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Molecular cloning and characterization of rock bream SOD cDNA especially with respect to the transcriptional response to acute cadmium exposure

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Introduction

Generation of reactive oxygen species (ROS) is an unavoidable consequence of oxygen metabolism in aerobic organisms and the excess of ROS can damage cellular components such as DNA, protein and membrane lipids. Organisms express antioxidant defense systems to protect themselves from the toxic effects caused by such oxidative stress. Superoxide dismutase (SOD) plays a key role in an antioxidant pathway as the first defense line against oxidative stresses by catalyzing reactive oxygen molecules to hydrogen peroxide that consequently converted to water by catalase. Many previous studies reported the tracing the SOD activity as a biomarker for monitoring the aquatic environment, because both metals and certain organic xenobiotics generate oxidative stress. However, those studies have claimed mostly based only the biochemical measurement of activity, rather than more fine molecular biological tools. The objective of this study is to characterize the cDNAs encoding SOD from rock bream and examine the potential usefulness of the transcriptional response of SOD at mRNA level as a molecular bio-indicator to address the cadmium exposure, a ubiquitous heavy metal toxicant.

Materials and Methods

1) cDNA library construction - rock bream liver
2) Isolation of rock bream SOD cDNA fragment by RT-PCR using degenerate primers
3) Sequence analysis -BLAST and CLUSTALW

4) Tissue distribution analysis of antioxidant enzyme transcripts using RT-PCR
5) *In vivo* experimental cadmium exposure - IP injection and/or immersion
6) Quantization of SOD transcripts- RNA blot hybridization and quantitative densitometry

Results and Discussion

The transcriptional levels of SOD gene in was deferentially affected by both concentrations (intraperitoneal injections of 0, 1.2, 2.5 or 5 mg cadmium per kg body weight) and durations (immersion of fish at 5 ppm cadmium solution for 0, 1, 4, 7, 14 days) of cadmium exposure, based on scanning densitometry of RNA dot blot and northern blot hybridization. Generally, higher concentrations and longer durations of cadmium exposure increased the transcriptional activity of SOD (Fig. 1). Results from the present study indicate that assessment of transcriptional activity of fish SOD gene at mRNA level might be useful for biomonitoring the environmental stress caused by heavy metal pollutants in a fine manner. The present study suggests that the nucleic acid hybridization-based assay of anti-oxidant enzyme transcript could be used as an alternative system to detect the pollution-causing stresses in marine animals in a fine manner. When considering that many previous studies reported frequently a poor correlation between pollution status and activity of antioxidant enzymes, the present strategy may provide important possibilities to improve the sensitivity for detecting the biological stresses in marine and aquatic animals.

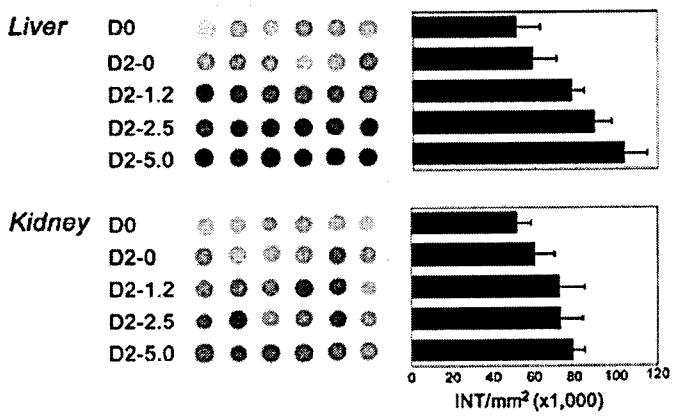

Fig. 1. Quantitative assay of SOD transcripts using RNA blot hybridization from the fish exposed to different concentrations of cadmium (0~5) for 48 hours.