# Purification and characterisation of ferritin from the Baltic blue mussel *Mytilus trossulus**

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**Abstract**

Baltic blue mussels *Mytilus trossulus* were collected from the Gulf of Gdańsk (southern Baltic Sea) in order to isolate ferritin from its soft tissues, as well as to purify and characterise this protein.

Proteins were isolated from the inner organs of *M. trossulus* (hepatopancreas, gills and soft tissue residue) by thermal denaturation (70°C) and acidification (pH 4.5) of the homogenates, followed by ammonium sulphate ($(NH_4)_2SO_4$) fractionation. The ferritin was then separated by ultracentrifugation ($100\,000 \times$ g, 120 min.). The protein content in the purified homogenates was determined by the Lowry method using bovine serum albumin (BSA) and horse spleen ferritin (HSF) as standards. PAGE-SDS and Western blotting analysis permitted identification of ferritin in the purified preparations. Additionally, the purified homogenates and mussel soft tissue were analysed for their heavy metal contents (especially cadmium and iron) in a Video 11 E atomic absorption spectrophotometer, following wet digestion of the samples ($HNO_3/HClO_4$).

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The electrophoregrams showed that the inner organs of *M. trossulus* contained ferritin, which, like plant ferritin, is characterised by the presence of subunits in the electrophoregram in the 26.6–28.0 kDa range. The highest ferritin content was recorded in the hepatopancreas, followed by the gills and the soft tissue residue. With regard to the sampling stations, the highest content of ferritin was noted in the animals sampled off Sopot (station D3), and in those collected by a diver off Jastarnia (W1) and Gdynia (W4). Ferritin isolated from the inner organs of mussels collected from these stations also contained the largest quantities of heavy metals (Cd and Fe). Ferritin isolated from the inner organs of mussels collected by a diver from wrecks – sites where the concentrations of iron and other trace metals in the sea water are high – contained higher quantities of heavy metals (Cd and Fe) than the ferritin isolated from the inner organs of mussels collected with the drag. This confirms that 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.

## 1. Introduction

Ferritin is a protein commonly found in living organisms: from bacteria (Penfold et al. 1996, Treffry et al. 1998), through invertebrates and vertebrates (Theil 1987, Bauchspieß et al. 1995, Winzerling et al. 1995, Rahman et al. 1999, Zhang et al. 2003), to higher plants, in which it is known as phytoferritin (Fobis-Loisy et al. 1996, Briat & Lobréaux 1997).

With respect to the role it plays in the organism, ferritin has been described as a protein essential for the metabolism of iron, exceptional for its ability to accumulate vast amounts of iron (up to 4500 ions of $Fe^{2+}$ per ferritin molecule), capable of regulating the iron content and of protecting the cell from toxic quantities of $Fe^{2+}$ ions (Theil 1987, Theil et al. 2000).

The distribution of ferritin in the organism is uneven and depends on the concentration of $Fe^{2+}$ ions (Uchida 1995, Worwood 1997). In the presence of excess iron, the system's supply of Fe increases substantially and very much more ferritin is detectable in the tissues – mainly in the liver and spleen (Chasteen 1998, Kong et al. 2003, Huang et al. 2004).

Ferritin is a large protein with a native molecular mass of around 400–500 kDa and a sedimentation constant of 17–18S; it consists of 24 sub-units each of 18–28 kDa in mass (Chasteen & Harrison 1999).

Studies of plant and animal ferritin have confirmed the existence of two sub-units, denoted H (heavy) and L (light), differing in mobility during electrophoretic separation; in bacteria only H-type sub-units have been found (Chasteen & Harrison 1999). The mass of the sub-units in plant ferritin has been determined electrophoretically at ca 28 kDa (H) and ca 26.5 kDa (L); characteristic values for animal ferritin are ca 21 kDa (H) and ca 19 kDa (L) (Theil 1987). The H and L sub-units are the translation products of various mRNA; the similarity of the amino-acid sequences in H

and L sub-units is of the order of 47% (Theil 1987, Ragland & Theil 1993). The difference results primarily from the H sub-unit's ability to rapidly oxidise $Fe^{2+}$ ions – this is because it contains a ferroxidase centre (Chasteen & Harrison 1999).

One molecule of ferritin can bind up to 4500 $Fe^{2+}$ ions. When fully loaded with iron, the ferritin can increase in mass up to ca 900 kDa; it then has a sedimentation coefficient of 24S (Chasteen & Harrison 1999). The iron is stored within the protein coat in the form of ferric hydroxide [$Fe^{3+}O(OH)$] combined with a variable amount of phosphates (Korcz & Twardowski 1989). The enzymatic combination of divalent iron ($Fe^{2+}$) with apoferritin (ferritin minus Fe) yields the complex compound – ferritin; during this reaction iron is oxidised to trivalent $Fe^{3+}$. Conversely, each time iron is split off from ferritin, it is reduced to divalent $Fe^{2+}$ (Theil & Sayers 1989). The ability of ferritin to catalyse the oxidation of $Fe^{2+}$ ions is due to the conservative ferroxidase centre situated inside the polypeptide chains of apoferritin (Chasteen & Harrison 1999).

Thanks to its compact structure, ferritin remains stable at elevated temperatures (from $-70°$C to $+70°$C) and is unreactive towards proteolytic enzymes in the pH range 4.5–10 (Aisen & Listkowsky 1980, Korcz & Twardowski 1992a). These properties are put to good use when ferritin is isolated from biological material.

The main biological functions of ferritin are to protect the cell from toxic concentrations of iron and to counteract the related stress; also, to store iron (which is the ferritin captured earlier while fulfilling its protective function), thus ensuring that this element is available for its basic cell requirements and for maintaining the iron balance in the body (Theil 1987). Excess iron can put the system in danger, principally by causing nucleic acids to decompose. To a certain extent, living organisms can protect themselves against toxic concentrations of Fe ions in that they biosynthesise ferritin, which is able to chelate the excess iron (Korcz & Twardowski 1992b, Chasteen & Harrison 1999). Ferritin acts as an iron sink for a number of metabolic processes in the cell that require this element (e.g. to transport electrons and oxygen, to reduce nitrogen and DNA synthesis (reduction of RNAs) (Theil 1987). In evolution, the role of ferritin took on a particular significance once oxygen had appeared as a product of photosynthesis – this oxidises the soluble $Fe^{2+}$ ion to the insoluble $Fe^{3+}$ ion. Ferritin ensures that the iron remains in a soluble form – its concentration in cells specialised in the accumulation of iron can reach $10^{-5}$ M (Korcz & Twardowski 1989).

Ferritins are protein nanocages with multiple sites that catalyse the oxidoreduction of Fe(II) and $O_2$ to produce diferric mineral precursors; the

mineral precursors migrate into a central cavity 8 nm in diameter and form an Fe(III) hydrated oxo mineral. Iron minerals in ferritins serve to concentrate iron to the high levels needed for the synthesis of iron-containing cofactors (Tosha et al. 2008). The Fe(II) oxidation reactions in ferritins consume dioxygen or hydrogen peroxide, thereby minimising dangerous Fenton chemistry (Liu & Theil 2005). Ferritin protein nanocages self-assemble from 24 or 12, 4-$\alpha$-helix-bundle subunits in maxiferritin or miniferritin respectively; historically, miniferritins were named Dps (DNA protection during starvation) proteins and, to date, are known only in bacteria and archaea; haem-containing ferritins in bacteria are called bacterioferritins (Bfr) (Frolow et al. 1994).

Ferritin is central to the effective control of iron and dioxygen chemistry (Lewin et al. 2005, Liu & Theil 2005). The soluble ferritin protein nanocages form a ferric oxy biomineral in the central cavity, consuming dioxygen (and sometimes peroxide) in the process and reversibly concentrating iron (Theil 2007).

The ability of ferritin to store Fe ions allows it to act as a detoxicant with respect to the genetic material in the cell (Theil 1998). The threat to the genetic material lies principally in the ability of iron to facilitate the formation of reactive oxygen species (ROS), which are capable of degrading both DNA and RNA. However, if the iron is bound to ferritin, it is rendered harmless to biological systems. The Fe-ferritin complex thus enables Fe, an important co-factor in many enzymatic processes, to be stored in a non-toxic form (Harrison & Arosio 1996).

Although ferritin is a protein that mainly stores and transports iron, it is also capable of extending these properties to other heavy metals. Ferritin's complexing properties with regard to trace metals have been examined in numerous studies. Kumar & Prasad (1999) elucidated the complexing properties of phytoferritin isolated from black gram beans (*Vigna mungo*) with respect to cadmium ($^{109}$Cd). Sczekan & Joshi (1989) did likewise with phytoferritin isolated from soya beans (*Glycine max*) with respect to $Cd^{2+}$, $Zn^{2+}$, $Al^{2+}$ and $Be^{2+}$ ions. Ferritin isolated from horse spleen displayed complexing properties towards zinc $Zn^{2+}$ and beryllium $Be^{2+}$ (Price & Joshi 1982, Price & Joshi 1983), and also $Cd^{2+}$, $Mn^{2+}$ and $Tb^{3+}$ ions (Wardeska et al. 1986).

The aim of the present work was to isolate ferritin from the soft tissues of the Baltic blue mussel (*Mytilus trossulus*), to purify it, and to examine its properties.

## 2. Material and methods

### 2.1. Sampling

Baltic mussels *Mytilus trossulus* were sampled during research cruises of the Department of Marine Chemistry and Biochemistry of IO PAS on r/v 'Oceania'. They were collected with a drag net in the autumn-winter and spring-summer seasons from the Gulf of Gdańsk (D1 – Mechelinki, D2 – Gdynia, D3 – Sopot, D4 – Hel) and in the open Baltic off the northern coast of the Hel Peninsula (D5). Mussels were also collected by a diver from a number of wrecks lying in the Gulf of Gdańsk – (W1 – the 'Groźny', W2 – the 'Delfin', W3 – a 'German gunboat', W4 – the 'Szmaragd') – sites where the concentrations of iron and other trace metals in the sea water are reported to be high. The location of the sampling sites is shown on the map in Figure 1.

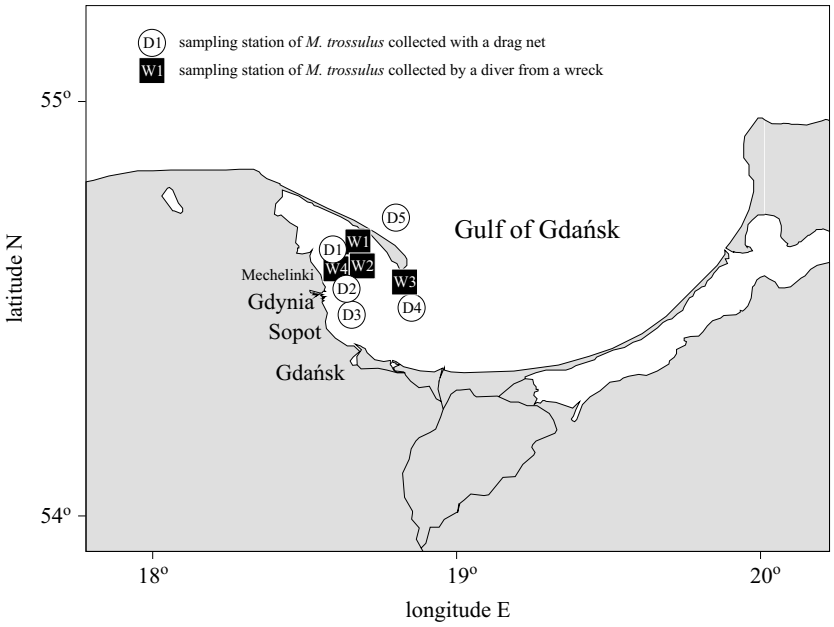



**Figure 1.** Sampling stations of mussels *Mytilus trossulus*

After collection, 50–60 mussels 35–40 mm in length were selected from each site for further processing. The soft tissues were then removed from the shells, and the hepatopancreas and gills dissected out. The residual soft tissue was referred to as 'residue'. The dissected internal organs and the residual soft tissues were mixed and divided into two subsamples, then

frozen at −80°C until further analysis. The next step was to isolate and purify the ferritin from these organs and tissues.

### 2.2. Ferritin isolation

The ferritin was salted out of the homogenised and defrosted organ samples with ammonium sulphate (Korcz & Twardowski 1992a, Geetha & Deshpande 1999). This protein was isolated in the following steps: homogenisation of the biological material in a buffer (50 $cm^3$ 1 M Tris-HCl, pH 8.0; 30 $cm^3$ 1 M NaCl); first centrifugation (5000 × g, +6°C, 60 min., Beckman type GS-6R centrifuge); precipitation of proteins by salting out the supernatant with ammonium sulphate [$(NH_4)_2SO_4$; 120 min., 75% solution saturation]; second centrifugation (5000 × g, +6°C, 60 min.); separation of ferritin proteins from the precipitate (dissolved in buffer) obtained, after centrifugation, by precipitation of the proteins decomposing at high temperatures and in an acid medium (the temperature was raised to +70°C for 15 min.; the pH was reduced to 4.5–5.0 by acidification); third centrifugation (5000 × g, +6°C, 30 min.); precipitation of ferritin proteins by salting out the supernatant with ammonium sulphate (120 min., 60% solution saturation); fourth centrifugation (5000 × g, +6°C, 30 min.); repeated precipitation of ferritin proteins by salting out the precipitate (dissolved in buffer) with ammonium sulphate (120 min., 50% solution saturation); the final centrifugation (5000 × g, +6°C, 30 min.). Suspended in the precipitate obtained after the final centrifugation, the ferritin proteins were dissolved in buffer and the solution preserved by freezing. These preparations constituted the starting material for the further purification of ferritin.

### 2.3. Ferritin purification

The ferritin proteins isolated from the soft tissues of mussels were purified by multiple ultracentrifugation using an Optima L-90K ultracentrifuge (Beckman, Gdańsk University). In this method, these proteins were subjected to multiple centrifugation at a velocity of 100 000 × g (+4°C, 120 min.). The supernatant thereby obtained was discarded, while the pellet was again centrifuged (5000 × g, +6°C, 60 min.), after which the precipitate was discarded. The supernatant was further purified by successive ultracentrifugation (100 000 × g; +4°C, 120 min.) and centrifugation (5000 × g; +6°C, 60 min.). This process was repeated four times. The supernatant obtained after the final centrifugation was the starting preparation of isolated and purified ferritin for further analysis.

## 3. Analysis

### 3.1. Protein content

The total protein content in the raw extracts and in the homogenates containing purified ferritin isolated from the mussels was determined spectrophotometrically after Lowry et al. (1951), on the basis of standard curves plotted by regression analysis for bovine serum albumin (BSA) and for horse spleen ferritin (HSF). Three replicates for each sample were made in order to obtain statistical data.

The standard curves were plotted for a range of 5–100 $\mu$g protein in the sample. Absorption was measured on a BECKMAN DU-62 ($\lambda = 650$ nm) spectrophotometer.

### 3.2. PAGE-SDS electrophoresis and transfer to a PVDF membrane

Ferritin was identified in the above homogenates by electrophoresis in 12% polyacrylamide gel (PAGE) in the presence of SDS with respect to the protein standards (Low Molecular Range; Merck) and to the ferritin standards isolated from HSF (Sigma). The replicas of the protein profiles from the PAGEs were then transferred to a PVDF membrane. The gel and PVDF membrane were stained with Coomassie Brilliant Blue.

### 3.3. Heavy metal content

Cd and Fe concentrations in the homogenates containing purified ferritin isolated from the soft tissues of Baltic mussels were measured in a Video 11E atomic absorption spectrophotometer (Thermo Jarrell Ash) after samples had been wet-digested with $HNO_3/HClO_4$. The concentrations of these metals were also determined in the soft tissues of the mussels.

## 4. Results and discussion

The method of isolating proteins from the soft tissues of the Baltic mussel (*Mytilus trossulus*) applied in this work yielded raw extracts containing a protein with the properties of ferritin, which resisted denaturisation both at a high temperature (+70°C) and in an acidic environment (pH 4.5). Purification of these extracts by multiple ultracentrifugation yielded homogenates containing preparative quantities of ferritin.

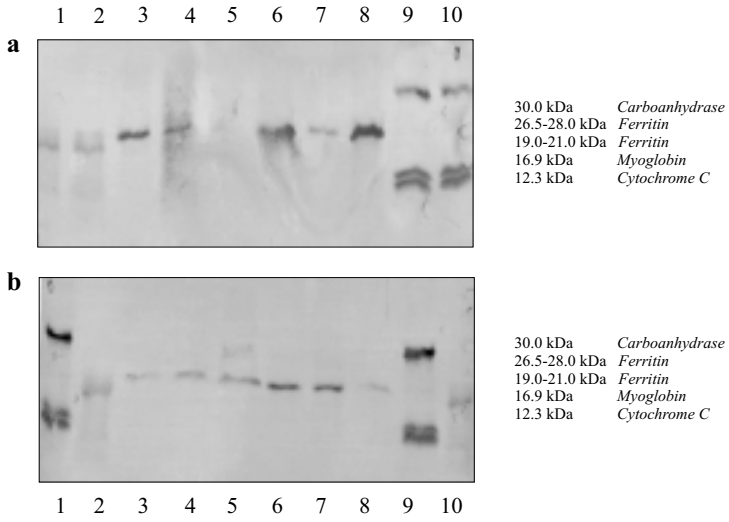
Table 1 gives the average (from three replicates of two subsamples) concentrations of these ferritin-like proteins in the raw extracts (after isolation and before purification) in mg per g wet weight (mg $g^{-1}$ w.w.) and the average concentrations of ferritin in the homogenates (after purification) in $\mu$g per g wet weight ($\mu$g $g^{-1}$ w.w.).

**Table 1.** Mean concentrations (± SD) of ferritin-like proteins in raw extracts following isolation from the soft tissues of *Mytilus trossulus* and in extracts following purification (Lowry's method, abs. $\lambda = 650$ nm)

| Mussel sampling station | FERRITIN-LIKE PROTEINS CONCENTRATION in raw extracts following isolation [mg $g^{-1}$ w.w.] | | | FERRITIN CONCENTRATION in extracts following purification [$\mu$g $g^{-1}$ w.w.] | | |
|---|---|---|---|---|---|---|
| | *Hpt | G | R | Hpt | G | R |
| D1 | 2.58±0.19 | 2.63±0.14 | 1.23±0.11 | 23.24±2.15 | 14.78±1.94 | 10.15±1.68 |
| D2 | 2.23±0.15 | 1.85±0.11 | 0.36±0.05 | 24.04±1.98 | 12.71±2.11 | 10.84±1.46 |
| D3 | 2.76±0.18 | 1.98±0.10 | 1.54±0.12 | 48.74±5.01 | 26.57±2.55 | 14.62±1.33 |
| D4 | 1.99±0.16 | 1.48±0.08 | 1.55±0.14 | 21.99±2.65 | 12.23±1.62 | 16.24±1.75 |
| D5 | 1.72±0.21 | 1.71±0.12 | 0.64±0.09 | 38.20±4.12 | 31.97±3.88 | 12.62±1.52 |
| W1 | 2.28±0.18 | 2.08±0.20 | 1.91±0.15 | 36.70±3.29 | 33.89±4.03 | 16.01±1.66 |
| W2 | 2.04±0.14 | 1.77±0.13 | 1.72±0.16 | 17.13±2.32 | 14.71±1.12 | 12.51±1.37 |
| W3 | 2.59±0.21 | 1.73±0.16 | 1.38±0.11 | 27.34±3.24 | 12.31±1.35 | 7.63±1.51 |
| W4 | 1.87±0.17 | 1.92±0.16 | 2.06±0.18 | 24.04±1.89 | 22.77±2.74 | 20.84±2.53 |

*Hpt – hepatopancreas; G – gills; R – soft tissue residue.

Among the analysed internal organs of the mussels, regardless of where these had been sampled, the hepatopancreas contained the largest amounts of ferritin, the gills less, and the soft tissue residue least of all. In the protein samples isolated from the hepatopancreas of Baltic mussels from the Gulf of Gdańsk (D1, D2, D3, D4), the ferritin content ranged from 21.99 to 48.74 $\mu$g $g^{-1}$ w.w. (1.1–1.8% of the proteins in this organ after isolation and before purification – $B_0$), that of the gills was 12.23–26.57 $\mu$g $g^{-1}$ w.w. (0.8–1.3% $B_0$), and that of the soft tissue residue was 10.15–16.24 $\mu$g $g^{-1}$ w.w. (0.8–1.1% $B_0$). The soft tissues of mussels from the open waters of the southern Baltic (D5) displayed a ferritin content ranging from 12.62 $\mu$g $g^{-1}$ w.w. (soft tissue residue) to 38.20 $\mu$g $g^{-1}$ w.w. (hepatopancreas), which was 2.0 and 2.2% of the isolated protein before purification ($B_0$) respectively. The gills contained 31.97 $\mu$g $g^{-1}$ w.w. ferritin (1.9% $B_0$). The soft tissues of mussels collected from the wrecks (W1, W2, W3, W4) contained similar amounts of ferritin: hepatopancreas – 17.13–36.70 $\mu$g $g^{-1}$ w.w. (0.8–1.6% $B_0$), gills – 12.31–33.89 $\mu$g $g^{-1}$ w.w. (0.7–1.6% $B_0$); residue 7.63–20.84 $\mu$g $g^{-1}$ w.w. (0.6–1.0% $B_0$). As far as the sampling location is concerned, the highest ferritin contents were found in the mussels sampled by drag net off Sopot (D3) and in those sampled from the wrecks of the 'Groźny' off Jastarnia (W1) and the 'Szmaragd' off Gdynia (W4).




**a**

| | |
|---|---|
| 1, 2 | Horse spleen ferritin standard (HSF; Sigma) |
| 3 | Ferritin isolated from gills of *M. trossulus* (D5) |
| 4 | Ferritin isolated from heaptopancreas of *M. trossulus* (D5) |
| 5 | Ferritin isolated from hepatopancreas of *M. trossulus* (D4) |
| 6 | Ferritin isolated from hepatopancreas of *M. trossulus* (D3) |
| 7 | Ferritin isolated from hepatopancreas of *M. trossulus* (D2) |
| 8 | Ferritin isolated from hepatopancreas of *M. trossulus* (D1) |
| 9, 10 | SDS-PAGE Standards (Low Molecular Range; Merck) |

**b**

| | |
|---|---|
| 1, 9 | SDS-PAGE Standards (Low Molecular Range; Merck) |
| 2, 10 | Horse spleen ferritin standard (HSF; Sigma) |
| 3 | Ferritin isolated from hepato-pancreas of *M. trossulus* (W2) |
| 4 | Ferritin isolated from gills of *M. trossulus* (W2) |
| 5 | Ferritin isolated from 'the rest' *M. trossulus* (W2) |
| 6 | Ferritin isolated from hepato-pancreas of *M. trossulus* (W3) |
| 7 | Ferritin isolated from gills *M. trossulus* (W3) |
| 8 | Ferritin isolated from gills of *M. trossulus* (W1) |

**Figure 2.** The pattern of protein migration during electrophoresis in 12% polyacrylamide gel in the presence of SDS (PAGE-SDS) and transfer to a PVDF membrane; staining with Coomassie Brilliant Blue

Multiple ultracentrifugation is suggested in the literature (Korcz & Twardowski 1992a, Kakuta et al. 1997, Kumar & Prasad 1999, Rahman et al. 1999, Suryakala & Deshpande 1999) as a technique enabling high-molecular-weight proteins such as ferritin (molecular mass 400 000–500 000 Da) to be isolated in a homogeneous state. Applied to the soft tissues of the mussel *M. trossulus*, this technique yielded homogenates containing preparative quantities of purified ferritin. These homogenates were compared with a ferritin standard isolated from horse spleen and with other protein standards by polyacrylamide gel electrophoresis in the presence of SDS

(PAGE-SDS). Obtained during electrophoresis and transfer to a PVDF membrane, the migration pattern of the proteins isolated from the mussel soft tissues (Figure 2) indicates the presence of proteins with plant ferritin properties, for which the molecular mass of the sub-units in the electrophoretic picture is 26.5–28.0 kDa (Theil 1987).

Table 2 shows the contents of heavy metals (iron and cadmium) in the mussel soft tissues prior to ferritin isolation (expressed in $\mu$g of metal per g dry weight) and in homogenates containing purified ferritin (expressed in $\mu$g Fe per mg ferritin and ng Cd per mg ferritin).

The soft tissues of mussels collected from the wrecks – sites where the concentrations of iron and other trace metals in the sea water are likely to be higher – contained larger quantities of Fe and Cd than the soft tissues of mussels collected from the bottom sediments with the drag.

Analysis of the Fe and Cd content in the homogenates containing purified ferritin confirmed that both these heavy metals are bound by this ferritin.

The purified ferritin preparations isolated from the inner organs of *M. trossulus* contained 5.7–36.5 $\mu$g Fe mg$^{-1}$ ferritin and 2.5–17.6 ng Cd mg$^{-1}$ ferritin. Regardless of sampling location, the largest quantities of both metals were found in ferritin isolated from the hepatopancreas of the mussels (22.6–36.5 $\mu$g Fe mg$^{-1}$ ferritin; 7.4–17.6 ng Cd mg$^{-1}$ ferritin), less was found in the gills (18.1–23.5 $\mu$g Fe mg$^{-1}$ ferritin; 6.9–14.2 ng Cd mg$^{-1}$ ferritin), and least of all in the soft tissue residue (5.7–18.0 $\mu$g Fe mg$^{-1}$ ferritin; 2.5–11.8 ng Cd mg$^{-1}$ ferritin).

Iron can be both in the protein subunits that make up the ferritin cage and within the mineral core inside the cage (Theil et al. 2000). Other metals, such as cadmium, are more likely to be bound to the protein cage itself (Ueno et al. 2009). In the case when only a small amount of iron is available (D1: $270.1 \pm 17.1$ $\mu$g Fe g$^{-1}$ d.w. of the hepatopancreas, $198.2 \pm 13.1$ $\mu$g Fe g$^{-1}$ d.w. of the gills and $176.3 \pm 8.2$ $\mu$g Fe g$^{-1}$ d.w. of the soft tissue residue), the amount of iron found in purified ferritin preparations is relatively low in the hepatopancreas of D1 ($24.7 \pm 1.5$ $\mu$g Fe mg$^{-1}$ ferritin) and even lower in other parts of the mollusc ($21.2 \pm 1.6$ $\mu$g Fe mg$^{-1}$ ferritin in the gills and $8.3 \pm 0.5$ $\mu$g Fe mg$^{-1}$ ferritin in the soft tissue residue). This indicates that the animal is mildly iron deficient, and that when more iron is available, as in the case of W1 ($661.7 \pm 31.2$ $\mu$g Fe g$^{-1}$ d.w. of the hepatopancreas, $747.4 \pm 47.2$ $\mu$g Fe g$^{-1}$ d.w. of the gills and $329.7 \pm 27.6$ $\mu$g Fe g$^{-1}$ d.w. of the soft tissue residue), the average iron content of ferritin remains similar ($27.9 \pm 1.9$ $\mu$g Fe mg$^{-1}$ ferritin in the hepatopancreas, $23.1 \pm 1.7$ $\mu$g Fe mg$^{-1}$ ferritin in the gills and $9.7 \pm 0.6$ $\mu$g Fe mg$^{-1}$ ferritin in the soft tissue residue), suggesting that it is used to synthesise iron proteins rather than

**Table 2.** Mean concentrations (± SD) of heavy metals (Fe and Cd) in the soft tissues of *Mytilus trossulus* and in ferritin isolated from the soft tissues of *M. trossulus*

| Mussel sampling station | CONCENTRATION OF IRON IN SOFT TISSUES in raw extracts following isolation [$\mu$g Fe g$^{-1}$ d.w.] | | | CONCENTRATION OF IRON IN FERRITIN in extracts following purification [$\mu$g Fe mg$^{-1}$ ferritin] | | |
|---|---|---|---|---|---|---|
| | *Hpt | G | R | Hpt | G | R |
| D1 | 270.1±17.1 | 198.2±13.1 | 176.3± 8.2 | 24.7±1.5 | 21.2±1.6 | 8.3±0.5 |
| D2 | 277.6±16.2 | 231.2±21.2 | 202.9±16.8 | 23.9±2.0 | 23.5±2.1 | 9.8±0.4 |
| D3 | 433.8±25.9 | 394.8±27.5 | 236.2±18.4 | 36.5±2.8 | 22.7±1.8 | 9.9±0.5 |
| D4 | 220.7±18.4 | 374.3±30.4 | 171.1±12.3 | 25.1±1.3 | 22.6±1.5 | 7.3±0.3 |
| D5 | 206.6±17.3 | 171.2± 9.2 | 145.5±13.8 | 22.6±2.1 | 18.1±1.1 | 5.7±0.4 |
| W1 | 661.7±31.2 | 747.4±47.2 | 329.7±27.6 | 27.9±1.9 | 23.1±1.7 | 9.7±0.6 |
| W2 | 658.1±29.8 | 632.6±26.6 | 509.1±39.1 | 24.4±1.6 | 21.6±1.9 | 10.7±0.8 |
| W3 | 581.3±27.2 | 567.0±23.4 | 283.6±20.2 | 23.6±2.0 | 20.2±1.4 | 10.3±0.7 |
| W4 | 557.5±30.9 | 613.3±46.1 | 397.2±36.1 | 27.9±2.2 | 21.7±1.3 | 18.0±1.2 |
| Mussel sampling station | CONCENTRATION OF CADMIUM IN SOFT TISSUES in raw extracts following isolation [$\mu$g Cd g$^{-1}$ d.w.] | | | CONCENTRATION OF CADMIUM IN FERRITIN in extracts following purification [ng Cd mg$^{-1}$ ferritin] | | |
| | Hpt | G | R | Hpt | G | R |
| D1 | 3.9±0.1 | 3.6±0.1 | 3.4±0.2 | 11.6±0.9 | 8.6±0.3 | 3.6±0.1 |
| D2 | 4.9±0.2 | 3.4±0.1 | 4.1±0.2 | 11.1±0.8 | 11.9±1.2 | 6.1±0.2 |
| D3 | 5.4±0.2 | 4.3±0.2 | 3.8±0.1 | 14.2±1.1 | 13.3±1.1 | 8.2±0.4 |
| D4 | 4.7±0.3 | 4.1±0.1 | 4.7±0.2 | 10.3±0.9 | 9.2±0.5 | 2.5±0.1 |
| D5 | 3.7±0.2 | 2.5±0.1 | 2.7±0.1 | 7.4±0.4 | 6.9±0.4 | 5.4±0.3 |
| W1 | 7.4±0.5 | 7.9±0.4 | 4.3±0.2 | 17.6±1.2 | 14.2±0.9 | 4.9±0.3 |
| W2 | 4.7±0.3 | 7.1±0.3 | 3.9±0.2 | 13.6±0.8 | 8.0±0.3 | 5.1±0.2 |
| W3 | 6.9±0.5 | 10.7±0.6 | 5.8±0.3 | 13.6±0.7 | 9.2±0.7 | 7.2±0.4 |
| W4 | 5.1±0.3 | 6.3±0.3 | 3.8±0.2 | 16.7±1.3 | 14.4±1.2 | 11.8±1.2 |

*Hpt – hepatopancreas; G – gills; R – residue.

being stored in ferritin (Chasteen 1998). In the case of cadmium, the ferritin isolated from the soft tissues of mussels collected from wrecks contained higher quantities of the metal than that isolated from the soft tissues of mussels collected from wrecks contained higher quantities of the metal than that isolated from the soft tissues of mussels collected from the sediment with the drag. This suggests that cadmium is stored in ferritin,

but in much smaller quantities, as in the case of metallothioneins, which play the main part in Cd binding proteins in mussels and oysters (Nair & Robinson 2000, Geret & Cosson 2002, Choi et al. 2008).

## 5. Conclusions

The results of this study indicate that ferritin is one of the proteins isolated from the soft tissues of the Baltic mussel *Mytilus trossulus*. It is resistant to elevated temperatures (+70°C) and acidic conditions (pH 4.5), and its electrophoretic pattern is characterised by sub-units in the range from 28 to 26.5 kDa.

The largest amounts of ferritin are contained in the hepatopancreas, less in the gills and least of all in the soft tissue residue.

There were differences in the ferritin content with respect to sampling location: the highest amounts were recorded in mussels sampled by drag net off Sopot (D3) and in those collected from the wreck of the 'Groźny' – (W1) (off Jastarnia – Gulf of Gdańsk) and the 'Szmaragd' – (W4) near Gdynia. The ferritin isolated from the soft tissues of these particular animals also contained the greatest amounts of iron and cadmium.

Determinations of the heavy metal content (Fe and Cd) in homogenates containing purified ferritin confirm the fact that both iron and cadmium are bound by ferritin. Ferritin isolated from the soft tissues of mussels collected from wrecks contained quantities of iron at the same levels, and slightly higher quantities of cadmium, than the ferritin isolated from the soft tissues of mussels collected from the sediment with the drag.

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