Available online at www.sciencedirect.com
SCIENCE DIRECT®
Archives of Biochemistry and Biophysics 435 (2005) 103–111
ABB
www.elsevier.com/locate/yabbi

Up-regulation of a thioredoxin peroxidase-like protein, proliferation-associated gene, in hibernating bats

Sean F. Eddy*, J.D. McNally[1], K.B. Storey

Departments of Chemistry and Biology, Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6

Received 15 October 2004, and in revised form 17 November 2004
Available online 7 December 2004

Abstract

Two-dimensional gel electrophoresis was used to assess differential protein expression between euthermic and hibernating states in heart of *Myotis lucifugus*. A hibernation-induced protein was identified by mass spectrometry as a thioredoxin peroxidase-like protein known as PAG. Western blotting confirmed up-regulation (>2-fold) and RT-PCR also revealed up-regulation (>5-fold) of *pag* mRNA. Cloning revealed a highly conserved sequence suggesting a conserved function for PAG. Oxidative stress markers, p-IκB-α (Ser 32) and p-HSP27 (Ser 78/82), were also up-regulated in heart and skeletal muscle during hibernation. Although there are selected increases in gene/protein expression during hibernation, general translation inhibition occurs as part of metabolic rate depression. This was confirmed by elevated levels of the inactive forms of the eIF2α (Ser 51) in both heart and skeletal muscle (2- to 5-fold higher than in euthermia) and the eEF2 (Thr 51) in skeletal muscle (a 15-fold increase). This study suggests that hibernators may use up-regulation of specific proteins to counteract oxidative stress.
© 2004 Elsevier Inc. All rights reserved

Keywords: 2D-PAGE; eEF2; eIF2α; Heart; HSP27; IκB-α; Mammalian hibernation; PAG; Skeletal muscle; Thioredoxin peroxidase

Hibernation allows many small mammals to survive when environmental temperatures are very low and food resources scarce or absent. By strongly suppressing metabolic rate and allowing body temperature to fall to near ambient, hibernators can save as much as 90% of the energy that would otherwise be required to remain euthermic throughout the winter [1]. Studies have begun to identify some of the molecular responses of animals to hibernation, including the up-regulation of selected genes/proteins and the central role of reversible protein phosphorylation in providing regulatory control over the activities of many enzymes and functional proteins during transitions to and from torpor [2]. For example, recent studies with ground squirrels have shown that overall rates of protein synthesis are strongly suppressed in organs of hibernators as evidenced by changes in vitro protein translation rates, the distribution of ribosomes between polysome and monosome fractions, and the phosphorylation state of ribosomal initiation and elongation factors [3–5].

The hibernation season consists of long bouts of torpor that are interrupted periodically by arousal back to the euthermic state for short periods of time. These torpor/arousal cycles result in major changes in oxygen consumption over short time frames; oxygen consumption in torpor can be only 1–5% of the normal euthermic rate but can rise by 100- to 300-fold within just a few minutes during the arousal process to fuel the massive thermogenesis by brown adipose tissue and skeletal muscle that rewarms the animal. Hence, the potential for oxidative damage due to the formation of reactive oxygen

* Corresponding author. Present address: Department of Biochemistry, Boston University School of Medicine, K624, 80 East Concord St., Boston, MA 02118, USA.
E-mail address: eddy@biochem.bumc.bu.edu (S.F. Eddy).
[1] Present address: Dalhousie University Faculty of Medicine, Halifax, NS, Canada B3H 4H7.

0003-9861/$ - see front matter © 2004 Elsevier Inc. All rights reserved
doi:10.1016/j.abb.2004.11.020

species (ROS)[2] varies widely over the hibernation–arousal cycle. To deal with this, hibernators must have effective antioxidant defenses in place; indeed, activities of several of the main antioxidant enzymes are known to be very high in hibernator organs, particularly brown adipose [6,7]. In addition, hibernators must have good antioxidant defenses in place to limit the accumulation of oxidative damage products over the long weeks of torpor [8].

Thioredoxin, and its associated redox enzymes, have recently gained attention for their roles in antioxidant defense [9]. The thioredoxin peroxidases are a family of enzymes that reduce ROS in cells using thiol groups on conserved cysteine residues (positions 52 and 173) through coupled reactions with thioredoxin proteins. One of the family members is a protein known as the proliferation-associated gene (PAG), which is over-expressed in disease states such as cancer [10]. Members of the thioredoxin peroxidase family also appear to be directly involved with the activation of nuclear factor κ B (NF-κB) [11,12], an important transcription factor that regulates gene expression in cells, often in response to oxidative stress. Classical NF-κB exists as a homo- or heterodimer of p65 and p50 subunits and, under normal cellular conditions; an inhibitor, IκB, sequesters NF-κB in the cytoplasm by capping the NF-κB nuclear localization signal. In response to one of a number of stresses, including oxidative stress, IκB is phosphorylated at two conserved serine residues located at positions 32 and 36 leading to its ubiquitination and degradation, and thereby freeing NF-κB for translocation into the nucleus. Another protein that is involved in the response to oxidative stress in cells is the small heat shock protein 27 (HSP27). Enhanced phosphorylation of HSP27 has been associated with oxidative stress. The present study evaluates a number of parameters associated with oxidative stress and antioxidant defense in muscles (heart, skeletal muscle) of hibernating little brown bats, *Myotis lucifugus*. We document hibernation-responsive up-regulation of PAG at both the mRNA and protein levels as well as hibernation-associated changes in the levels of the phosphorylated forms of IκB-α, HSP27, and ribosomal initiation and elongation factors.

Materials and methods

Little brown bats, *M. lucifugus*, were collected, experimentally hibernated, and processed as previously described [13,14]. Briefly, 20 bats were collected in late November from caves in which they had been hibernating for ∼2 months. Bats were aroused during collection and remained aroused during transport to the Université de Sherbrooke. Ten of the animals were maintained in this state at 23–24 °C; they remained alert and responsive and their body temperatures remained high (determined qualitatively by touch). These euthermic animals were sacrificed 48 h post-collection. The other 10 bats were placed in a cold room at 5 °C and allowed to re-enter torpor; they were sampled after 36–38 h of continuous torpor (mean rectal temperatures were 5.6 °C) to provide hibernating samples. Tissues from euthermic and hibernating animals were rapidly excised, immediately frozen in liquid nitrogen, and then transported to Ottawa on dry ice and stored at −80 °C until analysis.

Two-dimensional polyacrylamide gel electrophoresis (PAGE)

Heart samples (∼100 mg, 1 heart per extract) were homogenized with a Polytron homogenizer in 1.5 ml of buffer (25 mM Hepes, 25 mM KCl, 250 mM sucrose, 1 mM EGTA, and 1 mM EDTA) with ∼0.1 mM phenylmethylsulfonyl fluoride added immediately after homogenization. Samples were centrifuged at 15000 rpm for 15 min in a Biofuge 15 (Baxter Canlab) and supernatants were removed. 2D-PAGE was performed using the Mini-PROTEAN II system as outlined by the manufacturer (Bio-Rad Laboratories). For isoelectric focusing in the first dimension, duplicate 4% acrylamide gels were prepared in 75 mm × 1 mm capillary tubes with 1.6% ampholines, pH 4.0–6.0, and 0.4% ampholines, pH 3.5–10.0 (Sigma Chemical). Aliquots of sample extracts containing 50 μg of protein were combined with equal volumes of sample buffer (9.5 M urea, 2.0% v/v Triton X-100, 5.0% v/v β-mercaptoethanol, 1.6% ampholines, pH 4.0–6.0, and 0.4% ampholines pH 3.5–10.0) and loaded onto the surface of the first dimension gel. Samples were then covered with sample overlay buffer (9 M urea, 0.8% ampholines, pH 4.0–6.0, and 0.2% ampholines, pH 3.5–10.0, and 0.0025% w/v bromophenol blue). Isoelectric focusing was performed at 500 V overnight (12 h). Duplicate first dimension gels were removed from capillary tubes and loaded onto 12% SDS–PAGE gels. Duplicate second dimension gels were run at 100 V for 2 h and then one gel was silver-stained and the second was stained by colloidal Coomassie blue. Protein spots showing differential expression between euthermic and hibernating conditions were excised from the Coomassie blue-stained gels, placed in Eppendorf tubes, destained with 100 mM ammonium bicarbonate (BDH, Mississauga, ON) in 30% acetonitrile (Anachemica, Mississauga, ON), and then rinsed three times with distilled water.

[2] *Abbreviations used:* PAG and *pag*, proliferation-associated gene protein product and mRNA, respectively; NF-κB, nuclear factor κ B; IκB, inhibitor of NF-κB; eIF2α, eukaryotic initiation factor 2 α; eEF2, eukaryotic elongation factor 2; HSP27, heat shock protein 27; ROS, reactive oxygen species.

Download English Version:

https://daneshyari.com/en/article/9882312

Download Persian Version:

https://daneshyari.com/article/9882312

Daneshyari.com