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Short communication

Redox proteomics in the mussel, *Mytilus edulis*

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Abstract

Pollutants (e.g. PAHs, metals) cause oxidative stress (OS) by forming reactive oxygen species. Redox proteomics provides a means for identifying protein-specific OS effects in *Mytilus edulis*. Groups of mussels were sampled from a clean site in Cork Harbour, Ireland and exposed to 1 mM H_2O_2 in holding tanks. Protein extracts of gill and digestive gland were separated by two dimensional electrophoresis and similar protein expression profiles were found. Effects of OS on disulphide bridge patterns were investigated in diagonal gels by separating proteins in non-reducing conditions followed by a second reducing dimension. Immunoprecipitation selected carbonylated and glutathionylated proteins. These methodologies can contribute to redox proteomic studies of pollutant responses in marine organisms.

Keywords: Carbonylation; Glutathione; Oxidative stress; Proteomics; *Mytilus edulis*; Actin

Oxidizing changes in redox potential can modify amino acid side chains by carbonylation, glutathionylation or altered formation of disulphide bridges (Stadtman and Levine, 2000). Some modifications cause inactivation, some are protective and others allow the cell to "sense" altered redox status. Aquatic organisms such as mussels are constantly exposed to pro-oxidants in their natural environment since seawater contains appreciable amounts of H_2O_2 generated by photo-oxidation (O'Sullivan et al., 2005). Some environmental pollutants including polyaromatic hydrocarbons (PAHs) and metals can generate reactive oxygen species (Livingstone et al., 1993). When the antioxidant defences of a marine

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organism are overcome by excessive levels of reactive oxygen species, a state of oxidative stress (OS) results. Biochemical markers of OS may provide novel endpoints for exposure to environmental pollutants (Livingstone et al., 1993; McDonagh et al., 2005; Valavanidis et al., in press). Here, we describe approaches to identify specific *Mytilus edulis* proteins targeted by oxidative stress (OS).

Animals (50) approximately 4–6 cm in length were sampled from a reference site in Cork Harbour, Ireland (Lyons et al., 2003). Five groups (five individuals) were acclimated (1 week, 12 h light/dark cycle) with regular feeding (at intervals of 48 h) on Phytoplex™ phytoplankton feed (Kent Marine Inc., Acworth, GA, USA). They were then exposed to 1 mM H_2O_2 (24 h) and dissected after a recovery period (24 h). Comparison was made with unexposed controls (5×5 animals). A relatively short exposure time was used to gain insight into acute toxicity as a result of H_2O_2-induced OS (McDonagh et al., 2005). The twenty-four hour recovery period was to allow clearance of "unreacted" H_2O_2 by catalase since residual H_2O_2 might artefactually oxidize proteins in cell extracts. Gills/digestive glands were dissected, pooled and homogenized in 10 mM Tris/HCl, pH 7.2 containing 500 mM sucrose, 1 mM EDTA and 1 mM PMSF. Extracts were collected by centrifugation at 20,000*g* for 1 h at 4 °C and stored (−70 °C) until required.

Similar patterns/expression levels were found for control/exposed samples by two dimensional electrophoresis (2D SDS PAGE), so alternative analytical methods were explored. Proteins (50 μg) were separated by 12% non-reducing SDS-PAGE. The entire lane was excised, incubated in buffer containing 2% DTT (20 min) followed by 2.5% iodoacetamide (20 min). Slices were placed horizontally on a second 12% SDS-PAGE gel which was silver stained after electrophoresis. Proteins lacking disulphide bridges form a diagonal across the reducing gel (Fig. 1). Interchain disulphide bridges produce spots below the diagonal while intrachain disulphides produce spots above the diagonal. OS modifies actin (McDonagh et al., 2005), so we wondered if effects on disulphide bridge patterns of actin could be revealed in diagonal gels. Diagonal separations (Fig. 1) were electroblotted and probed with actin antibodies (Sigma, Poole, Dorset UK). This revealed

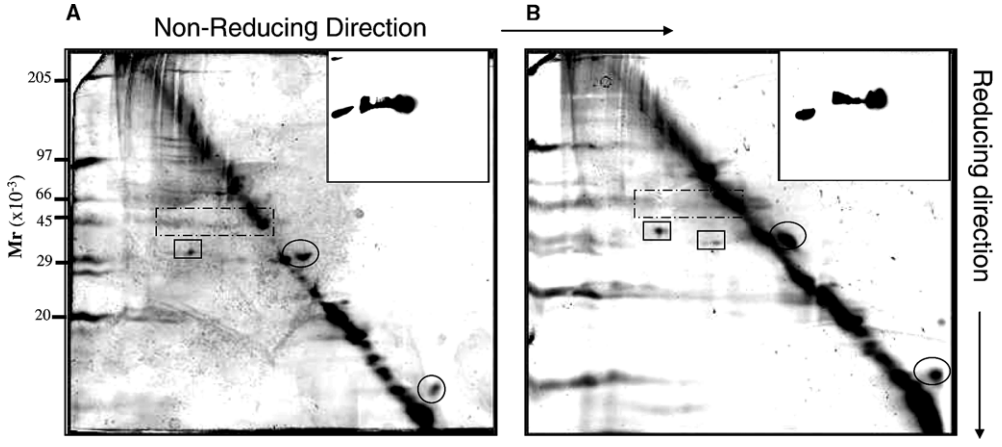

Fig. 1. Silver stained gels of gill (A) control (B) H_2O_2-exposed. Insets: Western blots probed with anti-actin. Protein spots below the diagonal (□) contain reduced intermolecular disulphide bridges while those above the diagonal (O) contain reduced intramolecular disulphide bridges. Location of actin is denoted by dashed boxes.