Comparative Biochemistry and Physiology, Part A 172 (2014) 52–56

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Oxidative stress decreases with elevation in the lizard *Psammodromus algirus*

Senda Reguera [a,*], Francisco J. Zamora-Camacho [a], Cristina E. Trenzado [a,b], Ana Sanz [a], Gregorio Moreno-Rueda [a]

[a] Department of Zoology, University of Granada, Avda Fuentenueva s/n, E-18071 Granada, Spain
[b] Department of Cell Biology, University of Granada, Avda Fuentenueva s/n, E-18071 Granada, Spain

ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form 20 February 2014
Accepted 25 February 2014
Available online 4 March 2014

Keywords:
Antioxidant enzymes
Antioxidant protection
Elevation gradient
Lipid peroxidation
Lizards
Ultraviolet radiation

ABSTRACT

Oxidative stress is considered one of the main ecological and evolutionary forces. Several environmental stressors vary geographically and thus organisms inhabiting different sites face different oxidant environments. Nevertheless, there is scarce information about how oxidative damage and antioxidant defences vary geographically in animals. Here we study how oxidative stress varies from lowlands (300–700 m asl) to highlands (2200–2500 m asl) in the lizard *Psammodromus algirus*. To accomplish this, antioxidant enzymatic activity (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione transferase, DT-diaphorase) and lipid peroxidation were assayed in tissue samples from the lizards' tail. Lipid peroxidation was higher in individuals from lowlands than from highlands, indicating higher oxidative stress in lowland lizards. These results suggest that environmental conditions are less oxidant at high elevations with respect to low ones. Therefore, our study shows that oxidative stress varies geographically, which should have important consequences for our understanding of geographic variation in physiology and life-history of organisms.

1. Introduction

Oxidative stress, the unbalance between the production of pro-oxidant substances and antioxidant defences (Halliwell, 2007), is considered one of the most important ecological and evolutionary forces (von Schantz et al., 1999; Costantini, 2008; Dowling and Simmons, 2009; Monaghan et al., 2009; Costantini et al., 2010; Metcalfe and Alonso-Alvarez, 2010). Aerobic metabolism implies the production of pro-oxidant substances (reactive oxygen/nitrogen species, RONS; Finkel and Holbrook, 2000), which may react with molecular components of the cell such as lipids, proteins and nucleid acids, producing damages in cells' machinery (Halliwell and Gutteridge, 1995; Sies, 1997; Halliwell, 2007). Organisms are protected against oxidative damage by enzymatic and non-enzymatic antioxidant defences, which work to maintain RONS levels at equilibrium and minimize RONS damages in the organism (Sies, 1997; Finkel and Holbrook, 2000; Blokhina et al., 2003). Production and maintenance of antioxidants implies energy and resources consumption and therefore it is costly (Halliwell, 2007). When this balance is lost and antioxidant mechanisms cannot face RONS, oxidative stress occurs in cells (Sies, 1997; Jones, 2008).

In the wild, oxidative stress is induced by a wide range of environmental factors including changes in oxygen availability (Storey, 1996; Hermes-Lima and Zenteno-Savín, 2002; Buttemer et al., 2010), high or low temperatures (Hermes-Lima and Storey, 1993; Voituron et al., 2006), contaminants (Regoli, 2000; Prevodnik et al., 2007; Labrada-Martagón et al., 2011), and ultraviolet (UV) radiation (Dahms et al., 2011). These environmental factors vary geographically, and thus levels of oxidative stress and antioxidant defences should vary along a cline of these environmental factors. Nevertheless, there is a lack of studies examining geographic variation in oxidative stress in animals (Prevodnik et al., 2007; Costantini et al., 2010).

Sunlight radiation, whose UV-B wavelength component is the most harmful (Dahms et al., 2011), is an important causes of oxidative stress (Chang and Zheng, 2003; Chuang and Chen, 2013). UV-radiation initiates a series of redox reactions ending in free radical formation and leading to oxidative stress in cells (Dahms and Lee, 2010). Moreover, reduction of O_2 by photolytic reactions results in negative effects on oxidative balance, increasing oxygen radicals and producing lipid peroxidation as well as changes in antioxidant enzyme activities (Dahms and Lee, 2010). Damages produced by UV-radiation negatively affect organisms' fitness, by reducing sperm motility, hatching success and growth rates, as well as by increasing embryo malformation and

Abbreviations: BCI, Body Condition Index; CAT, catalase; DTD, DT-diaphorase; MDA, malondialdehyde; m asl, metres above sea level; GPX, glutathione peroxidase; GR, glutathione reductase; GST, glutathione transferase; DCPIP, 2,6-dichlorophenol indophenol; RONS, reactive oxygen/nitrogen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reacting substance; UV-radiation, Ultraviolet radiation.

* Corresponding author at: Department of Zoology, University Granada, E-18071 Granada, Spain. Tel.: +34 958 249854.
E-mail addresses: sreguera@ugr.es (S. Reguera), zamcam@ugr.es (F.J. Zamora-Camacho), ctrenzad@ugr.es (C.E. Trenzado), anasanz@ugr.es (A. Sanz), gmr@ugr.es (G. Moreno-Rueda).

http://dx.doi.org/10.1016/j.cbpa.2014.02.018
1095-6433/

mortality (e.g. Blaustein et al., 1998; Pahkala et al., 2002; Marquis et al., 2008; Hylander et al., 2014). As a consequence, UV-radiation is an important abiotic selective agent shaping physiology and life histories of organisms (Merilä et al., 2000). Because UV-radiation increases with elevation (Blumthaler et al., 1997; Sola et al., 2008), we expect that organisms from high elevations will be more prone to suffer oxidative stress than organisms from low elevations.

However, along an elevation gradient there are other environmental factors that gradually vary, such as temperature and oxygen partial pressure (PO_2; Körner, 2007; Graae et al., 2012). Both temperature and PO_2 are involved in biochemical reactions and both decrease along elevation gradient. These environmental factors may have antagonistic effects with solar radiation regarding to oxidative stress generation. Oxygen plays an important role in oxidative metabolism and oxidative damages can be reduced in the presence of low PO_2 (Buttemer et al., 2010). Low temperatures slow down biochemical reactions in ectotherms (but do the reverse in endotherms), which lead to low oxidants production and, for instance, low oxidative damage in cold environments (Jena et al., 2013). Moreover, in cold environments many animals hibernate, a period in which animals decrease metabolism to the minimum. Considering that PO_2 and temperature decrease with elevation, organisms from highlands might show less oxidative stress levels than organisms from lowlands.

In this work, we studied how oxidative stress damage and antioxidant enzymatic activity vary in the lizard *Psammodromus algirus* along an elevation gradient of 2200 m. *P. algirus* is an abundant lizard in Mediterranean landscapes of south-western Europe and north-western Africa, inhabiting along a wide elevation gradient (0–2700 m above sea level; m asl), therefore, facing a wide range of environmental conditions of temperature, PO_2 and UV-radiation (Salvador, 2011).

Oxidative stress processes combine various components such as free radical production, antioxidant defences, oxidative damage, and repair mechanisms (Monaghan et al., 2009). In this study, two of these components were assayed, lipid peroxidation (as an indicator of oxidative stress; Del Rio et al., 2005; Monaghan et al., 2009; Hõrak and Cohen, 2010), and activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione transferase (GST), DT-diaphorase (DTD) (as indicators of antioxidant defences), to guess possible variation in oxidative stress of lizards along a strong environmental gradient.

2. Material and methods

2.1. Study area and field procedures

Samplings were performed in Sierra Nevada Mountain (SE Spain), during the second half of July, in 2011. Sampling was concentrated in a short time period in order to avoid possible biases due to seasonal changes in oxidative stress (Hermes-Lima et al., 2012). Two sample sites were located at lowlands (36°53′N, 3°24′W, 300 m asl and 36°55′N, 3°26′W, 700 m asl) and two at highlands (36°58′N, 3°19′W, 2200 m asl and 37°01′N, 3°19′W, 2500 m asl). In Sierra Nevada, mean annual temperature goes from 3.5 to 17.6 °C (in the highest and lowest elevation respectively; REDIAM; http://www.juntadeandalucia.es/medioambiente/site/rediam), and in the study area, environmental temperature during *P. algirus* activity season (March to October) differs 8 °C on average between the lowest (300 m asl; mean ± sd = 25.0 ± 5.09 °C) and the highest sampling plot (2500 m asl; 17.2 ± 4.87 °C; Zamora-Camacho et al., 2013). Relative irradiance also varies with elevation, increasing on average 6–8% km^{-1} for UV-A and 7–11% km^{-1} for UV-B radiations (Sola et al., 2008). In the study area, UV-B radiation went from 1.28 ± 0.03 to 4.8 ± 0.37 μW cm^{-2} nm^{-1} between the lowest and the highest sampling plot (measured with the 305 nm wavelength channel of a BIC compact 4-channel radiometer, Biospherical Inc., CA, USA).

We captured 19 individuals (9 from highlands and 10 from lowlands) to measure oxidative stress under natural conditions at different elevations. Only adult males were used in this study, in order to avoid confounding effects due to age or sex (Olsson et al., 2012). Specimens were captured by hand. We measured snout-vent length (SVL) with a metal ruler (accuracy 1 mm) and weighted with an electronic balance (Model Radwag WTB200, accuracy of 0.01 g). To avoid killing specimens, tissue samples were taken from the tail because lizards can regenerate it. Taking a tissue sample from tail has small or null impact on lizard survival (Niewiarowski et al., 1997). We took tail sample in situ, preserving samples in liquid nitrogen until carried to the laboratory facilities, where they were keep in a freezer at −80 °C.

2.2. Biochemical analyses

Tissue samples were homogenized in ice-cold buffer (100 mM Tris–HCl, 0.1 mM EDTA and 0.1% Triton X-100 (v/v), pH 7.8) at a ratio of 1:9 (w/v). Homogenates were centrifuged at 30,000 *g* for 30 min in a Centrikon H-401 centrifuge. After centrifugation, the supernatant was collected and frozen at −80 °C until analysed. All enzymatic assays were carried out at 25 ± 0.5 °C using a PowerWavex microplate scanning spectrophotometer (Bio-Tek Instruments, USA) in duplicate in 96-well microplates (UVStar®, Greiner Bio-One, Germany). The enzymatic reactions were started by the addition of the tissue extract, except for SOD, where xanthine oxidase was used. The specific assay conditions were as follows.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 concentration at 240 nm according to Aebi (1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and freshly prepared 10.6 mM H_2O_2.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured spectrophotochemically by the ferricytochrome C method using xanthine/xanthine oxidase as the source of superoxide radicals. The reaction mixture was consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome C and 0.024 IU mL^{-1} xanthine oxidase. One activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome C reduction rate measured at 550 nm (McCord and Fridovich, 1969).

Glutathione peroxidase (GPX; EC 1.11.1.9) activity was measured following the method of Flohé and Günzler (1984). A freshly prepared glutathione reductase solution (2.4 U mL^{-1} in 0.1 M potassium phosphate buffer, pH 7.0) was added to a 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM sodium azide, 0.15 mM NADPH and 0.15 mM cumene hydroperoxide. After the addition of 1 mM GSH (reduced glutathione), the NADPH-consumption rate was monitored at 340 nm.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Carlberg and Mannervik (1975) with some modifications, by measuring the oxidation of NADPH at 340 nm. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.63 mM NADPH, and 0.15 mM GSSG.

Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined by the method of Habig et al. (1974) adapted to microplate. The reaction mixture consisted of 0.1 M phosphate buffer (pH 6.5), 1.2 mM GSH and 1.23 mM solution of 1-chloro-2,4-dinitrobenzene in ethanol were prepared just before the assay. GST activity was monitored at 340 nm by the formation of glutathione-CDNB-conjugate.

DT-diaphorase (NADPH): quinone oxidoreductase; EC 1.6.99.2) activity was measured according to Sturve et al. (2005) and adapted by Sanz et al. (2010). The reaction mixture contained 50 mM Tris–HCl (pH 7.3), 50 μM DCPIP (2,6-dichlorophenol indophenol) and 0.5 mM NADH. Control reaction was measured with the addition of distilled water instead of sample extract. DTD activity was defined as the difference between sample and control DCPIP reduction.

Except for SOD, for which the arbitrary units have already been mentioned, for other enzymatic activities, one unit of activity is defined as