Primary structure and tissue-specific expression of blue crab (*Callinectes sapidus*) metallothionein isoforms

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In aquatic animals, synthesis of the metal-binding protein metallothionein (MT) can be induced through exposure to elevated levels of metals in food or water. Whether the different routes of exposure lead to expression of different metallothionein isoforms in different tissues is unknown. In this study we examined the induction of metallothionein isoforms in the hepatopancreas and gills of the blue crab *Callinectes sapidus*. When blue crabs are exposed to cadmium in their diet, the metal accumulates in the hepatopancreas. Size-exclusion and anion-exchange chromatography show the presence of five low-molecular-mass cadmium-binding proteins. All of the observed cadmium-binding proteins belong to the class I MT family. They are designated as MT-Ia, MT-Ib, MT-Ic, MT-IIa and MT-IIb. All purified proteins run as single peaks upon rechromatography on anion-exchange HPLC, except for MT-Ic, which segregates

into two peaks corresponding to MT-Ia and MT-Ic. The amino acid sequence of MT-Ia and MT-Ic is identical. MT-Ib differs from MT-Ia and MT-Ic only in having an extra N-terminal methionine. The 18 cysteine residues in MT-Ia and MT-IIa occur in identical positions; however, of the remaining 40 amino acids, 15 are found to be different. MT-IIb is identical with MT-IIa, except for an extra methionine residue at its N-terminal position. It appears therefore that, of the five observed CdMTs, only two are the products of distinct genes. CdMT-Ia and -IIa are post-translationally modified forms of Ib and IIb, respectively, and CdMT-Ia and -Ic appear to be conformational isomers. Cadmium-induced expression of the two genes is tissue-specific. When crabs are exposed to cadmium in the water, the metal accumulates in the gills, where it is bound to MT-II. MT-I is virtually absent.

INTRODUCTION

Copper, iron and zinc are essential for the biological function of numerous proteins [1–3]. However, when ‘free’ in solution, both copper and iron can catalyse the production of highly toxic hydroxyl radicals from physiologically generated reduced oxygen species, which contribute to many pathological disorders [4–8]. Organisms have therefore designed (different) strategies to limit the concentration of free copper and iron to a minimum. Intracellular levels of iron are controlled through iron-regulated synthesis of the iron-storage protein, ferritin, and synthesis of the transferrin receptor, which is responsible for the uptake of transferrin-bound iron [2]. Little is known about the control of intracellular levels of copper and zinc. The recent discovery of ATP-dependent pumps that play a role in the cellular uptake, distribution and export of copper is an important step toward further elucidating the mechanisms that underlie the regulation of this metal [9,10]. Once inside the cell, copper and zinc induce the synthesis of metallothionein (MT), which may act as the functional equivalent of ferritin. However, whether CuMT and/or ZnMT can deliver their metals for copper/zinc-dependent processes is still controversial.

The class I MTs constitute a family of low-molecular-mass (6500 Da; 57–62 amino acids), cysteine-rich (18–20 cysteines/molecule), metal-binding proteins [11], which are expressed in many different cell lines and tissues following exposure to heavy metals, glucocorticoid hormones, interferon, interleukin-I, bacterial endotoxin, UV radiation and oxidative stress [12–16]. In mammals there are at least two major class I MT isoforms,

designated MT-I and MT-II, whose induction can be metal- and hormone-specific [17]. A third and a fourth member of the class I MT family, designated MT-III and MT-IV, are present in mammalian brain [18,19] and in stratified epithelia in mice and humans [20]. The biological function of MT is still controversial. For example, yeast mutants that lack the *CUP1* gene that encodes the copper-inducible MT, and mice containing inactivated MT-I and MT-II genes are more susceptible to copper and cadmium poisoning, respectively, but show normal growth and development [21,22], suggesting that MTs are not essential for development (under copper- and zinc-adequate conditions). However, the localization of MT in the nucleus of fetal- and newborn-rat liver and in early S-phase rat hepatocytes is suggestive of a fundamental role of this protein as a zinc donor during growth and development [23,24], whereas studies with Ehrlich ascites-tumour cells have shown that ZnMT can donate zinc to other sites in the cell by ligand-substitution reactions, under zinc-deficient conditions [25,26].

Unfortunately little is known of the fate of metals after binding to MT. This obvious problem is due, at least in part, to the fact that ZnMT is the major form of MT in most organisms. Since zinc is a necessary part of many important metalloenzymes, this makes it difficult to select or trace a single zinc-dependent protein with respect to changes in ZnMT. A great advantage of the use of the blue crab, *Callinectes sapidus*, for studies on the biological function of MT is that it has been possible to monitor CuMT and its changes in conjunction with the changes in a single protein, haemocyanin, which represents 50% of the proteins synthesized in the hepatopancreas, and is the pre-

dominant secretory protein which accounts for > 90% of haemolymph proteins [27–34].

We have identified three CuMTs and two CdMTs in blue crab hepatopancreas [33,35–37]. In addition to the two CdMTs, two other ill-defined, cysteine-rich, low-molecular-mass cadmium-binding proteins have been observed in the hepatopancreas of cadmium-exposed crabs [37]. This study was undertaken to characterize unequivocally the multiple cadmium-binding proteins that are present in blue crab hepatopancreas after dietary exposure to cadmium. Since aquatic organisms may derive metals from diet or water, we also evaluated the effects of cadmium on MT expression in crabs when exposed to metals dissolved in the water. These studies demonstrate that post-translational modification of two distinct MT isoforms results in the presence of five apparent MTs, and that the effect of cadmium on MT expression is tissue-specific.

METHODS

Metal-exposure conditions

Male blue crabs (carapace width 8–12 cm) were obtained from local crab fishermen and kept in tanks with flow-through seawater at ambient temperature (approx. 22 °C). Crabs were exposed to elevated levels of cadmium in their diet by feeding them on fish (approx. 10 g pieces) injected with 5 mg of CdCl₂ at 2-day intervals, for 20–30 days (10–15 dosages). Crabs exposed to cadmium in the water were kept for 5–7 days in 10 litres of well-aerated seawater containing 1 p.p.m. (9 µM) cadmium. After exposure, crabs were anaesthetized on ice, and their hepatopancreas and gills were dissected and processed immediately or frozen in liquid nitrogen.

Purification of blue crab CdMT isoforms

About 30 g of hepatopancreas tissue, obtained from 4–5 animals was homogenized with a Brinkman Polytron homogenizer in 1 vol. (w/v) of ice-cold buffer (10 mM Tris/HCl, pH 7.6, containing 0.1 mM PMSF). The homogenate was saturated with nitrogen and centrifuged for 70 min at 200 000 g. The supernatant was passed through glass wool and then subjected to chloroform/ethanol extraction and acetone precipitation [56]. The resulting pellets were taken up in a total of 6 ml of 10 mM Tris/HCl, pH 7.6, and applied to a Sephadex G-75 column (2.5 cm × 50 cm). The column was eluted with 10 mM Tris/HCl, pH 7.6 (pH refers to room temperature), at a flow rate of 20 ml/h. All buffers were saturated with nitrogen, and fractions were collected at 4 °C in a nitrogen atmosphere. Cadmium content was measured with a Perkin-Elmer 5000 atomic-absorption spectrophotometer. Cadmium-containing fractions corresponding to 10 000 Da ($V_e/V_0 = 2.5$) were pooled and directly applied to a DEAE-cellulose anion-exchange column (2.5 cm × 20 cm). MT isoforms were eluted with a linear gradient generated from 500 ml of 10 mM Tris/HCl, pH 7.6, and 500 ml of 200 mM Tris/HCl, pH 7.6, at a flow rate of 35 ml/h. CdMTs were concentrated on an Amicon YM-2 membrane. Homogeneity of the CdMT isoforms was ascertained by anion-exchange HPLC using a Beckman Gold system and a DEAE 5PW column (0.75 cm × 7.5 cm), eluted with 10 mM Tris/HCl, pH 7.0, for 2 min, followed by a linear gradient from 10 to 200 mM Tris/HCl, pH 7.0, for 30 min at a flow rate of 1 ml/min. The absorbance of the eluate was monitored at 250 and 280 nm.

Gill tissues were treated in a similar way. A 1 g portion of tissue was cut into small pieces with a scalpel, homogenized with a Tissue Tearor, subjected to low- (15 min at 21 000 g) and high-speed (70 min at 200 000 g) centrifugation, and applied directly

to a 1.5 cm × 70 cm Sephadex G-75 column, equilibrated with 10 mM Tris/HCl, pH 7.6, containing 0.5 mM β-mercaptoethanol. Fractions of volume 2 ml were collected at a flow rate of 10 ml/h. A second tissue homogenate was applied to a DEAE-cellulose column (2.5 cm × 10 cm). MTs were eluted with a linear gradient from 250 ml of 10 mM Tris/HCl, pH 7.6, to 250 ml of 200 mM Tris/HCl, pH 7.6, at a flow rate of 35 ml/h. Both buffers contained 0.5 mM β-mercaptoethanol.

Reduction and alkylation

A 1 mg sample of CdMT was dissolved in 1 ml of 6 M guanidinium chloride in 50 mM Tris/HCl (pH 8.0)/1 mM EDTA. Dithiothreitol was added to a final concentration of 5 mM, and the sample was incubated for 60 min in the dark before the addition of 20 µCi of iodo[¹⁴C]acetic acid (55 mCi/mmol). After a 60 min incubation, iodoacetic acid was added to a final concentration of 10 mM, and this reaction was continued for an additional 60 min. Excess reagent was removed by reverse-phase HPLC.

Peptide purification

Carboxymethylated CdMT-Ia (100 µg) was digested with *Staphylococcus aureus* V8 proteinase (SPase V8) (Sigma) and papaya proteinase IV (PP4; a gift from Dr. David Buttle, Strangeways Laboratory; [38]), at an enzyme/substrate ratio of 1:50 (w/w) in 50 mM Tris/HCl/0.1 M NaCl, pH 7.4, for 4 h at 37 °C. CdMT-Ic and CdMT-IIa were digested with SPase V8 (1:50, w/w) for 20 h at 37 °C. The peptides were separated by reverse-phase HPLC on a Vydac C18 column, by using a gradient generated from 0.1% trifluoroacetic acid (solution A) and 0.07% trifluoroacetic acid/9.93% water/90% acetonitrile (solution B; 1% B/min) [39,40].

Amino acid sequence analysis

Automated Edman degradation of 200–500 pmol of protein or peptides was carried out in an Applied Biosystems 477A pulsed liquid-phase sequencer with on-line PTH (phenylthiohydantoin) analysis using an Applied Biosystems 120A HPLC apparatus. The samples were applied to Porton peptide or protein sample support disks, by employing the modified cycles PI-BGN and PI-1 recommended by Porton Instruments. Samples (100 µl) of the PTH derivatives were analysed in the 120A HPLC instrument, and the remaining sample was delivered directly into scintillation vials and the ¹⁴C radioactivity in each fraction was measured in a Beckman LS 5000 TD liquid-scintillation system.

Mass spectrometry

A portion of the undigested protein and the purified peptides was analysed by time-of-flight (TOF) and/or plasma-desorption MS. The mass spectrum was recorded by using nitrocellulose targets in an Applied Biosystems Biolon 20 plasma desorption instrument. The spectrum was recorded for 1 × 10⁶ fission events or overnight. Further details of the instrumentation and spectral analysis have been described elsewhere [41].

RESULTS

Purification of CdMT from hepatopancreas after exposure to cadmium from the diet

When the Cd-containing fractions corresponding to the elution volume of MT in the Sephadex chromatogram ($V_e/V_0 = 2.5$)

Figure 1 Anion-exchange chromatography of MTs

(a) Anion-exchange elution profile of the pooled MT fractions obtained after Sephadex G-75 chromatography ($V/V_0 = 2.5$) of hepatopancreas cytosol from blue crabs exposed to elevated levels of dietary cadmium. A DEAE-cellulose column (2.5 cm $\times$ 20 cm) was developed with a linear gradient generated from 500 ml of 10 mM Tris/HCl and 500 ml of 200 mM Tris/HCl, pH 7.6, at a flow rate of 35 ml/h. (b) Same procedure, but different batch of hepatopancreas tissues. The peaks designated as MT-Ia, MT-Ib, MT-Ic, MT-IIa and MT-IIb are eluted at Tris concentrations of approx. 18, 26, 45, 100 and 110 mM Tris respectively.

were subjected to anion-exchange chromatography, the number of Cd-binding proteins observed in the elution profile varied from one preparation to another. In some cases four cadmium-containing components were resolved (Figure 1a), whereas others

Table 1 Amino acid sequence of blue crab MT isoforms (Figures 1a and 1b)

Sequence of MT-Ia and MT-Ic are identical. The sequences of MT-Ib and MT-Ia are identical except for an additional N-terminal Met in MT-Ib. MT-Ia and MT-IIa differ by 15 amino acids (8 in the N-terminal domain and 7 in the C-terminal domain). MT-IIb is identical with MT-IIa, except for an extra N-terminal Met in MT-IIb.

	10	20	
MT-Ia	P-G-P-C-C-N-D-K-C-V-C-Q-E-G-G-C-K-A-G-C-Q-C-T-S-C-R-C-		
MT-IIa	P-D-P-C-C-N-D-K-C-E-C-K-E-G-E-C-K-T-G-C-K-C-K-S-C-R-C-		
	30	40	50
MT-Ia	S-P-C-Q-K-C-T-S-G-C-K-C-A-T-K-E-E-C-S-K-T-C-T-K-P-C-S-C-C-P-K		
MT-IIa	P-P-C-D-K-C-S-S-E-C-K-C-T-S-K-E-E-C-S-K-T-C-S-K-P-C-S-C-C-P		

Table 2 Observed and predicted masses of carboxymethylated MT isoforms and the peptides used to determine their amino acid sequences

Observed masses of intact MT and peptides were determined by mass spectrometry as described in the text.

		Mass (Da)	
		Observed	Calculated
MT-Ia	1–58	7056.9	7060.3
	1–19	2159.4	2161.1
	20–44	3153.5	3155.6
	45–58	1780.0	1780.0
MT-IIa	1–57	7201.2	7199.0
	1–13	1715.6	1715.6
	14–36	2859.4	2858.0
	37–44	1043.2	1043.4
MT-IIb	47–57	1637.0	1637.0
	1–58	7322.0	

showed the presence of five cadmium-binding proteins (Figure 1b). In the latter case, peak 1 (Figure 1a) was resolved into two components (Figure 1b: Ia and Ib). In still other cases MT-Ia and MT-Ib were present, but poorly resolved. Possible reasons for this phenomenon will be addressed in the Discussion section. Both MT-Ia and MT-Ib isoforms chromatograph as single, sharp, symmetrical peaks on analytical size-exclusion HPLC, corresponding to monomeric MT. The retention times on DEAE 5PW HPLC were 3.31 and 3.64 min respectively. The third peak (Figure 1b; CdMT-Ic) runs as a single monomeric species on analytical size-exclusion HPLC, but, when subjected to DEAE 5PW HPLC, this peak splits into two components with retention times of 3.33 (CdMT-Ia) and 8.12 (CdMT-Ic) min. The fourth and fifth cadmium-containing peaks (Figure 1b) behave as pure components on analytical anion-exchange HPLC, with retention times of 16.59 and 17.52 min.

Amino acid sequence of CdMT-I

The complete amino acid sequence of CdMT-Ia (Figure 1b; Table 1) was derived from direct N-terminal sequence determination of the intact protein (residues 1–36) and of peptides purified from a SPase V8 digest (residues 14–43) and a PP4 digest (residues 1–19), and two SPase V8 subdigests (residues 20–44 and 45–58) of fragment 20–58 obtained after PP4 digestion. To verify the deduced amino acid sequence, the intact alkylated polypeptide chain and the PP4 peptides covering the entire sequence were analysed by TOF MS. The observed and calculated masses are given in Table 2. The sum of the masses of the three peptides is equal to that of the entire polypeptide chain (plus two water molecules), indicating that the derived amino acid sequence represents the complete sequence of CdMT-Ia.

The amino acid sequence of CdMT-Ic was determined by the same techniques as described in the previous paragraph. The sequence of CdMT-Ic is identical with that of CdMT-Ia. The sequence of the first 51 amino acids of CdMT-Ib was determined by Edman degradation of the intact polypeptide chain. MT-Ib was identical with MT-Ia, with the exception of an additional methionine in the N-terminal position.

Amino acid sequence of CdMT-II

A SPase V8 digest of carboxymethylated CdMT-IIa was

Figure 2 DEAE-cellulose (2.5 cm $\times$ 10 cm) anion-exchange profile of MT fractions obtained from the gills of blue crabs exposed to cadmium in the seawater

MTs were eluted with a linear gradient from 250 ml of 10 mM Tris/HCl to 250 ml of 200 mM Tris/HCl, pH 7.6, at a flow rate of 35 ml/h. The major cadmium-containing peak is eluted at 100 mM Tris.

chromatographed on Vydac C18. Collected fractions were analysed by plasma-desorption MS and the peptides were sequenced. The complete sequence of the protein was derived from four peptides: residues 1–13, 14–36, 37–44 and 45–57. The theoretical mass of the peptides matches the data obtained by MS (Table 2). The TOF MS of the intact protein gave a value of 7199 Da, which agreed with the calculated theoretical mass for the intact protein. The amino acid sequence of CdMT-IIa is given in Table 1.

The sequence of the first 43 amino acids of CdMT-IIb was determined by subjecting the intact polypeptide chain to Edman degradation. The analysis showed that CdMT-IIb is identical with CdMT-IIa, except for a N-terminal methionine residue in CdMT-IIb, similar to what is found for CdMT-Ia and -Ib. The atomic mass of CdMT-IIb is 7322 Da (Table 2). The atomic mass of the N-terminal Met is 133 Da. The difference in mass between CdMT-IIa and CdMT-IIb is 123 Da. We conclude therefore that CdMT-IIa and CdMT-IIb are identical, except for the N-terminal Met in CdMT-IIb.

Purification of CdMT from gills after exposure to cadmium in the water

Cadmium accumulates in the gill tissues when crabs are exposed to cadmium in the water. Only two CdMT isoforms are observed in an anion-exchange elution profile of gill homogenate (Figure 2). The peaks are eluted at a conductivity of approx. 3 mMho (100 mM Tris/HCl and 110 mM Tris/HCl respectively) which corresponds to the elution positions of hepatopancreas CdMT-IIa and CdMT-IIb. CdMT-Ia, -Ib and -Ic are virtually absent. Analysis of the hepatopancreas of the same crabs showed that the amount of CdMT in these tissues (expressed as μ g of Cd bound to MT per g wet wt. of tissue), determined by Sephadex chromatography, was about 20-fold less than the CdMT in the gill. Very little cadmium appears to be transported from the gills to the hepatopancreas.

DISCUSSION

Most vertebrates have at least two major class I MT isoforms, designated as MT-I and MT-II [42]. A third and a fourth member of the class I MT family, MT-III and MT-IV, are present in mammalian brain [18,19] and in stratified epithelia in mice and humans [20]. The low-molecular-mass cadmium-binding proteins from blue crab hepatopancreas can be separated into four or five components. All of these proteins have now been identified as class I MTs. MT-I (fraction 70 in Figure 1a) sometimes segregates into two components (MT-Ia and MT-Ib; Figure 1b). MT-Ia and MT-Ib differ only by a single methionine residue that is present in the N-terminal position in MT-Ib. MT-Ia starts with proline instead. Similarly, MT-IIb is identical with MT-IIa, except for an additional N-terminal methionine residue. The removal of the initiator methionine from CdMT-I and CdMT-II in cadmium-exposed hepatopancreas tissues appears to be incomplete. The degree of processing was variable, as indicated by the observation that the ratios of CdMT-Ia/Ib and CdMT-IIa/Ib differed considerably from one preparation to another. There was a striking correlation between the amounts of CdMT-Ib and CdMT-IIb (Figures 1a and 1b). Small amounts of CdMT-IIb, indicative of a high degree of methionine removal, were always accompanied by small amounts of CdMT-Ib. It seems therefore likely that the absence of CdMT-Ib from some of our MT preparations was, at least in part, due to complete conversion of MT-Ib into MT-Ia. A similar phenomenon was found in the shore crab *Carcinus maenas*, which has two charge-separable MTs which are identical, except for an N-terminal methionine [43]. In other marine crustacea, however, the N-terminal Met is processed completely [44,45]. Whereas the N-terminal Met in crustacean MTs is unblocked, vertebrate MTs have a blocked N-terminus which is usually the initiator Met [42]. Yet another modification is found in the oyster *Crassostrea virginica*, whose MTs can be separated into two components which are identical except for a N-acetyl group bound to an N-terminal serine [46].

The second component in Figure 1(a) and the third component in Figure 1(b) (MT-Ic) are eluted at the same Tris concentration (45 mM). Surprisingly, the amino acid sequence of this protein is identical with that of MT-Ia. In addition, when this MT isoform is re-run on an analytical DEAE-HPLC column, the protein segregates into MT-Ia and MT-Ic, but MT-Ia never converts into MT-Ic. Size-exclusion HPLC shows that MT-Ic is a monomeric species. MT-Ia and MT-Ic are thus not monomers and dimers of the same protein. In addition, both proteins were found to contain six cadmium atoms per molecule. It appears therefore that CdMT-Ic is an unstable conformational isomer of CdMT-Ia, perhaps with a different arrangement of the cadmium-thiolate cluster(s) due to interconversion of terminal and bridging cysteines, as observed in metallothionein-like inorganic complexes [47]. In summary, our data show that the number of charge-separable MTs observed in an ion-exchange profile do not simply represent distinct gene products. In our case, the five MTs observed appear to be derived from two distinct genes, whose products are modified post-translationally.

The three-dimensional solution structure of *C. sapidus* CdMT-I has been determined by homonuclear and heteronuclear magnetic resonance spectroscopy [48,49]. These structural studies revealed the presence of two separate domains. Each domain contains a cluster of three metal ions, tetrahedrally co-ordinated to the sulphur atoms of the nine cysteine residues present in each domain. In total, six Cys residues participate in bridging two metal ions and 12 participate as terminal ligands. The only element of regular secondary structure in either of the two

domains is a short segment of helix in the C-terminal domain between Lys-42 and Thr-48. This stretch of amino acid residues is completely conserved within the two MT isoforms (Table 1). This structural element seems to be conserved in all crustacean MTs sequenced so far. In *Scylla* MT-I and -II, and *Homarus* and *Carcinus* MT, residue 42 is a lysine, followed by a glutamic or aspartic acid in positions 43 and 44, a cysteine in 45, a variable residue in 46 and a lysine and a threonine in positions 47 and 48 [43–45].

In general, the primary structures of the six MTs from decapod crustacea that have been sequenced are highly conserved, with the greatest homology occurring in the N-terminal domain (19 out of 27 residues; 18 out of 30 in the C-terminal domain). Variable residues are distributed randomly throughout both domains. Despite the homology between the MTs of different crustaceans, blue crab MT-I is very distinct from MT-II. The two isoforms differ in 15 positions, 7 of which are in the N-terminal domain and 8 in the C-terminal domain. The most striking homology between the MTs from different crustaceans is found between the N-terminal domains of *Callinectes* MT-I and *Scylla* MT-I, which differ in only two positions. The same is found for the N-terminal domains of *Callinectes* MT-II and *Scylla* MT-II. The C-terminal domains of these proteins are much less conserved, with the two MT-I proteins differing in six positions and the two MT-II proteins in eight positions. The observation that the N-terminal β domain seems to be subject to greater structural constraint than the C-terminal domain suggests that the two domains may have evolved to perform different structural roles. Comparison of the structural elements of blue crab MT-I domains shows that the metals in the β domain are more exposed than in the α domain [49]. In addition, the line widths of the assigned ^{113}Cd NMR resonances from the two clusters predict a more ready exchange of metal ions in the crab β domain compared with the α domain [48], similar to what has been found for the β domain of mammalian MT [50,51]. The continual breaking and re-forming of co-ordination bonds allows for ready intramolecular metal exchange within the N-terminal cluster and intermolecular exchange between clusters of different MT molecules [51]. This suggests that the kinetically labile N-terminal domain may function in metal-exchange processes, whereas the kinetically more stable C-terminal domain may be important in detoxification. Studies on mutant MTs in which cysteine residues in either domain have been replaced corroborate this hypothesis [52,53].

When crabs are exposed to cadmium in the food, the majority of the metal is found in the hepatopancreas, with only a small portion in the gills [54]. When blue crabs are exposed to cadmium in the water, the metal accumulates in the gill tissues, with only a small fraction transported to the hepatopancreas. The major MT isoform in the gills is eluted at 100 mM Tris from the DEAE-cellulose (Figure 2), in the same position as hepatopancreas MT-II. The amino acid composition of gill MT-II [55] matches closely that of hepatopancreas MT-II and differs from MT-I. For example, hepatopancreas MT-I and MT-II can be distinguished by their Asp/Asn, Thr and Ser content, which is 3, 5 and 5 residues/molecule for MT-I and 4, 3 and 7 for MT-II. The values for gill MT are 4, 3 and 7, which unequivocally defines gill MT as a MT-II. The cadmium-induced expression of blue crab MT is thus tissue-specific. Hepatopancreas expresses both MT-I and MT-II, but the gill expresses MT-II only (Figures 1 and 2).

In summary, we have shown that the five CdMT isoforms observed in an anion-exchange chromatogram obtained from the hepatopancreas of cadmium-exposed blue crabs represent only two distinct gene products. The other three arise from covalent post-translational modification and from non-covalent confor-

mational changes. The two isoforms are expressed to different extents in different tissues. The biological significance for the observed polymorphism of MT and the differential expression of the different isoforms in response to cadmium remains to be established.

M.B. acknowledges support from U.S. Public Health Service Grants ES04074 and ES06422 from the National Institutes of Health.

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Received 8 March 1995/5 June 1995; accepted 21 June 1995