Comparison of short-term cadmium poisoning in the shrimp *Crangon crangon* from the Baltic Sea and the shrimp *Palaemon serratus* from the Atlantic Ocean with cadmium bioaccumulation and malic enzyme activity in abdomen muscle

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Abstract

Shrimps were exposed to various concentrations of $CdCl_2$ under laboratory conditions for 96 h. Although kept at the same Cd^{2+} concentration but at different salinities – *C. crangon* in Baltic seawater (6 PSU) and *P. serratus* in Atlantic water (36 PSU) – the shrimps accumulated a Cd^{2+} level in abdomen muscle which was much higher in the animals kept at the lower salinities than in those at the higher

ones. The same Cd^{2+} bioaccumulation of *ca* 80 ng g^{-1} w.w. of abdomen muscle were found in *C. crangon* kept in 0.2 mg dm^{-3} of $CdCl_2$ in Baltic seawater and in *P. serratus* kept in 2 mg dm^{-3} of $CdCl_2$ in Atlantic water. In both cases the NADP-dependent malic enzyme activity per g w.w. of shrimp abdomen muscle was higher by *ca* 150% in animals kept at a $CdCl_2$ concentration close to LC_{50} as compared to the control groups.

1. Introduction

Of all of the toxic metals found in the environment and used in industry, cadmium occupies a special place because of the generally intractable nature of cadmium (Cd) poisoning. Unlike other metals, Cd has a long biological half-life and is excreted very slowly from its soft tissue storage site in the body (Bernard and Lauwerys, 1984; Bryan, 1984). Cd has a broad range of toxic effects. It can, for example, interfere with the metabolism of essential divalent cations regulating a large number of cellular processes (Simkiss and Mason, 1983). NADP-dependent malic enzyme from shrimp abdomen muscle utilises manganese more efficiently than magnesium or other divalent cations, as cadmium and cobalt are effective for expressing the reaction activity (Biegniewska and Skorkowski, 1983). However, antagonism between cadmium chloride and divalent metal cations in the activation of *Crangon crangon* abdomen muscle malic enzyme was observed *in vitro*. At a Cd concentration of 45 μM the enzyme reaction was no longer activated (Biegniewska *et al.*, 1993). Recently it has also been demonstrated that the total adenylate pool decreases significantly in shrimps exposed to Cd concentrations of 4 mg dm^{-3} (Thebault *et al.*, 1996).

The present investigation was undertaken to establish the effect of short-term Cd poisoning on two species of decapod crustaceans kept at different salinities. Exposure of the shrimps to Cd led to (in a concentration-dependent manner) an increase in (1) cadmium bioaccumulation, (2) NADP-dependent malic enzyme activity per g fresh abdomen muscle, and (3) total soluble protein extracted per g wet weight (w.w.) of abdomen muscle. However, some specific and environmental differences were also observed.

2. Materials and methods

Animals

The shrimps *C. crangon* 3–4 cm in length were caught in the Gulf of Gdańsk in July and kept in aerated seawater (6 PSU). Young common prawns *P. serratus* 3–4 cm in length were collected in Concarneau Bay in July and kept in aerated seawater (36 PSU). The animals were regularly fed with mussels.

Toxicity tests

The shrimps, of average length 3–4 cm, were divided equally into groups of ten animals and exposed to concentrations of 0.02, 0.06, 0.2, 0.6, 2 and 4 mg dm^{-3} $CdCl_2$ in aerated water at $18 \pm 1°C$. The experimental solutions were changed regularly at 24 h intervals. The shrimps were kept under a natural light regime. The animals were not fed during the experiment and died after 96 h.

Tissue preparation

The abdomen muscles were dissected free of cuticule and divided into two parts. One part was dried at 60°C during two days for the dry weight (d.w.) determination and trace metal analysis. The other, to be used in enzyme and protein assays, was homogenised at 4°C for 15 s at 22 000 rpm in a top-drive Polytron PT 2000 homogeniser with 4 vol. of 10 mM Tris-HCl buffer pH 7.8 containing 2 mM EDTA. The homogenate was centrifuged for 20 min at 20 000 g. The precipitate was discarded and the supernatant used for the malic enzyme activity and protein assays.

Trace metal analysis

Samples of abdomen muscle were analysed for Cd after wet digestion (HNO_3 – $HClO_4$) and solution in 0.1 M HCl, and analysed for total Cd in a Video 11E atomic absorption spectrometer (Thermo Jarrell Ash, USA).

Enzyme assays

Malic enzyme (L–Malate: NADP oxidoreductase (decarboxylating) EC 1.1.1.40) activity was followed spectrophotometrically on a recording spectrophotometer by observing the appearance of NADPH at 340 nm and 30°C. The incubation medium for the assay activity contained 50 mM Tris-HCl buffer pH 7.5, 10 mM L-Malate, 0.5 mM NADP and 1 mM $MnCl_2$. Enzyme activities were calculated using $E\ mM \times 340^{-1} = 6.22$ for NADPH in a 1 cm light-path quartz cell.

Protein assays

Protein concentration was determined by the Coomassie Blue method (Spector, 1978).

Chemicals

NADP, Tris, $MnCl_2$, $CdCl_2$ and Brilliant Blue G were from Sigma Chemical Co., St. Louis, Mo, USA. All other chemicals were analytical reagent grade materials from Prolabo, Paris, France or POCh, Gliwice, Poland.

3. Results

Cadmium toxicity

Shrimps *C. crangon* and *P. serratus* were exposed to different concentrations of cadmium chloride under laboratory conditions for a short period (96 h). In the case of *C. crangon* kept in seawater of 6 PSU, mortality was high at a Cd concentration of 0.6 mg dm^{-3} (Fig. 1a). After 96 h, 100% of the shrimps died. The no-effect level determined under our test conditions

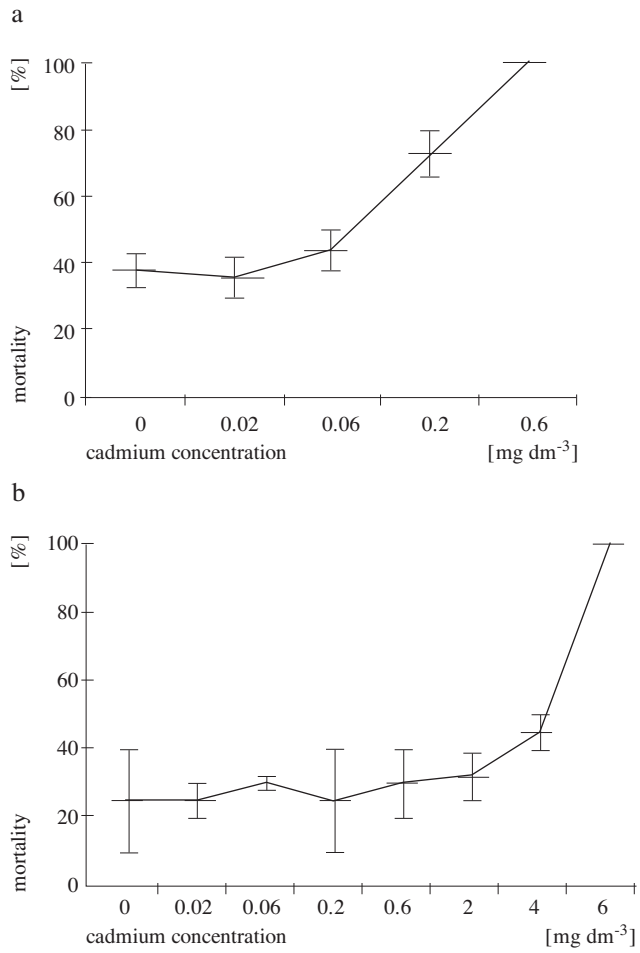


Fig. 1. Cadmium toxicity expressed as a percentage of shrimp mortality after 96 h of exposure to different cadmium concentrations: *C. crangon* in Baltic seawater (6 PSU) (a); *P. serratus* in Atlantic water (36 PSU) (b). Each point represents the average of two independent experiments

was *ca* 0.06 mg dm^{-3}. The LC_{50} was around 0.2 mg dm^{-3} on the 4th day. On the other hand, for *P. serratus* kept in seawater of 36 PSU, the no-effect level determined under our test conditions was *ca* 2–3 mg dm^{-3}. The LC_{50} was *ca* 4 mg dm^{-3}. The lethal concentration was *ca* 6 mg dm^{-3} (Fig. 1b).

Cadmium accumulation

Shrimps were exposed to different concentrations of cadmium chloride for 96 h. The abdomen muscles were then excised and the Cd concentration

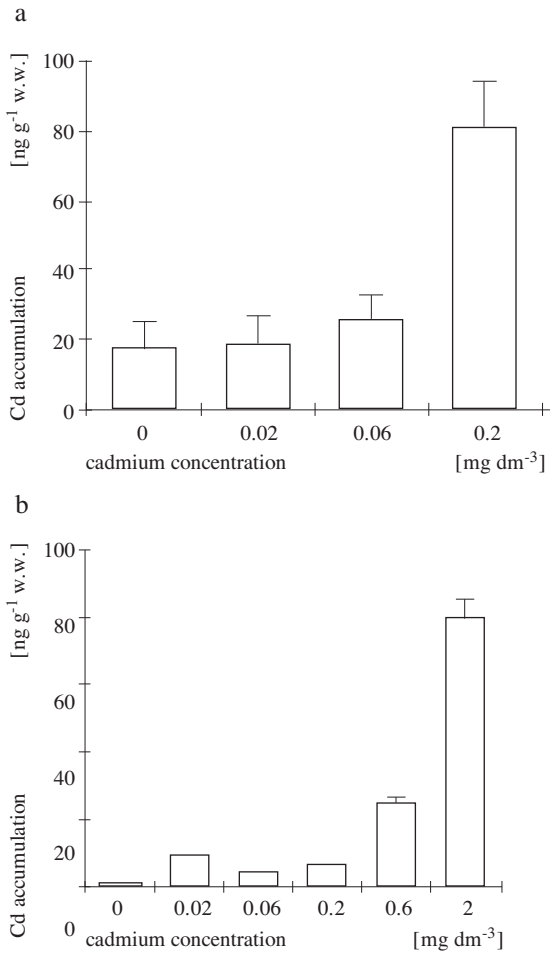


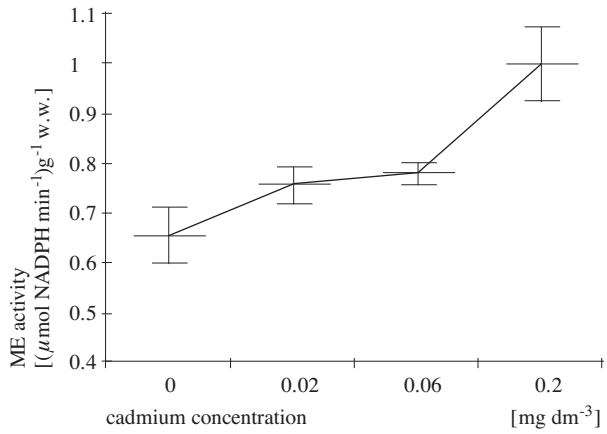
Fig. 2. The relationship of cadmium bioaccumulation in abdomen muscle of shrimps to different cadmium concentrations after 96 h of exposure: *C. crangon* in Baltic seawater (6 PSU) (a); *P. serratus* in Atlantic water (36 PSU) (b). Each value represents the average of three assays

estimated. Cd accumulation increased with dosage, and was much higher in the abdomen muscle of *C. crangon* from 6 PSU seawater (Fig. 2a) than in that of *P. serratus* (Fig. 2b) from 36 PSU seawater. The same Cd accumulation of *ca* 80 ng g^{-1} w.w. of abdomen muscle was found in *C. crangon* kept at a Cd concentration of 0.2 mg dm^{-3} in Baltic seawater and in *P. serratus* kept at a Cd concentration of 2 mg dm^{-3} in Atlantic water. This shows that salinity may control Cd bioaccumulation and content in shrimp abdomen muscle. A Cd concentration of *ca* 80 ng g^{-1} w.w. of shrimp abdomen muscle appears to be sublethal in this tissue.

Effect of cadmium on malic enzyme activity

To examine the effect of cadmium accumulation on NADP-dependent malic enzyme activity in abdomen muscle, shrimps were exposed to various Cd concentrations for 4 days. In the two species of shrimps investigated, NADP-dependent malic enzyme activity in abdomen muscle was induced by Cd in a concentration-dependent manner (Figs. 3a and 3b). In both cases NADP-dependent malic enzyme activity per g w.w. of shrimp abdomen muscle was higher by *ca* 150% in animals kept at a Cd concentration close to LC_{50} as compared to that in the controls. Previous observations had shown that Cd could cause *in vitro* inhibition of malic enzyme activity, which suggested that the enzyme molecules were inactivated by this compound (Biegniewska *et al.*, 1993). Cd bioaccumulation in shrimp abdomen muscle suggested an *in vivo* inhibition of the NADP-dependent malic enzyme. Hence, this malic enzyme inhibition may induce the synthesis of new enzyme molecules and in turn a higher malic enzyme activity *in vivo*.

a



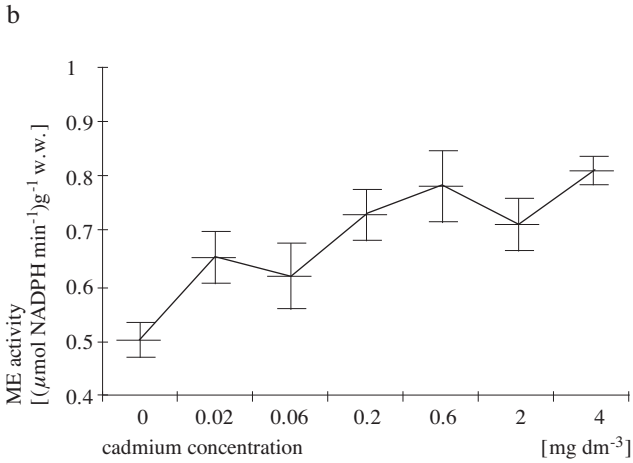
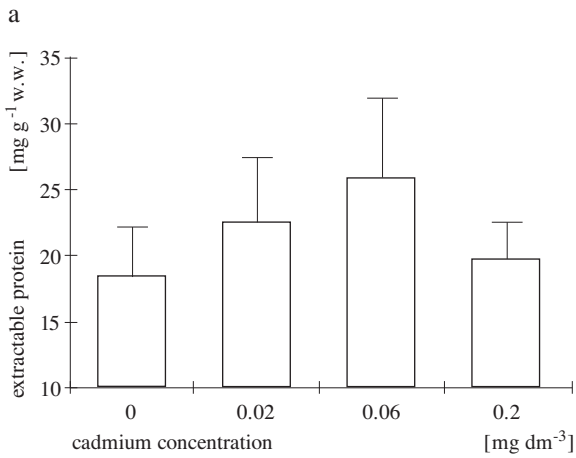


Fig. 3. NADP-dependent malic enzyme activity in shrimp abdomen muscle extract after 96 h of exposure to different cadmium concentrations: *C. crangon* in Baltic seawater (6 PSU) (a); *P. serratus* in Atlantic water (36 PSU) (b). Each point represents the average of three measurements

Soluble protein extracted

The effect of Cd accumulation on the soluble protein extracted from shrimp abdomen muscle was investigated after 96 h exposure. Figs. 4a and 4b show the effect of Cd concentration on the increase in the level of soluble protein extracted from this tissue in the two shrimp species. Protein synthesis was usually decelerated by short-term exposure to Cd. The cytotoxity of Cd may be due to interaction with a variety of cellular



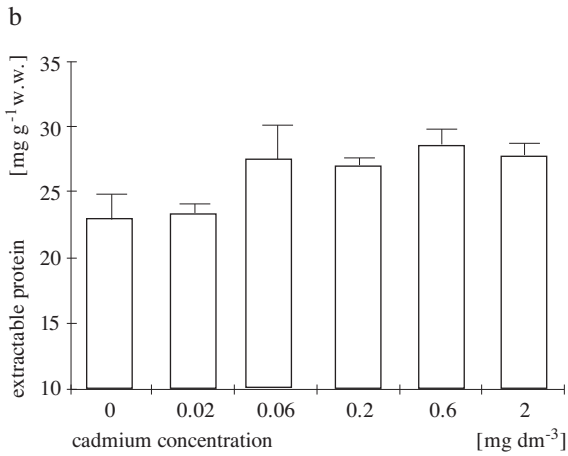


Fig. 4. Soluble protein extracted from shrimp abdomen muscle after 96 h of exposure to different cadmium concentrations: *C. crangon* in Baltic seawater (6 PSU) (a); *P. serratus* in Atlantic water (36 PSU) (b). Each value represents the average of three assays

pathways where essential cations such as zinc and calcium help to sustain normal cellular functions. It has recently been shown that Cd induces apoptosis (el Azzouzi *et al.*, 1994). This process is a programmed cell death which also stimulates proteolysis, and may be responsible for the increase in soluble protein extracted from abdomen muscle.

4. Discussion

The toxic effects of Cd can affect two major mechanisms. Firstly, the metal ion can bind to the sulphydryl groups in protein and to nucleic acid bases, thus affecting the structure and function of these macromolecules (Vallee and Ulmer, 1972). Secondly, Cd can also interfere with the metabolism of essential divalent cations involved in the regulation of a large number of physiological processes (Brostrom and Brostrom, 1990). The activity of malic enzyme in crustacean muscle is substantially higher than that observed in most terrestrial species (Skorkowski *et al.*, 1977). Malic enzyme is particularly interesting since it uses pyruvate as a substrate and provides an alternative route for pyruvate catabolism with potential roles in both anaerobic and oxidative metabolism. Malic enzyme catalyses the reversible decarboxylation of malate to form pyruvate in the presence of NADP coenzyme and the divalent cation Mn^{2+} or Mg^{2+}. Over the pH range 6.5–7.0 the rate of pyruvate carboxylation is equal to the rate of malate decarboxylation, suggesting an anaplerotic function for *C. crangon* abdomen muscle malic enzyme (Biegniewska and Skorkowski, 1983).

Short-term exposure of shrimps to Cd inhibited the synthesis of some proteins while inducing the expression of specific proteins, *e.g.* metal-thionein-like proteins and stress proteins. These differences were observed in the intensity of Coomassie Blue staining of the gel after SDS-PAGE (Napierska *et al.*, 1996). The present data show that NADP-dependent malic enzyme activity in shrimp abdomen muscle is increased by short-term exposure to Cd *in vivo* (Fig. 3). A possible mechanism for an increase in malic enzyme activity may include Cd bioaccumulation in shrimp abdomen muscle. The inhibition of malic enzyme activity by Cd, which interferes with the divalent cations essential for the activation of the enzyme reaction, could induce new enzyme synthesis. Our previous study (Biegniewska *et al.*, 1993) showed that malic enzyme is a molecule highly sensitive to Cd exposure *in vitro* and that this ion strongly inhibits malic enzyme activity. In cation combinations *in vitro*, Cd inhibits enzyme activity up to the value this would have if Cd were added alone; in the presence of both cations (Cd^{2+} and Mn^{2+}) Mn^{2+} does not activate malic enzyme activity (Biegniewska *et. al.*, 1993).

The increase in malic enzyme activity correlated well with the effect of short-term Cd poisoning on the level of soluble protein extracted from the abdomen muscle of both shrimp species (Fig. 4). However, further research is required in order to discover more about the mechanism of the action of Cd on malic enzyme synthesis in shrimp abdomen muscle.

Our study confirms the evidence that Cd is toxic to *C. crangon* and *P. serratus*, as this ion diminishes the capacity of shrimps to survive (Fig. 1). While the Cd concentration needed to bring about this effect in *C. crangon* is relatively low for this species, that required to produce the same effect in *P. serratus* is *ca* 10 times greater. When shrimps were kept at the same Cd concentration but at different salinities, much higher levels of Cd accumulated in abdomen muscle at the lower than at the higher salinity (Fig. 2). It has already been suggested that salinity is important in Cd uptake and accumulation by *C. crangon* body tissues (Szaniawska, 1985).

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