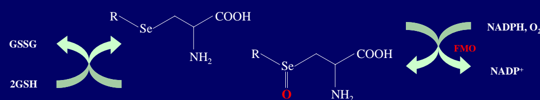
OXIDATION OF SELENOMETHIONINE BY FLAVIN-CONTAINING MONOOXYGENASES (FMOs)

Dalin Shi and Daniel Schlenk, *Department of Environmental Sciences, University of California, Riverside*

INTRODUCTION

SELENIUM biotransformation into organoselenium forms by primary producers and subsequent accumulation by higher food chain organisms such as fish are critical for mechanistically understanding the adverse effects of selenium in aquatic organisms. Although previous laboratory and field studies have both demonstrated a good correlation between adverse effects and total selenium in fish tissue, direct confirmation of the most toxicologically important forms of organoselenium is lacking, with nearly all measurements being total selenium. To illustrate this, we hypothesize that following accumulation by fish organoselenides are oxidized by flavin-containing monooxygenase (FMO) to selenoxides consuming the antioxidant glutathione (GSH) in sensitive targets. In this study, an *in vitro* substrate dependent NADPH oxidation assay was performed to test the first step of the hypothesis, the oxidation of organoselenium by FMO. Previous studies have shown that hyperosmotic conditions induce expression and activity of FMO in euryhaline fish, therefore the effects of osmotic pressure on the oxidation of organoselenium by FMO was examined.

HYPOTHESIS



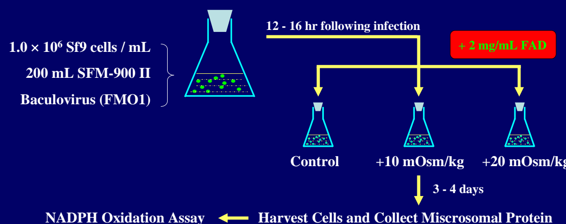
MATERIALS & METHODS

I. NADPH Oxidation Assay

The organoselenium, selenomethionine (SeMet) oxidation was measured by substrate-dependent NADPH oxidation. The reaction mixture contained 100 µg human microsomal FMO1 or FMO3 proteins in 50 mM phosphate buffer (pH 8.4) containing 0.2 mM NADPH. The sample was allowed to equilibrate at 37 ° C for 3 min before adding the substrate. A series of SeMet concentration of 0.05 – 2.0 mM was used in a total reaction volume of 1 mL. All incubations were carried out in triplicate. The absorbance of NADPH (340 nm) of the reaction mixture was measured over 30 min at 5 min intervals using a spectrophotometer (UV - 1601, Shimadzu, Kyoto, Japan), and an NADPH extinction coefficient of 6220 M^{-1} cm^{-1} was used in calculating catalytic constants.

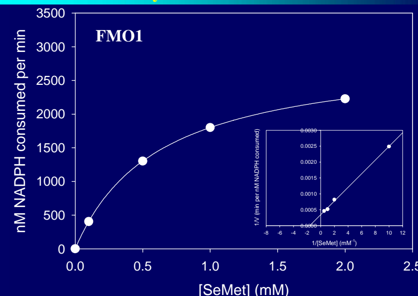
II. Effects of Salinity on the oxidation of SeMet by FMO1

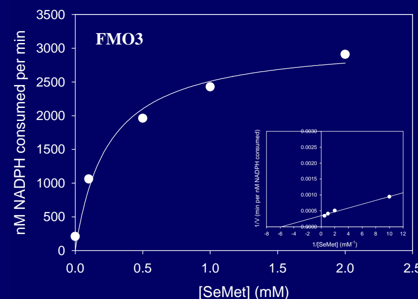
The effects of salinity on enzymatic activity of FMO1 expressed using a baculovirus system was evaluated with 600 µM SeMet as substrate.



RESULTS

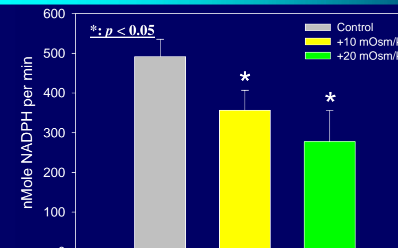
I. Oxidation of SeMet by FMO1 and FMO3



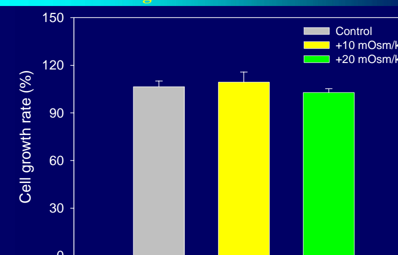


	K_m (µM)	V_{max} (nmol/min)	V_{max}/K_m
FMO1	617	2.882×10^3	4.671
FMO3	172	2.865×10^3	16.657

II. Effects of Osmotic Pressure on the Oxidation of SeMet by FMO1



III. Cell Growth during Infection



CONCLUSIONS

- Both FMO1 and FMO3 demonstrated catalytic activity for SeMet;
- FMO3 was more effective and had a higher affinity for SeMet;
- Hyperosmotic conditions diminished catalytic activity of FMO1 but failed to alter cell growth indicating down regulation of FMO1 enzymatic activity for oxidation of SeMet in Sf9 cells.

ACKNOWLEDGEMENTS

This research was supported by UCR/AES (CSREES-ENS-6853-H) and the UCR Graduate Division.

UNIVERSITY of CALIFORNIA Riverside