Journal of Insect Physiology 58 (2012) 1424–1431

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

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

Genome-wide analysis of transcriptional changes in the thoracic muscle of the migratory locust, *Locusta migratoria*, exposed to hypobaric hypoxia

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ARTICLE INFO

Article history:
Received 10 June 2012
Received in revised form 10 August 2012
Accepted 16 August 2012
Available online 15 September 2012

Keywords:
Hypobaric hypoxia
Altitude hypoxia
Microarray
Gene expression
Locusta migratoria

ABSTRACT

Hypobaric hypoxia has both beneficial and detrimental effects on living organisms in high altitude regions. The impact of hypobaric hypoxia has been investigated in numerous vertebrates. However, it is still not well characterized how invertebrates respond to hypobaric hypoxia. In this study, we examined the transcriptional profiles of locust thoracic muscles using microarrays to disclose their strategies to cope with hypobaric hypoxia. We found that hypoxia-inducible factor (HIF) and its target genes did not respond significantly to hypobaric hypoxia. As with severe, normobaric hypoxia, mitochondrial activities were systemically suppressed, mainly involving in energy production and mitochondrial biogenesis. The surveillance processes, involving in clearance of dysfunctional proteins in endoplasmic reticulum, were activated, e.g. endoplasmic reticulum-associated degradation, protein glycosylation, and protein folding. In contrast to severe, normobaric hypoxia, glycolysis was suppressed and the pentose phosphate pathway strengthened. Our data suggested that hypobaric hypoxia induced an oxidative stress rather than an energy crisis in locust thoracic muscles. Our research provides a different perspective of biological responses to hypoxia, complementing the well-studied biological responses to extreme, normobaric hypoxia.

1. Introduction

Hypobaric hypoxia has both beneficial and detrimental effects on animal and human health. It can damage lipids and proteins (Magalhães et al., 2005), interfere with rat spermatogenesis (Liao et al., 2010), and increase the damage of reactive oxidative species (ROS) even in native Tibetans (Gelfi et al., 2004). Hypobaric hypoxia can also be beneficial for human beings. For instance, body weight was reduced when obese people were exposed to hypobaric hypoxia (Lippl et al., 2010). However, most studies on hypobaric hypoxia focus on vertebrates (Murray, 2009; Storz et al., 2010). How invertebrates respond to hypobaric hypoxia is poorly understood.

Hypoxia-inducible factor 1 (HIF-1) is a master regulator of hypoxic responses, which is a heterodimeric transcription factor comprising HIF-1α and HIF-1β. HIF-1α protein is constantly expressed and regulated post-transcriptionally by oxygen sensors with prolyl-hydroxylase domain (PHD1–3). In normoxia, HIF-1α is hydroxylated by PHDs and directed to ubiquitination and proteasomal degradation. In hypoxia, HIF-1α is stabilized with the existence of ROS which act upstream of PHDs in regulating HIF-1α in the oxygen-sensing pathway (Brunelle et al., 2005; Guzy et al., 2005; Kaelin, 2005; Mansfield et al., 2005). HIF-1β is constitutively expressed without dependence of oxygen contents. Under hypoxic conditions, HIF activates the expression of various proteins controlling glucose metabolism, cell proliferation, and vascularization. For instance, pyruvate dehydrogenase kinase 1 was activated by HIF-1 in human cell lines subjected in 0.1% or 0.5% oxygen, phosphorylating and inhibiting pyruvate dehydrogenase to prevent pyruvate from fueling the mitochondrial TCA cycle (Kim et al., 2006; Papandreou et al., 2006). Glycolytic enzymes, e.g. phosphoglycerate kinase 1, pyruvate kinase M and lactate dehydrogenase A, were activated by HIF-1 in Hep3B or HeLa cells exposed to 1% oxygen (Firth et al., 1994; Semenza et al., 1994), promoting ATP production via substrate-level phosphorylation in glycolysis.

Insects, the largest taxa of invertebrates, are extremely tolerant of hypoxia and have been used as models to illuminate the morphological, behavioral, physiological and molecular adaptation to normobaric hypoxia (Law and Wells, 1989; Hoback and Stanley, 2001). A comparison of metabolic rates in adult insects including mosquitos, flies, termites and grasshoppers showed that the critical partial oxygen pressure (pO_2) for insects was below 5 kPa, much lower than that of mammals (Greenlee and Harrison, 2004). Measurement of respiration in locusts indicated that they could withstand 5 kPa pO_2 and survive 0.7 kPa pO_2

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0022-1910/$ - see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jinsphys.2012.08.006

(Arieli and Lehrer, 1988), demonstrating the extraordinary capacity of locusts to cope with hypoxia. Only few studies measured the physiological parameters of insects under hypobaric hypoxia. For example, the critical partial oxygen pressures of the honeybee *Apis mellifera* and the moth *Carpocapsa saltitans* were measured under hypobaric conditions and they appeared similar to those measured under normobaric conditions (Harrison et al., 2006). Nevertheless, to our knowledge, there is no large-scale analysis of gene expression of insects under hypobaric hypoxia.

Locusts are distributed widely in both low altitude plains and high altitude plateaus, such as Tibetan plateau with an average altitude exceeding 4 km. To understand the locust responses to the naturally-existing altitude hypoxia, we treated the migratory locust, *Locusta migratoria*, in simulated 4 km-altitude hypoxia and examined their transcriptional responses using customized microarrays in our lab (Guo et al., 2011; Ma et al., 2011; Wang et al., 2012). The data indicated that the migratory locusts utilized glucose in a different way under hypobaric hypoxia as compared to that has been observed for a variety of animals under severe, normbaric hypoxia.

2. Material and methods

2.1. Insects and treatment

Locusts were collected from a gregarious population in Huanghua, Hebei Province, China, raised gregariously under a 14-h:10-h light:dark photoperiod regime at 30 ± 2 °C, and fed on fresh wheat seedlings and wheat bran (Kang et al., 2004). Female adult locusts of 10–14 days after eclosion were selected and treated in a hypobaric chamber. Locusts were placed within a glass tank in which wheat seedlings and wheat bran were provided. The glass tank was covered with an iron mesh and transferred into the hypobaric chamber. Thirty minutes later when locusts were quiescent, the air was pumped out from the chamber to lower the pO_2 down to 13 kPa simulating 4 km altitude hypoxia. The locust ventilation frequencies were counted before and during the hypobaric treatment. After 48-h treatment, locust thoracic muscle was immediately sampled and frozen in liquid nitrogen. Six groups of locusts, treated identically, were used with ten individuals in each group. Six groups of ten adult locusts in normoxia were also sampled in parallel, serving as controls.

2.2. Microarray hybridization

Total RNA was extracted following the protocol of the RNeasy Mini Kit (Qiagen). Forty microgram RNA was reversely transcribed to prepare cDNA probes. Probes from hypoxia-treated locusts were labeled with Cy3 and the others with Cy5. Then the probes were purified, mixed, and hybridized to customized locust microarrays (Guo et al., 2011; Ma et al., 2011; Wang et al., 2012). Microarrays were scanned by using a GenePix 4000B microarray scanner and image analysis was performed by using GenePix Pro 6.0 (Axon Instruments). Six independent hybridizations with biological replicates were conducted with a direct-comparison strategy.

2.3. Microarray data analysis

Differentially expressed genes (DEGs) were identified with the limma package (Smyth, 2004). After background correction with *normexp* method (Ritchie et al., 2007) and within-array normalization, data were fitted to a linear model. The cutoff for DEGs was set as FDR < 0.05 and fold change > 1.5. DEGs were mapped to gene ontology (GO) terms by using WEGO (Ye et al., 2006). A chi-square test was conducted to determine the significantly different GO terms. Fisher's exact test was used when any expected value of count was below 5, which causes inaccurate chi-square test. Significantly enhanced or suppressed pathways were identified by using KOBAS (Wu et al., 2006) and delineated by using iPath (Letunic et al., 2008). All microarray data were MIAME compliant. Both raw and processed data were deposited in the NCBI gene expression omnibus (GEO ID: GSE33898).

2.4. Gene expression of HIF-α and HIF target genes

Both HIF-α and HIF target genes present on the locust microarrays were analyzed to evaluate the role of HIF system under hypobaric hypoxia. The gene expression of HIF-α was analyzed by using QPCR because there was no probe representing HIF-α on the locust microarray. Three mammalian HIF-α proteins, namely human HIF-1α (GenBank ID: NP_001521), HIF-2α (GenBank ID: NP_001421), HIF-3α (GenBank ID: NP_690007), and fruitfly HIF-1α SIMA (GenBank ID: NP_524584) were used as baits to search for the locust homologues in the *de novo* transcriptome of the migratory locust (Chen et al., 2010) with TBLASTN algorithm. Two candidate transcripts (MergeConsensus50436 and MergeConsensus5770) were obtained and further merged into a longer one. Primers were designed according to the merged sequence and only part of the merged sequence was confirmed by PCR and Sanger sequencing. Then, the sequence was extended by RACE experiments with SMART™ RACE cDNA Amplification Kit (Clontech Laboratories, Inc.) and deposited in GenBank (GenBank ID: JX110862). Gene expression of HIF-α was quantified with QPCR in six biological replicates (forward primer: GCTGACTTAATGTGGGGTCC; reverse primer: TCCTATGGTGGTTCGTGCT), following the same protocol as in Section 2.5. Differences between treatments and controls in normoxia were analyzed by using Student's *t*-test implemented in SPSS 15.0 software (SPSS Inc., Chicago, IL).

The gene expression levels of HIF target genes were also analyzed. The validated and predicted HIF target genes were obtained from a study combining both computational and experimental strategies to identify HIF-target genes (Benita et al., 2009). The locust homologues of these HIF target genes were identified with TBLASTN algorithm and only the best hit was adopted with identity over 30% and *E* value smaller than 1e-5 (Camacho et al., 2009).

2.5. QPCR analysis

The stand curve method (Guo et al., 2010) was used to measure the relative RNA expression level. PCR amplifications were conducted with an MX3000P spectrofluorometric thermal cycler (Stratagene) and a RealMasterMix (SYBR Green) kit (Tiangen). Melting curve analysis was performed to confirm the specificity of amplification. Expression levels of target genes were normalized by the gene LM05710 which was identified as the most stably expressed gene by geNorm (Vandesompele et al., 2002) and NormFinder (Andersen et al., 2004). Primer sequences were presented in Table S5.

3. Results

3.1. Locust responses to hypobaric hypoxia

Locusts are widely distributed on Tibetan plateau with an average altitude over 4 km. To evaluate the locust responses to altitude hypoxia, we subjected locusts to a simulated 4 km-altitude condition. Locust activities were suppressed during the treatment. For instance, the locusts tended to stay quietly on wheat seedlings and rather than crawled around under normoxia. They continued feeding but ate less, as judged from the remaining wheat seedlings