Archives of Biochemistry and Biophysics 636 (2017) 90–99

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Regulation of glutamate dehydrogenase (GDH) in response to whole body freezing in wood frog liver linked to differential acetylation and ADP-ribosylation

Stuart R. Green, Kenneth B. Storey*

Institute of Biochemistry & Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, K1S 5B6 Ontario, Canada

ARTICLE INFO

Keywords:
Glutamate dehydrogenase
Freeze tolerance
Amino acid metabolism
Rana sylvatica
Liver
Nitrogen metabolism

ABSTRACT

Glutamate dehydrogenase (GDH) represents a critical enzyme catalyzing the reaction spanning amino acid catabolism, the Krebs cycle, and urea production in the wood frog. GDH breaks down glutamate and NAD^+ to generate α-ketoglutaric acid (α-KG), NADH and ammonium that can be metabolized to form urea. Purification of GDH from control and frozen male wood frog livers was performed using a two-step column chromatography procedure with a cation exchange column and a GTP-agarose affinity column. Analysis of kinetic parameters of the purified GDH showed several notable differences between the control and stress. Under standard assay conditions, the affinity of GDH for its substrates was significantly higher for the freeze-exposed enzyme than for the control (glutamate K_m: 41% decrease, NAD^+ K_m: 40% decrease). The maximal activity for the control enzyme was also noted to be lower than the frozen. This suggests that the frozen form of the GDH was activated relative to the control form. Western blot analysis of common posttranslational modifications indicated that the frozen enzyme had a lower degree of acetylation and ADP-ribosylation than its control counterpart. These results suggest that GDH is regulated in the wood frog liver by means of altering post-translational modifications in response to freezing.

1. Introduction

During the winter months when air temperatures drop below freezing, the biochemical pathways in the North American wood frog, *Rana sylvatica,* undergo large-scale changes in order to alter the production of metabolites needed for survival of whole body freezing. During freezing the heartbeat, breathing, and brain activity cease but the frogs still manage to revive themselves during the springtime. Freeze tolerance is relatively rare among vertebrates and represents one of the most extreme hypometabolic strategies in nature [1]. During the winter when the water in the body cavity freezes, approximately 65% of the animal's body water content becomes extracellular ice and the animal experiences global tissue anoxia [1]. To survive this, the wood frog reworks much of its internal biochemical processes to reduce metabolic expenditure during extended periods of freezing and produces metabolites that act as cryoprotectants, preventing damage to the frog's cellular components and internal organs [2]. The role of elevated glucose concentrations to serve as a cryoprotectant, thereby preventing damage to the internal organs, is widely understood [3,4]. More recently, the role of urea in wood frog winter survival has gained more attention [5,6].

Due to the highly permeable nature of amphibian skin, many species are particularly prone to water-loss and commonly accumulate the nitrogenous waste urea as an osmoprotectant in the event of dehydrating conditions or elevated salinity [7]. During freezing in the wood frog, critical internal organs such as the liver are particularly dehydrated and may lose around 25% of their water content to ice in the body cavity [3]. This helps to prevent organ damage due to intracellular ice formation by increasing the concentration of cryoprotectants and thereby enhancing their colligative properties. In preparation for winter freezing, the wood frog, much like other amphibians, accumulates large amounts of urea; up to 85 mM plasma urea via regulation of urea transporters in the urinary bladder and kidney [5,8]. In addition to pre-freezing accrual of urea in the tissues, another study has demonstrated that over a 48 h freezing period wood frogs from Ohio showed a 40% increase in liver urea levels compared to unfrozen frogs from the same population, whereas Alaskan wood frogs demonstrated an 80% increase in urea [9]. This study suggests that the increase of urea by members of this species is relevant to freeze-tolerance since the Alaskan populations can survive lower winter temperatures and produce more urea.

* Corresponding author.
E-mail address: kenneth_storey@carleton.ca (K.B. Storey).

https://doi.org/10.1016/j.abb.2017.10.010
Received 8 September 2017; Received in revised form 11 October 2017; Accepted 14 October 2017
Available online 19 October 2017
0003-9861/ © 2017 Elsevier Inc. All rights reserved.

Abbreviations:

α-KG	α-ketoglutaric acid
CM	Carboxymethyl cation exchange material
DSF	Differential Scanning Fluorimetry
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
GDH	Glutamate dehydrogenase
GTP	Guanosine triphosphate
K_A	Activator constant (concentration causing half maximal activation)
K_m	Michaelis constant
PTM	Posttranslational modification
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
T_m	Unfolding temperature

Growing evidence has suggested that urea plays two overlapping roles in the survival of the wood frog as both a cryoprotectant alongside glucose preventing ice from forming intracellularly during freezing and as an osmoprotectant in regulating cell volume during freezing and thawing (for review; Storey and Storey, 2017). Osmoprotection is an important aspect of freeze tolerance since formation of extracellular ice crystals excludes solute molecules and thereby causes an increase in the osmolality of the remaining extracellular fluid that if left unchecked can cause fatal organ dehydration [10,11]. The urea cycle is an ATP dependent process and therefore, continued urea synthesis in the liver during freezing, when other metabolic processes are reduced, further supports the notion that it plays a critical role in freeze survival [9]. The cryoprotective properties of urea in the wood frog have been demonstrated in several studies that have shown that urea significantly reduces damage during freezing to organs (heart and muscle), improves viability of erythrocytes and is associated with improved survival rates and post-thaw recovery [6,12]. The mechanism of the cryoprotective function of urea in the wood frog can be partly explained by the simple increase in its concentration and the resulting freeze point depression but also is important in preventing excessive organ dehydration by altering osmotic balance. However, urea has also been suggested to have other mechanisms of action such as improving membrane stability during freezing [6].

Although evidence suggests that urea production is critical for freezing survival, the underlying enzymatic mechanisms behind the regulation of urea production in the wood frog in response to freezing have not been thoroughly explored. Understanding the function of glutamate dehydrogenase (GDH) in response to hypometabolic conditions is important since this enzyme is central to the generation of mitochondrial ammonium, the main substrate for carbamoyl phosphate synthetase I; the first step in the urea cycle. GDH carries out this role though oxidative deamination of glutamate (referred to as the forward reaction) through the use of either NAD^+ or $NADP^+$ to generate α-ketoglutaric acid (α-KG) accompanied by the release of ammonium. GDH can also catalyze the incorporation of ammonium to generate glutamate. However this is not commonly seen under most physiological conditions as the concentration of ammonium needed to elicit a substantial reaction rate in this direction would result in cellular toxicity due to the high K_m (low affinity) of GDH for ammonium [13]. While GDH is important in regulating influx of materials through the citric acid cycle, the production of ammonium ions via the forward GDH reaction can lead to cellular toxicity [14]. To cope with this, many terrestrial animals sequester excess ammonium by incorporating it into urea via the urea cycle which spans both mitochondrial and cytosolic spaces [15]. Regulation of GDH therefore represents a possible step that could offer upstream control over the production of urea during freezing or other metabolic stress in the wood frog.

GDH is a homohexamer composed of subunits that have a molecular weight of approximately 56 kDa [16]. Allosteric sites on GDH help to regulate its activity in response to GTP/ATP and GDP/ADP concentrations [17]. Since high concentrations of GTP and ATP are indications of an abundance of cellular energy, these molecules act as potent inhibitors of GDH activity to reduce flux of α-KG into the Krebs cycle [13]. Conversely, ADP is an allosteric activator of GDH due to its role in signaling low cellular energy [18]. Expression of GDH can be found in many organs of the body, but the liver expresses large quantities of the enzyme and due to its role in urea metabolism [15], it was of particular interest in studying GDH regulation. A previous study from our lab showed that GDH is regulated in a mammalian hibernator, the Richardson's ground squirrel (*Spermophilus richardsonii*), to cope with hypometabolic conditions placed on the animal. The study showed that a reduction in K_m for substrates in both directions of the reaction were present concurrently with a reduction in GDH phosphorylation in the hibernating animal suggesting that GDH is more active during hibernation [19]. Wood frog GDH has also been explored in relation to differences between geographically distinct populations. The results of the study demonstrated that an Alaskan population had a significantly higher activity of GDH within skeletal muscle in the glutamate consuming direction compared to GDH from an Ohio population of wood frogs during winter acclimation. This suggested that catabolism of muscle tissue may be of increasing importance in more northern populations in preparation for winter to increase ammonium available for urea production in the liver [9].

Due to the importance of urea as both a cryoprotectant and an osmoprotectant to reduce freezing and/or dehydration stress on cells [6], it was hypothesized that GDH is regulated in wood frog tissues in order to favour production of urea to benefit freezing survival. The research presented here investigates the regulation of GDH in wood frog liver to identify the molecular mechanism that can promote this GDH function.

2. Materials and methods

2.1. Animals and chemicals

The chemicals used to perform the experiments described here were purchased from BioShop (Burlington, ON), or from Sigma Chemical Company (St. Louis, MO) unless otherwise stated. The water used in the experiments was distilled water filtered with a Milli-Q (Millipore Corporation) water purifier.

Male wood frogs, *Rana sylvatica*, were collected from spring breeding ponds in the Ottawa area. Animals were first washed with tetracycline and then kept alive for two weeks at 5 °C in moist containers with sphagnum moss to help simulate the natural environment. The control groups were subjected to these conditions until they were euthanized by pithing. Prior to euthanizing and dissecting, frogs in the frozen group were treated according to the protocols described by Dieni and Storey [20]. The animals were dissected quickly and their tissues of interest, including liver, were frozen in liquid nitrogen and then transferred to −72 °C for storage until use. The treatment of the animals was approved by the Carleton University Animal Care Committee (protocol 13683) under the guidelines set out by the Canadian Council on Animal Care.

2.2. Enzyme purification

Liver samples were quickly weighed and homogenized in a 1:9 w:v ratio with homogenate buffer A (50 mM MES buffer, 2 mM EDTA, 2 mM EGTA, 25 mM β-GP, 10 mM β-mercaptoethanol, 10% glycerol v:v, pH 6.2) with several crystals of phenylmethylsulfonyl fluoride (PMSF) serine protease inhibitor. Samples were kept cool on ice while being

Download English Version:

https://daneshyari.com/en/article/8288888

Download Persian Version:

https://daneshyari.com/article/8288888

Daneshyari.com