Stress proteins induced by cadmium in the abdominal muscle of the shrimp *Crangon crangon**

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Abstract

Shrimps were exposed to various concentrations of $CdCl_2$ under laboratory conditions for 96 h. Abdominal muscles were isolated from exposed and control animals. The induction of stress proteins (heat shock proteins of the Hsp70 family and metallothionein) was detected following polyacrylamide gel electrophoresis in the presence of SDS (SDS–PAGE) and specific staining (Western blotting method in the case of the Hsp70 or Coomassie blue and silver staining in the case of metallothionein). The short-term cadmium poisoning in the shrimp *Crangon crangon* resulted in the induction, in a concentration-dependent manner, of metallothionein and a new protein with an approximate molecular weight of 70 kDa in abdominal muscle. This protein was immunologically cross-reactive with the 70 kDa heat shock protein of the mouse.

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In a variety of organisms and cultured cells cadmium ions inhibit the overall protein synthesis, while the synthesis of specific proteins, called stress proteins or heat shock proteins (hsps), can be enhanced. The function of the heat shock proteins is not completely known (Lindquist and Craig, 1988; Lipińska, 1989). Many stress proteins are responsible for immediate stress protection or conduct cellular repair processes (Pelham, 1986). It has been shown that exposure to cadmium evokes the expression of stress proteins in fish (Heikkila *et al.*, 1982) and invertebrates (Veldhuizen-Tsoerkan *et al.*, 1990).

Stress proteins with molecular masses between 70 and 90 kDa are seen in response to most situations of stress; others, including metallothionein (MT), appear to be induced more selectively. Metallothionein is a cysteine-rich, heat-stable, low-molecular-weight protein involved in the detoxification, storage and regulation of heavy metals (Hamer, 1986; Glaven *et al.*, 1991).

Our previous study (Napierska *et al.*, 1997) showed that NADP-dependent malic enzyme activity in shrimp abdomen muscle was increased by short-term exposure to Cd *in vivo*. Differences in the protein expression pattern were also observed (Napierska *et al.*, 1996). The present investigation was undertaken to establish the effect of short-term Cd poisoning on the synthesis of specific stress proteins in the abdominal muscle of *Crangon crangon*.

Shrimps *C. crangon* were caught in the Gulf of Gdańsk in the summers of 1996 and 1997. The animals were kept in aerated seawater (salinity 6 PSU) at 18 ± 1°C under a natural light regime. The shrimps were divided equally into groups of ten animals and exposed for 4 days to concentrations of 20, 60 and 200 μg dm^{-3} $CdCl_2$. The animals were not fed during the experiment. The abdominal muscles were dissected free of cuticle and homogenised at 4°C. The homogenate was centrifuged for 20 min at 20 000 g. The supernatant was diluted 1:1 in SDS-sample buffer and heated for 5 min in a boiling water bath. One-dimensional sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described by Laemmli (1970) using 12% polyacrylamide gels. Protein concentration in the samples were determined by the Coomassie Blue method (Spector, 1978).

Identification of metallothionein by gel electrophoresis and silver staining was carried out following the procedure described by McCormick and Lih-Yuan Lin (1991). We routinely employed the concentrate solutions provided by Sigma (U.S.) and followed the manufacturer's recommended procedure.

Following electrophoresis, the proteins were transferred on to immobilon-P membranes (Millipore) at a constant 1.5 mAmps per cm^2 of gel for 1.5 h in a Biometra Fastblot semi-dry transfer unit. After transfer the membranes were incubated overnight in 3% non-fat dry milk in Tris buffer saline (TBS), and then placed for 1h in an appropriately diluted solution of monoclonal mouse Anti-Heat Shock Protein 70 Antibody (IgG_1) in 3% non-fat dry milk in TBS. The antibody was obtained from Affinity Bioreagents (U.S.). The antibody was produced from a hybridoma, the product of a fusion between SP2/o myeloma cells and splenocytes from BALB/c mice immunised with recombined human Hsp70 previonsly induced in *E. coli*. Following brief washing with TBS the membranes were incubated for 1 h in a diluted antibody solution of sheep anti-mouse conjugated with alkaline phosphatase. After the final wash the immunocomplex was detected with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP).

As can be seen in Fig. 1, Western blot analysis revealed the induction of proteins with an approximate molecular weight of 70 kDa. This induction was detected most clearly at the highest cadmium concentration (200 μg $CdCl_2$ dm^{-3}; Fig. 1e). The new protein was immunologically cross-reactive with the 70 kDa heat shock protein of the mouse (Figs. 1c, 1d and 1e). The protein was not present at a detectable level in unstressed animals (Fig. 1b).

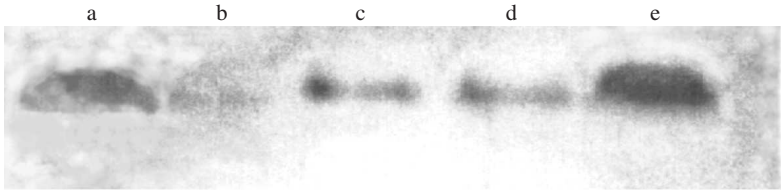

Fig. 1. Effect of cadmium exposure on the synthesis of Hsp70 as revealed by Western blot analysis in the abdominal muscle of the shrimp *C. crangon*. Lanes: a – Hsp70 (DnaK) from *E. coli*; b – control group; c – animals exposed to 20 μg $CdCl_2$ dm^{-3}; d – 60 μg $CdCl_2$ dm^{-3}; e – 200 μg $CdCl_2$ dm^{-3}

Elevated synthesis of metallothionein in cadmium-treated animals was detected after SDS–PAGE electrophoresis and staining with Coomassie Blue (Fig. 2a) and enhancing with silver stain (Fig. 2b). The different colour of MT after silver staining (Fig. 2b, lanes b, c, d) positively identified this protein among other proteins of shrimp abdominal muscle homogenate.

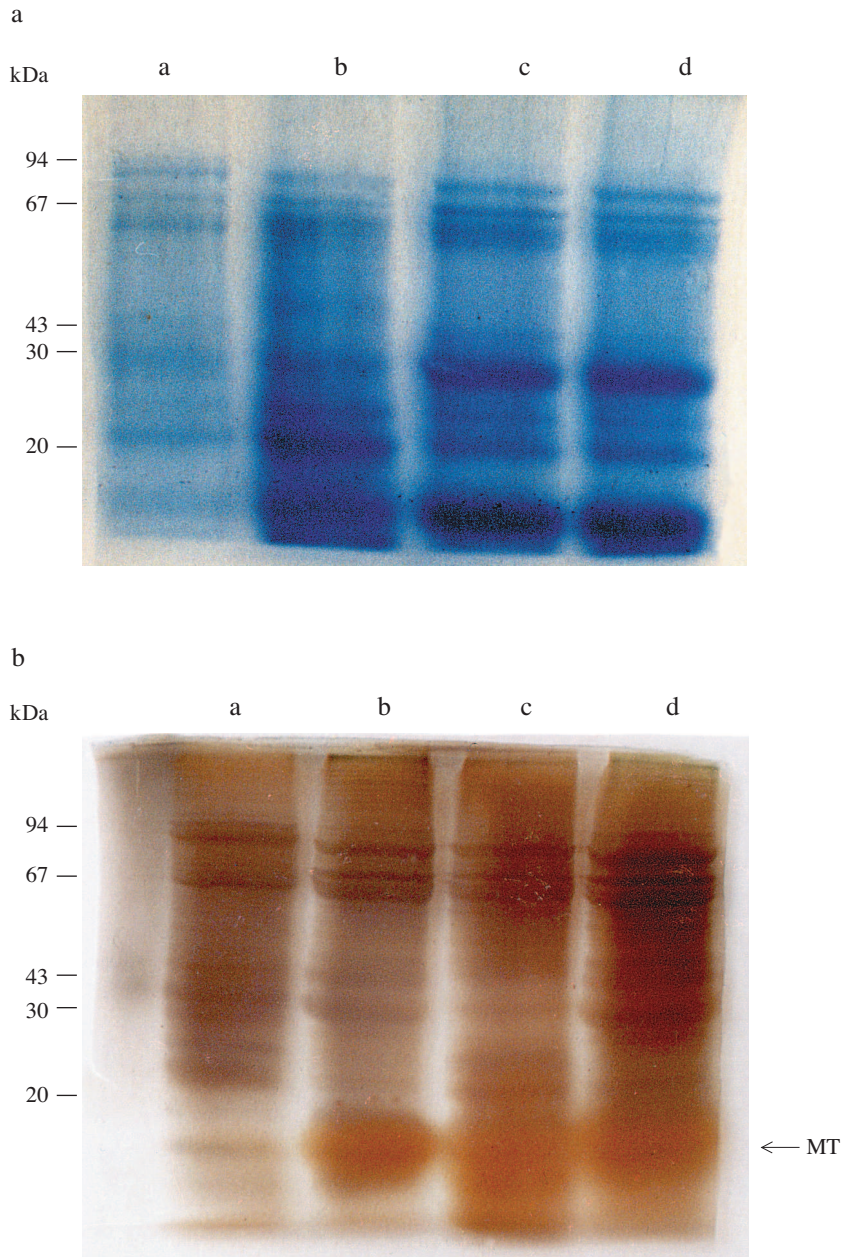

Fig. 2. Polyacrylamide gel electrophoresis in the presence of SDS (SDS–PAGE) of control and cadmium-treated shrimp *C. crangon* abdominal muscle homogenate. Elevated synthesis of metallothionein (MT) was detected after staining with Coomassie Blue (a) and enhancing with silver stain (b). Lanes: a – control group; b – animals exposed to 20 μg $CdCl_2$ dm^{-3}; c – 60 μg $CdCl_2$ dm^{-3}; d – 200 μg $CdCl_2$ dm^{-3}

The animals used in the experiments live in brackish coastal waters (salinity 6 PSU) where cadmium levels have increased considerably in recent years (Bolałek *et al.*, 1989). The susceptibility of many marine and estuarine invertebrates to the toxic effects of some trace metals increases as the salinity of seawater decreases (McLusky *et al.*, 1986). It has already been observed that salinity is important in Cd uptake and accumulation by shrimp body tissues (Szaniawska, 1985).

As shown previously, Cd bioaccumulation in shrimp abdominal muscle depended on the Cd concentration in water and increased with dosage (Napierska *et al.*, 1997). In this study, linear accumulation of cadmium in the abdominal muscles of the animals was also recorded (data not given). Like most organisms, shrimps *C. crangon* undergo a change in the pattern of protein synthesis in response to increased metal concentrations. The analysis of the presence and intensity of bands revealed by SDS–PAGE electrophoresis and specific staining indicates that the new proteins could be stress proteins induced by Cd bioaccumulation in shrimp abdominal muscle.

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