Journal of Thermal Biology 76 (2018) 107–114

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Effect of selenomethionine on cell viability and heat shock protein 70 levels in rainbow trout intestinal epithelial cells at hypo-, normo-, and hyperthermic temperatures

John J. Kim[a], Phuc H. Pham[a], Mark E. Hamilton[a], Lucy E.J. Lee[b], Niels C. Bols[a,*]

[a] Department of Biology, University of Waterloo, Waterloo, Canada N2L 3G1
[b] Faculty of Science, University of the Fraser Valley, Abbotsford, Canada BC V2S 7M8

ARTICLE INFO

Keywords:
Rainbow trout
Intestinal cell line
Selenomethionine
Cytotoxicity
HSP70
Thermal response

ABSTRACT

As global warming and environmental pollution modify aquatic environments, the thermal biology of fish could be affected by interactions between temperature and pollutants, such as selenium (Se). Therefore, selenomethionine (SeMet) was studied for effects on cell viability and on heat shock protein 70 (HSP70) levels in the rainbow trout intestinal epithelial cell, RTgutGC, at hypothermic (4 °C), normothermic (14 and 18 °C) and hyperthermic (26 °C) temperatures. RTgutGC cultures remained viable for at least a week at all temperatures, although energy metabolism as measured with Alamar Blue (resazurin) was appreciably diminished at 4 °C. Over a 7-day incubation, HSP 70 levels in cultures remained steady at 4 °C, declined at 18 °C, and increased slightly at 26 °C. When 125 μM SeMet was present, cultures remained viable and HSP70 levels were neither increased nor decreased relative to control cultures, regardless of the temperature. With 500 and 1000 μM SeMet, cell viability was profoundly impaired after 7 days in cultures at 14, 18 and 26 °C but was unchanged at 4 °C. Overall the results suggest that only hypothermia modulated the response of rainbow trout cells to SeMet.

1. Introduction

How selenium (Se) acts on fish is an important issue in both aquaculture and ecotoxicology. Se is a micronutrient that appears essential for fish to grow and to maintain physiological functions, including the response to stress (Ilham and Fotedar, 2016; Thomas and Janz, 2011). In aquaculture, supplementing feeds with organic Se is being explored in order to raise Se tissue levels in cultivated salmonids to levels of their wild counterparts and to enhance the responses of salmonids to stress and pathogens (Pacitti et al., 2016; Rider et al., 2009). Although Se is essential for fish, too much Se is toxic (Hodson and Hilton, 1983) and can be considered an ecotoxicant (Lemly, 2002). Anthropogenic activities, such as ash from coal-fired power plants and agricultural irrigation, causes Se to accumulate in aquatic biota, leading to the impairment of fish growth and reproduction (Hamilton, 2004; Miller et al., 2009).

With climate change, efforts are being made to develop feeds that optimize fish performance at higher temperatures (Norambuena et al., 2016) and to understand how temperature influences the actions of ecotoxicants (Noyes et al., 2009), but only a few studies have investigated how temperature might modulate Se effects on fish. Se/temperature interactions have been studied at a cold temperature for bluegill (Lemly, 1993), at a warm temperature for yellowtail kingfish (Ilham and Fotedar, 2016), and at a stressful temperature for green and white sturgeon (Silvestre et al., 2010). The results suggest interactions occur between temperature and selenium but too few studies and too few species have been examined for mechanisms and generalizations to emerge.

Among fish and Se compounds, especially important are rainbow trout, Oncorhynchus mykiss and L-selenomethionine (SeMet). The importance of rainbow trout lies in its intensive use in aquaculture and in research. SeMet is of interest because this is the main dietary form of Se for fish (Fan et al., 2002) and has been used recently as surrogate for other Se compounds in fish toxicity testing (Rigby et al., 2014). The effects of SeMet on rainbow trout have been examined from different perspectives, including ecotoxicology (Miller et al., 2009), physiology (Thomas et al., 2013), reproduction (Wiseman et al., 2011), nutrition (Knight et al., 2016; Schlenk et al., 2003), and parasitology (Hursky and

Abbreviations: SeMet, selenomethonine; HSPs, heat shock proteins; RT, rainbow trout; FBS, fetal bovine serum; L15, Leibovitz's medium; AB, Alamar Blue; CFDA-AM, carboxyfluorescein diacetate acetoxymethyl ester; RFUs, relative fluorescence units

* Corresponding author.
E-mail address: ncbols@uwaterloo.ca (N.C. Bols).

https://doi.org/10.1016/j.jtherbio.2018.07.011
Received 7 February 2018; Received in revised form 7 June 2018; Accepted 21 July 2018
Available online 23 July 2018
0306-4565/

Pietrock, 2015). In human health research, the actions of Se compounds have been studied intensively on cell lines in order to understand their possible anti-carcinogenic and chemotherapeutic actions (Brozmanova et al., 2010; Wallenberg et al., 2014). By contrast, for rainbow trout, only a few studies have been done at the cellular level and these have been with primary hepatocyte cultures (Misra et al., 2012).

Yet, an assemblage of rainbow trout (RT) cell lines, which is referred to as the RT invitrome (Bols et al., 2017), is available and includes an intestinal epithelial cell line, RTgutGC (Kawano et al., 2011). As with other fish cell lines, these can be studied at normothermic, hyperthermic, and hypothermic temperature ranges (Bols et al., 1992). Temperatures that support the proliferation of cells from a species constitute the normothermic range, although the upper and lower boundaries are difficult to define absolutely. For RT, normothermic is between 5 and 26 °C (Bols et al., 1992). Hyperthermic and hypothermic temperatures are those at and beyond the upper and lower temperatures of the proliferation zone. At 4–5 °C, RT cell cultures persist for months (Bols et al., 1992; Plumb and Wolf, 1971). At 26 °C, RT cell cultures begin synthesizing heat shock proteins (HSPs) and endure for a week or more, while at temperatures above 26 °C the time for HSP synthesis and cell survival decreases as temperature increases (Bols et al., 1992).

HSPs consists of four conserved protein families, small HSPs, HSP60, HSP70 and HSP90, and have been widely studied in fish (Roberts et al., 2010), especially for salmonids, like rainbow trout (Basu et al., 2002), and particularly with the Hsp70 family (Yamashita et al., 2010). The salmonid HSP70 family includes the major stress-inducible form, HSP70, the heat-shock cognate 70, Hsc70, and the glucose-regulated protein 78, GRP78 (Yamashita et al., 2010; Ojima et al., 2005). The general function of HSP70 is to maintain protein homoeostasis (Bukau et al., 2006). For a wide range of plants and animals, including fish, HSP70 protein levels have been considered as a biomarker of anthropogenic stress (Webb and Gagnon, 2009). In birds and mammals, the expression of HSP70 has been shown to be modulated by Se (Gan et al., 2013; Mahmoud and Edens, 2005; Rivera et al., 2005).

Therefore, in this study, the effects of SeMet on cell viability and HSP70 levels were examined in RTgutGC cultures incubated for a week at either normothermic (14 or 18 °C), hyperthermic (26 °C), or hypothermic (4 °C) temperatures. SeMet at 125 μM did not affect cell viability or HSP70 levels in cultures at any temperature. However, at 500 and 1000 μM, SeMet was cytotoxic in cultures at 14, 18 and 26 °C but not at 4 °C. Therefore, incubation at 4 °C protected RTgutGC from the cytotoxic actions of SeMet, possibly by the diminished metabolism at 4 °C.

2. Materials and methods

2.1. Routine culturing of RTgutGC

The RTgutGC cell line was developed in this laboratory (Kawano et al., 2011) and was routinely maintained at 18 °C in L15/FBS. L15/FBS consists of basal medium Leibovitz's L15 (Leibovitz, 1963) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution (Sigma-Aldrich, Oakville, ON, Canada). The L15 and FBS were obtained from Thermo Fisher Scientific (Burlington, ON, Canada). The cells were grown in 75 cm^2 flasks with non-vented caps at 18 °C. Falcon flasks manufactured by BD Bioscience (San Jose, CA, USA) and distributed by VWR (Mississauga, ON, Canada) were used. When the cultures became confluent, they were subcultivated such that the contents of one flask were split into two or three flasks. Trypsin was used to subcultivate cultures as described previously (Bols and Lee, 1994). Cells from passages between 80 and 100 were used in the following experiments.

2.2. Assessing energy metabolism of RTgutGC at different temperatures

Energy metabolism by RTgutGC in confluent monlayer cultures that had been set up in multiwell plates and maintained for a week at different temperatures was assessed with the fluorescent indicator dye, Alamar Blue (Dayeh et al., 2013; Rampersad, 2012). Alamar Blue (AB) is a commercial preparation of resazurin (Life Technologies Inc, Burlington, ON). Two auxiliary assessments were made to support the AB assay. The cultures were observed for up to 7 days under a phase-contrast microscope to be assured that no cells were detaching from the well surface, which would have removed them from the AB assay. The cultures were also evaluated with another fluorescent indicator dye, 5-carboxyfluorescein diacetate, acetoxymethyl ester (CFDA-AM) (Molecular Probes, Eugene, OR). In situations where the cells remain viable and attached to the surface of culture wells, CFDA AM assays non-specific esterase activity (Dayeh et al., 2005). This provides an alternative and supplementary assessment of cellular activities at different temperatures.

The AB and CFDA AM assays were initiated by adding RTgutGC cells in L15/FBS to multiwell plates with each well receiving approximately 40, 000, 100,000 and 1,000,000 cells for respectively 96, 48, and 6 well plates. These were incubated at 18 °C for 24 h by which time the cells had formed confluent monolayers in the wells. At this point, some plates were kept at the 18 °C, while the others were shifted to 4 °C, 14 °C and 26 °C. After 7 days of incubation, the AB and CFDA AM assays were performed on these plates as described previously (Dayeh et al., 2005, 2013), with the plates being incubated with the indicator dyes for 60 mins at room temperature before being read in a fluorescence plate reader for relative fluorescence units (RFUs). For each incubation condition, 6–12 wells were read. The RFU values for 18 °C plate constituted the 18 °C 7-day control. Some additional plates that had been held for 7 days at 4 °C were assayed in exactly the same way except that they were incubated with the indicator dyes for 1 h at 4 °C rather than at room temperature. The RFUs for cultures at 4 °C, 14 °C and 26 °C for 7 days were expressed as percentages of the RFUs for cultures at 18 °C at the start (zero-time control) and had been kept for 7 days (7-day control), providing the % change in energy metabolism and esterase activity at hypothermic and hyperthermic temperatures. The RFUs for assays done at 4 °C, 14 °C, and 26 °C were expressed as percentages of the RFUs done at room temperature. Further calculations with the RFUs are described in the statistical Section (2.7).

2.3. Exposing RTgutGC to selenomethionine at different temperatures

RTgutGC cells were exposed to seleno-L-methionine (SeMet) for up to 7 days at different temperatures. SeMet from Sigma-Aldrich (cat # S3132) was used to prepare a stock solution, as described previously (Bloch et al., 2017), and from the stock, SeMet was added to L15/FBS to give different test concentrations up to 1000 μM. Exposures were done in 96 well plates for assessing effects on cell viability and in 6 well plates for investigating HSP70 levels. Approximately, 40,000 cells and 1,000,000 cells in L15/FBS were added per well for respectively 96 and 6 well plates and allowed to attach at 18 °C. After 24 h, cell monolayers had formed and the medium was then removed. L15/FBS with varying SeMet concentrations was then added. For cell viability, each SeMet concentration was applied to six replicate wells; and six wells received just L15/FBS and constituted the control wells. The multiwell plates were then wrapped with parafilm and placed at 4 °C, 18 °C, or 26 °C. Some 96 well plates were also placed at 14 °C.

2.4. Evaluating the effects of selenomethionine on cell viability

Cultures with and without SeMet were periodically observed under a phase contrast microscope and found to be deteriorating after 7 days at high concentrations. The loss of cell viability at the end of 7 days was quantified with the AB and CFDA AM assays by following the step-by-