Opinion

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The universality of enzymatic rate–temperature dependency

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Organismal adaptation to extreme temperatures yields enzymes with distinct configurational stabilities, including thermophilic and psychrophilic enzymes, which are adapted to high and low temperatures, respectively. These enzymes are widely assumed to also have unique rate–temperature dependencies. Thermophilic enzymes, for example, are considered optimal at high temperatures and effectively inactive at low temperatures due to excess rigidity. Surveying published data, we find that thermophilic, mesophilic, and psychrophilic enzymes exhibit indistinguishable rate–temperature dependencies. Furthermore, given the nonenzymatic rate–temperature dependency, all enzymes, regardless of their operation temperatures, become >10-fold less powerful catalysts per 25°C temperature increase. Among other factors, this loss of rate acceleration may be ascribed to thermally induced vibrations compromising the active-site catalytic configuration, suggesting that many enzymes are in fact insufficiently rigid.

Adaptation of enzymes to temperature

Temperature is a dominant environmental component that affects all living organisms. It affects the kinetic energy of molecules, including biomolecules such as proteins, their collision and reaction rates, the strength of molecular interactions, and other physic-chemical properties. The relations between the growth temperature of organisms and the biophysical properties of their proteins have been extensively explored [1]. The most dominant effect of temperature is on protein stability: proteins unfold (i.e., lose their distinct 3D structures) beyond a certain temperature (the melting temperature, T_m). Environmental adaptation to extreme temperatures resulted in enzymes with appropriate melting temperatures (i.e., high overall configurational stability, ΔG_{U-N}). However, by the ruling paradigm, temperature adaptation also leads to distinct rate–temperature profiles such that enzymes exhibit maximal rates at the organismal growth temperature. Thermophilic enzymes comprise the most studied example. They are highly thermostable and exhibit maximal rates at $\geq$60°C [2]. In agreement with their high configurational stability, thermophilic enzymes exhibit a higher degree of structural packing [1,3,4] and lower configurational flexibility relative to mesophilic enzymes; enzymes from organisms living at moderate temperatures (20–45°C) [1,5]. Psychrophilic enzymes, isolated from organisms adapted to cold environments, comprise the other extreme. They are considered highly labile in terms of configurational stability and are sufficiently flexible near 0°C [5–8].

At their respective operation temperatures, the catalytic efficiencies (k_{cat}/K_M values) of thermophilic, mesophilic, and psychrophilic enzymes appear similar [9]. Thus, from an evolutionary point of view, each of the three classes of enzymes is equally metabolically competent within its own operational, physiological temperature. However, at moderate temperature, the rates of thermophilic enzymes are much lower than at high temperature, and lower compared to the rates of their mesophilic counterparts [1,5]. This is usually interpreted as thermophilic enzymes being 'nearly inactive at ambient temperature as a result of their compactness and rigidity' [5] (see also [1,10–12]). In other words, it is generally assumed that the conformational dynamics of thermophilic enzymes are specifically optimized for high temperature [5,11]. Likewise, psychrophilic enzymes are considered to exhibit high flexibility, making them optimized for low temperature [5]. Thus, the rate dependencies of enzymes and their configurational dynamics are considered to vary according to their operation temperatures.

What needs to be considered, however, is that the rates of all reactions, including nonenzymatic ones, drop down with a temperature decrease (Box 1). Indeed, mesophilic enzymes exhibit systematically weaker rate–temperature dependencies relative to the corresponding nonenzymatic reaction. This difference is attributed to the lower activation enthalpies of the enzymatic versus nonenzymatic reactions [13].

Here, we jointly address the rate–temperature dependencies of all enzyme classes, and compare them to the dependency of nonenzymatic reactions. We challenge the generally accepted paradigm that thermophilic, mesophilic, and psychrophilic enzymes have distinct rate–temperature dependencies, and that temperature–rate dependencies relate, by default, to enzyme dynamics. We suggest a common theme that underlines the temperature–rate dependency of all enzymes as long as they maintain their folded state. Also, we re-examine the generally

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Box 1. Effects of temperature on reaction rates and temperature coefficient Q_{10}

Reactions occur when colliding reactant molecules have sufficient kinetic, thermal energy to cross the energy barrier of the reaction. The fraction of molecules with thermal energy above a certain threshold increases exponentially with temperature (Maxwell–Boltzmann distribution). Reaction rates (k) therefore relate to temperature (T, in Kelvin) and to the activation energy (E_a) of the reaction, as described by the Arrhenius equation:

$$k = Ae^{-E_a/(RT)} \quad [I]$$

where A is the pre-exponential factor and R is the universal gas constant.

Given this exponential dependency, the reaction rate multiplies by a certain factor (fold increase) per linear increases in temperature. Empirically, the most often used description is Q_{10} – the fold-increase in rate per 10°C increase in temperature, or the temperature coefficient. Given two measured rates, at low temperature (T_1) and high temperature (T_2), Q_{10} is given by [56]:

$$Q_{10} = \left(\frac{k_{T_2}}{k_{T_1}}\right)^{\frac{10}{T_2-T_1}} \quad [II]$$

The smaller slope for the enzymatic (Figure I, blue, dashed line) versus the nonenzymatic reactions (black line) indicates the different rate–temperature dependencies of these reactions. The different slopes result in the catalytic power exhibited by an enzyme, or the rate acceleration, which corresponds to the factor by which an enzyme accelerates the reaction rate relative to the spontaneous, nonenzymatic reaction (k_{cat}/k_{non}), decreasing with temperature. The loss of rate enhancement factor, L_{10}, marks the loss of rate acceleration per temperature increase of 10°C, as defined by equation (3), where k_{cat}/k_{non1} and k_{cat}/k_{non2} are the rate acceleration at low (T_1) and high temperature (T_2), respectively.

$$L_{10} = 1/\left(\left(\frac{k_{cat}/k_{non_2}}{k_{cat}/k_{non_1}}\right)^{\frac{10}{T_2-T_1}}\right) = \frac{Q_{10}^{non}}{Q_{10}^{enz}} \quad [III]$$

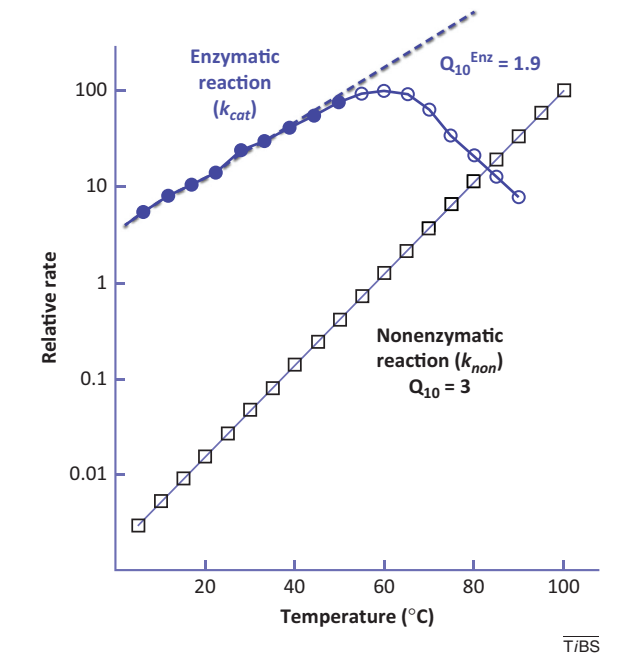

Figure I. Reaction rates as a function of temperature – a schematic representation. The rates of ordinary chemical reactions generally follow exponential relations within a wide temperature range (black line; note that reaction rates are plotted on a logarithmic scale). Enzymatic reactions (blue line) show more complex rate–temperature relations. Foremost, the fraction of folded enzyme molecules decreases as the temperature approaches the melting temperature of the enzyme. Consequently, the rate–temperature dependency curve of an enzyme is typically bell shaped. The linear range (in full circles, dashed blue line) corresponds to the temperature range under which ≥95% the enzyme molecules are in the folded state, and is thus used to assign the enzymatic temperature coefficient, Q_{10}^{Enz}. The second part of the curve (open circles) shows a decline of the reaction due to an increasing fraction of enzyme molecules become unfolded. The nonenzymatic reaction rates exhibit a more pronounced temperature dependency (black, open squares), and consequently a higher Q_{10} value.

assumed linkage between the overall rigidity of the fold and active site of an enzyme.

Universality of enzymatic Q_{10} values

Rate–temperature dependencies are compared using the empirical measure of Q_{10} (Box 1). We systematically explored the existing literature, and extracted Q_{10} values for >150 enzymatic reactions from the three environmental classes, including reactions for which Q_{10} values were available for both the enzymatic and nonenzymatic reactions (Figure 1; Text S1 and Tables S1–S4 in the supplementary material online).

The observation that mesophilic enzyme rates roughly double per 10°C (Q_{10}^{enz} = 2) [13] is confirmed in this larger dataset; the average Q_{10}^{enz} value for mesophilic enzymes is 1.8 (Figure 1). However, unexpectedly, the Q_{10}^{enz} values of all classes, including thermophilic and psychrophilic enzymes, do not seem to significantly differ. The universality of enzymatic Q_{10} values observed here is surprising: thermophilic enzymes being considered highly active at high temperature and nearly inactive at ambient temperature [1,5,11], are expected to show higher Q_{10} values than mesophilic enzymes. Psychrophilic enzymes were also ascribed a unique rate–temperature dependency [14–16], and distinctly low Q_{10} values, owing to their high flexibility at low temperatures. However, our survey indicates that the temperature dependency of enzymatic rates (k_{cat}, or k_{cat}/K_M) is essentially the same for all three classes.

Nonenzymatic 'Q10' values and rate accelerations

How do the rate–temperature dependencies of enzyme reactions compare to those of nonenzymatic reactions (Q_{10}^{non})? A direct comparison (i.e., having both the enzymatic and nonenzymatic Q_{10} values) is available for only a small set (n=9) that exhibits an average Q_{10}^{non} of 3.4. A larger set of reactions (n=18) indicates an average Q_{10}^{non} of ~4.4 (Table S5). The prevailing rule of thumb is that reaction rates double per 10°C increase. We could not, however, find any systematic exploration of this rule. As previously noticed [17], the Q_{10}^{non} values for enzyme-catalyzed reactions appear to be much higher than 2. It thus remains unclear whether the rule of thumb should become Q_{10} = 3, or possibly 4, or whether the current sample of Q_{10}^{non} is biased, particularly for reactions in water, where dramatic changes in water properties such as density and ionic concentration occur at high temperatures [18].

As is obvious from the average Q_{10}^{non} (3.4) versus Q_{10}^{enz} (1.8) values, the rates of the nonenzymatic reactions