

IDENTIFICATION OF SELENOPROTEINS IN SELENIUM-SENSITIVE TISSUES IN JAPANESE MEDAKA (*O. LATIPES*) USING TANDEMLY COUPLED HPLC/ICP-MS

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INTRODUCTION

Selenium is a naturally occurring element that is an essential micronutrient for many organisms incorporated as selenocysteine at the active site of a wide range of selenoproteins, such as glutathione peroxidase (Brown, K.M., Arthur, J.R., 2001). Although selenium is essential to these organisms, at slightly elevated levels, selenium contamination can cause devastating effects to oviparous organisms (Ohlendorf, H.M., 2002; Lemly, A.D., 2002; Fan, et al., 2002). In aquatic systems, selenium biogeochemistry can play a major role in selenium toxicity, particularly biotransformation into organoselenium forms (Maier, K.J., Knight, A.W., 1994; Fan, et al., 2002). In the water column, selenium can exist as several different forms, including selenite (Se³⁺) and selenate (Se⁶⁺), which are reported to be the most dominant forms of dissolved Se in both non-contaminated and Se-contaminated waters (Presser, T.S., Ohlendorf, H.M., 1987). These oxyanions can then be readily transformed by plants and microorganisms into various organoselenium forms including selenomethionine (SeMet). Proteinaceous SeMet has been frequently found within organisms (Fan et al., 1998a,b), and is believed to be the key form of Se that leads to Se biomagnification and ecotoxicity in aquatic ecosystems (Alaimo et al., 1994; Fan et al., 2002).

The mechanistic action of selenium is believed to be via replacement of sulfur (S) within proteins, as Se has similar chemical and physical properties. Sulfur is a fundamental constituent of many proteins, and is found in the disulfide bond linkages (S-S linkages) between amino acid strands. The replacement of S by Se is hypothesized to exert its toxicity by interfering with these disulfide bonds, resulting in distorted, dysfunctional proteins and enzymes, ultimately impairing normal cellular biochemistry. Yet, the proteins involved in selenium toxicity have yet to be determined. As selenium toxicity in fish has been shown to target certain organs, mainly the liver and the ovary (Fan et al., 2002; Schlenk et al., 2003), it is believed that vitellogenin (Vtg), the yolk precursor protein, is involved in selenium transport and disposition.

In oviparous organisms, yolk resources accumulated into the oocytes during oogenesis and mobilization during embryogenesis are fundamentally important processes for successful reproduction. These yolk proteins are derived from the enzymatic cleavage of precursors such as Vtg. Vitellogenesis is a process involving the synthesis of Vtg, its transport to the ovary, as well as its uptake into maturing oocytes. This is a key process as it provides Vtg to the egg and developing larvae (Byrne et al., 1989; Arukwe and Goksøyr, 2003). This may also be key to the transfer of Se-contamination from the maternal organism to the offspring, causing the detrimental effects that are characteristically seen in organisms within selenium-contaminated regions.

MATERIALS AND METHODS

Exposures of Brine shrimp (*Artemia*)

- Cultured *Artemia* were reared in salt water for 48 hours. Upon hatching, *Artemia* were separated into the control *Artemia* and the treated *Artemia*.
- Treated *Artemia* were subjected to 100 µg/L L-(+)-Selenomethionine (SeMet) for 1 hour.
- Resulting concentrations of 40.19 ± 3.61 µg Se/g dry weight was observed in SeMet-treated animals and 3.79 ± 0.032 µg Se/g dry weight was observed in the controls.
- Treated *Artemia* were then put through a rinsing regime to remove extrinsic SeMet. Control and treated *Artemia* were subsequently fed to control and treated *O. latipes*, respectively.

Exposures of Japanese Medaka (*O. latipes*)

- Cultured *O. latipes* were exposed to selenium via dietary exposure using SeMet-treated *Artemia*.
- The SeMet-treated *Artemia* were prepared daily following the above procedure, washed thoroughly to remove extrinsic Se, and subsequently fed to *O. latipes*. Maximum feeding dose averaged 6.8 ± 0.56 mg Se/fish.
- Replicates of 15 fish per treatment (10 females, 5 males) were utilized using a 16:8 light:dark photoperiod to promote egg laying.
- Exposures lasted a period of two weeks. Following the exposure, the female fish were sacrificed after a 24hr depuration period; liver, gonad/egg, and extruded/fertilized egg samples were taken.
- Samples were either homogenized and subjected to a centrifugation procedure to obtain the cytosolic fraction or dried and acid digested.

Selenium Incorporation into Japanese Medaka (*O. latipes*)

- Selenium incorporation into *O. latipes* within the control and treated organisms was determined via ICP-MS (Perkin Elmer™ #100 DRC, dual quadrupole) analysis for metal analysis, carried out at California State University, Long Beach.
- ICP-MS metal quantification was performed via standard curve of known-concentration NIST-certified multi-elemental metal standards.
- Selenium incorporation into proteins
- Selenium incorporation into proteins within the control and treated organisms was determined via size-exclusion HPLC (BioSep SEC S2000, 300 x 7.8 mm) tandemly coupled to an ICP-MS for concurrent metal analysis. To compensate for polyatomic Ar₂ dimer interference at m/z 80, Se was monitored at m/z 82.
- ICP-MS metal quantification was performed via flow-injection analysis (FIAs) of known-concentration NIST-certified multi-elemental metal standards.

Statistical Analysis

- Statistical analysis was performed using two-sample, equal-variance t-test.

RESULTS

Selenium Incorporation into Japanese Medaka (*O. latipes*)

- Differences between the acid digested control and SeMet-treated sample means of liver, gonad/egg, extruded/fertilized egg, and whole-body samples were shown to be statistically significant (p<0.05). (Figure 1)

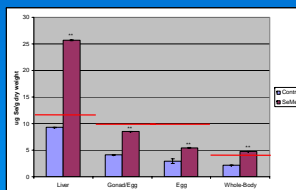


Figure 1. Acid digested samples of Control and SeMet-treated *O. latipes* analyzed by ICP-MS showing µg Se/g dry weight. The red lines depict the biological effects thresholds for fish for each target organ (12, 10, and 4 µg/g) of liver, ovary and egg, and whole body, respectively.

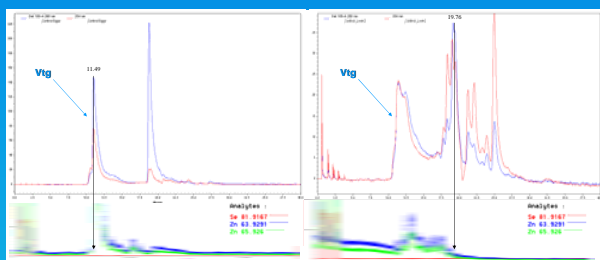


Figure 2. UV and metal chromatograms of egg cytosol from untreated *O. latipes*. Upper graph contains the HPLC UV spectra displaying an overlay of 280 and 254nm. The lower graph shows the ICP-MS metal intensities corresponding to the HPLC run.

Figure 3. UV and metal chromatograms of liver cytosol from untreated *O. latipes*. Upper graph contains the HPLC UV spectra displaying an overlay of 280 and 254nm. The lower graph shows the ICP-MS metal intensities corresponding to the HPLC run.

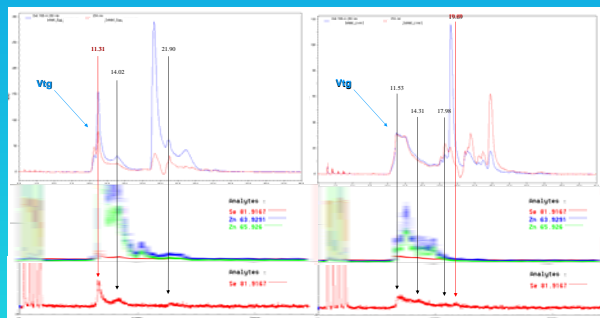


Figure 4. UV and metal chromatograms of egg cytosol from SeMet-treated *O. latipes*. Upper graph contains the HPLC UV spectra displaying an overlay of 280 and 254nm. The lower graph shows the ICP-MS metal intensities corresponding to the HPLC run. Numbers displayed in red depict corresponding Se peaks in control samples.

Figure 5. UV and metal chromatograms of liver cytosol from SeMet-treated *O. latipes*. Upper graph contains the HPLC UV spectra displaying an overlay of 280 and 254nm. The lower graph shows the ICP-MS metal intensities corresponding to the HPLC run. Numbers displayed in red depict corresponding Se peaks in control samples.

Sample	Peak Number	Molecular Weight (Da)	Average Peak Time (min.)	Average µg Se/g Protein	Average µg Zn/g Protein
Control Egg	1**	360K	11.48	37.94	227.52
SeMet Egg	1**	360K	11.31	625.50	2369.10
SeMet Egg	2**	50K	14.02	806.43	2136.61
Total SeMet Egg	3	245	22.52	1599.79	3031.72
Control Liver	1	8.4K	19.76	99.71	271.36
SeMet Liver	1**	360K	11.53	628.22	640.11
SeMet Liver	2	50K	14.31	207.16	
SeMet Liver	3	17K	17.98	556.76	
SeMet Liver	4	8.4K	19.69	65.17	
Total SeMet Liver				1476.85	

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RESULTS (cont.)

- Concentrations of Se within liver and whole-body samples of SeMet-treated exceeded biological effects thresholds (12 and 4 µg Se/g dry weight, respectively) for health and reproductive success of fish (Lemly, 1997). Extruded/fertilized egg samples did not exceed the biological effects threshold (10 µg Se/g dry weight). (Figure 1)
- Developmental effects were also evaluated in offspring of SeMet-treated organisms, yet no detrimental effects were seen.
- Se concentrations ranged from Liver > Extruded/fertilized Egg > Whole-Body.

Selenium Incorporation into proteins

- Selenium incorporation into cytosolic proteins within the control organisms determined via size-exclusion HPLC tandemly coupled to ICP-MS for concurrent metal analysis revealed a Se peak at 11.49 min. and 19.76 min. for control egg and control liver samples, respectively (Figures 2-3).
- In treated organisms, Se peaks were observed at 11.31, 14.02, 21.90 min. and 11.53, 14.31, 17.98, 19.69 min. for SeMet-treated egg and SeMet-treated liver samples, respectively (Figure 4-5). Approximate molecular weight of these proteins ranges from 360K, 50K, 245Da and 360K, 50K, 17K, and 8.4KDa for SeMet-treated egg and SeMet-treated liver samples, respectively.
- Zinc (Zn) is an important marker for Vtg as Vtg is a Zn-containing protein. Zn peaks correlating with Se peaks occurred around 11.31 and 14.02 min. for egg samples, and 11.53 min. for liver samples. (Table 1)
- HPLC/ICP-MS analysis revealed that purified *O. latipes* Vtg eluted at the time of the Se/Zn peaks.
- Proteinaceous Se concentrations within the egg and liver were 4.20 and 3.34 fold higher than that of the control counterparts. (Table 1)

CONCLUSIONS

- Selenium concentrations within the SeMet-treated liver and the whole-body tissue digestions showed levels exceeding the biological effects threshold for freshwater fish. Selenium concentrations within the SeMet-treated egg tissue digestions showed levels lower than the biological effects threshold. This may be attributed to the fact that *O. latipes* produced multiple egg clutches within the two-week exposure period.
- Multiple proteins with molecular weights ranging from 360KDa to 245Da within the liver and egg showed Se incorporation.
- The Se/Zn-containing proteins within both SeMet-treated egg (11.31 and 14.02 min.) and SeMet-treated liver (11.53 min.) correlated with purified *O. latipes* Vtg.
- Combining all proteinaceous Se, the Se within the SeMet-treated egg and SeMet-treated liver samples had 4.20 and 3.34 times higher amounts of Se than that of their control counterparts.
- Se within the SeMet-treated egg showed 2.05 times higher amount of proteinaceous Se than the liver in SeMet-treated organisms, yet in the untreated samples, the liver showed 2.63 times higher amounts of proteinaceous Se than did the egg.

LITERATURE CITED

Alaimo, J., Ogle, R.S., Knight, A.W. 1994. Selenium uptake by larval *Chironomus decorus* from a *Ruppia maritima*-based benthic/detrital substrate. Arch. Environ. Contam. Toxicol. 27, 441-448.

Arukwe, A., Goksøyr, A. 2003. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption. Comparative Hepatology 2:4.

Brown, K.M., Arthur, J.R. 2001. Selenium, selenoproteins and human health: a review. Public Health Nutr. 4(2B), 593-599.

Byrne, B.M., Gruber, M., Ab, G. 1989. The evolution of egg yolk proteins. Prog. Biophys. Molec. Biol. 53, 33-69.

Fan, T.W.-M., Teh, S.J., Hinton, D.E., Higashi, R.M. 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquatic Toxicology 57, 65-84.

Fan, T.W.-M., Lane, A.N., Martens, D., Higashi, R.M. 1998a. Synthesis and structure characterization of selenium metabolites. Analyst 123 (5), 875-884.

Fan, T.W.-M., Higashi, R.M., Lane, A.N. 1998b. Biotransformations of selenium oxyanion by filamentous cyanophyte-dominated mat cultured from agricultural drainage waters. Environ. Sci. Technol. 32, 3185-3193.

Lemly, A.D. 2002. Symptoms and implications of selenium toxicity in fish: the Belweys Lake case example. Aquatic Toxicology 57, 39-49.

Lemly, A.D. 1997. Environmental implications of excessive selenium: A review. Biomed. Environ. Sci. 10, 415-435.

Maier, K.J., Knight, A.W. 1994. Ecotoxicology of selenium in freshwater systems. Rev. Environ. Contam. Toxicol. 134, 31-48.

Ohlendorf, H.M. 2002. The birds of Kesterson Reservoir: a historical perspective. Aquatic Toxicology 57, 1-10.

Presser, T.S., Ohlendorf, H.M. 1987. Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. Environ. Manage. 11, 805-821.

Schlenk, D., Zubcov, N., Zubcov, E. 2003. Effects of salinity on the uptake, biotransformation, and toxicity of dietary seleno-L-methionine to rainbow trout. Toxicol. Sciences 75, 309-313.