Communications

Induction of stress proteins in the presence of cadmium in the Baltic blue mussel *Mytilus trossulus**

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Abstract

The exposure of organisms to environmental stressors affects the expression levels of certain 'stress proteins' that play an important role in protein homeostasis and stress tolerance. Examining the protein profiles by SDS–PAGE and Western blotting analysis, mussels *Mytilus trossulus* were exposed to cadmium, which induced a number of Hsp70 proteins in accordance with the metal concentrations. In immunodetection two commercial monoclonal antibodies were used to monitor this response in gill tissue. It follows that Hsp70, which is typically induced by moderate heat-shock treatment, is in most cases also induced in the presence of cadmium.

The impact of pollutants on ecosystems and their components is attracting increasing attention. Currently, there are intensive, cost-effective biomarkers that can be used in ecological risk assessment (Depledge, 1994). It has been shown that stress proteins (heat shock proteins) are induced

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by exposure to pollutants (McCarthy and Shugart, 1990). The biomarker is a ubiquitous response to a number of stressors, whereas the stress proteins are mainly induced by exposure to trace metals. Most studies to date have involved laboratory experiments where correlations have been established between trace metal exposure and the level of stress proteins (Radłowska and Pempkowiak, 1996).

Among the stress proteins, the Hsp70 family is the most prominent one (Sanders and Martin, 1993) because it is the largest and most highly conserved evolutionary group.

We have previously described the immunological detection of stress-70 isoforms in the blue mussel *Mytilus trossulus* using two commercially available antibodies:

- Affinity BioReagents, isotype IgG1, clone 3a3;
- Sigma, isotype IgG1, clone BRM–22.

The aims of the present study were (1) to examine the stress reaction to a metal stressor and (2) to compare the immunodetection using two different antibodies with a view to their suitability for a monitoring study.

Conditions and heavy metal exposure

Baltic Sea mussels *M. trossulus* were collected from the Gulf of Gdańsk in May 1996. The mean shell length of the mussels selected for experiment was 2.5–3.5 cm. Twelve mussels were kept in an aquarium containing 15 dm^3 of aerated seawater (salinity 7 PSU) at 10°C without being fed for one week's acclimatisation prior to the experiment. After acclimatisation the animals were exposed for 7 days to cadmium chloride at concentrations ranging from 0.05 to 0.4 ppm Cd (II) in seawater. Composite samples of gills dissected from 5 mussels were prepared for analysis. The tissues were homogenised in Tris-HCl buffer and aliquots of homogenate were centrifuged. The extracted proteins were solubilised in SDS-sample buffer and heated at 80°C for 5 minutes. The protein concentration was determined by Bradford's method using bovine serum albumin as standard (Bradford, 1976).

SDS–PAGE and Western blot analysis

One-dimensional sodium dodecasulphate–polyacrylamide gel electrophoresis was performed according to the Laemmli (1970) procedure. Proteins were separated on 10% resolving gel with 5% stacking gel. Total proteins – 22 μg – were applied. The proteins were transferred to Immobilon using a semi-dry unit, after which the membranes were stained for 5 minutes with Ponceau S to visualise and mark the position of the proteins used as molecular-weight standards. After removing the Ponceau S by rinsing

with water, the blots were incubated in TBS buffer containing 3% non-fat dry milk to block non-specific bonding sites. Immunological detection of stress-70 proteins was performed by using in parallel two commercial antisera against Hsp70 – clone 3a3 (Affinity BioReagents) and clone BRM–22 (Sigma) overnight in a 1:5.000 dilution in TBS-milk solution. The blots were then reacted with commercial goat anti-mouse antibody conjugated to alkaline phosphatase. The immunocomplex was developed using the p-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate visualisation system, as described by Smerdon *et al.*, (1995). Western blots were quantitated by scanning with a Laser Densitometer (Epson GT) and the absorbance of the samples expressed the percentage of the standards.

The effect of cadmium exposure on levels of stress-70 proteins in gill tissue

The data showed an increase in Hsp70 expression, which was dependent on the metal concentration the animals were exposed to. Although one-dimensional SDS–PAGE of the gill extract did not allow direct visualisation of Hsp70 in the gel, Western blot analysis revealed an increase in the amount of Hsp70 protein after 7 days' exposure. This induction was detected at the highest cadmium concentration used – 0.4 μg dm^{-3}. It was also evident at lower concentrations.

Comparison of two commercial antibodies with immunodetection of Hsp70

The densitometric analysis as measured with the image analyser is summarised in Fig. 1. This shows the cross-reactivity of the two monoclonal antibodies clone 3a3 (Affinity Bioreagents) and clone BRM–22 (Sigma) with the protein extract from the gill tissue of control and metal-stressed *M. trossulus* individuals fractionated by one-dimensional SDS–PAGE and Western analysis.

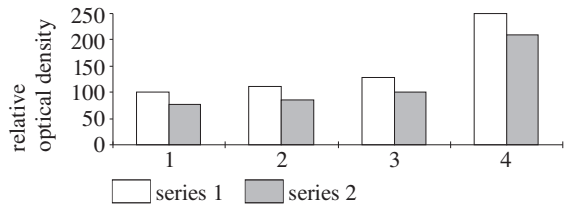

Fig. 1. Mean intensities of Hsp70 in the gills of the blue mussel *M. trossulus*. Series 1 – clone 3a3, series 2 – clone BRM–22; 1 – control, 2 – 0.05 ppmCd, 3 – 0.1 ppmCd, 4 – 0.4 ppmCd

The results show that in untreated individuals (controls), proteins of apparent molecular weights 70 kDa were recognised by both antibodies. In the animals exposed to cadmium, there was evidence of Hsp70 family induction.

In this study we used two commercially available monoclonal antibodies raised against human Hsp70 to further investigate the stress response in mussels. To study this response, a number of different antibodies are needed which react to different subsets of the Hsp70 family. During immunoblotting, these antibodies localise both the constitutive and inducible forms of Hsp70 and react with Hsp70 family members across a board range of species (Affinity BioReagents and Sigma data sheet). Furthermore, these two antibodies recognise Hsp70 in the ELISA immunoassay (Radłowska, unpublished data). We have demonstrated conclusively that monoclonal antibodies can be used in the simultaneous study of the differential expression stress of the Hsp70 family in response to man-made environmental contamination in mussels and other marine organisms. We are currently investigating the sensitivity of the antibodies to shorter periods of stress, and are using the system to study tissue-specific responses to a range of chemical stressors.

References

Bradford M., 1976, *A rapid and simple method for quantitation of microgram quantities of protein using the principle of dye binding*, Anal. Biochem., 72, 248–254.

Depledge M. H., 1994, *The rational basis for the use of biomarkers as ecotoxicological tools*, [in:] *Non-destructive biomarkers in vertebrates*, M. C. Fossi and C. Leonzio (eds.), Lewis Publ., Boca Raton, Florida, 271–295.

Laemmli U. L., 1970, *Cleavage of structure proteins during the assembly of bacteriofage T4*, Nature, 277, 680–685.

McCarthy J. F., Shugart L. R., (eds.), 1990, *Biomarkers of environmental contamination*, Lewis Publ., Boca Raton, Florida, 335 pp.

Radłowska M., Pempkowiak J., 1996, *Qualitative analysis of stress proteins induced by cadmium in the blue mussel Mytilus trossulus*, Oceanol. Stud., 3, 133–137.

Sanders B. M., Martin L. S., 1993, *Stress proteins as biomarkers of contaminant exposure in archived environmental samples*, Sci. Total Environm., 139/140, 459–470.

Smerdon G. R., Chapple J. P., Hawkins J. S., 1995, *The simultaneous immunological detection of four stress-70 protein isoforms in Mytilus edulis*, Mar. Environm. Res., 40, 399–407.