Evaluation of using metallothionein as a biomarker of Hg pollution in *Scatophagus argus* for marine pollution biomonitoring

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Abstract

The effect of mercury exposure on total MT response and bioaccumulation under control and acute Hg exposure were investigated in scats (*Scatophagus argus*). scats were exposed to different Hg concentrations (10,20,30 μg/L) during 24,48,72 hours. Total MT levels were determined by Enzyme-linked Immunosorbent assay (ELISA) method. Mercury contents were determined through cold Vapour atomic Absorption spectrometry. Induction of MT during exposure was tissue specific, displaying different response pattern in gill and liver. Mercury accumulated much stronger in liver than gill and the latter also showed lower MT level. MT biosynthesis in liver showed a significant increase after exposure to different Hg concentration during different times. This increase was significantly correlated with Hg bioaccumulation. By contrast, in gills presence of different Hg concentration during different times did not significantly modify total MT except for 72hrs at 30μg/L. Our results suggest that this form of MT existing in *S.argus* was Hg-inducible. This could be extended the use of MT in *S.argus* as a biomarker of mercury in marine pollution biomonitoring.

Keywords: Scatophagus argus, metallothionein, Bioaccumulation , Biosynthesis, mercury ,Biomarker

Introduction

Marine ecosystems are contaminated by different pollutant especially metals due to human activities. Mercury pollution is one of the world’s most serious environmental problems \[1\]. Mercury exposure is the second most common cause of toxic metal poisoning. It exists in both organic and inorganic forms \[21\] and is among the few pollutants that exhibit biomagnification in aquatic food chain \[20\]. Public health concern over mercury exposure due to contamination of fish with methyl mercury has long been a topic of political and medical debate. Existing situation in the environment has prompted numerous investigations to consider effect of mercury on the biological functions of marine organisms, particularly defense mechanism in fish. Different fish species show differences in metal accumulation and metallothionein biosynthesis as a detoxification mechanism. Tissue also accumulate metals to a different extend due to the differences in physiological and biochemical functions.

Overall distribution of scats are in the pacific region in harbours, natural embayments, brackish estuaries and lower parts of freshwater streams, frequently among mangroves. This species often exist in small aggregation.

Metallothionein among a class of proteins with relatively low molecular weight 6-8(Kda) characterized by the intrinsic presence of 20 cysteine group in their structure which confers unique metal binding properties to the molecules \[8\], \[6\]. MT occurs mainly in the cytosol and is also present in the nucleus \[12\]. Metallothioneins involves in sequestration of toxic (Cd ,Hg) and essential (Cu.Zn) metals have been proposed as sensitive biomarker in assessing metal exposure and prediction of potential detrimental effects induced by metal contamination \[15\].The induction of MT or similar metal binding proteins in fish was demonstrated either under experimental or environmental conditions \[11\], \[14\].

In the present report, *S.argus* were exposed to different contamination level of mercury during different period of times. Bioaccumulation of Hg and MT Biosynthesis in tissues (gill and liver) were measured in such experimental series at the end of each exposure period. The aim of our study was to 1) To assess Hg-binding capacity of two organs (liver and gill) to biosynthesis metallothionein 2)Examining the relationship between MT and Hg concentrations for each tissue,3)Determining effects of time and dose on MT response in these tissues,4) Evaluation of using metallothioneien as a biomarker of heavy metals specially mercury in marine pollution biomonitoring.
Material and method

Fish holding condition

Fishes (*S. argus*) were collected from west coast of Persian Gulf during summer 2007. Male and Female of scats (with a mean weight of 143±7g and total length 13-15cm) were transferred to laboratory of Khoramshahr Marine Science and Technology University (KMSU, IRAN). They were maintained in two 200-L aquaria for 2 weeks before initiation of the exposure study. They were acclimatized to aerated and standard OECD water [19] at a temperature of 26±1°C under a photoperiod of 12-14hrs. Hardness was 250mg CaCO3/L and pH 7.4±0.2. Water was filtered and the levels of NH4, NO2 and NO3 in the water were maintained below 0.1 mg/L, 0.1 mg/L and 20mg/L. During acclimatization, scats were being fed once a day with Biomar Co.fish food at a ratio of 1% of fish biomass.

Waterborne Hg exposure experiment

Examined scats (n=5 for each concentration) were exposed to three contamination levels (0),(10),(20),(30) μg/L for 24, 48, 72 hour at 26±1°C exposure duration by addition of Hg from a Hg stock prepared in deionised water (HgCl2,20, extra pure, Merck). No mortality was observed during the experiment. Water replacement was carried out every day and the Hg concentrations in the water were determined by CVAAS. The water hardness was 250 mg/L expressed as mg/L CaCO3 and the water pH varied between 7.37 to 7.68. Scats were not fed during the experiment and starved for 24 hours before it. After each exposure period Scats were anesthetized by dry extract of clove pink, then liver & gill samples were dissected on ice. The samples were divided into two parts, weighted and stored at -80°C for further processing.

Mercury analysis

Total mercury levels were determined using cold vapour analysis techniques. After thawing, one gram of tissue was digested in 20 mL of 3:1 concentrated redistilled HNO3 and concentrated H2SO4, further oxidized with 10mL of saturated solution of KMnO4. Excess oxidizing agents and mercury ions were reduced by 10mL of reducing solution (3%NaBH4 in 1%NaOH) in hydride generator apparatus, then mercury was vaporized and measured in the atomic absorption spectrophotometer (Unicam 919). The instrument was calibrated with standard solution prepared from commercial material. Analytical blank(n=3), that were run in the same way as the sample and determined using standard prepared in the same acid matrix, did not show significant metal contamination.

MT analysis

After thawing, the gill and liver samples were prepared by individually homogenizing in homogenization buffer (10mM cold Tris-HCL pH7.0) Containing 5m M2-mercaptoethanol (To prevent oxidation) with phenylmethanesulfonfonylfluoride (PMSF, protease inhibitor) in a 1:2.5-3.0 (w/v) volume using a Teflon homogenizer at 1000-1200 rpm. The homogenates were centrifuged at 12000×g for 40 min at 4°C. The supernatant was heated at 80°C for 10min in order to denature thermolabile proteins, and then centrifuged again at 12000×g for 40 min at 4°C. 96-well palates were coated with 100μl of the different samples for 12hrs at 4°C. The saturation was realized for 2hrs at ambient temperature with 200μl of a 3% bovine serum albumin (BSA) in 0.01 mol/L-1 phosphate-buffered serum albumin (PBS) at pH 7.4. After four rinses with 0.01%BSA, 0.05% Tween 20 in PBS,10μl of polyclonal antibody (Rabbit Anti–cod metallothionein diluted 1:1000) were added to each well and incubated for 2hrs at 37°C. After four rinses with previous buffer, 100μl of HRP (Peroxidase labeled goat anti-Rabbit IgG)diluted 1:3000 in TBS-Tween were added and incubated for 2hrs. After 4 washes,100μl/well of the ABTS peroxidase substrate (Kirkegaard and perry lab, USA) was added followed by incubation at room temperature for 20-30 min. Colour development was measured at 405 nm with an automatic micro-titer plate ELISA reader. The linear regression coefficient (Microsoft excel 97 SR-1,1997, Microsoft corp, seattle WA, USA) for the logarithm for the MT standard concentrations was -0.99 and the slope was -0.2.

Statistical analysis

Statistical analysis of data was carried out using SPSS statistical package programs (version 13). Data were tested for homogeneity of variance and normal distribution. ANOVA was calculated. A post host comparison was done using Tukey’s tests. Differences between means were done at the 5% probability level. Diagrams were drawn with Microsoft Excel.
Results

Mercury bioaccumulation

Hg concentration in the liver was significantly higher than gills. Hg bioaccumulation was strongly dependent on the contamination level of water and exposure duration. No interaction was found between these two factor (concentration and exposure duration) (p<0.05)

In the liver, Hg bioaccumulation was rapid during 24hrs exposure then a plateau tendency between 24 to 48hrs for 10μgHg/L and a shoulder ing for 20 and 30μgHg/L at 48hrs. Hg bioaccumulation for 30μgHg/L/24hrs condition was lower than 20μgHg/L after 72hrs exposure duration (Fig 1A).

In the gills, the accumulation tendencies showed significant increases in Hg concentration with time close to linearity but here Hg bioaccumulation in two contamination levels (20 and 30μgHg/L) after 24 and 48hrs exposure duration were lower than 72hrs respectively for 10 and 20μgHg/L (Fig 1B).

MT biosynthesis

MT biosynthesis strongly depends on tissues that was significantly higher in the liver than gill (Fig 3). In liver results showed significant differences between control and treatments. A two way ANOVA (contamination level and exposure duration) performed on MT concentrations is significant (p<0.05), there is an effect of contamination level of Hg and effect of exposure duration on MT biosynthesis. The interaction contamination × Exposure duration is significant (p<0.05).

Our data showed any significant differences at 10μgHg/L during 24 and 48hrs exposure with each other and with two other contamination levels for 24hrs (p>0.05) (Fig 4A). In the gill, Results showed an effect of exposure duration and contamination level (p<0.05) on MT biosynthesis but no interaction was found. No significant differences was found between control and treatments except for 30μgHg/L/72hrs condition. Between treatments no significant differences was found except for 10μgHg/L during 24 and 48hrs and 20μgHg/L/24hrs condition with 30μgHg/L for 72 hours (Fig 4B).
Fig 4: Mean concentration (±standard deviation) of MTs in liver (A) and gill (B) of scats: unexposed and exposed to Hg. The values followed by the same letter are not statistically different among the treatment.

Correlation between metal accumulation in *S. phagus* and the corresponding MT level

Increase in MT biosynthesis in the liver of scats for 72 hours to Hg exposure were highly correlated ($R^2=0.79$, $p \leq 0.001$) with increasing Hg concentration in this tissue (Fig 5A). Although no significant difference was found in the gills between control and exposed scat except for 30μg/L after 72 hours, high correlation ($R^2=0.83$, $p \leq 0.001$) between MT and Hg was found (Fig 5B).

Fig 5. Correlation between MT concentration and Hg bioaccumulation in the liver (A) and gill (B) of *S. argus* for 72hrs to Hg. $R^2$ values $\geq 0.46$ are significant.
Discussion

Short term exposure of scat to different Hg concentrations ranging from 0 to 30μg/L during different time resulted in increasing Hg accumulation in the gills and liver with increasing exposure duration and concentration. Hg was accumulated significantly in the liver compared to gill. This accumulation order was also found by several other authors and may be attributed to the lower metal-binding capacity of the gills as a consequence of the low gill MT concentrations present [7], [10], [16]. Olsvik [18] showed that the Cd present in the gills of trout is rapidly cleaned via the circulation system to the liver and kidney where it can be retained for a longer time.

MT contents of these tissues appeared to be time and dose dependent during 24-72hrs period. Much research has shown that the MT content appears to have time and dose dependent responses in in vitro and in vivo studies. Wu Su-Mei [23] also showed time and dose response of the liver and gill tissue of Tilapia (Oreochromis mossambicus) after exposure to Cd during 24-72 hours. All these results imply that dose–related response of MT expression only occur with doses of heavy metals that do not cause detrimental effects to the physiological functioning of the fish [22]. Correlation among MT expression, heavy metal accumulation, and tolerance of fish to heavy metals are complicated, especially in in vivo systems [22] similar changed appeared in this in vitro study.

Results show clear tissue specific differences of MT induction in response to Hg exposure. Liver can induce MT biosynthesis much higher than gill. Gill MT level showed only a moderate increase during the exposure experiment. Indeed no significant Hg related induction of gill MT could be detected in the scat even at the highest exposure level and duration reflecting apparently low capacity of the gill tissue for Hg-induction after a short water-borne Hg exposure. Some authors have suggested that the gill do not constitute a good organ for MT quantification [5], [18] perhaps because MT induction is dependent on the cell type and occur primarily in the chloride cell [4], [9] but our result show that a clear correlation exists between Hg and MT level in the gill tissue. It is remarkable that scat which show the best survival rate under Hg exposure has the fastest and target organ during exposure. Hg toxicity in fish shows a typical "shock phase" with the extensive damage in the first hours or days of exposure and repair thereafter [17] and a fast protective response is thus a clear advantage.

Our data also show a good correlation between Hg and MT levels in the liver of scat. This positive correlation have also been observed in roach liver exposed to Cd [3] and in common carp and gibel carp [11] as well as gad geon exposed to increased Zn concentrations in the field [2]. Several authors have interpreted the positive correlations between metal and metallothionein content in fish tissue as the metal sequestration by MT and a poor correlation as metal exceeding the binding capacity of MT or the involvement of non-MT proteins [13]. According to this interpretation metal binding capacity of liver metallothionein is not exceeded at any mercury concentration and exposure duration measured in our study and provides the mechanism for the high mercury bioaccumulation capacity of this organ.

The results from this study show that liver is more efficient organ than gill to induce MT biosynthesis. There is a significant and early increase of MT biosynthesis in the gills and liver with increasing exposure duration and concentration. Hg was accumulated significantly in the liver compared to gill. This accumulation order was also found by several other authors and may be attributed to the lower metal-binding capacity of the gills as a consequence of the low gill MT concentrations present [7], [10], [16]. Olsvik [18] showed that the Cd present in the gills of trout is rapidly cleaned via the circulation system to the liver and kidney where it can be retained for a longer time.

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The results from this study show that liver is more efficient organ than gill to induce MT biosynthesis. There is a significant and early increase of MT biosynthesis in S. argus after exposure to Hg in liver but in gill only after higher contamination level occur. Results also show significant effect of contamination level and exposure duration on MT concentration in S. argus. The increase was significantly correlated to Hg bioaccumulation. These may be used to evaluate MTs as a biomarker of mercury exposure. The advantage in using scat is the rapid response within 24hrs. This could extend use of MT in S. argus as a biomarker of mercury pollution in marine ecosystems.

References


