

GLYCEROL PRODUCTION
BY RAINBOW SMELT (*OSMERUS MORDAX*)
AT SUB-ZERO TEMPERATURES IS DEPENDENT UPON
BUT NOT CONTROLLED BY G3PDH ACTIVITY

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Rainbow smelt (*Osmerus mordax*) is a teleost that tolerates temperatures close to the freezing point of sea water through the use of proteins (Ewart and Fletcher, 1990) and glycerol (Raymond, 1992) as antifreeze agents. The lower the temperature, the higher the glycerol level such that at -2°C serum glycerol may approach 400 mM (Raymond, 1992). Although effective at lowering the freezing temperature glycerol is lost at a rate that may exceed 10% of glycerol stores per day (Raymond, 1993). Therefore, there must be vigorous glycerol synthesis at very low temperatures.

Liver is an active site of glycerol synthesis and one source of carbon for glycerol is probably dietary amino acids that are degraded to glyceraldehyde 3-phosphate (G3P) (Raymond and Driedzic, 1997; Driedzic et al., 1998). It appears that carbon flow is $\text{G3P} \rightarrow \text{dihydroxyacetone-P (DHAP)} \rightarrow \text{glycerol-3-P} \rightarrow \text{glycerol}$. Blood glycerol levels and liver enzyme activity levels were determined in rainbow smelt, Atlantic tomcod (*Microgadus tomcod*) and smooth flounder (*Liposetta putmani*). All animals were sampled simultaneously from sub-zero water temperatures. Blood glycerol was 109, 0.29 and 0.17 mM in smelt,

tomcod, and flounder, respectively. Glycerol-3-phosphate dehydrogenase (G3PDH), the enzyme that catalyzes the conversion of DHAP to glycerol-3P was 156, 5.6 and 12.5 $\mu\text{mol min}^{-1} \text{g}^{-1}$ in smelt, tomcod, and flounder, respectively. Similarly, the activity of glycerol-3-phosphatase was approximately 3 to 6-fold higher in smelt than in tomcod or flounder liver (Driedzic et al., 1998). These findings may be considered in the framework of a large literature related to osmotic stress in yeast cells where hyperosmotic challenge results in glycerol production and increased expression of both G3PDH and glycerol-3-phosphatase (Norbeck and Blomberg, 1997).

More recently, smelt have been examined under controlled laboratory conditions to test the hypothesis that G3PDH is a key regulatory enzyme in glycerol production. Animals were collected in October and held in the laboratory under natural photoperiod conditions. All animals were fed frozen brine shrimp on a daily basis and populations ($N = 5$) were sampled on Dec. 15, Jan. 11, Feb. 29, and Mar. 30. One group of animals was maintained at 4°C. A second group was held at ambient water temperature that decreased to 0.8°C by Jan. 11, to 0°C by Feb. 29, and to -0.8°C by Mar. 30.

Animals initially sampled in Dec. had plasma glycerol levels of $80 \pm 26 \mu\text{mol ml}^{-1}$. In smelt maintained at 4°C, plasma glycerol decreased to 68 ± 18 , 31 ± 20 , and $10 \pm 6 \mu\text{mol ml}^{-1}$, on the sample dates stated above. In smelt living at ambient water temperature plasma glycerol levels were 234 ± 56 , 217 ± 33 , and $110 \pm 13 \mu\text{mol ml}^{-1}$, on the sample dates stated above.

Animals initially sampled in Dec. had liver G3PDH activities of $159 \pm 24 \mu\text{mol min}^{-1} \text{gm}^{-1}$ when assayed at 15°C. In smelt maintained at 4°C, liver G3PDH activities were 84 ± 14 , 67 ± 8 , $78 \pm 10 \mu\text{mol min}^{-1} \text{gm}^{-1}$, on the sample dates stated above. In smelt living at ambient water temperature liver G3PDH activities were 103 ± 30 , 64 ± 5 , $67 \pm 6 \mu\text{mol min}^{-1} \text{gm}^{-1}$, on the sample dates stated above. In both groups, liver G3PDH activities were lower following the initial sample date and there was no difference in enzyme activity between the two groups at any sample date.

In summary, smelt produce glycerol in response to a decrease in temperature that approaches 0°C. The activity of liver G3PDH is elevated relative to other species. High G3PDH activity in smelt liver is not induced by low temperature but instead is probably high, compared to other species, at all times. Although high activities of G3PDH are necessary for high rates of glycerol synthesis in

smelt, the induction of this enzyme is not a key element in the control process as it is in yeast cells that produce glycerol to increase osmotic pressure.

References

- Driedzic, W.R., J.L. West, D.H. Sephton, and J.A. Raymond. 1998. Enzyme activity levels associated with the production of glycerol as an antifreeze in liver of rainbow smelt (*Osmerus mordax*). *Fish Physiol. Biochem.* 18: 125-134.
- Ewart, K.V. and G.L. Fletcher. 1990. Isolation and characterization of the antifreeze proteins from smelt (*Osmerus mordax*) and the Atlantic herring (*Clupea harengus harengus*) *Can. J. Zool.* 68: 1652-1658.
- Norbeck, J. and A. Blomberg. 1997. Metabolic and regulatory changes associated with growth of *Saccharomyces cerevisiae* in 1.4 M NaCl. *J. Biol. Chem.* 272: 5544-5554.
- Raymond, J.A. 1992. Glycerol is a colligative antifreeze in some northern fishes. *J. Exp. Zool.* 271: 425-431.
- Raymond, J.A. 1993. Glycerol and water balance in a near-isosmotic teleost, winter-acclimatized rainbow smelt. *Can. J. Zool.* 71: 1849-1854.
- Raymond, J.A. and W.R. Driedzic. 1997. Amino acids are a source of glycerol in cold-acclimated rainbow smelt. *Comp. Biochem. Physiol.* 118B: 387-393.

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