Analysis of ferritin-type proteins in the hepatopancreas of Baltic blue mussel (*Mytilus trossulus*)*

OCEANOLOGIA, 39 (2), 1997.
pp. 157–162.

KEYWORDS
Mytilus trossulus
Ferritin
Capillary electrophoresis

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Manuscript received May 13, 1997, in final form June 20, 1997.

Abstract

Ferritin is a protein able to store and transport not only iron, but also, though to a lesser extent, some other heavy metals, including cadmium. This study concerned the analysis of ferritin-type proteins in a dominant species of the southern Baltic zoobenthos, the blue mussel *Mytilus trossulus*. Proteins were isolated by precipitation from homogenised mussel hepatopancreases. The protein samples were heated (70°C) and acidified (pH 4.5). Then the proteins with a molecular weight close to that of plant ferritin were separated by ultracentrifugation. These preparations were compared using capillary electrophoresis with plant ferritins isolated from lupin (*Lupinus luteus*) and amaranth (*Amaranthus hybridus*). The

* This study was financially supported by the Polish Committee for Scientific Research, grant No. 705/P04/95/09, and the University of Gdańsk, grant No. DS/8000–4–0026–7.

electrophoregrams obtained revealed that the hepatopancreas of the Baltic blue mussel (*Mytilus trossulus*) contains a protein displaying the properties of plant ferritin.

1. Introduction

Heavy metals represent a significant hazard to living organisms, one of their most dangerous properties in the ecosystem being their ability to accumulate in the tissues of living organisms. In the marine environment they are passed along the trophic chain to fish and finally to humans. Marine benthic organisms, especially mussels, are characterised by exceptionally high coefficients of heavy metal accumulation, including cadmium (Raibow and Phillips, 1993).

Heavy metal accumulation is subject to various regulatory mechanisms. The high concentration of cadmium in mussel tissues and internal organs is due, among other things, to the presence of metallothioneins, proteins capable of complexing absorbed metals (Ray, 1984; Bebianno and Langston, 1991). However, cadmium also accumulates in tissues in which metallothioneins have not been found. In such cases, the biosynthesis of other proteins capable of complexing metals, *e.g.* ferritins, may be responsible for cadmium accumulation in cells.

Ferritin is a protein which primarily stores and transports iron; to a lesser extent, however, it also acts in a similar manner on some other heavy metals, including cadmium (Murno and Linder, 1978; Korcz and Twardowski, 1992a). Hence, the binding of cadmium accumulated in mussel tissue by ferritin seems feasible. This, however, has not been documented so far and requires confirmation.

This study aimed to identify and to analyse quantitatively and qualitatively ferritin-type proteins in a dominant species of the southern Baltic zoobenthos – the blue mussel *Mytilus trossulus*.

2. Materials and methods

Materials

Blue mussels *Mytilus trossulus* were collected with a dredge from the bottom of the southern Baltic in the Gulf of Gdańsk. The organisms within the 25 to 35 mm length class were selected, their soft tissues separated from shells and the hepatopancreases excised. These were stored in a deep-freeze.

Protein isolation

The mussel hepatopancreases were thawed and thoroughly homogenised in electrophoretic buffer 'A' (50 mm 1M Tris–HCl, pH 8, 30 mm 1 M NaCl), after which the proteins were isolated by precipitation (Korcz and Twardowski, 1992b). The homogenised hepatopancreas samples were centrifuged at 1000 × g for 60 min using a Beckman type GS –6R centrifuge. The pellet was discarded and the supernatant precipitated with ammonium sulphate (400 mg of sulphate per 1 mm of the supernatant) for 2 h with constant stirring. The solution was centrifuged again at 1000 × g for 60 min. The supernatant was discarded and the pellet was dissolved in buffer 'A': 1 mL of buffer per 400 mg of soft tissue. The protein preparations were heated slowly up to 70°C for 15 min., then chilled. The denatured proteins were removed from the solution by centrifugation at 1000 × g for 30 min. The resulting supernatant was acidified with 1M sodium acetate to pH 4.5 and again precipitated as described above. The precipitate was collected by centrifugation (1000 × g, 60 min) and dissolved in buffer 'A' using 0.2 ml of the buffer per 400 mg of soft tissue used for protein isolation.

Protein fractionation

The solutions of proteins isolated from mussel hepatopancreases were fractionated by ultracentrifugation at 100000 × g for 120 min at 4°C in a Beckman L7–65 type ultracentrifuge, type L7–65 (Korcz and Twardowski, 1992a). The supernatant was discarded, while the pellet was dissolved in buffer 'A' and centrifuged at 1000 × g for 60 min. The resulting pellet was discarded and the supernatant was again subjected to ultracentrifugation as described above. This procedure was repeated 4 times. After the final centrifugation, the protein concentration in the supernatant was measured by Bradford's method (Bradford, 1976) with bovine serum albumin (BSA from Sigma) as standard.

Ferritin analysis by SDS–PAGE electrophoresis

The protein preparations obtained were compared with plant ferritin isolated from lupin (*Lupinus luteus*) shoots (Poznań Plant Breeding Station at Wiatrowo near Poznań). For this purpose, polyacrylamide gel electrophoresis (10% PAGE) in the presence of sodium dodecylsulphate (SDS) was used (Laemmli, 1970). The homogeneous proteins were then blotted onto Millipore PVDF membranes (Mayer and Walker, 1980).

Ferritin analysis by capillary electrophoresis

The protein preparations obtained from blue mussel hepatopancreases were also compared using capillary electrophoresis with plant ferritins derived from lupin (*Lupinus luteus*) shoots and amaranth (*Amaranthus hybridus*) roots (both ferritins were obtained from the Poznań Plant Breeding Station at Wiatrowo near Poznań). A Beckman PACE System 2100 capillary electrophoresis instrument with the cathode on the detection side was employed. All solutions and samples were filtered through 0.22 μm teflon membrane filters prior to analysis. Electrophoresis was performed in 25 mM phosphate buffer, pH 7.0. The capillary cassette used was fitted with a 75 μm i.d. BioCAP uncoated fused silica capillary 57 cm in length (50 cm to the detector). Runs were carried out at a constant voltage of 10 kV. Electrokinetic injections of samples at 10 kV for 20 s were used. The temperature of the capillary was maintained at 28°C. The separation of proteins was monitored at 254 nm. The electrophoretic data were acquired and stored on an IBM 386 DX–40 computer using the System Gold software package. The data were exported as DIF (data interchange file) to the Sigma Plot version 5.01 program (Jandel Corporation) and plotted.

3. Results and discussion

The method developed for the isolation of plant ferritin (Korcz and Twardowski, 1992b) applied to the isolation of proteins from hepatopancreases of the blue mussel *M. trossulus* enabled preparations containing proteins to be obtained which displayed ferritin properties and which were

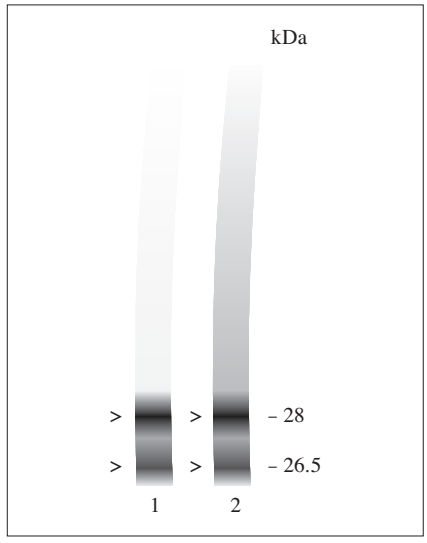

Fig. 1. SDS–PAGE electrophoresis of preparations containing ferritin type proteins isolated from *L. luteus* shoots (1) and *M. trossulus* hepatopancreas (2)

resistant to elevated temperature (70°C) and acidic conditions (pH 4.5). From 1 g of wet mussel hepatopancreas 0.05 mg ferritin was obtained as determined by Bradford's method, which was used to measure protein concentration in purified preparations. This constitutes about 2% of the total protein in this organ.

Fig. 1 shows the results of gel electrophoresis of ferritin isolated from lupin *L. luteus* shoots and proteins isolated from hepatopancreas of the blue mussel *M. trossulus*. These results demonstrate that among the mussel

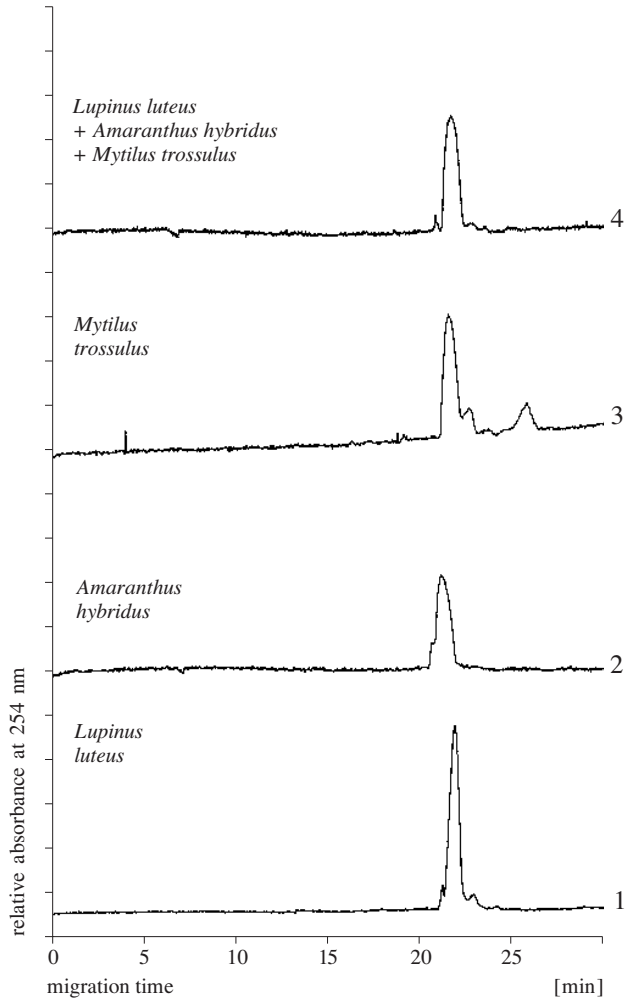

Fig. 2. Capillary electrophoresis of ferritin isolated from *L. luteus* shoots (1), *A. hybridus* roots (2), *M. trossulus* hepatopancreas (3), and coinjection of the three proteins analysed (4)

proteins there is one which, like plant ferritin, is characterised by the presence of two subunits in the electrophoregram: 28 kDa and 26.5 kDa (Lauelhere *et al.*, 1988).

Fig. 2 presents the capillary electrophoresis of ferritin preparations isolated from lupin (*L. luteus*) shoots (Fig. 2 (1)), amaranth (*A. hybridus*) roots (Fig. 2 (2)) and mussel hepatopancreas (Fig. 2 (3)). The electrophoregrams obtained confirm the presence of a protein displaying the properties of plant ferritin in the hepatopancreas of the blue mussel (*M. trossulus*). All the ferritins studied exhibit basically identical migration times and elute as one peak when coinjected (Fig. 2 (4)). The identity of the preparations studied is additionally confirmed by such properties as resistance to high temperature and low pH.

References

Bebianno M. J., Langston W. J., 1991, *Metallothionein induction in Mytilus edulis exposed to cadmium*, Mar. Biol., 108, 91–96.

Bradford M. M., 1976, *A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding*, Analyt. Biochem., 72, 248–254.

Korcz A., Twardowski T., 1992a, *Lupine ferritin: purification and characterization, biosynthesis and regulation of in vitro synthesis in plant*, J. Plant Physiol., 141, 75–81.

Korcz A., Twardowski T., 1992b, *The effect of selected heavy metal ions on the in vitro translation system of wheat germ – protective function of plant ferritin*, Acta Physiol. Plant., 14, 185–190.

Laemmli U. K., 1970, *Cleavage of structural proteins during the assembly of the head of bacteriophage* T_4, Nature, 227, 680–685.

Lauelhere J. P., Lescure A. M., Briat J. F., 1988, *Purification and characterization of ferritin from maize, pea and soybean seeds*, J. Biol. Chem., 263, 10289–10294.

Mayer R. J., Walker J., 1980, *Immunological methods in the biological sciences: enzymes and proteins*, Academic Press, London.

Murno H. N., Linder M. C., 1978, *Ferritin: structure, biosynthesis and role in iron metabolism*, Physiol. Rev., 58, 317–396.

Raibow P. S., Phillips D. J. H., 1993, *Cosmopolitan biomonitors of trace metals*, Rev. Mar. Pollut. Bull., 26, 593–601.

Ray S., 1984, *Bioaccumulation of cadmium in marine organisms*, Experientia, 40, 13–23.