

**EARLY DEVELOPMENTAL EXPRESSION OF TWO GLUTAMINE  
SYNTHETASE GENES IN RAINBOW TROUT  
(*ONCORHYNCHUS MYKISS*).**

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**Introduction and Objectives**

Most adult teleosts excrete nitrogenous wastes primarily as ammonia, mainly across the gills. Ammonia diffusion from the teleost embryo, however, may be hindered by the lack of gill ventilation and the presence of an unstirred boundary layer (Rahaman-Noronha *et. al.*, 1996), resulting in a potentially toxic accumulation of this molecule (Wright *et. al.*, 1995). Alternatively, ammonia may be converted to less toxic compounds, such as glutamate, glutamine or urea. Glutamine synthetase (GSase) catalyzes the conversion of glutamate and ammonia to glutamine, and in fish, functions in ammonia detoxification, and urea and pyrimidine synthesis (Ip *et. al.*, 2001). Besides its roles in urea and pyrimidine synthesis, we propose that GSase plays a critical role in the detoxification of ammonia *via* storage in the glutamine molecule. Our study attempts to answer the following questions: 1. When are GSase genes first expressed and what is the pattern of expression during development and in different adult tissues? 2. How does the increase in expression of GSase genes correlate with activities and with increases in tissue ammonia and glutamine?

Recent work has revealed the presence of at least four GSase genes in rainbow trout (Murray *et.al.*, manuscript submitted). We measured the expression of two of these GSase genes, *Onmy-GS01* and *Onmy-GS02*, in early life stages of rainbow trout, ranging from 3 - 80 days post fertilization (dPF), compared to expression in six rainbow trout adult tissues. As well, GSase enzyme activities, ammonia excretion rates, and ammonia concentrations were measured.

## Materials & Methods

Rainbow trout embryos were purchased on the day of fertilization. Hatching occurred 26 - 30 dPF and total yolk absorption occurred at 50 dPF. To characterize the pattern of mRNA expression of *Onmy-GS01* and *Onmy-GS02* during early development and in adult tissues, we designed and constructed radiolabelled RNA probes for these transcripts for use in ribonuclease protection assay (RPA) (Ambion, Austin, Texas). Total RNA from six early developmental stages (3, 10, 21, 31, 60 and 80 dPF) and from six adult tissues (brain, spleen, white muscle, large intestine, skin and liver) was also extracted for use in the RPA. GSase enzyme assays for early developmental stages were conducted on whole animals, as described by Chadwick and Wright (1999). Ammonia excretion rates were measured over 3 h, at each of the 6 early developmental stages (Wright *et. al.*, 1995). Ammonia levels were measured using a Sigma diagnostic kit on frozen tissue. Ammonia concentrations were measured on either whole animals or separate yolk and tissue fractions.

## Results

### *Early Developmental Stages*

Expression of *Onmy-GS01* mRNA during early developmental stages resembled one oscillation, with a peak at 21 dPF. The pattern of expression of *Onmy-GS02* during early developmental stages also oscillated, with two peaks, one at 21 dPF and another at 60 d PF. Overall, *Onmy-GS02* was expressed at a higher level than *Onmy-GS01*. GSase activities were observed at the first early developmental stage examined, rising steadily to a maximum value of 0.49  $\mu\text{mol/g/min}$  at 80 dPF, with a significant increase in activity at 31 dPF. Ammonia concentrations in whole embryos increased gradually from 3 dPF (0.97 nmol N/g) to 21 dPF (1.29 nmol N/g). When embryos were dissected from yolk at 31 dPF, ammonia tissue levels were 1.11 nmol N/g, whereas yolk ammonia levels were significantly higher, at 2.47 nmol N/g. Ammonia excretion steadily rose during development to 0.67  $\mu\text{mol N/g/h}$  at 80 dPF, with a significant increase observed at 21 dPF.

#### *Adult Tissues*

Overall, *Onmy-GS02* was more highly expressed in adult tissues than *Onmy-GS01*. Expression of both *Onmy-GS01* and *Onmy-GS-02* was higher in the brain of adult rainbow trout when compared with all other tissues examined. Adult tissue expression of *Onmy-GS01* was, in descending order of intensity, brain >> white muscle > skin > large intestine > spleen/liver, and of *Onmy-GS02*, brain > white muscle > large intestine > liver/skin > spleen. The largest difference in expression of these two GSase genes was in the brain. GSase activity was higher in the brain than any other adult tissue assayed (~200 times greater than the next highest tissue, the liver). After the brain, activities were next highest in the liver, followed by spleen > large intestine > skin > white muscle.

#### **Summary & Conclusions**

We have characterized the early pattern of expression of two GSase genes in rainbow trout, and the data indicate that *Onmy-GS01* and *Onmy-GS02* are differentially expressed during early development, as well as in adult tissues. Significant increases in both *Onmy-GS01* and *Onmy-GS02* mRNA expression at 21 dPF, coupled with increasing levels of GSase activities at 31 dPF (just after hatching) may reflect the importance of this enzyme, particularly in early developmental stages, in ammonia detoxification. Low levels of ammonia in embryonic tissue, but not in yolk, at 31 dPF support this proposal. GSase may be important in both glutamine storage and urea synthesis (*via* the O-UC) in the embryo (Wright *et. al.*, 1995). Further work on tissue glutamine and urea levels during development will be valuable.

#### **References**

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