Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part B 142 (2005) 153 – 163
CBP
www.elsevier.com/locate/cbpb

Expression and tissue distribution of astacin-like squid metalloprotease (ALSM)

Nobuyuki Kanzawa *, Shuntaro Tatewaki, Ryousuke Watanabe, Ikuko Kunihisa, Haruka Iwahashi, Kaori Nakamura, Takahide Tsuchiya

Department of Chemistry, Faculty of Science and Technology, Sophia University, 102-8554, Tokyo, Japan

Received 28 January 2005; received in revised form 14 May 2005; accepted 15 May 2005
Available online 2 August 2005

Abstract

Astacin metalloprotease family members function in a wide variety of biologic events, including cell differentiation and morphogenesis during embryonic development and adult tissue differentiation. We previously isolated and characterized an astacin-like squid metalloprotease (ALSM). To elucidate the embryonic expression of ALSM, we performed immunohistochemical analysis with specific antibodies and examined the expression profiles of ALSM isoforms by in situ hybridization analysis. Tissue distribution and expression were also examined in adult spear squid. mRNA expression of ALSM isoforms I and III was first detected in newly hatched squid and was restricted to the liver. No mRNA signals were detected in other tissues even in adult squids. At the protein level, both isoforms were prominent in the liver of embryos and later in digestive organs of adult squid. Both isoforms were also detected in muscle tissues, including mantle and tentacle muscle. Staining for ALSM III was also identified in the iris and in tissues near the eye in squid embryos. However, no reactive bands were detected by immunoblotting of adult squid eyes. Thus, ALSM is initially expressed at the late stage of embryogenesis in spear squid, and expression is restricted to the liver. Thereafter, ALSM isoforms function in various tissues in an isoform-dependent manner.

Keywords: ALSM; Astacin; Embryo; Expression; Liver; Metalloprotease; Squid; Tissue distribution

1. Introduction

Astacin-like squid metalloprotease (ALSM) has a high substrate specificity for myosin heavy chain (MyHC) and was originally identified in squid mantle muscle (Okamoto et al., 1993; Tamori et al., 1999). Primary sequence analysis has shown that ALSM is a member of the astacin family (Yokozawa et al., 2002). Astacins were first identified as digestive enzymes (EC 3.4.24.21) in the stomach of the freshwater crayfish *Astacus astacus* (Titani et al., 1987; Dumermuth et al., 1991). Astacin family members have unique consensus sequences, including a zinc-binding sequence (HEXXHXXGFXHEXXRXDRD) and a Met-turn sequence (SXMHY) (Bode et al., 1993), and have been identified in various organisms from mammals to hydras (Bond and Beynon, 1995; Sarras, 1996). Studies of domain structure have revealed that astacins possess signal and prosequence domains along with a conserved protease domain characterized by the zinc-binding motif as well as a unique C-terminal domain specific to each family member. ALSM possesses signal, prosequence, and protease domains, followed by a MAM (meprin, A5 protein, receptor protein-tyrosine phosphatase μ) domain, which is a conserved domain in the C-terminus of meprin, an astacin family member (Bond and Beynon, 1995; Yokozawa et al., 2002). Astacins are involved in a wide variety of physiologic events, including digestion (Vogt et al., 1989), development (Takahara et al., 1994; Piccolo et al., 1996), hatching (Yasumasu et al., 1992a; Lee et al., 1994; Katagiri et al., 1997; Fan and Katagiri, 2001), regeneration (Yan et al., 2000a,b), and activation of hormones and some peptides (Yamaguchi et al., 1991). For example, human bone

* Corresponding author. Tel.: +81 3 3238 3363; fax: +81 3 3238 3361.
E-mail address: n-kanza@sophia.ac.jp (N. Kanzawa).

1096-4959/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2005.05.018

morphogenetic protein 1 (huBMP1) induces ectopic bone formation in adult vertebrates (Wozney et al., 1988); *Drosophila* tolloid is required for normal dorsal patterning (Shimell et al., 1991; Marques et al., 1997); and meprins are found in mammalian kidney and intestinal tissues and play crucial roles in the processing of biologically active peptides and extracellular matrix (ECM) proteins (Craig et al., 1987; Bond and Beynon, 1995). An astacin-like protein from the freshwater polyp *Hydra vulgaris*, described as hydra metalloprotease I (HMP-1), is localized in the ECM in a head-specific manner and has a functional role during development (Yan et al., 1995). The fish hatching enzymes, high and low choriolytic enzymes (HCE/LCE), are secreted from hatching glands and digest the egg envelope (Yasumasu et al., 1992b; Inohaya et al., 1995). Thus, astacins have unique functions in each tissue in which they are expressed. ALSM can hydrolyze MyHC; therefore, it is thought to be involved in the metabolism of skeletal muscle proteins (Okamoto et al., 1993). Tissue distribution analysis of ALSM activity with the use of MyHC as an in vitro substrate revealed that ALSM exists in a wide variety of tissues, particularly in digestive organs (Tajima et al., 1998; Tamori et al., 1999). Two groups of ALSM isoforms are present in squid: types I and III in spear squid and types I and II in Japanese common squid. These isoforms are classified according to specificity for hydrolytic sites in rabbit skeletal muscle MyHC. Differentially regulated expression and distribution of the two groups of astacins have been reported in hydra (Yan et al., 2000a,b). However, the exact expression profiles of astacin isoforms have not been reported.

In the present study, we obtained specific antibodies for ALSM I and III isolated from spear squid (*Loligo bleekeri*) and examined the tissue distributions of ALSM isoforms in squid by immunohistochemistry. Expression of ALSM mRNA in the squid was examined by in situ hybridization and northern blotting and RT-PCR analysis.

2. Materials and methods

2.1. Materials

Spear squid (*Loligo bleekeri*) were purchased from the Tokyo Central Wholesale Fish Market. Spear squid eggs and embryos were a kind gift from Dr. Yuzuru Ikeda, Ryukyu University, and were fixed immediately in 4% paraformaldehyde for histochemical and immunohistochemical analyses or frozen in liquid nitrogen for biochemical analysis. Embryos were collected from eggs incubated in a small aquarium after approximately 6, 8, and 10 weeks of incubation. Eggs incubated for 6 or 8 weeks were extracted from the egg capsules, and the embryos were removed from the eggs. Embryos after 10 weeks of incubation were at the hatching stage. Embryos were inspected under light microscopy (Fig. 1), and embryonic stages were determined on the basis of morphologic characteristics according to the criteria of Baeg et al. (1992). Embryos after 6 weeks of incubation had large yolk sacs compared to the embryonic body; red retinas and red chromatophores were evident on the mantle surface but not on the tentacles, as is typical of stage 23 of embryonic development. In embryos after 8 weeks of incubation, the yolk sac and head were approximately the same size, which is typical of stage 26. Another marker of this stage, a filled ink sac, was also visible through the mantle muscle. Newly hatched embryos were classified as stage 28 embryos.

2.2. Expression of recombinant proteins

Recombinant ALSM I and III of spear squid were expressed in *Escherichia coli* for antibody specificity tests. ALSM I and III cDNAs were amplified from isolated clones (Yokozawa et al., 2002) by PCR with primer pairs (summarized in Table 1) of Y1-20 and T3 primer, and Y3-1 and Y3-2, respectively. Amplified fragments were

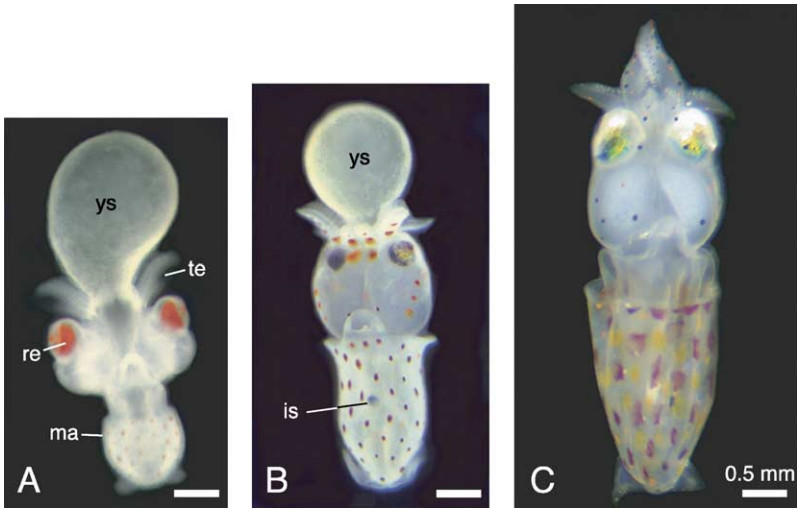

Fig. 1. Light micrographs of spear squid embryos at stages 23 (A), 26 (B), and 28 (C) is, ink sac; ma, mantle; re, retina; te, tentacle; ys, yolk sac.