© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 4273-4274 doi:10.1242/jeb.103192

An affinity for biochemical adaptation to temperature

Alex Gunderson and Jonathon Stillman

discuss Peter Hochachka and George Somero's classic paper 'The adaptation of enzymes to temperature', published in *Comparative Biochemistry and Physiology* in 1968.

If you have had the pleasure of diving under sea ice in Antarctica, you've probably noticed that Channichthyid icefish swim just as fast as your pet goldfish. This is a curious observation. Icefish are in extremely cold water (–2°C), while your goldfish is at balmy room temperature. We learn in high school chemistry courses that chemical reactions slow down as temperatures decrease. Since movement is based on chemical reactions, shouldn't the icefish move much more slowly than the goldfish? How is it possible that organisms from such disparate thermal environments can maintain similar physiological performance? That is the over-arching question addressed in the seminal 1968 paper by Hochachka and Somero (Hochachka and Somero, 1968), which is the subject of this Classics article.

However, before we get to their study, some additional background is warranted. Early physiologists noted that rates of whole-organism physiological processes, such as digestion and locomotion, change predictably with temperature, as would be expected based on fundamental chemical principles. However, they also noted another pattern when looking across species, exemplified by the above discussion of fishes: namely, that organisms tend to have optimal physiological performance at the temperatures that they typically experience in their natural habitats. The challenge was to reconcile these observations with a mechanistic model of thermal adaptation. To do so, physiologists in the 1950s and 1960s turned to comparative enzyme biochemistry.

Where to begin? As a natural starting point, one might expect enzymes of cold-adapted species to have lower energy barriers to catalysis (i.e. lower activation energies, thus overcoming the inherently low energy in cold systems) and/or to have their highest reaction velocities (i.e. maximum V_{max}) at low temperatures that reflect field body temperatures. Many early investigations focused on these enzyme properties. However, at the time, results from such studies were surprisingly mixed. For example, Paul Licht compared temperatures at maximum V_{max} (which he referred to as 'optimal' temperatures) of myosin ATPase and alkaline phosphatase orthologs (homologous gene products found in different organisms) from lizards from a wide range of thermal habitats (Licht, 1964). Licht found that optimal temperatures of myosin-ATPase orthologs did correlate with thermal environments, but were generally several degrees higher than the preferred temperature of each species. In addition, optimal temperatures for alkaline phosphatase were similar for all orthologs, and in some cases were higher than the upper lethal temperature of the species from which they were isolated (Licht, 1964). Some portion of the story was clearly missing.

Hochachka and Somero surmised that substrate affinity may be an important missing piece of the thermal adaptation puzzle. Affinity is measured as the Michaelis–Menten constant K_m, the amount of substrate necessary for a reaction to occur at 50% of V_{max} (thus, the lower the K_m value, the higher the affinity). Their reasoning was based on the simple fact that substrate concentrations in living tissues are typically far below the saturation point. Affinity has an enormous effect on reaction rates at low substrate levels due to the non-linearity of Michaelis–Menten kinetics. As demonstrated in Fig. 1, when a substrate is limiting, an enzyme with a low K_m value will have a greater reaction velocity than another enzyme with a higher K_m value, even if both share the same V_{max}. Thus, Hochachka and Somero hypothesized that natural selection would favor the minimization of K_m under thermal conditions that reflect environmental temperatures.

To test their hypothesis, they measured the temperature dependence of K_m of the enzyme lactate dehydrogenase (LDH) isolated from an array of fishes from different thermal habitats: an Antarctic fish (*Trematomus borchgrevinki*), lake and brook trout (*Salvelinus* spp.), bluefin tuna (*Thunnus thynnus*) and a tropical lungfish (*Lepidosiren paradoxa*). They tested three specific predictions. First,

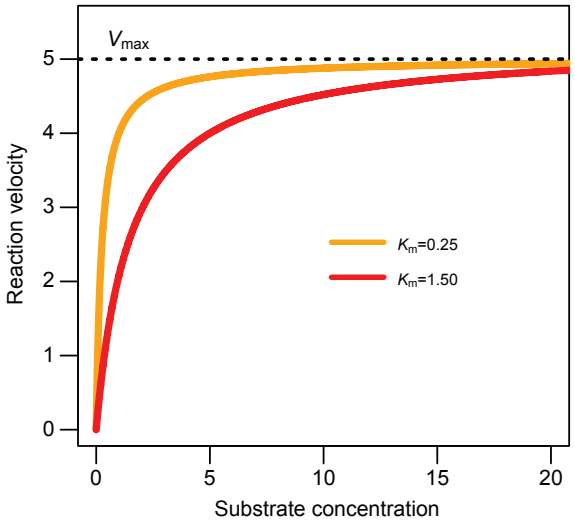

Fig 1. Reaction velocities are faster for high-affinity (low K_m) enzymes at low substrate concentration. Both hypothetical enzymes have the same V_{max}.

Classics is an occasional column, featuring historic publications from the literature. Written by modern experts in the field, these articles discuss each classic paper's impact on the field of biology and their own work.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.103192

that LDH isolated from warm-habitat fish would have K_m minima at higher temperatures than those from cold-habitat fish. Second, that fish acclimated to warm (or cool) conditions would upregulate LDH isozymes with K_m minima at higher (or lower) temperatures. Third, in heterothermic species (i.e. species whose tissues differ in temperature within the body), there would be tissue-specific expression of LDH isozymes, with warm tissues expressing isozymes with K_m minima at higher temperatures. Bluefin tuna were ideal for this test because they maintain muscle temperatures that are several degrees higher than the rest of the body through counter-current heat-exchange mechanisms.

Their predictions were substantiated across the board. In the interspecific comparisons, they found that muscle LDH from the Antarctic fish had maximum affinity at the coldest temperatures, tuna LDHs had maximum affinity at intermediate temperatures, and tropical lungfish muscle LDH had maximum affinity at the warmest temperatures (Fig. 2). Within species, cold-acclimated trout upregulated the expression of LDH subunits that had maximum affinity at low temperatures. Furthermore, within tuna the LDH isozymes from cool heart tissue had maximum affinity at lower temperatures than LDH from warm muscle tissue (Fig. 2).

Hochachka and Somero's paper demonstrated that enzyme–substrate affinity is a major target for selection during adaptation to different thermal environments. Subsequent investigations, many spearheaded by Hochachka, Somero and their students, confirmed the importance of K_m in thermal adaptation and extended the scope of their initial discovery beyond temperature: enzyme–substrate affinity has been demonstrated to be a target of selection in response to most environmental parameters considered, including ionic/osmotic strength (Hochachka and Somero, 2002), pH (Yancey and Somero, 1978) and hydrostatic pressure (Hochachka and Somero, 1984).

Although a sled-pulling adult Siberian husky bounds regally through the snow, its first puppy steps are typically less than graceful; so too were the first steps in understanding the role of substrate affinity (K_m) in thermal adaptation. The model put forth by Hochachka and Somero (Hochachka and Somero, 1968) needed to be refined, and in the process some components were discarded. For example, we now know that K_m is not necessarily minimized at physiological temperatures, nor does minimum K_m vary widely among orthologs (i.e. Fig. 2). Instead, selection seems to favor the maintenance of K_m within a relatively narrow range during evolution [for examples, see figs 7.7 and 7.8 in another Hochachka and Somero classic, *Biochemical Adaptation* (Hochachka and Somero, 2002)], which is probably related to conservation of cellular metabolite concentration or flux. Subsequent studies also rarely found the U-shaped relationship between K_m and temperature shown in Fig. 2, possibly because later studies used pH buffers with more realistic temperature sensitivity (e.g. Yancey and Somero, 1978).

Yet despite these minor shortcomings, the enduring legacy of Hochachka and Somero's 1968 *Comparative Biochemistry and Physiology* paper is that it shifted the paradigm for how physiologists conceptualized adaptation at the molecular level. Their discovery provided an important cornerstone for the development of an integrated model of biochemical adaptation that incorporates enzyme–substrate affinity, enzyme structural stability, activation energy and V_{max} into a coherent whole [for an authoritative treatment of this model, see *Biochemical Adaptation* (Hochachka and Somero, 2002)].

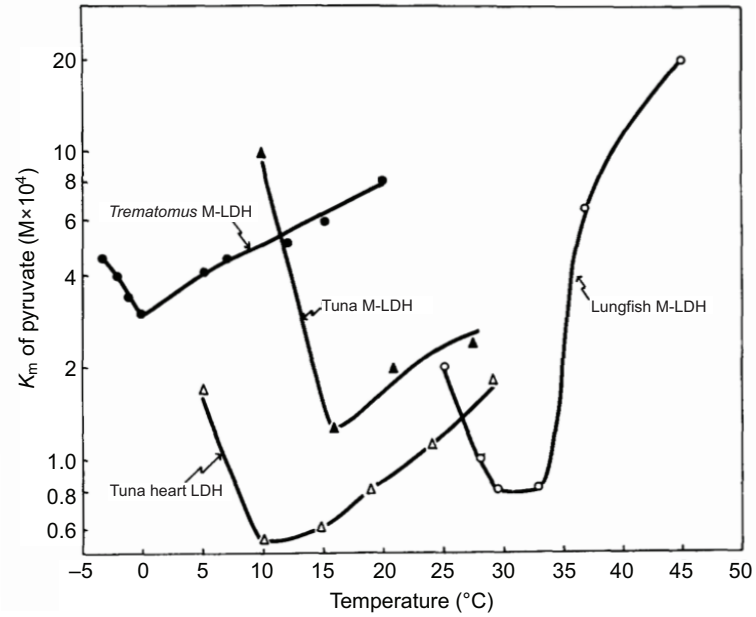

Fig 2. Figure taken from Hochachka and Somero's classic paper showing the temperature dependence of K_m values of lactate dehydrogenase (LDH) from different fishes and tissues. Minimum K_m, or maximum affinity, for the substrate pyruvate occurs at body temperatures typical for each species in their natural habitats. In addition, the K_m minimum for tuna heart LDH occurs at a lower temperature than the K_m minimum for tuna muscle LDH, which is warmed by countercurrent heat exchange mechanisms. Reprinted from *Comparative Biochemistry and Physiology*, vol. 27, Hochachka, P. and Somero, G. (1968), with permission from Elsevier.

In summary, in their 1968 paper, Hochachka and Somero opened the door for a deeper, unified understanding of the fundamental ways in which living things adapt to their physical environments.

Alex R. Gunderson
San Francisco State University and University of California Berkeley
alexrgunderson@gmail.com

Jonathon H. Stillman
San Francisco State University and University of California Berkeley
stillmaj@sfsu.edu

References

Hochachka, P. and Somero, G. (1968). The adaptation of enzymes to temperature. *Comp. Biochem. Physiol.* **27**, 659-668.

Hochachka, P. W. and Somero, G. N. (1984). *Biochemical Adaptation*. Princeton, NJ: Princeton University Press.

Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and process in physiological evolution*. Oxford, UK: Oxford University Press.

Licht, P. (1964). The temperature dependence of myosin-adenosinetriphosphatase and alkaline phosphatase in lizards. *Comp. Biochem. Physiol.* **12**, 331-340.

Yancey, P. H. and Somero, G. N. (1978). Temperature dependence of intracellular pH: Its role in the conservation of pyruvate apparent K_m values of vertebrate lactate dehydrogenases. *J. Comp. Physiol.* **125**, 129-134.