant in La Paz, Baja California Sur, in February 2014. The Blue crab *Callinectes bellicosus* and the Whiteleg shrimp *Penaeus vannamei* were caught in the Gulf of California and at the Mexican Pacific coast in August and October 2013, and September 2016. Specimens were processed in the laboratories of the Centro de Investigaciones Biológicas del Noroeste in La Paz. BCS, Mexico.

After collection, the specimens were maintained without food for 24 h in running seawater to level their dietary status and to empty their stomachs of food remains. The gastric fluids from *H. americanus*, *A. astacus*, *P. interruptus*, *Pagurus bernhardus*, *Liocarcinus depurator*, *C. bellicosus*, and *Cancer pagurus* were sampled from the gastric chambers by inserting a flexible tube through the oesophagus which was attached to a 10 ml syringe. Depending on the specimens' size, 0.5 to 2 ml of gastric fluid was obtained. Each specimen was sampled only once. Immediately after sampling the gastric fluid was transferred into 1.5-ml reaction tube and stored at −80 °C until used. It was not possible to obtain gastric fluid from *Pandalus montagui*, *Crangon crangon*, *P. vannamei*, and *Nephrops norvegicus*.

Subsequently, tissue samples of midgut gland (MG), muscle (M), and gills (G) were taken. All samples were divided into two subsamples, one was transferred into a 1.5-ml tube and frozen at − 80 °C for further protein and enzyme analysis. The other was transferred into 1.5-ml tubes containing 0.5 ml of RNAlater (AM7020, Life Technologies, ONT, Canada) for future RNA extraction.

2.2. Enzyme preparation

Samples of gastric fluid were thawed and centrifuged for 15 min at 10,000 × *g* and 4 °C to discard solids. Samples of midgut gland, muscle, and gills (75 to 100 mg) were thawed and homogenates of individual tissues were prepared on ice by ultrasonication (Branson Sonifier Cell Disruptor) with three bursts of 5 s and 10 s breaks in between. Homogenates were centrifuged for 30 min at 10,000 × *g* and 4 °C, and the supernatants, which contained the soluble protein, were aliquoted and stored at −80 °C. The sample was aliquoted to avoid repeated thawing and freezing. Each aliquot was used only once. During the assays the samples were cooled on ice. No loss of activity was detected between fresh and frozen samples.

2.3. Quantification of soluble protein and cathepsin D activity

Protein concentration was quantified after Bradford (1976) with serum bovine albumin as the standard (B-4287, Sigma-Aldrich). Cathepsin D activity was measured in a microplate reader using the fluorogenic substrate 7-methoxycoumarin-4-acetyl-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys-(DNP)-DArg-amide (M0938, Sigma-Aldrich) in 50 mM sodium acetate buffer at pH 4.0 (Rojo et al., 2010b). Excitation and emission wavelengths were 320 nm and 380 nm, respectively. Rates of hydrolysis of the substrate were recorded by measuring the increase of fluorescence every 30 s for 10 min and expressed as change of relative fluorescence units per minute (RFU min^{-1}). The linear part of the kinetic curve was used to calculate activity. A calibration curve was established by measuring the fluorescence of increasing concentrations of the fluorochrome 7-methoxycoumarin-4-acetic acid (MCA) (235,199, Sigma-Aldrich). One unit of activity was expressed as one nmol of MCA liberated per minute per mg of protein in 50 mM of sodium acetate buffer at pH 4.0 and room temperature.

2.4. Inhibition of cathepsin D with pepstatin A

Specific inhibition of cathepsin D was done using pepstatin A (Sigma, P 5318), an inhibitor specific for aspartic peptidases (Navarrete del Toro et al., 2006). Ten µl of enzyme preparation were incubated with 10 µl of 10 mM pepstatin A at room temperature and the remaining activity was measured after 30 min as above.

Download English Version:

https://daneshyari.com/en/article/8318859

Download Persian Version:

https://daneshyari.com/article/8318859

Daneshyari.com