Journal of Thermal Biology 54 (2015) 47–55

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Thermal acclimation in the perch (*Perca fluviatilis* L.) immunity

Pertti Marnila [a,b,*], Esa-Matti Lilius [c]

[a] Natural Resources Institute Finland, FIN-31600 Jokioinen, Finland
[b] Department of Biology, Laboratory of Animal Physiology, University of Turku, FIN-20014 Turku, Finland
[c] Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland

ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form
31 December 2014
Accepted 15 January 2015
Available online 23 January 2015

Keywords:
Temperature acclimation
Temperature
Immunity
Phagocyte
Respiratory burst
Opsonin recognition
Perch
Perca fluviatilis

ABSTRACT

Fish immune systems must be able to cope with pathogens over a wide temperature range. Earlier research suggest that fish are more dependent on innate immune responses based on pattern recognition than acquired functions with specific recognition. If this applies to phagocytes, then opsonins (serum factors that augment phagocytosis e.g. immunoglobulins and complement proteins) attached on zymosan (Z) particles should be recognized better at higher temperatures than Z only. Z is recognized by glucan receptor representing pattern recognition. In this study perch were acclimated to 5 °C or 16 °C for 3–5 weeks. The recognition and activation of respiratory burst reaction of peripheral blood phagocytes was examined at seven different measurement temperatures (5, 10, 16, 20, 24 27, and 30 °C) when the cells were stimulated with Z and serum opsonized zymosan (OZ). Respiratory burst was measured as luminol chemiluminescence (CL) from diluted whole blood. OZ-induced CL per volume of blood was on average approximately 4.6 times higher in 16 °C acclimated fish than 5 °C acclimated perch ($P < 0.0001$). Z-induced CL was approximately 3 times higher at lower temperatures in 16 °C acclimated perch than 5 °C acclimated fish and 6–9 times higher at 27 °C and 30 °C ($P < 0.001$), respectively. CL reaction kinetics were faster in perch acclimated to 5 °C than 16 °C -acclimated fish, especially at low temperatures ($P < 0.001$). Thermal acclimation caused a 3–4 °C shift in temperature response curves of CL towards the acclimation temperature ($P < 0.0001$ and $P < 0.053$ in Z and OZ-induced CL, respectively).

Serum opsonins activated perch phagocytes substantially better at higher temperatures in both acclimation groups, which is consistent with an earlier study in rainbow trout (*O. mykiss*). However, opsonin recognition was significantly better in 16 °C acclimated perch than 5 °C acclimated fish, which was seen as higher CLs for OZ compared to Z, especially at higher temperatures. This is opposite to previously reported results in rainbow trout.

Differences between rainbow trout and perch in opsonin recognition by blood phagocytes suggest that the living habits of perch, which prefers approximately a 10 °C higher temperature than rainbow trout, may be reflected in immune cell functions. Results of the present examination suggest that also in fish phagocytes pattern recognition is the prevailing system at low temperatures, and specific recognition is more effective at high temperatures.

1. Introduction

The immune system of poikilothermic animals, such as fish, living in boreal or subarctic regions must be able to protect the host against pathogens over a wide temperature range. In general, many animals that live in boreal regions show natural seasonal acclimatization and have a capacity for thermal acclimation under laboratory conditions. Seasonal variations in water temperatures in boreal regions often exceed 20 °C. Waters in summer are substantially warmer near the surface than in deeper layers beneath the thermocline, and fish live in a temperature gradient. The fish immune system undergoes substantial seasonal changes, and factors, such as the temperature and photoperiod, affect immune functions partially via the neuroendocrine system (for reviews, see Bly and Clem, 1992; Zapata et al., 1992; Bowden et al., 2007). Therefore, the thermal history of an individual fish should be considered in the measurement of immune functions.

Phagocytic leukocytes that form the first line of immune defense, play a crucial role in protection against microbial infections (Secombes and Fletcher, 1992; Dzik, 2010; Grayfer et al., 2014). Phagocytes kill microorganisms by means of lysosomal degradative enzymes and highly toxic reactive oxygen intermediates.

Abbreviations: CL, chemiluminescence; Z, zymosan; OZ, opsonized zymosan; Ig, immunoglobulin; IgM, M class immunoglobulin

* Corresponding author at: Natural Resources Institute Finland, FIN-31600 Jokioinen, Finland.
E-mail address: pertti.marnila@luke.fi (P. Marnila).

http://dx.doi.org/10.1016/j.jtherbio.2015.01.002
0306-4565/

The reactive oxygen compounds are produced, in a process called the 'respiratory burst', by the NADPH–oxidase complex in plasma membrane, and by myeloperoxidase enzyme released during degranulation from azurophilic granules into phagolysosomes and extracellular space. These processes generate electronically excited states which, on relaxation, emit photons, giving rise to phagocyte chemiluminescence (CL). The CL emission can be amplified with a chemical enhancer, luminol by a factor of $\sim 10^4$. Luminol-amplified CL of phagocytes has been shown to be almost completely dependent on the release of myeloperoxidase from azurophilic granules. CL activity correlates well with the ingestion and killing of bacterial pathogens in salmonids (Stave et al., 1984; Ottinger et al., 1999; Nikoskelainen et al., 2005) and in human (Lilius and Nuutila, 1993; 2006).

Zymosan (Z), a cell wall preparation from yeast *Saccharomyces cerevisiae,* is commonly used to activate phagocyte respiratory burst. Z contains glucan and mannan, which are in mammals recognized by the complement receptor 3 complex (CD11b/CD18, Mac-1). This complex recognizes also complement C3bi and fibrinogen (Lilius and Marnila, 1992; Boshra et al., 2006). Also in fish recognition of Z is mediated by glucan receptors (Ainsworth, 1994), which belong to pattern recognition receptors. The pattern recognition receptors recognize widely conserved motifs of various pathogens and initiate a rapid innate immune response.

The process of opsonization is a means of identifying invading microbes by phagocytes. Binding of the serum complement components C3b and C3bi and the specific binding of the serum antibodies to the invading pathogen are normally required for a successfull recognition and destruction of the pathogen by phagocytes. Opsonization accelerates phagocytosis and killing of bacteria. Human phagocytes killed 41% of phagocytozed non-opsonized *Escherichia coli* K-12-bacteria in 180 min but, when the bacteria were pre-incubated in 0.4% (v/v) serum 96% were killed by phagocytes in same time (Atosuo and Lilius, 2011). Specific binding of immunoglobulins (Ig) on microbe surface accelerates killing of microbes also by the complement system (serum bacteriolytic activity). The reaction velocity rates of fish and human antibody dependent complement pathways were about two and five times higher than those of non-antibody-dependent alternative pathways, respectively (Kilpi et al., 2009). In opsonization of Z serum complement compounds and Igs are attached to Z particles. Serum opsonized Z (OZ) is recognized by human phagocytes partly via the glucal receptor (CD11b/CD18), partly by complement receptor 1 (CD35) which bind to C3b and partly by Ig-receptors (CD16 and CD64). Specific Igs and receptors recognizing the Igs and complement factors attached to Ig–antigen complexes, represent specific acquired immunity. Also in fish specific M-class Igs (IgM) and complement factors enhance phagocytosis and killing (Boshra et al., 2006; Nikoskelainen et al., 2005, 2007; Verho et al., 2005). In rainbow trout (*O. mykiss*) specific IgM for the bacterium *Aeromonas salmonicida* without an active complement was a relatively inefficient opsonin, but specific IgM with an active complement increased the magnitude of ingestion-mediated CL activity and accelerated the ingestion of target bacteria (Nikoskelainen et al., 2005).

In fish the complement receptors have not yet been characterized in detail, but accumulating evidence suggests that also fish phagocytes have receptors for complement proteins and IgM (Ainsworth, 1994; Couso et al., 2001; Esteban et al., 2004; Nakao et al., 2004; Boshra et al., 2006; Stafford et al., 2006a, 2006b). Thus, we assume that also in fish OZ is recognized by IgM, complement and glucan receptors, and Z only by the glucan receptors.

Low temperatures suppress humoral immunity, especially the primary antibody response, and many T-cell mediated immune functions in most fish species studied (Sypek and Burreson, 1983; Bly and Clem, 1991;1992; Ainsworth et al., 1991; Collazos et al., 1995a; Le Morvan-Rocher et al., 1995; Alcorn et al., 2002; Magnadóttir et al., 1999; Nikoskelainen et al., 2004; Grayfer et al., 2014). It has been proposed that at low temperatures fish rely more on non-specific innate immune responses, while at higher temperatures specific immunity is used to a greater extent (Ainsworth et al., 1991; Le Morvan et al., 1998; Alcorn et al., 2002; Bowden et al., 2007). If this hypothesis is valid in phagocytic leukocytes, then at low temperatures the activation of respiratory burst via glucan receptors (which recognize Z; pattern recognition) should be functional and activation via receptors that recognize serum opsonins on Z particles (acquired specific immunity) should be relatively inactive. In that case opsonins on Z particles would not increase or accelerate phagocyte activation much but, at high temperatures opsonization of Z particles should result to substantially stronger phagocyte activation than Z alone. It is probable that thermal acclimation may alter the expression and function of different receptor types.

To best of our knowledge fish phagocyte activation by Z or OZ at different temperatures has this far been examined only in rainbow trout (*O. mykiss*) (Nikoskelainen et al., 2004). Rainbow trout were acclimated to 5, 10, 15 and 20 °C. The recognition of opsonins was more effective at higher (15 and 20 °C) than colder temperatures (5 and 10 °C) in all acclimation groups. This is in agreement with the hypothesis above. However, phagocytes of rainbow trout that were acclimated to 5 °C or 10 °C recognized serum opsonins better at all temperatures than phagocytes of fish that were acclimated to 15 °C or 20 °C. This is somewhat controversial with the hypothesis. The authors suggested that this improved opsonin recognition due to cold acclimation could be an adaptive compensation reaction to low temperature, in which opsonin recognition is impaired (Nikoskelainen et al., 2004). Rainbow trout like other salmonids prefer cold water. It is not known if temperature or thermal acclimation has similar effects on phagocyte recognition in fish species that prefer warmer water. This information is needed in order to know if hypothesis presented above is valid in phagocyte recognition patterns. It would add on understanding on the effects of temperature on fish immunity and what sets the thermal limits in host defense. Therefore, in the present study we examined the effects of temperature and thermal acclimation of perch (*Perca fluviatilis*) on the ability of peripheral blood phagocytes to recognize Z and serum opsonins on Z particles and on the respiratory burst activity. Perch prefer approximately 10 °C warmer water than rainbow trout. It is a relatively eurythermic pelagial species that tolerate high water temperatures up to 30 °C.

2. Materials and methods

2.1. Animals and acclimation

Perch of both sexes were captured from the Baltic Sea near the island of Seili in the archipelago of Turku (southwest coast of Finland). Fish were captured using a fishing net in winter and spring between February and early May. After capturing the perch were transported to the Archipelago Research Institute, University of Turku. The salt content of water in the capturing area is 0.6% (w/v). In winter the water temperature is +0.2 °C under the ice and +4 °C at deeper levels. Ice breakup occurs in April. The water temperature in May varies from +4 °C in deep water to +10 °C on the surface.

The mean weight of captured perch was 85 g (range 43–181). Fish were kept in 70 l brown plastic containers filled with charcoal-filtered tap water that was changed daily. A total of 5–7 perch were kept in each container, and the biomass varied approximately from 300 to 900 g per 70 l. Illumination was kept dim, and the light/dark cycle was 12/12 h. Perch were fed chironomid larvae