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Properties of cod metallothionein, its presence in different tissues and effects of Cd and Zn treatment

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Abstract.

One isoform of the low-molecular-weight metal-binding protein metallothionein (MT) has been isolated from the liver of Atlantic cod by size-exclusion and ion-exchange chromatography. Cod MT contained 33% cysteine, no aromatic amino acids or arginine. As is the case for other piscine MTs, the N-terminus of cod MT lacked the asparagine in position 4 which is present in mammalian MTs. In addition, cod MT differed from all other vertebrate MTs described in that the N-terminal methionine was not acetylated. Antibodies were raised in rabbits against hepatic MT from cod by repeated injections of native protein mixed with adjuvant. Anti-cod MT antisera cross reacted with similarly-sized proteins in liver, brain, anterior kidney, posterior kidney, spleen, intestine, gills and ovaries. The putative MT in cod brain migrated differently to that of the other tissues in native gel electrophoresis. Intraperitoneally injected Cd (1 mg/kg) was nearly entirely associated with the MT-peak in hepatic and renal cytosols, whereas a single injection of Zn (10 mg/kg) resulted in increases in all cytosolic Zn pools of the liver and no apparent change in cytosolic Zn, Cu, Ni or Cd in kidney.

Introduction

Metallothionein (MT) is a low-molecular-weight, cysteine-rich protein present in all vertebrates investigated and many invertebrates (Kägi and Schäffer 1988; George 1990). The protein is primarily a Cu- and Zn-binding protein under normal metabolism and its synthesis is induced by those and other metals such as Cd and Hg (Baksi *et al.* 1988; Bremner 1991). In mammals, MT is also induced by other transition metals such as Pb and Ni, although they are generally less effective inducers than the above metals (Eaton *et al.* 1980; Arizono *et al.* 1985; Cartaña *et al.* 1991). The latter group of metals have been shown to bind to MT *in vitro* (Nielson *et al.* 1985), but do not associate with the protein to any extent *in vivo* (McCormick and Dietert 1991). In addition, MT in mammals will be induced by other factors, such as exposure to free radicals (Thornally and Vasak 1985; Hidalgo *et al.* 1988; Sato 1991) or

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radical-generating agents (Lohrer and Robson 1989; Sato and Sasaki 1991), starvation (Bremner and Davies 1975), restrainment (Hidalgo *et al.* 1987), inflammation (Fleet *et al.* 1990) and exposure to cold or burns (Oh *et al.* 1978). Induction by factors other than Cu, Zn, Cd and Hg appear to be less prominent in fish and lower vertebrates than in mammals (Overnell *et al.* 1987; Hyllner *et al.* 1989; Kille *et al.* 1992).

The prevailing nomenclature lists three classes of MT, embracing all "phenotypically related metal thiolate polypeptides" (Fowler *et al.* 1987). Class I MTs, to which all vertebrate MTs would appear to belong, are polypeptides with the location of cysteine residues closely related to those in equine renal MT. In mammals, there are two main groups of MT isoforms, separable by charge (Kägi and Schäffer 1988), whereas both one, two and three such isoforms have been reported for the fish, reptile, amphibia and bird species that have been studied (Yamamura and Suzuki 1984; McCormick *et al.* 1988; Kille *et al.* 1992). Metallothionein has been isolated and characterized from several freshwater and marine fish species, *i.e.* dogfish, *Scyliorhinus caniculatus* (Hidalgo and Flos 1986), eel, *Anguilla anguilla* (Noël-Lambo *et al.* 1978), carp, *Cyprinius carpio* (Kito *et al.* 1982), goldfish, *Carassius auratus* (Carpenè and Vasak 1989), rainbow trout, *Oncorhynchus mykiss* (Ley and Cherry 1983), stone loach, *Neomacheilus barbatulus*, pike, *Esox lucius* (Kille *et al.* 1991), plaice, *Pleuronectes platessa* (Overnell *et al.* 1981), winter flounder, *Pseudopleuronectes americanus* (Shears and Fletcher 1984), perch, *Perca fluviatilis* (Olsson and Hogstrand 1987a), skipjack, *Katsuwonus pelamis* (Takeda and Shimizu 1982b) and largemouth bass, *Micropterus salmoides* (Weber *et al.* 1992). The N-terminal or entire amino acid sequence is also known for some species, *i.e.*, rainbow trout (Bonham *et al.* 1987), stone loach and pike (Kille *et al.* 1991), plaice (Overnell *et al.* 1981; George *et al.* 1989) and winter flounder (Chan *et al.* 1988). Common to the sequence of all vertebrate MTs are the invariant positions of all but one of the 20 cysteine residues present in most isoforms and an acetylated methionine at the N-terminus of the protein. In addition, all described fish MTs lack the position 4 residue (asparagine) of mammalian MT. The N-terminal sequence of this protein is of particular interest as it constitutes the main antigenic determinant of the protein (Winge and Garvey 1983; Kikuchi *et al.* 1990). Although MT has been isolated and characterized from a number of fish species, information is still scarce on its occurrence and levels in tissues other than liver, kidney or gill. Further, there have been reports of an apparent lack of MT in specific fish tissues (Baer and Thomas 1991) and species (Scudiero *et al.* 1992).

The present study forms two parts; the first concerns the purification of cod hepatic MT and the immunochemical identification of the protein in various tissues of Atlantic cod (*Gadus morhua* L.). The second part of the study compares the effects of intraperitoneal injections of Zn, Cd and saline on the cytosolic distributions of Cu, Zn, Ni and Cd in liver and anterior kidney of cod.

Materials and methods

Treatment of fish and samples

Experiment 1: Isolation and characterization of MT

Atlantic cod (*Gadus morhua* L.) weighing 200–500 g were collected in the Oslo Fjord using beach seine. They were kept in 400 l tanks with recycled sea water and a 12h: 12h day-night light regimen. The temperature of the water was 15–16°C and salinity 34–35 ‰ during the experiment. One group of 15 cod was injected *i.p.* with solutions of $CdCl_2$ in 0.9% saline in the following manner: 1 mg/kg (day 1), 1 mg/kg (day 5), 2 mg/kg (day 8), 2 mg/kg (day 11). A second group of 5 cod were not injected. All individuals from both groups were sacrificed on day 14; they were stunned by a blow to the head and blood withdrawn by heart puncture using a heparinized syringe. Plasma and blood cells were separated immediately by centrifugation. The blood cells were diluted 1:3 (v/v) with homogenization buffer before freezing. Bile was withdrawn from the bladder by a syringe and brain, liver, anterior kidney, posterior kidney and spleen dissected

out and transferred to beakers containing ice-cold homogenizing buffer (100 mM Tris-HCl, pH 8.1 with 5 mM 2-mercaptoethanol). The above tissues were removed from the injected group, whereas gills, intestine and ovaries were removed from the second group (not injected). The various tissues were pooled and immediately homogenized in at least two volumes (w/v) of ice-cold homogenizing buffer using a Potter-Elvehjem type glass homogenizer with teflon pestle. Gill and intestinal samples were homogenized using a Tenbroek type homogenizer with a ground-glass pestle. The samples were then centrifuged at 4°C at 10 000 × *g* for 30 minutes. Aliquots of tissue supernatants, plasma and blood cells were transferred to polypropylene vials and stored at −70°C until use.

Experiment 2: Cytosolic distribution of metals

Atlantic cod weighing 1000–1100 g were collected in the outer Oslo Fjord using traps. They were kept in a 2000 l tank with continuous supply of sea water; water temperature was 4–8°C and the salinity 35‰ during the experiment. Pairs of cod were injected once *i.p.* with 1 mg/kg $CdCl_2$ in 0.9% saline, 10 mg/kg $ZnCl_2$ in 0.9% saline and one pair with 0.9% saline only. The fish were sacrificed on day 14 by a blow to the head. Liver and anterior kidney were immediately removed and treated as in experiment 1.

Chromatographic procedures

From experiment 1, 10 000 × *g* supernatants from liver and posterior kidney were loaded directly on a Sephadex G-75 column and eluted with 10 mM Tris-HCl, pH 8.1. The eluate was continuously monitored at 254 nm. Fractions containing components with M_r 3–20 kDa were pooled and concentrated using an Amicon stirred cell ultrafiltration unit with a DP5 filter (Amicon, MA, USA). The concentrate (30 ml) was loaded on Neobar AQ (Dynochrom, Norway) or Mono-Q (Pharmacia, Sweden) anion-exchange columns and eluted with a gradient of 10 to 400 mM Tris-HCl, pH 8.1, controlled by a Pharmaia FPLC-system. The eluate was monitored continuously for absorption at 254 and 280 nm and fractions analyzed for Cd, Cu and Zn using flame or electrothermal atomic absorption spectrometry (Varian SpectrAA 10). Selected fractions were pooled and the sample desalted on a Sephadex G-25 column equilibrated with 10 mM ammonium carbonate buffer, pH 7.5. The eluate was then lyophilized and the purified cod MT stored at −20°C until use.

In experiment 2, frozen aliquots (100 µl) were thawed and applied on a column packed with Superose 12 preparative grade (Pharmacia, Sweden) equilibrated with 100 mM Tris-HCl, pH 8.1. The eluate was monitored as above, and the content of Cd, Cu, Zn and Ni in fractions determined. The protein content of the 10 000 × *g* supernatant was determined by the method described by Bradford (1976) using a kit from Bio-Rad (CA, USA).

Amino acid composition and N-terminal sequence analysis

The lyophilized MT was dissolved in 0.2 M ammonium bicarbonate buffer, pH 7.5. Aliquots of the protein-solution were hydrolyzed for 24 h in 6 M HCl, with or without prior oxidation by performic acid (Hirs 1967), and subsequently applied on a Biotronic Amino Acid Analyzer LC 5000. Three parallel analyses were made of samples without and two analyses with performic acid treatment. N-terminal analysis was performed using an automatic sequence analyser (Applied Biosystems 477A protein/peptide sequencer) coupled to a HPLC apparatus (Applied Biosystems 120A analyser). Attempts to remove Cu from the protein were unsuccessful and cysteine-residues in the N-terminal sequence were therefore not positively identified.

Electrophoretic procedures and immunoblots

Samples of plasma, blood cells, brain, liver, anterior kidney, posterior kidney and spleen from Cd-injected cod and gill, intestine and gonad from untreated fish were subjected to SDS and native polyacrylamide gel-electrophoresis using 15% gels and

a discontinuous buffer system (Laemmli 1970). After thawing, the samples were centrifuged at 10,000 × *g* for 3–5 min and the supernatant mixed 1:4 (v/v) with the appropriate sample buffer. Samples to be applied to native gels were incubated with sample buffer (0.1 M Tris-HCl, pH 6.8, 20% glycerol and 1 mM Cd^{++}) for 30 min and then again centrifuged at 10 000 × *g* for 1 min. Samples to be applied to SDS gels were mixed with reducing sample buffer (60 mM Tris-HCl, pH 6.8, 20% glycerol, 10% SDS, 0.75 M 2-mercaptoethanol) and heated at 90°C for 5 min. The gels were run in a Bio-Rad Mini-Cell at 180 V for about 50 min. Following electrophoresis, gels were incubated for 5–10 min in transfer buffer (10 mM $NaHCO_3$, 3 mM Na_2CO_3, 20% methanol, 0.1% SDS) at 4°C. The separated proteins were then transferred to a PVDF-membrane (45 µm, Immobilin P, Millipore, MA, USA) according to Towbin *et al.* (1979) and the instructions of the manufacturer (Immobilon TP006, Millipore, MA, USA). The transfer was effected in the transfer buffer at 50–60 V for 90–120 min. The membranes were then probed with antibodies raised in rabbit against cod MT. All steps of the staining and immunochemical procedures were done at room temperature on a rocking table. Immunochemical identification of proteins on the membrane was by a double-antibody procedure. The membrane was incubated in TBS (Tris buffered saline; 20 mM Tris, 0.5 M NaCl), pH 8.5, for 10–15 min immediately after the electrotransfer and thereafter blocked in 3% gelatin/TBS, pH 8.5, for 45 min. The membrane was then incubated with primary antibody in 1% gelatin/TBS (1:2000 dilution), pH 8.5 for 12–18 h (overnight). Following 5 × 5 min washes in TBS, pH 8.5, the membrane was incubated with secondary antibody conjugated with horseradish peroxidase (1:2000 dilution; Sigma, MO, USA) in 1% gelatin/TBS, pH 8.5, for 2 h. The membrane was again washed 5 × 5 min in TBS, pH 7.5, and incubated with the appropriate substrate (4-chloro-1-naphtol, Sigma, MO, USA) in TBS, pH 7.5, for 5 to 15 min. The staining was terminated by immersing the membrane in dH_2O.

Production of antiserum

Six-month old female rabbits (*Oryctolagus cuniculus*, Chbb: CH, 2–2.5 kg) were injected with a solution containing 200–300 µg cod MT mixed with an equal volume of Freund's incomplete adjuvant at two-week intervals for two months and then again after an additional two months. The antibody titre was checked using immunoblots as described above. One month after the last injection the rabbits were bled through the *arteria femoralis* and the serum retained by allowing the blood to clot. The resulting anti-cod MT antiserum was then divided into appopriate aliquots and frozen at −70°C.

Results

Purification and characterization of cod MT

One major metal-binding peak eluted from the ion-exchange columns when concentrated samples of liver and anterior kidney were applied. The profiles were similar for the two different columns and for samples from liver and kidney, and only one is shown (Figs. 1A, B). The major peak was found to be metallothionein by its amino acid composition (Table 1). In liver, this peak contained most Cu, followed by Zn and Cd (Fig. 1B), whereas the peak contained almost exclusively Cd in kidney samples (results not shown). A minor metal-binding component with higher absorbance at 254 nm than at 280 nm eluted earlier than the major peak from the column (Fig. 1A, B). Clearly not MT, there were only minor amounts of cysteine in the latter protein, but much aspartic and glutamic acid. This protein also contained arginine and the aromatic amino acids phenylalanine and histidine (results not shown).

Metallothionein from cod contained 20 cystein-residues and no aromatic amino acids or arginine (Table 1). The protein contained more asparagine/aspartic acid and glutamine/glutamic acid, and less serine, than other vertebrate MTs. As noted by others (Hunziker 1991), total removal of Cu from MT for N-terminal sequence analysis was not successful. Therefore, cysteine-residues could not be

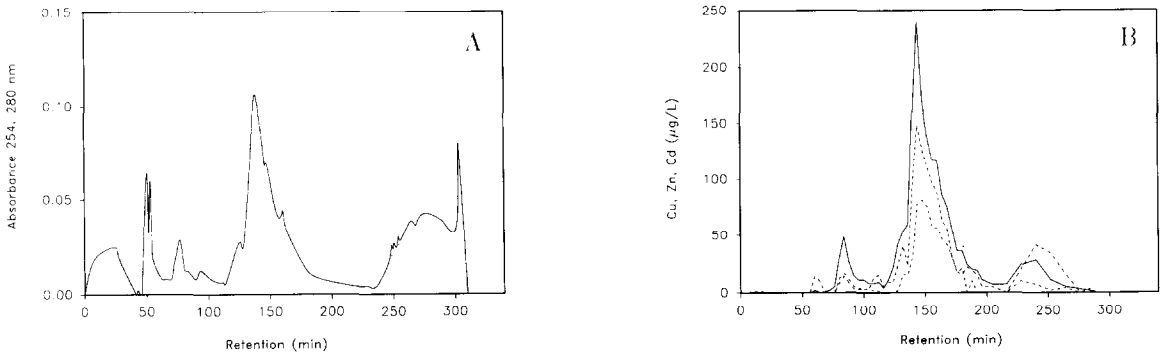

Fig. 1. Separation of fractions pooled from gel-infiltration on a Mono-Q column eluted with a gradient of 10-400 mM Tris-HCl, pH 8.1. **A.** Absorbance at 254 nm (solid line) and 280 nm (dotted line), **B.** Cd (solid line), Cu (dotted line), Zn (dashed line).

Table 1. Amino acid composition of selected vertebrate metallothioneins

Amino acid	cod*	perch		carp		winter flounder*	pigeon		rat
		MT-1	MT-2	MT-1	MT-2		MT-1	MT-2	MT-2
Asp/Asn	7	4	4	5	6	6	5	5	4
Thr	6	7	6	4	5	8	3	1	2
Ser	4	6	6	7	6	6	5	12	10
Glu/Gln	4	2	2	2	2	2	2	3	3
Pro	2	5	5	3	3	4	4	3	2
Gly	4	8	7	6	7	6	7	3	4
Ala	3	3	3	3	3	1	6	5	5
Val	2	1	2	1	1	1	1	1	1
Cys	20	17	18	20	20	19	20	20	20
Met	1	1	1	1	1	1	1	1	1
Lys	7	6	6	7	6	6	9	6	8
Ile	–	–	–	–	– 1	–	–	–	1
Leu	1	–	–	–	–	–	–	–	–
Tyr	–	–	–	–	–	–	–	–	–
Phe	–	–	–	–	–	–	–	–	–
His	–	–	–	–	–	–	–	1	–
Arg	–	–	–	–	–	–	–	2	–
total	61	60	60	59	61	60	63	63	61

Compositions were compiled from Olsson and Hogstrand (1987): perch; Kito *et al.* (1982): carp; Chan *et al.* (1988): winter flounder; Lin and Huang (1990): pigeon; Winge and Rajagopalan (1972): rat; * The number of residues were calculated assuming one methionine in each molecule and a size of 6000–7000 Da.

positively identified and their positions were assumed from alignment with MT from other fish species (Fig. 2). In contrast to all other vertebrate MTs described, the N-terminus of cod MT was not acetylated. Furthermore, cod MT deviated from other fish MTs by containing an aspartic acid rather than glutamic acid in position 5 and an alanine rather than a serine in position 7. Cod MT also differs from one or more of the other sequenced fish MTs at positions 11, 16, 17, 19 and 21. The latter residues appear to be variable among fish MTs, however, as there were differences between two or more of the other species in those positions.

Immunological identification of MT in tissues

Metallothionein was detectable by the immunoblotting procedure in samples from brain, liver,

Position (fish MTs)	1	2	3			4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Atlantic cod	M	D	P	-	-	C	D	C	A	K	T	G	T	C	N	C	G	T	S	C	T	C	A	N	...
plaice, winter flounder	AcM						E		S									G					K		...
rainbow trout, pike	AcM						E		S				S					G			K		S		...
stone loach	?M						E		S									A	T		K		S		...
pigeon MT-1	AcM		S	Q	D		P			A	G							D	N		K		K		...
pigeon MT-2	AcM		S	Q	D		T			A	G	D	S		S		A	G			K		K	D	...
rat MT-2	AcM			-	N		S			T	D		S		S		A	G			K		K	Q	...

Fig. 2. N-terminal amino acid sequence of selected vertebrate metallothioneins. The sequences have been compiled from: Bonham *et al.* (1987): rainbow trout; Overnell *et al.* (1981): plaice; Chan *et al.* (1988): winter flounder; Kille *et al.* (1991): pike and stone loach; Lin and Huang (1990): pigeon; Winge and Rajagopalan (1972): rat. Space denote residues similar to cod MT. The sequence for stone loach was derived from the cDNA-sequence and it is therefore not known if the N-terminal methionine is acetylated. AcM: acetylated methionine; M: methionine; D: aspartic acid; P: proline; S: serine; Q: glutamine; D: asparagine; C: cysteine; A: alanine; K: lysine; G: glycine; E: glutamic acid; T: threonine.

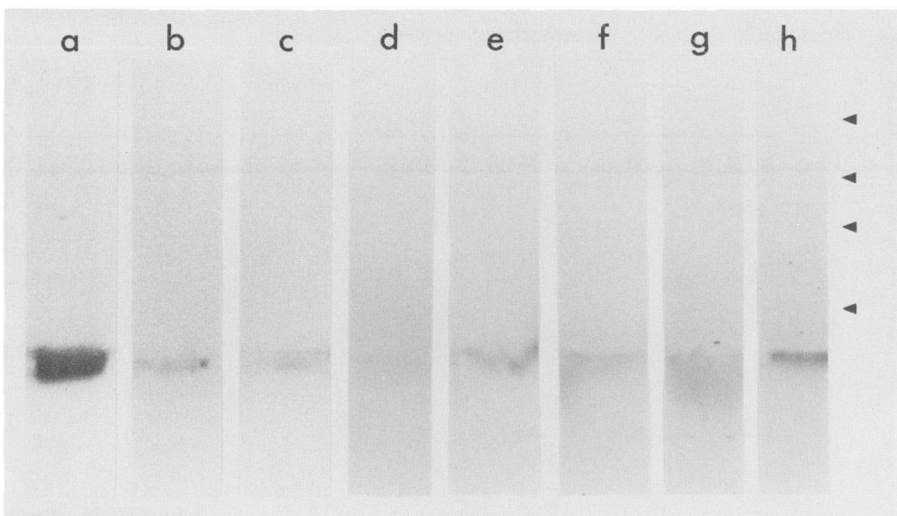

Fig. 3. SDS-PAGE electrophoresis of cytosolic extracts of tissues from cod. **Lanes a-h**: liver, ovary, intestine, gill, anterior kidney, posterior kidney, spleen, brain. Biotinylated molecular weight markers indicated by arrows: hen egg white ovalbumin (45 kDa), bovine carbonic anhydrase B (31 kDa), soybean trypsin inhibitor (21.5 kDa), hen egg white lysozyme (14.4 kDa).

anterior and posterior kidney, spleen, gonads, gill and intestine (Fig. 3A). The protein could not be detected in plasma, bile or blood cell samples of cod by this procedure (results not shown). Metallothionein in the different tissues migrated identically to purified cod hepatic or renal cod MT, with the exception of spleen and kidney samples, for which a broader band was observed. Brain MT migrated differently to that of the other tissues under native conditions (Fig. 4).

Cytosolic distribution of metals

Nearly all cytosolic Cu and Cd in the liver of control cod was associated with the peak corresponding to MT after gel-filtration (Fig. 5A). A substantial part of cytosolic Zn was also found associated with that peak. Cytosolic Ni was mainly associated with high molecular weight components in the livers of control fish. The Zn-level of all cytosolic pools appeared to increase following Zn-injection (Fig. 5B). In the liver of Cd-injected cod, cytosolic Cd was nearly exclusively found in the MT-peak with minor amounts associated with the high molecular weight pool (Fig. 5C). The levels of both

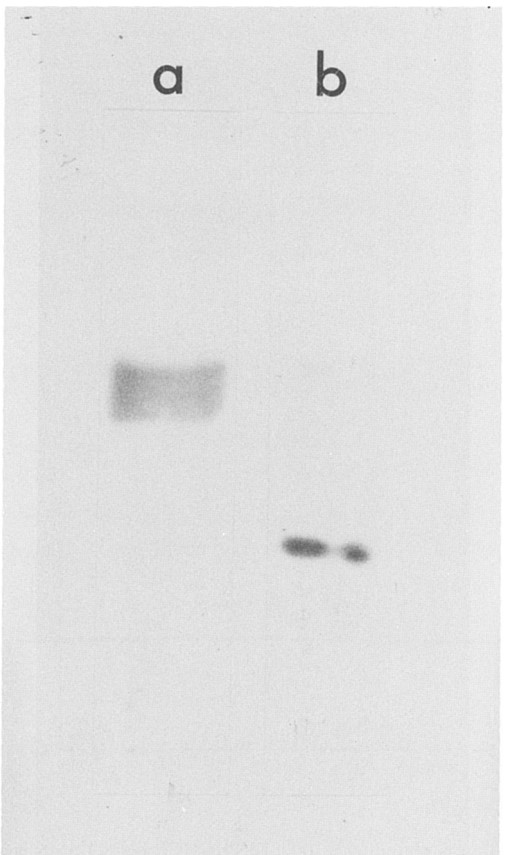

Fig. 4. Native polyacrylamide electrophoresis of cytosolic extracts of tissues from Cd-injected cod. **Lane a**: liver, **lane b**: brain.

Cu and Cd were one or two orders of magnitude lower in the kidney compared to liver, but a share of both metals were associated with the peak corresponding to the size of MT in control cod kidney (Fig. 6A). Zinc was mainly bound to components of high molecular weight (M_1 > 40 kDa), whereas Ni was found associated with both high and very low (M_r < 3 kDa) molecular weight components in the kidney. There were no obvious effects of Zn-injection on the cytosolic distribution of the metals in anterior kidney (Fig. 6B). Similarly, cytosolic Cd in the kidney of Cd-injected fish was mainly associated with the MT-peak (Fig. 6C). There was also a major increase in cytosolic Cu in Cd-injected fish, most of which was found associated with this peak. All binding of metals to very low molecular weight components appeared to be abolished by this treatment. Zinc and Ni were thus only associated with components of high molecular weight in the kidney of Cd-injected cod.

Discussion

Only one isoform of cod metallothionein (MT) was isolated from liver and anterior kidney by anion-exchange chromatography. Both one and two major isoforms of the protein have been reported for other fish species (Overnell and Coombs 1979; Chatterjee and Maiti 1987; Olsson and Hogstrand 1987a; Krezoski *et al.* 1988; Kille *et al.* 1992). The presence of a single MT-encoding gene in some fish species further supports the notion that fish generally have fewer isoforms of the protein than mammals (Kille *et al.* 1992). The amino acid composition suggests that cod MT contains more acidic amino acids than other fish MTs and this has been verified by its later elution from anion-exchange columns (Hylland, unpublished results). In addition to MT, another low-molecular-weight metal-binding protein was also present in cod liver. This protein contained little cysteine, much aspartic and glutamic acid, as well as the aromatic amino acids phenylalanine and histidine. Clearly not MT, the composition of this protein appeared to be similar to that of the low-molecular-weight Cd-binding proteins reported by Baer and Thomas (1991) to be present in the ovaries of seatrout (*Cynoscion nebulosus) and Atlantic croaker (Micropogonias undulatus*). Interestingly, this protein was not apparent in hepatic cytosols from juvenile cod (Hylland, unpublished results).

The N-terminus of cod MT was found to be distinct from that of other vertebrate MTs. Apart from canine lysosomal MT, which lacks the N-terminal methionine altogether (Lerch *et al.* 1985), cod MT is the first described vertebrate MT with a non-acetylated N-terminus. Furthermore, two of the first seven N-terminal amino acids of cod MT were different compared to MT from other fish species. Both those deviant amino acids, glutamic acid in position 5 and serine in position 7, are identical in the other fish MTs (Fig. 2). The fish species from

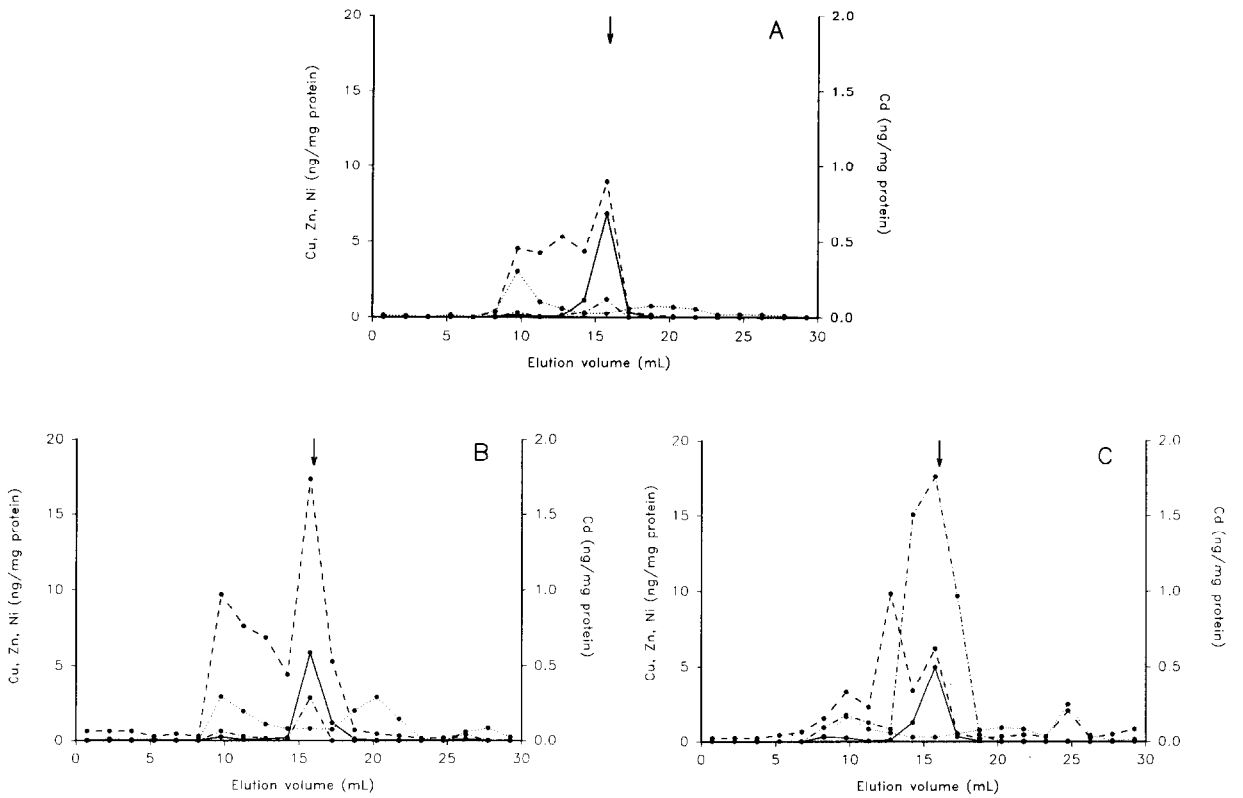

Fig. 5. Metal content of cytosolic components from cod liver on a Superose column, eluted with 100 mM Tris-HCl, pH 8.1 (dot-dashed line), Zn (dashed line), Cu (solid line), Ni (dotted line). **A.** Saline-injected cod, **B.** Zn-injected cod. **C.** Cd-injected cod. The arrow indicates the elution volume of purified cod MT.

which the N-terminal sequence is known belong to separate euteleostean superorders (Lauder and Liem 1983): Ostariophysi (stone loach), Protacanthopterygii (rainbow trout and pike), Acanthopterygii (flounder and plaice) and Paracanthopterygii (Atlantic cod). Although the small number of fish for which the N-terminal sequence is known precludes any universal conclusions, it would nevertheless appear that MT from other fish species are remarkably similar compared to cod MT.

Metallothionein was present in detectable amounts in all the tissues investigated, but the level in plasma, bile and blood-cells was too low to be identified by the method used. Chan *et al.* (1988) has previously established the presence of MT mRNA in liver, kidney, heart, brain, intestine and gills of Cd-injected winter flounder (*P. americanus*). Similarly, Zafarulla *et al.* (1989) found MT mRNA in liver, kidney, spleen, gills, ovaries and testes of rainbow trout, *O. mykiss*. The liver and brain of Atlantic cod contained more MT than the other tissues and there was surprisingly little MT in the kidney of Cd-injected fish. Furthermore, haematopoietic tissue from Cd-injected cod (spleen and anterior kidney) appeared to contain more MT than renal tissue (posterior kidney). The relative amounts of MT in different tissues are in contrast with what was found for catfish (*Heteropneustes fossilis*), in which the order was liver > kidney > pancreas > brain (Chatterjee and Maiti 1987). However, the presence of substantial amounts of MT in cod brain is in accordance with the observations by Chan *et al.* (1988) of high constitutive levels of MT mRNA in the brain of winter flounder. The reason for the different migration of brain and hepatic MT under native conditions is not

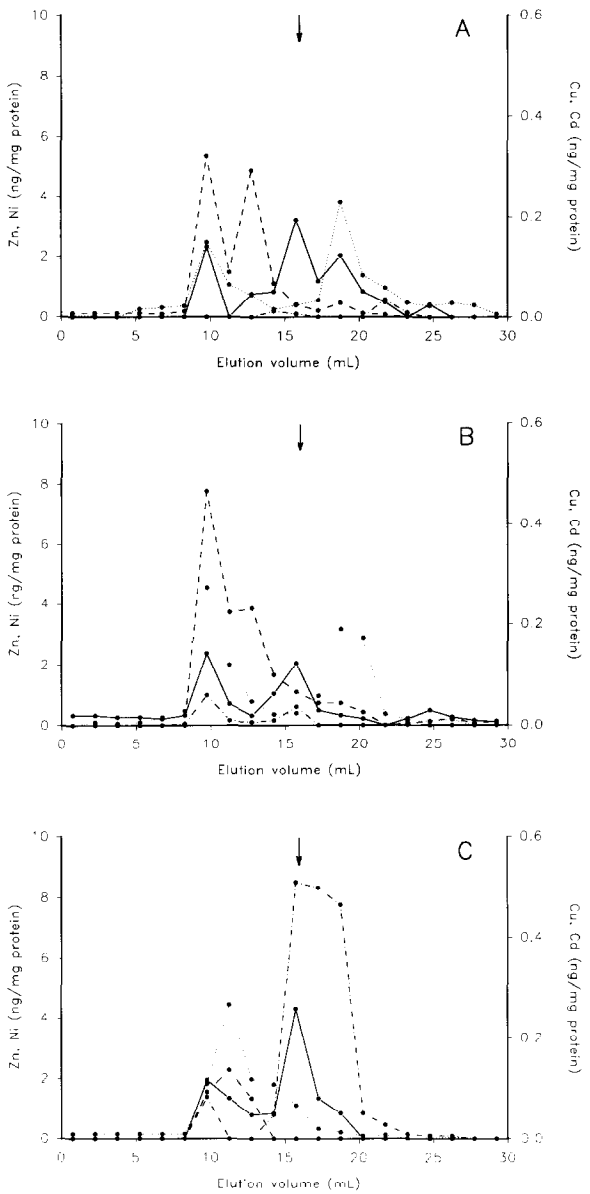

Fig. 6. Metal content of cytosolic components from cod anterior kidney on a Superose column, eluted with 100 mM Tris-HCl, pH 8.1. Cd (dot-dashed line), Zn (dashed line), Cu (solid line), Ni (dotted line). **A.** Saline-injected cod, **B.** Zn. injected cod, **C.** Cd-injected cod. The arrow indicates the elution volume of purified cod MT.

clear, but the recent results of Palmiter *et al.* (1992) of a novel MT isolated from mammalian brain (MT III) certainly provides an incentive for further exploration into the nature of cod brain MT. A role in signal transduction has been envisaged for Zn and brain MT in mammals (Ou and Ebadi 1992), but the relevance of those findings for fish is uncertain as the distribution of Zn in fish brains differ from the pattern found in mammals (Piñuela *et al.* 1992). The last tissue studied, the ovaries, were also found to contain detectable amounts of MT, in contrast to the results reported by Baer and Thomas (1991) for two other marine fish species, seatrout and Atlantic croaker, but is in accordance with what has been found for rainbow trout (Zafarullah *et al.* 1989; Olsson *et al.* 1990).

In the liver of both control and metal-treated cod, MT was the major cytosolic pool of Cu and Cd, but it bound variable amounts of Zn. Such cytosolic metal distribution is not unique to cod, but is commonly found in fish livers (*e.g.*, Takeda and Shimizu 1982a). Anterior kidney in control cod contained far less cytosolic Cu and Cd than the liver, and MT was only one of many pools of those metals. The introduction of Cd by intraperitoneal injection resulted in an accumulation of Cu and Cd in the MT-pool of both liver and anterior kidney, which is similar to what has been found for other fish species (Olsson and Hogstrand 1987b; Brown *et al.* 1990). Cytosolic Ni was associated with components of both high (M_r > 40 kDa) and very low (M_r < 3 kDa) molecular weight in both liver and kidney of all groups. A possible ligand for Ni associated with the very-low-molecular-weight pool is the tripeptide glutathione (*cf.* Cartaña *et al.* 1992). Cd-treatment will not change cellular glutathione content in rat kidney (Eaton *et al.* 1980) and it is therefore interesting that Cd-treatment apparently removes all Ni (and Cu) associated with the low-molecular-weight pool in cod kidney. However, the anterior kidney of cod (and other marine fish species) is a composite organ containing haematopoietic, renal and endocrine tissue, and a proper interpretation of those results will have to await further study.

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