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Comparative enzymology—new insights from studies of an "old" enzyme, lactate dehydrogenase☆

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ABSTRACT

Comparative enzymology explores the molecular mechanisms that alter the properties of enzymes to best fit and adapt them to the biotic demands and abiotic stresses that affect the cellular environment in which these protein catalysts function. For many years, comparative enzymology was primarily concerned with analyzing enzyme functional properties (e.g. substrate affinities, allosteric effectors, responses to temperature or pH, stabilizers, denaturants, etc.) in order to determine how enzyme properties were optimized to function under changing conditions. More recently it became apparent that posttranslational modifications of enzymes play a huge role in metabolic regulation. At first, such modifications appeared to target just crucial regulatory enzymes but recent work is showing that many dehydrogenases are also targets of posttranslational modification leading to substantial changes in enzyme properties. The present article focuses in particular on lactate dehydrogenase (LDH) showing that stress-induced changes in enzyme properties can be linked with reversible posttranslational modifications; e.g. changes in the phosphorylation state of LDH occur in response to dehydration stress in frogs and anoxia exposure of turtles and snails. Furthermore, these studies show that LDH is also a target of other posttranslational modifications including acetylation, methylation and ubiquitination that change in response to anoxia or dehydration stress. Selected new methods for exploring posttranslational modifications of dehydrogenases are discussed and new challenges for the future of comparative enzymology are presented that will help to achieve a deeper understanding of biochemical adaptation through enzyme regulation.

1. Introduction

The field of comparative biochemistry and physiology has grown exponentially since the 1960s, and has fostered the subdiscipline of comparative enzymology that seeks to understand how the protein catalysts of cells are adapted to serve different metabolic needs, respond to extracellular signals, and preserve functionality under changing parameters of abiotic stress (e.g. temperature, pressure, salinity, etc.). For myself and for my long term friend and colleague, Dr. Tom Moon, our entry to this fascinating field came from deliberately (Tom) or accidently (me) landing in the lab of Dr. Peter W. Hochachka at the University of British Columbia for graduate studies. We were both lucky to enter Peter's lab at a crucial time in the development of comparative biochemistry when mainline biochemists had learned enough about the catalytic and regulatory properties of mammalian enzymes to give us the framework to dive into fascinating questions about how these catalysts respond/adapt to the challenges presented by diverse environmental circumstances (e.g. heat/cold, high/low hydrostatic pressure, with/without oxygen). Peter's passion for understanding the "strategies of biochemical adaptation", set out in his seminal book of the same name (Hochachka and Somero, 1973) led Tom into important studies of temperature effects on enzyme function and myself into the enzymatic regulation of anoxia tolerance in turtles. I never met Tom in the UBC lab since he preceded me somewhat. We first crossed paths scientifically bobbing around on the RV Alpha Helix off Hawaii, ostensibly to study pressure effects on the enzymes of abyssal fish but ultimately producing many more papers on the enzymology of oceanic squid that were much easier to catch.[1] We met again on the Alpha Helix trip to study air versus water breathing fish on the Amazon River. Then, probably to Tom's despair, I moved to Ottawa and he has had to put up with

Abbreviations: ADH, alanopine dehydrogenase; LDH, lactate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; PDH, polyol dehydrogenase; PTM, posttranslational modification; RPP, reversible protein phosphorylation.

☆ Contribution to a special issue celebrating the work of Dr. Thomas W. Moon on the occasion of his retirement after 45 years in comparative biochemistry and physiology.

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[1] Tom participated in 3 research expeditions of the RV Alpha Helix (myself just the latter 2), trips that produced huge amounts of foundational data on aquatic physiology and biochemistry of animals facing unique challenges in environments that were virtually unstudied previously. Collected papers from each trip were published in special issues of *American Zoologist* [Vol. 11(3), 1971], *Comparative Biochemistry and Physiology B* [Vol. 52(1), 1975], and *Canadian Journal of Zoology* [Vol. 56(5, part 2), 1978].

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me for the past 35 years, providing an invaluable source of calm, sane advice to me and many of my graduate students.

With enzymology as the foundation of both our futures, Tom built a brilliant career centered mainly on freshwater fish in which he built upwards from an enzymology base to explore many aspects of fish intermediary metabolism, muscle and liver functionality, hormonal control of metabolism, and aquatic toxicology (endocrine disruptors, nanoparticles), rising to become a highly respected authority on fish biochemistry and toxicology (e.g. recent reviews: Massarsky et al., 2011; Mennigen et al., 2011; Polakof et al., 2012; Massarsky et al., 2014). My career moved more laterally across enzymology, metabolic regulation, gene and protein expression, and cell protective strategies. My lab has explored multiple adaptive strategies of animals including freeze tolerance, anoxia survival, estivation and hibernation, among others (e.g. recent reviews: Storey, 2015; Storey and Storey, 2010, 2012a, 2013). Both of our labs produced many studies of the adaptive regulation of enzymes, relating these properties to the cellular milieu in which enzymes must function and the stresses imposed on cells by changing environmental conditions.

Much research in my lab has focused on the regulation of metabolic rate depression, the mechanisms used by cells/tissues to both coordinate the overall suppression of metabolism and reprioritize ATP use in hypometabolic states. These studies have included metabolic enzymes, signal transduction enzymes, and ion motive ATPases to understand how metabolism can be coordinately suppressed by 90% or more, as well as studies of enzyme regulation that achieve specific stress-responsive outcomes (e.g. cryoprotectant synthesis in cold-hardy species) (Storey and Storey, 2010, 2012b). In particular, my lab has shown that posttranslational modification (PTM) via reversible protein phosphorylation (RPP), achieved via the stress-responsive actions of protein kinases and protein phosphatases, is crucial to coordinating metabolic responses during hypometabolism, affecting regulatory enzymes of many pathways, signal transduction enzymes, transporter proteins, transcription factors, ribosomal translation, and cell cycle suppression, among others (Storey and Storey, 1990, 2004, 2007). RPP can alter many enzyme properties: e.g. activity (frequently providing on/off control), substrate affinities, sensitivity to activators or inhibitors, enzyme location in the cell, and protein stability. Indeed, RPP is now known to be a core molecular mechanism of metabolic regulation in animals (Humphrey et al., 2015) and to exert crucial controls on many enzymes/proteins that are involved in coordinating the descent into hypometabolic states (Bickler et al., 2002; Storey and Storey, 2004; Valcourt et al., 2012; Chen et al., 2015).

2. New horizons in phosphorylation control of enzymes

Recently we began exploring the potential for a greatly expanded role for PTMs in the control of metabolic enzymes and biochemical adaptation to environmental stress in animals. This involved two ideas: (1) that RPP controls a much greater number of enzymes than previously suspected, and (2) that a regulatory interplay between multiple types of PTMs can modulate and coordinate enzyme response to stress signals. These are the main subjects of this article and I draw many of the examples discussed below from our recent studies of lactate dehydrogenase (LDH).

As all students learn in their first biochemistry course, LDH catalyzes the reversible reaction:

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \leftrightarrow \text{lactate} + \text{NAD}^+.$$

LDH is the terminal enzyme of anaerobic glycolysis in the cytosol and, by producing lactate and regenerating NAD^+, it facilitates continued ATP production by glycolysis. LDH is a tetramer with two main subunit types, A (originally called M for muscle) and B (originally called H for heart). In mammals LDH-A4 is specific to skeletal muscle and liver, B4 occurs in heart, red blood cells and brain, and isozymes with mixed A and B subunits are found in various other tissues. A third form, LDH C, is unique to testes (Goldberg et al., 2010). Kinetic differences between A4 and B4 LDH, chiefly a lack of inhibition of A4 by high pyruvate, led to the general idea that LDH-A4 is best suited for tissues that need to make and accumulate lactate rapidly under oxygen-limited conditions, whereas LDH B4 suits tissues that use lactate as an aerobic substrate.

In comparative biochemistry, and beginning from the early work of Peter Hochachka, LDH has been widely used as a model enzyme in the study of biochemical adaptation. In particular, LDH has been used extensively to investigate temperature effects on enzyme catalysis including to identify the changes to a protein that optimize catalytic function in diverse thermal environments and the gene/protein mutations that underlie enzyme evolution in populations along thermal gradients (Schulte, 2001; Fields et al., 2015). For example, studies that combine both analysis of native LDH-A from fish inhabiting different thermal environments with site-directed mutagenesis and bioinformatics modeling have shown that protein modifications that optimize LDH-A for low temperature function (e.g. maintaining K_m pyruvate in a narrow 0.15–0.35 mM range) can involve as few as 1–2 amino acid substitutions (Fields and Houseman, 2004; Fields et al., 2015).

Both Tom Moon and I have contributed to the comparative analysis of LDH properties in a variety of animal species. We both studied temperature and pressure effects on LDH from abyssal fish (Moon et al., 1971; Baldwin et al., 1975). Tom also analyzed LDH from cestodes, lampreys, and heterothermic mammals (fossorial mole rats, hibernating bats) (Moon et al., 1977; Moon, 1978; Mustafa et al., 1981; Baldwin et al., 1987) whereas my lab has investigated LDH from cephalopods, snails, turtles and frogs (Fields et al., 1976; Storey, 1977a,1977b; Xiong and Storey, 2012; Abboud and Storey, 2013; Dawson et al., 2013; Shahriari et al., 2013; Katzenback et al., 2014). Historically, LDH and many other dehydrogenases have been considered to be nonregulatory near-equilibrium enzymes that mainly respond to changes in substrate or product concentrations such as rising pyruvate levels during burst muscle exercise or an influx of lactate to be used as a substrate for gluconeogenesis. Indeed, apart from differences in sensitivity to inhibition by high pyruvate between LDH-A (largely insensitive) and LDH-B (heart form; inhibited by rising pyruvate) few kinetic controls on LDH have been identified, particularly when examined at physiological levels of putative regulators. However, new evidence indicates that metabolic regulation of LDH and various other dehydrogenases might instead be exerted more effectively via PTMs.

Beginning several years ago, we wondered if RPP also applied to so-called nonregulatory enzymes, exemplified by various dehydrogenases, and if this modification could contribute to their function. Our first investigation of this idea led to the discovery that glucose-6-phosphate dehydrogenase (G6PDH), the enzyme that gates the pentose phosphate cycle, was subject to RPP control (Ramnanan and Storey, 2006). G6PDH from hepatopancreas of the land snail (*Otala lactea*) was present in low and high phosphate forms that dominated in active and estivating snails, respectively, with the high phosphate estivating form showing higher activity (mU/mg) and a higher affinity for substrate (i.e. lower K_m for G6P) than its counterpart in active snails. Incubation studies to promote the action of protein kinases or phosphatases indicated that the enzyme may be controlled *in vivo* by protein kinase G and protein phosphatase 1. We found another example of RPP control in polyol dehydrogenase (PDH), the enzyme that produces the cryoprotectant, glycerol, in cold-hardy insects. PDH from freeze-avoiding larvae of the gall fly (*Epiblema scuddderiana*) also showed interconversion between high and low phosphate forms associated with a 2-fold difference in the K_m for glyceraldehyde between larvae acclimated to +5 °C (permissive for glycerol cryoprotectant synthesis) and versus −15 °C (synthesis halted) (Holden and Storey, 2011). Finally, although a well-known regulatory enzyme, our recent studies of glutamate dehydrogenase (GDH) have also shown that this enzyme is subject to RPP control. Liver GDH showed reversible changes in its phosphorylation state, affecting multiple kinetic properties, in response to both torpor in hibernating ground squirrels or anoxia exposure in turtles (Bell and Storey, 2010, 2012).