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Purification and biochemical characterization of a cadmium metallothionein from the digestive gland of the Antarctic scallop *Adamussium colbecki* (Smith, 1902)

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Abstract A cadmium-binding protein was purified from the digestive gland of the Antarctic scallop, *Adamussium colbecki*, and biochemically characterized. Purification procedures included gel permeation and anion exchange chromatography, followed by preparative polyacrylamide gel electrophoresis. Our results demonstrate that the *A. colbecki* cadmium-binding protein has the general properties of metallothioneins: low molecular weight of about 10 kDa, spectroscopic features typical of cadmium thiolate clusters and high metal (cadmium) content. Analysis of amino acid composition reveals the absence of aromatic amino acids, histidine, methionine and arginine. Asparagine and glutamine are also absent. The *A. colbecki* metallothionein shows high levels of glycine (14%), aspartic acid (14%), glutamic acid (11%) and a low lysine content (4%); the *A. colbecki* metallothionein shows a lower cysteine content (12%) compared to other metallothioneins (17–30%) purified from both vertebrate and invertebrate organisms. The presence of a metallothionein in the digestive gland of *A. colbecki* suggests that in cold-ocean-adapted molluscs the heavy metal homeostasis mechanisms may have evolved similarly to those of organisms living in temperate marine environments, although the *A. colbecki* cadmium-binding protein shows a typical amino acidic composition that might reflect a peculiar physiological role.

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Introduction

In recent years many authors have focused their attention on the study of biochemical and physiological processes in Antarctic organisms which are evolutionarily adapted to peculiar environmental conditions such as low temperature, high dissolved oxygen concentrations and a characteristic photoperiod. Among these organisms the Antarctic scallop, *Adamussium colbecki* (Smith 1902), has received increasing attention, since this lamellibranch mollusc is particularly representative of the Antarctic epibenthonic fauna. In fact, *A. colbecki* is an endemic species which represents the only survivor of a diversified Pectinidae fauna living in the early Quaternary. Moreover, it shows a circumantarctic and wide bathymetric distribution (between 4 and 805 m) (Dell 1974). At McMurdo Sound (Ross Sea) this pectinid is present at especially high levels of density and biomass, reaching 2 kg/m^2 (Stockton 1984).

Previous studies on heavy metal cation homeostasis in *A. colbecki* have demonstrated that, since at Terranova Bay (Ross Sea) cadmium is present at relatively high concentrations (Capodaglio et al. 1991), this mollusc accumulates extremely high Cd concentrations into its tissues (Mauri et al. 1990; Berkman and Nigro 1992; Viarengo et al. 1993). We have previously shown that in *A. colbecki* digestive gland cells, cadmium is found in both the particulate and the cytosolic fractions, mostly bound to a soluble, low molecular weight, sulphydryl-rich protein which may belong to the metallothionein superfamily (Viarengo et al. 1993). Metallothioneins are soluble, low molecular weight, cysteine-rich proteins, which have a high affinity and binding capacity for heavy metal cations. A remarkable property of metallothioneins is their inducibility: heavy metal ions accumulated within the cells cause metalloprotein neosynthesis by enhancing metallothionein gene transcription (Squibb and Cousin 1977; Viarengo and Nott 1993). Therefore, these proteins play a role in regulating the cytosolic concentration of zinc and copper ions to

physiological levels and in detoxifying noxious metal cations, such as cadmium and mercury, which have penetrated the cells (Viarengo and Nott 1993).

Metallothioneins are ubiquitous proteins that occur in most phyla with a relatively high phylogenetic conservation of cysteine residues. Although metallothioneins have been widely investigated in vertebrates (Suzuki 1991; Kägi 1993) and invertebrates living in temperate environments (Roesijadi and Fowler 1991; Roesijadi 1992; Kägi 1993; Viarengo and Nott 1993; Brouwer et al. 1995), few data have been reported concerning these metalloproteins in Antarctic organisms. Evidence for the presence of metallothioneins in Antarctic organisms has been reported for fish belonging to the Nothothenioidea suborder (sequences are available at European Molecular Biology Laboratory (EMBL) databases as both protein and DNA sources) (Scudiero et al. 1997a; Carginale et al. 1998) and for the sea urchin, *Sterechinus neumayeri* (Scudiero et al. 1997b).

To clarify the nature of the cadmium-rich protein present in the cytosol of the digestive gland cells of *A. colbecki*, this protein was purified to homogeneity by a three-step chromatographic/electrophoretic procedure and biochemically characterized. The amino acid composition was compared with that of other metallothioneins extracted by organisms living in temperate and Antarctic waters.

Finally, we investigated the inducibility of metallothioneins in the tissues of *A. colbecki* exposed to sublethal concentrations of heavy metal, to establish if this protein may be used as a biomarker of exposure to heavy metal pollution along the Antarctic coastal areas.

Materials and methods

Animals and cadmium treatments

Specimens of the Antarctic scallop, *A. colbecki* (Smith, 1902), 6 cm shell length, were obtained from Terranova Bay (74°S, 163°E; Antarctic Ross Sea) during the Italian expedition to the Antarctic region in the austral summer (January 1990). For metallothionein purification, the digestive glands from eight to ten animals were dissected, pooled and kept at −70 °C.

For metal exposure experiments, specimens of *A. colbecki* were kept for 7 days in aquaria containing filtered, aerated seawater (1 l per animal) at −1 °C. The seawater was changed daily. Cadmium was added daily as $CdCl_2$, at the concentration of 200 μg/l. A group of control non-exposed molluscs was kept under similar conditions (except for cadmium treatment). The tissues from pools of eight to ten individuals were dissected and immediately frozen at −70 °C.

Specimens of *Mytilus galloprovincialis* were purchased from the farm of La Spezia (Italy) and kept in aquaria containing artificial, aerated seawater (1 l per animal) (Viarengo et al. 1985). The seawater was changed daily. Cadmium was added daily for 7 days in the form of $CdCl_2$ at the concentration of 200 μg/l. A group of non-exposed control animals was kept under similar conditions.

Purification of Cd-binding proteins

The digestive glands from pools of *A. colbecki* were homogenized in three volumes of 20 mM Tris-HCl buffer, pH 8.6, containing 0.5 M sucrose, 6 μM leupeptin, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM dithiothreitol (DTT), as an antiproteolytic and antioxidant mixture. The homogenate was centrifuged at × 30,000 *g* for 1 h. The supernatant, containing the metalloprotein, was partially purified by acetonic fractionation (45–80%) as previously described (Viarengo et al. 1984). Briefly, cold acetone (−20 °C) was added to the supernatant to a final concentration of 45%. The sample was maintained at 4 °C for 30 min under magnetic stirring and then centrifuged at ×14,500 *g* for 10 min. The pellet was discarded and the acetone concentration of the supernatant was raised to 80%. The preparation was maintained at 4 °C for 40 min under magnetic stirring and centrifuged again at ×14,500 *g* for 10 min. The 80% acetonic pellet was resuspended in 10 mM Tris-HCl buffer, pH 8.6, containing 0.01% NaN_3 and 1 mM DTT. The sample (3 ml) was applied to a 4 × 85 cm Sephadex G-75 column calibrated with standard proteins (bovine serum albumine, carbonic anhydrase, cytochrome c, aprotinin) as molecular weight markers, and eluted at 60 ml/h with the same buffer. During chromatographic separation, the eluate was analysed for absorbance with an LKB UV detector set at 280 nm. The fractions (12 ml) were analysed for Cd content by atomic absorbtion spectroscopy (AAS). Further purification of the 10–25 kDa molecular weight cadmium-containing proteins was carried out on a diethylaminoethanol (DEAE)-cellulose ion exchange column (Millipore DEAE MemSep 1010). Cadmium-containing fractions were pooled and applied directly onto the DEAE column. The column was equilibrated with 20 mM Tris-HCl buffer, pH 8.6, containing 1 mM DTT and 0.01% NaN_3. A linear gradient of 150 ml 20–400 mM Tris-HCl buffer, pH 8.6, containing 1 mM DTT and 0.01% NaN_3 was applied to the column, followed by 45 ml 400 mM Tris-HCl buffer, pH 8.6, containing 1 mM DTT and 0.01% NaN_3. Absorbance at 254 nm was measured utilizing an LKB UV-diode array detector. Fractions were analysed for cadmium content by AAS. The peak-fractions containing cadmium were pooled and proteins precipitated by adding cold ethanol (−20 °C)/chloroform to a final concentration of 87%/1% as described by Viarengo et al. (1997). The pellet was resuspended in deionized H_2O containing 1 mM DTT and then incubated at 60 °C for 10 min, followed by purification on a 10% polyacrylamide gel in the presence of 8 M urea, according to the conditions described by Perrie and Perry (1970), using a BRL (Bethesda Research Laboratories) preparative gel electrophoresis apparatus, set at 270 V. Electrophoresis was performed in 20 mM Tris-HCl, pH 8.6, 120 mM glycine using 100 mM Tris-HCl, pH 8.6, as an eluting buffer at a flow rate of 30 ml/h. During separation, the absorbance was measured at 254 nm. Fractions of 2 ml were collected and analysed for cadmium content by AAS. The isolated cadmium-rich protein fraction was analysed by polyacrylamide gel electrophoresis (PAGE) (10% gel), both under the denaturing conditions already described (Perrie and Perry 1970) and by SDS-PAGE (Laemmli 1970).

Amino acid analysis

Amino acid analysis was performed on samples hydrolyzed in vacuum in boiling HCl for 24 h. Performic acid oxidation was carried out before hydrolysis, according to the method of Hirs (1967).

Metal determination

The metal content was determined by AAS, as described by Mazzucotelli et al. (1976).

Metallothionein evaluation

The metallothionein content in the two molluscs was evaluated as previously described by Viarengo et al. (1997).

Materials

Sephadex G-75 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden) and the DEAE MemSep 1010 column was obtained from Millipore (Milan, Italy). All the electrophoresis reagents were from Biorad (Richmond, Calif.). DTT, leupeptin, PMSF, standard Cd,Zn-thionein from rabbit liver and chromatographic molecular weight markers were purchased from Sigma (Milan, Italy). All other reagents were of analytical grade.

Results

Metallothionein purification

The purification of metallothionein was performed under reducing conditions to prevent oxidation of sulphydrylic groups of cysteine residues and to avoid partial loss of metals bound to the metalloproteins (Viarengo et al. 1984). The acetonic fractionation applied to the ×30,000 *g* supernatant containing metallothioneins allows the separation of highly stable low molecular weight protein from high molecular weight proteins, denaturated by organic solvents. In Fig. 1 is shown the protein elution profile (absorbance at 280 nm) of the resuspended pellet containing metallothioneins obtained after the 80% acetonic fractionation, separated by Sephadex G-75 chromatography. It shows an initial small peak (fractions 20–30), representing a minimal amount of residual, high molecular weight proteins (M_r > 50,000) present in the extract, and a low molecular weight peak (M_r < 5,000) (fractions 70–90), probably representing amino acids, glutathione (GSH), nucleotides, etc. Cadmium, as evaluated by AAS, was entirely found in a double peak, bound to proteins having apparent molecular weights ranging from 10 to 25 kDa and showing a minimum absorbance at 280 nm. Such an elution profile resembles that obtained by different authors using the Sephadex G-75 permeation chromatography step for purification of metallothioneins from tissues of other molluscs (Frazier et al. 1985; Mackay et al. 1993; Wang et al. 1994). The Cd-containing fractions belonging to the 10- to 25-kDa molecular weight range were pooled and subjected to further purification on a DEAE-cellulose ion exchange chromatography column. Eluted fractions (Fig. 2) were monitored at 254 nm for cadmium mercaptide bonds, collected and analysed for Cd content by AAS, resolving two Cd-containing peaks. The two peaks eluted at Tris-HCl concentrations of 170 mM and 350 mM, respectively. The first Cd-containing peak appeared to be composed of two partially overlapping peaks showing an absorbtion spectrum typical of Cd-thionein, while the second Cd-containing peak (eluting at a Tris-HCl concentration of 350 mM) showed an absorbtion spectrum different from that of a Cd-thionein (data not shown). Therefore, only the main Cd-containing peak eluting at a Tris-HCl concentration of 170 mM was subjected to further purification by preparative polyacrylamide gel electrophoresis, according to Perrie and Perry (1970). Eluted fractions were monitored at 254 nm and analysed for Cd content by AAS. Two peaks with proteins containing high cadmium content were resolved (Fig. 3). The main Cd-containing peak corresponding to fractions 38–45 showed a characteristic absorbance spectrum of Cd-thioneins in the 220 to 280 nm region, with a broad shoulder at 254 nm, typical of metallothionein cadmium thiolate clusters. Moreover, the apoprotein spectrum analysis of the Cd-rich fractions after acidification with HCl at pH 1, revealed the lack of the shoulder of absorbance at 254 nm, which may be related to disruption of Cd-thiolate clusters (Fig. 4).

The Cd-containing peak corresponding to fractions 38–45 was subjected to analytical polyacrylamide gel electrophoresis under urea denaturating conditions (Perrie and Perry 1970). The *A. colbecki* metallothioneins run as a single band (Viarengo et al. 1997), with an electrophoretic mobility typically found for proteins with a high charge/mass ratio such as metallothioneins, and similar to that of the standard Cd,Zn-thionein purified from rabbit liver (Fig. 5).

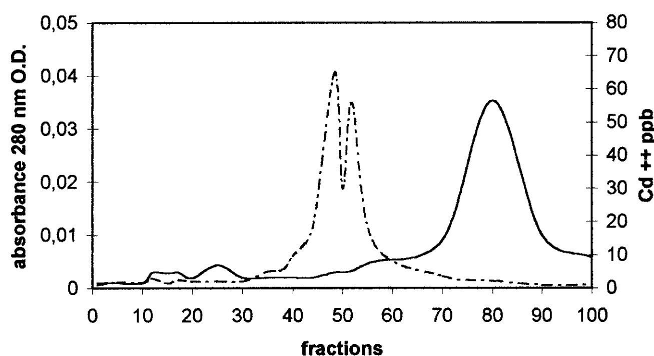

Fig. 1 Elution profile of Sephadex G-75 chromatography of acetonic extracts obtained from the digestive gland of the Antarctic scallop, *Adamussium colbecki* (— absorbance at 280 nm; – · – ppb Cd^{++})

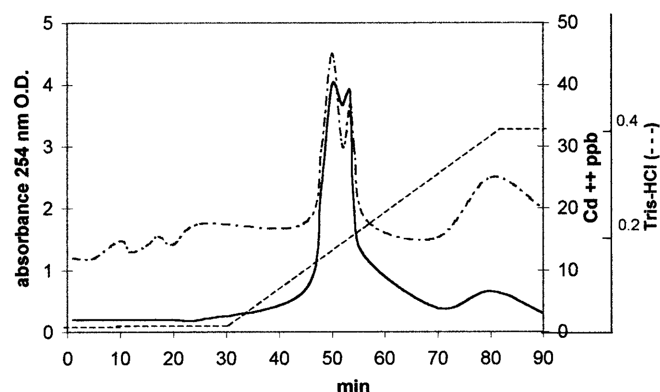

Fig. 2 Elution profile (optical density) of ion exchange chromatography on DEAE-cellulose column of the Cd-containing fractions obtained after Sephadex G-75 separation. The linear gradient (- - -) was developed with 20–400 mM Tris-HCl pH 8.6, containing 1 mM DTT and 0.01% NaN_3 (— absorbance at 254 nm; – · – ppb Cd^{++})

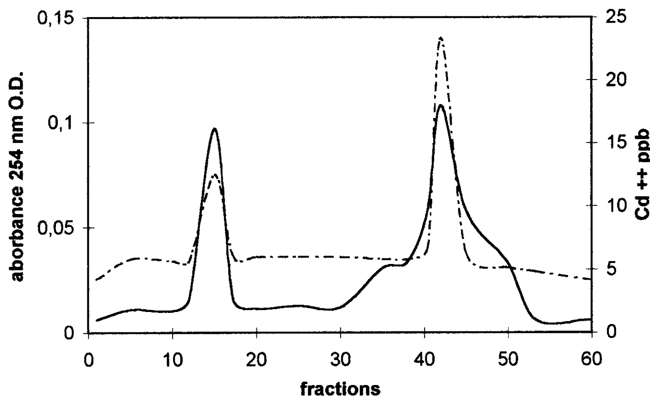

Fig. 3 Elution profile of preparative electrophoresis on a 10% polyacrylamide gel in the presence of 8 M urea of the Cd-containing fractions obtained after ion exchange separation. The Cd content of the collected fractions was evaluated by AAS (— absorbance at 254 nm; – · – ppb Cd^{++})

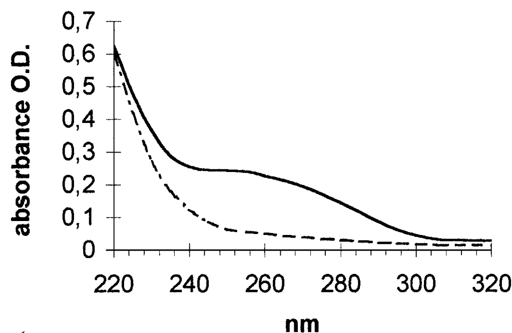

Fig. 4 Absorbance spectrum of the *Adamussium colbecki* Cd-thionein (—) and of the apoprotein prepared by HCl treatment (pH 1) (– · –)

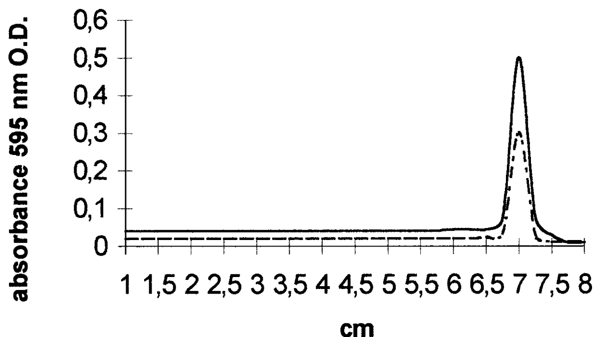

Fig. 5 Densitometric analysis (optical density) of the analytical electrophoresis of *Adamussium colbecki* Cd-thionein (– · –) obtained by preparative electrophoresis compared with Cd,Zn-thionein from rabbit liver (—). Proteins were stained with coomassie brilliant blue and analysed at 595 nm

Amino acid analysis

According to the biochemical features of metallothioneins, the *A. colbecki* metallothionein amino acid composition revealed the lack of aromatic residues such as phenylalanine and tyrosine. Histidine, arginine and methionine were not detected (Table 1). Interestingly, the protein is also lacking in asparagine and glutamine. The cysteine content was 12%, which is quite low with respect to the 17–30% that is generally found in metallothionein isoforms purified from different organisms (Kägi 1993; Viarengo and Nott 1993). Of additional interest was that the content of the positively charged amino acid, lysine, was only 4%. In contrast, high amounts of glutamic acid (11%), glycine (14%) and aspartic acid (14%) were detected.

Cd inducibility of *A. colbecki* metallothioneins

Previous data from Viarengo et al. (1997) demonstrated that copper exposure can stimulate metallothionein neosynthesis in *A. colbecki* gill cells. To test the inducibility of metallothioneins by cadmium, scallops were exposed to Cd at a concentration of 200 μg $animal^{-1}$ l^{-1} in a 7-day experiment. As a reference experiment, the common mussel, *M. galloprovincialis*, was subjected to the same treatment.

The assessment of metallothionein inducibility by Cd in *A. colbecki* appears of particular interest, considering that Cd is present in the Antarctic marine environment at relatively high concentrations (Capodaglio et al. 1991). For this experiment we exposed the animals to high levels of cadmium, since it had been previously demonstrated that *A. colbecki* accumulates extremely high Cd amounts into its tissues (Viarengo et al. 1993). Moreover, 200 μg Cd/l (1.9 μM) is a high metal concentration, but is appropriate because, although sublethal, it is known to induce metallothionein neosynthesis in mollusc tissues during short time-scale experiments (3–7 days) (Viarengo et al. 1985).

Cadmium exposure, as shown in Fig. 6, elicited a significative increase, up to 2.5-fold, of the metallothionein concentration evaluated as μequivalent sulphydrylic group (SH) of metallothionein/g wet weight in the gills of *A. colbecki* (from 0.19 to 0.46 μequivalent SH of metallothionein/g wet weight). A similar increase in metallothionein content was observed in the gills of *M. galloprovincialis* (from 0.22 μequivalent to 0.49 μequivalent SH of metallothionein/g wet weight).

Discussion

We have purified to homogeneity and calculated the amino acid composition of a novel cadmium-binding protein from the digestive gland cells of the Antarctic scallop, *A. colbecki*. Our results demonstrate that its biochemical and biological features match those of a metallothionein. This Antarctic molluscan metallothionein shows some unique features. The amino acid composition shows that, besides the typical molluscan metallothionein features of a high glycine residue content (14%) and lack of methionine (Viarengo et al. 1984;

Table 1 Comparison of the *Adamussium colbecki* Cd-thionein amino acid composition with that exhibited by different organisms

	Adamussium colbecki Cd-thionein	*Mytilus edulis* Cd-thionein[a]	*Ostrea virginica* Cd-thionein[b]	*Callinectes sapidus* Cd-thionein[c]	*Helix pomatia* Cd-thionein[d]	*Oryctolagus cuniculus* Cd-thionien[e]
Cys	12.0	32.4	28.0	30.5	27.3	32.8
Gly	14.0	16.9	16.0	8.5	15.2	6.6
Asp	14.0	2.8	5.3	1.7	3.0	3.3
Glu	11.0	2.8	4.0	5.1	6.1	1.6
Thr	8.0	7.0	8.0	8.5	7.6	6.6
Ser	8.0	8.4	9.3	8.5	13.6	14.7
Pro	10.0	4.2	5.3	8.5	3.0	3.3
Ala	8.0	4.2	5.3	3.4	4.5	9.8
Val	5.0	4.2	1.3	1.7	–	–
Tyr	–	–	–	–	–	–
Phe	–	–	–	–	–	–
Met	–	–	1.3	1.7	–	1.6
His	–	–	–	–	–	–
Arg	–	1.4	–	1.7	1.5	–
Ile	2.0	2.8	1.3	–	–	1.6
Leu	4.0	–	–	–	–	–
Lys	4.0	8.4	13.3	13.6	13.6	13.1
Asn	–	4.2	1.3	1.7	1.5	3.3
Gln	–	–	–	5.1	3.0	1.6
Trp	–	–	–	–	–	–

[a] Mackay et al. (1993) (Genbank accession no. S39421)
[b] Unger et al. (1991) (Genbank accession no. P23038)
[c] Brouwer et al. (1995) (Genbank accession no. P55949)
[d] Dallinger et al. (1997) (Genbank accession no. P33187)
[e] Hunziker et al. (1995) (Genbank accession no. P11957)

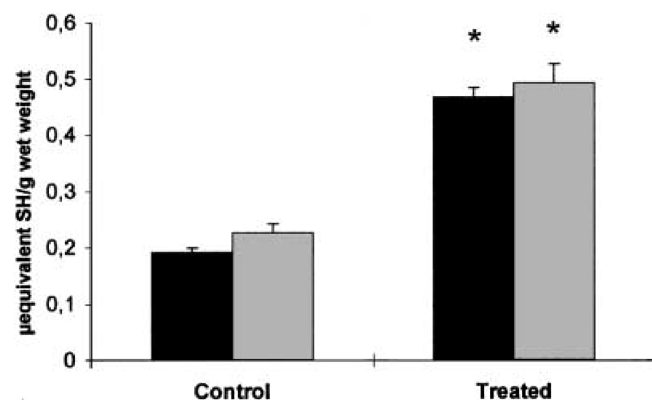

Fig. 6 Sulphydrylic group content of metallothioneins in the gills of control and Cd-exposed (200 µg animal^{-1} for 7 days) specimens of *Adamussium colbecki* (*black shading*) and *Mytilus galloprovincialis* (*grey shading*). Data are the means ± SD of at least six replicates (* significantly different from the control set of samples; $P < 0.01$, Mann-Whitney test)

Frazier et al. 1985; Roesijadi et al. 1989; Mackay et al. 1993), also revealed by our results were a high aspartic and glutamic acid content (14% and 11%, respectively), a low lysine content (4%), and the absence of arginine, which results in a highly negative charge of the *A. colbecki* cadmium metallothionein (Fig. 5). Furthermore, the cysteine content was 12% (of total amino acids), which is quite low with respect to the values found in most metallothioneins extracted from invertebrate and vertebrate organisms (Table 1). Together with its physiological and unusual cadmium content, the amino acid composition seems to be a remarkable feature of this Antarctic metallothionein. A possible explanation of such high binding of cadmium ions is that metallothioneins may exist as specific isoforms dedicated to the binding of specific metal ions. This is the case in the common snail, *Helix pomatia*, for which Dallinger et al. (1997) discovered the presence of two isoforms having different primary structures and a difference in their metal-binding properties: a cadmium-induced Cd-thionein that had a clear role in cadmium detoxification in the gut gland, and a Cu-thionein responsible for the regulation of physiological copper concentrations, which is present within the mantle cells of this Antarctic mollusc. In a similar way, the *A. colbecki* metallothionein amino acid composition might confer a preferential binding capacity for cadmium, a metal that occurs at high concentrations in the digestive gland cells. It is well established that Cd ions are toxic for the cell, due to their ability to react with nucleophilic groups of proteins and nucleic acids (Viarengo and Nott 1993), so that *A. colbecki* may have adapted a specific system for regulating the free cadmium concentration within the cell. Furthermore, it was demonstrated that only a part (30%) of the cadmium is present in the cytosol bound to metallothioneins, while most cadmium (70%) is associated with the particulate fraction, probably with membrane components (Viarengo et al. 1993). Thus, it can neither be excluded that *A. colbecki* evolved unique mechanisms to scavenge the excess of cadmium ions nor that Cd could play any biological role in the membrane of the digestive gland cells. Moreover, a certain amount of Cd is bound to other cytosolic components different from metallothioneins (Fig. 2). Therefore, further investigation of this Cd-binding cytosolic component could better clarify the Antarctic scallop mechanism of

Cd homeostasis and detoxification. A comparison of *A. colbecki* cadmium metallothionein with those found in several cadmium-exposed organisms (Table 1) shows that, with a cysteine content of 12%, the *A. colbecki* metallothionein has the lowest value. This peculiar composition of the *A. colbecki* Cd-binding protein seems to contradict its predicted role in cadmium detoxification, since few cysteine residues (less than half the value reported for the other organisms in Tables 1, 2) might not confer a high affinity for heavy metals, and therefore cadmium might be easily displaced from the thionein in a dynamic steady state. Also, the lack of aromatic amino acids such as tyrosine, phenylalanine, tryptophan, together with histidine and methionine, is typical of metallothioneins, and here asparagine and glutamine are also absent, rendering characteristic the amino acid composition of this protein. It could be argued that the low cysteine content might depend on the presence of contaminant proteins. Indeed, the occurrence of contaminant proteins with a low cysteine content and lack of aromatic amino acids and the other four amino acids are very unlikely.

However, further investigation on the *A. colbecki* metallothionein will be necessary to establish its affinity for Cd, Zn and Cu ions and to clarify the correct stoichiometry of the metals bound to this thionein. Table 2 shows the comparison of the amino acid composition obtained from *A. colbecki* metallothionein with that of two other Antarctic organisms. However, we could not establish similarities, indicating that although living in the same environment these organisms could show different amino acid compositions due to their different metabolism.

Table 2 Comparison of the amino acid composition of the *Adamussium colbecki* metallothionein with that deduced from two Antarctic organisms, the sea urchin *Sterechinus neumayeri* and the fish *Trematomus bernacchi*

Amino acid residue content (%)			
Aa	*Adamussium colbecki*	*Sterechinus neumayeri*[a]	*Trematomus bernacchi*[b]
Cys	12.0	31.2	33.0
Gly	14.0	14.0	10.0
Asp	14.0	4.7	3.3
Glu	11.0	7.8	–
Thr	8.0	7.8	10.0
Ser	8.0	3.1	15.0
Pro	10.0	1.6	5.0
Ala	8.0	7.8	1.7
Val	5.0	3.1	1.7
Tyr	–	–	–
Phe	–	–	–
Met	–	1.6	1.7
His	–	–	–
Arg	–	–	–
Ile	2.0	–	–
Leu	4.0	–	–
Lys	4.0	14.0	11.5
Asn	–	3.1	3.3
Gln	–	–	3.3
Trp	–	–	–

[a] Scudiero et al. (1997b) (Genbank accession no. 2497879)
[b] This sequence is unpublished (Genbank accession no. 3676240)

To better understand the role of metallothionein in the homeostasis of heavy metals in the Antarctic scallop, *A. colbecki*, the inducibility of metallothionein in the scallop tissues was evaluated by quantification of the metallothionein content, using the spectrophotometric method described by Viarengo et al. (1997). As shown in Fig. 6, the uptake of the scallop for 7 days cadmium (1.9 μM) was sufficient to induce metallothionein neosynthesis in *A. colbecki* gill cells up to 2.5 fold. Notwithstanding the different cysteine content of the *A. colbecki* and *Mytilus* sp. metallothionein, similar levels of metallothionein induction were observed in both species. It should be noted that the spectrophotometric method evaluates the metallothionein content in terms of sulphydrylic groups capable of reacting with 2,2-dithio-bis-nitrobenzoic acid (DTNB). Therefore, it seems that the increase in the amount of metallothionein cysteine residues available for cadmium detoxification is similar in the gills of the two molluscs and this is in accord with the fact that both of them were exposed to the same Cd concentration during the experiment.

In conclusion, the results presented in this paper demonstrate the presence of a cadmium metallothionein in *A. colbecki* digestive gland cells, suggesting that the Antarctic scallop *A. colbecki* employs a heavy metal homeostasis mechanism, as well as animals living in a temperate environment.

Furthermore, it was demonstrated that cadmium is able to induce metallothionein neosynthesis several fold in the gill cells in a short-term exposure experiment. Considering these data and the fact that copper is a good inducer of metallothioneins in scallop gills (Viarengo et al. 1997), metallothioneins could be proposed as a biomarker of exposure to heavy metals in Antarctic coastal areas, which can be subjected to anthropogenic pollution.

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