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.

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

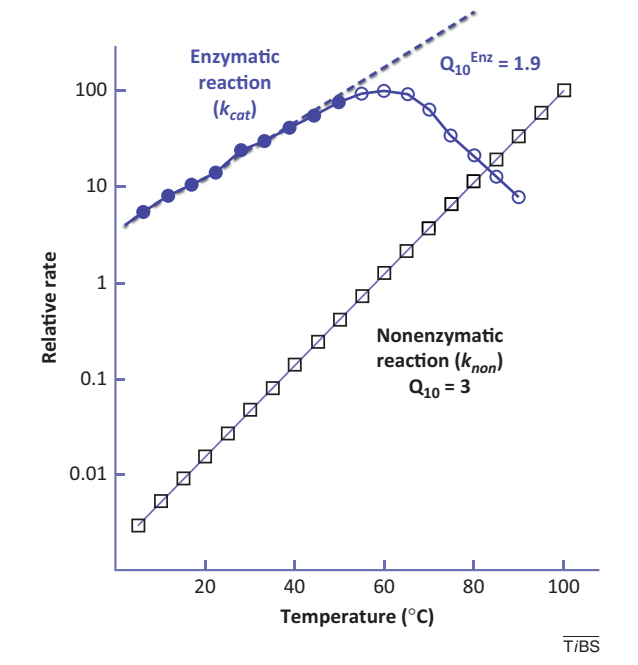

Figure I. Reaction rates as a function of temperature – a schematic representation. The rates of ordinary chemical reactions generally follow exponential relations within a wide temperature range (black line; note that reaction rates are plotted on a logarithmic scale). Enzymatic reactions (blue line) 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

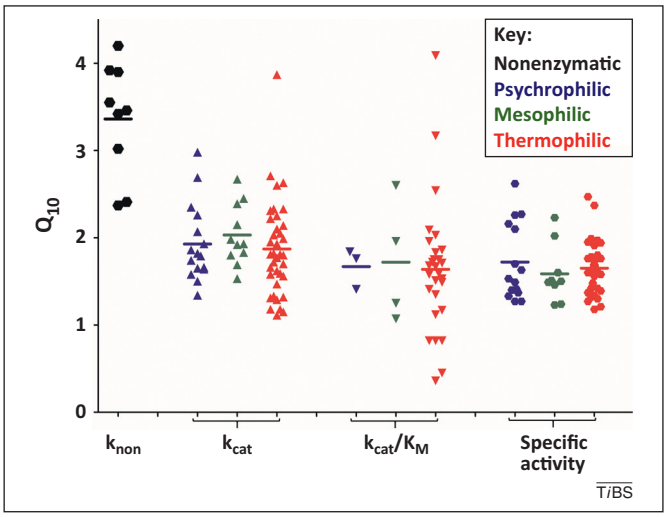

Figure 1. Temperature dependencies of the rate of enzymatic reactions. Q_{10} values (average fold-increase in reaction rate per 10°C temperature increase) for reaction rates (k_{cat}), catalytic efficiencies (k_{cat}/K_M), and specific activities of psychrophilic enzymes (in blue; *n*=34; mean=1.81; SD=0.43), mesophilic enzymes (in green; *n*=24; mean=1.81; SD=0.44), and thermophilic enzymes (in red; *n*=100; mean=1.73; SD=0.56), and for the corresponding nonenzymatic reactions (in black; *n*=9; mean=3.36; SD=0.65).

increase faster than the enzymatic reactions with temperature. Consequently, the rate enhancements (i.e., the ratio of the catalyzed versus uncatalyzed reaction rates, k_{cat}/k_{non}) exhibited by enzymes from all three classes consistently decrease with increasing temperature (Figure 2). Catalytic proficiencies ($k_{cat}/K_M/k_{non}$) show a similar, possibly even larger decline (discussed below). That enzymatic rate accelerations decline with temperature also implies that thermophilic enzymes need not be as catalytically efficient as mesophilic enzymes, and that psychrophilic enzymes comprise the most efficient catalysts. It is, however, the absolute values of k_{cat} and k_{cat}/K_M that are under evolutionary optimization, and not rate acceleration per se [19].

Why do rate accelerations decrease with temperature?

The activation energy of an enzymatic reaction is lower than the nonenzymatic one (Equation 1; Box 1), thus, Q_{10}^{enz} is obviously lower than Q_{10}^{non}. The most parsimonious explanation is therefore that each enzyme evolved for a given operational temperature, and upon temperature decrease, rates drop down as expected for any catalyzed reaction. However, because the Q_{10}^{enz} for all classes are essentially the same, the commonly accepted hypothesis that thermophilic and psychrophilic enzymes exhibit unique rate–temperature dependencies or unique active-site dynamics, finds no support.

For mesophilic enzymes, the differences in Q_{10} values, and the resulting decline in catalytic power with increasing temperature, are ascribed to the thermodynamics of activation; if the primary driving force of catalysis is reduced activation enthalpy, the enzymatic rate shows lower temperature dependency than the nonenzymatic one [20]. In this respect, because the mechanisms of enzymatic catalysis are common to all three classes, a similar rate–temperature dependency is also expected. However, to what degree can the enthalpic versus entropic contributions to rate enhancements be separated, particularly when the only available data are rate–temperature dependencies (Arrhenius plots)?

We suggest that factors other than differences in activation energies may also contribute to reduction in Q_{10}^{enz} relative to Q_{10}^{non} (discussed in: Temperature effects on k_{cat} versus k_{cat}/K_M, below). Following the argument that enzymatic rates drop less than expected, and considering the trend upon heating instead of cooling, we argue that enzymatic rates may fail to track down the expected rate–temperature increases.

Temperature dependency and enzyme floppiness

The observed decline in rate acceleration is largely continuous with the increase of temperature (Figure 2), and relates to temperatures much below the denaturation temperature (Box 1). This decline is therefore incompatible with a two-state model; namely, with a cooperative transition between a folded, enzymatically active state, and an unfolded, completely inactive one [21]. It is, however, readily accounted for with an alternative model whereby

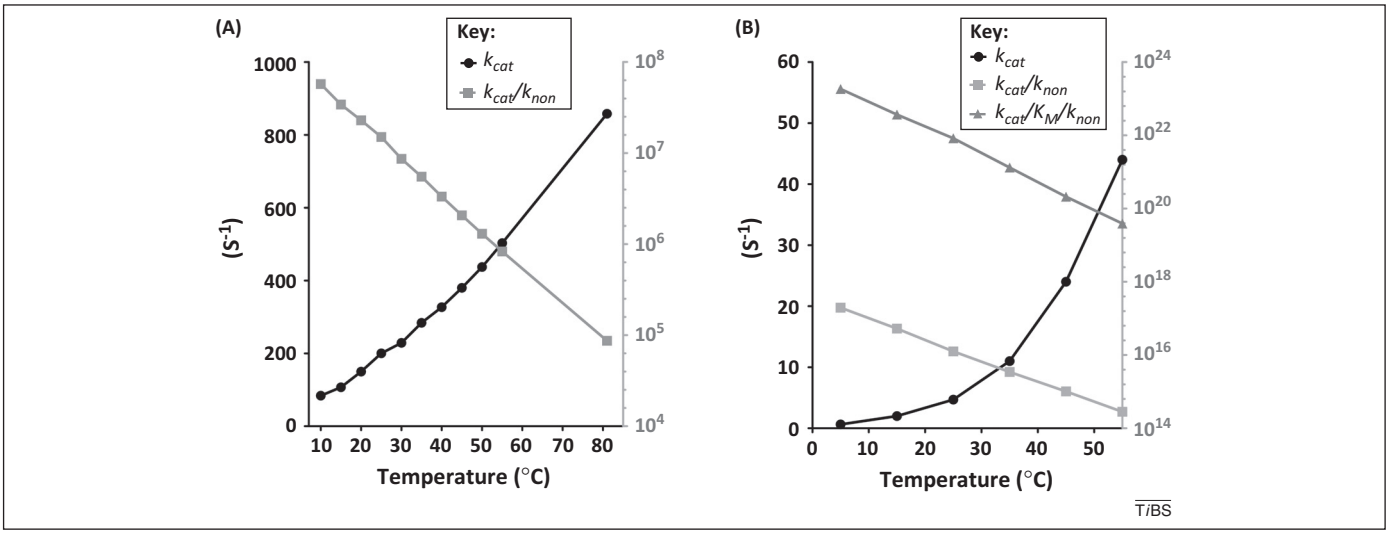

Figure 2. Catalytic power of enzymes decreases with temperature. **(A)** Temperature dependencies of k_{cat}, and k_{cat}/k_{non} (rate enhancement) for acyl-phosphatase from *Sulfolobus solfataricus*. Data at 81°C for acyl-phosphatase activity was extrapolated by the authors [57]. **(B)** Temperature dependencies of k_{cat}, k_{cat}/k_{non}, and $k_{cat}/K_M/k_{non}$ (catalytic proficiency) for orotidine 5′-monophosphate decarboxylase from *Methanothermobacter thermautotrophicus*. Data can be found in Table S2.

Box 2. Temperature-induced active-site floppiness

The effects of thermal vibrations on proteins vary in magnitude and time scales. The gradual, continuous perturbation in the catalytically optimal configuration is likely to be noncooperative (i.e., occur locally and independently at individual residues) and at the sub-Å distance scale. Such perturbations can be simulated by molecular dynamics at the ns timescale. We used three crystal structures of the triose phosphate isomerase (TIM) from a psychrophilic (*Vibrio marinus*), a mesophilic (*Leishmania mexicana*), and a thermophilic (*Geobacillus stearothermophilus*) organism. In particular, we examined the amplitude of motion of the key catalytic residues that are identical in the three orthologs, at increased temperature (Figure I). These simulations illustrate that the slope corresponding to the temperature-induced increase in thermal motion of the catalytic residues is essentially identical for the psychrophilic, mesophilic, and thermophilic representatives. Thus, the magnitude of temperature-induced floppiness, that is, the slope in terms of root mean square deviation (RMSD; in Å) per degree, seems to be independent of overall protein stability; the underlining reasons might be: (i) high overall, fold stability results in a rigid scaffold, but may have no effect on the rigidity of the active site [40,58]; and (ii) the more highly densely connected a structure is, the more destabilizing are its perturbations (e.g., as seen in the destabilizing effects of mutations [59]).

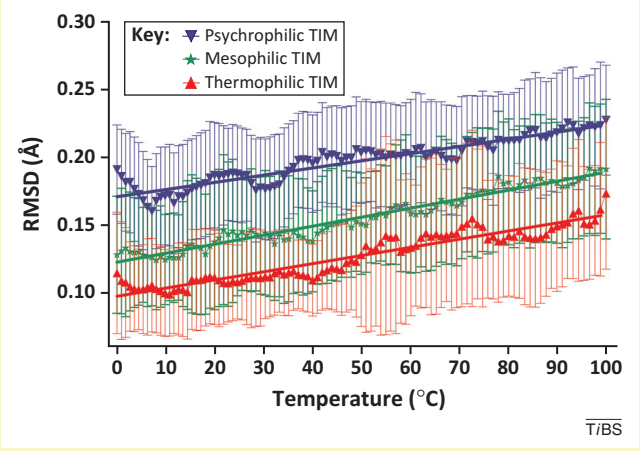

Figure I. Increased conformational ensembles simulated by molecular dynamics at different temperatures. Simulations were performed using replica exchange molecular dynamics (REMD; see Text S2 in the supplementary material online). The simulated time for each temperature was 1.5 ns, and thus unfolding, or other large cooperative changes were not observed. Although realizing the preliminary nature and inherent drawbacks of these simulations, they illustrate the notion of temperature-induced floppiness, and are consistent with the observation of the same Q_{10}^{enz} and loss factors (L_{10} values) observed for all three classes of enzymes.

the active enzyme is in a reversible equilibrium with folded yet inactive, or partially active forms [22,23]. Indeed, proteins should be considered as conformational ensembles, with reversible transitions between a range of substates that are structurally nearly identical to the folded state of the enzyme [24,25] that may exhibit different catalytic abilities [26,27]. These ensembles relate not only to discrete backbone configurations or side-chain rotamers, but also include sub-Ångstrom perturbations in bond lengths and small bond angle differences. As temperature rises, this ensemble becomes continuously larger in terms of both the number and structural variability of substates (ensemble widening is termed here floppiness). The catalytically optimal substate becomes gradually diluted, and k_{cat}, and/or k_{cat}/K_M fail to track the expected increase with temperature.

Active-site preorganization, a highly precise and rigidly held active-site configuration, is indeed a pre-requisite for efficient catalysis of the rate-determining chemical step [28,29]. Accordingly, the key catalytic residues are amongst the least mobile residues in enzyme crystal structures [30]. In thermophilic enzymes, a larger number of interactions and/or stronger interactions maintain the overall integrity of the fold and active site, and thereby enzymatic activity persists at temperatures under which psychrophilic and mesophilic enzymes are completely denatured. Nonetheless, the continuous, noncooperative temperature-induced increase in active-site floppiness might similarly affect all enzymes; each class within the range of temperatures that accommodate the folded state (Box 2). This trend was seen in the evolutionary optimization of a designed enzyme: a gradual increase of ~200-fold in k_{cat}/K_M was accompanied by a loss in configurational stability (>30°C drop in T_m) and a decrease in Q_{10}^{enz}, from ~2 to 1.3 ($Q_{10}^{non} \approx 2.8$); furthermore, the loss of stability and drop in Q_{10}^{enz} were correlated throughout the evolutionary process [31].

Alternatively, one can address enzymatic rate–temperature dependencies as follows. Reaction rates increase with temperature because an exponentially larger fraction of reactant molecules have enough thermal, kinetic energy to cross the activation-energy barrier (Box 1). However, this factor only partly applies to enzymatic reactions, foremost because the enzyme does not benefit from having higher thermal energy; rather, higher thermal energy results in lower representation of the catalytically proficient, optimally preorganized substate.

We also note that the loss of rate acceleration with temperature varies significantly between enzymes, and the L_{10} values (Box 1) range from ~1 up to nearly 5. The variation, however, shows no correlation with the different classes. Rather, enzymes among the most proficient characterized so far, such as cytidine deaminase or orotidine 5′-monophosphate decarboxylase [32], exhibit the largest loss factor (L_{10}) values (Table S2). This could be ascribed to these enzymes having the largest differences between the enzymatic and nonenzymatic activation energies. However, high rate enhancements and catalytic proficiencies (k_{cat}/k_{non}, $k_{cat}/K_M/k_{non}$, respectively) are not correlated with low Q_{10}^{enz} values (Figure S1, Table S6).

Temperature effects on k_{cat} versus k_{cat}/K_M

As previously reported for mesophilic enzymes [13], catalytic efficiency (k_{cat}/K_M) increases less steeply with temperature than turnover rate (k_{cat}). This trend is apparent in all enzyme classes (Figure 1), although with weaker differences than reported for certain mesophilic enzymes [13].

The weaker temperature dependency of k_{cat}/K_M has been ascribed to an increase in transition state affinity at lower temperature [13]. This interpretation is consistent with the above-discussed model of narrower substates ensembles at low temperature; the catalytically optimal substate also binds the transition state with the highest

affinity. In our view, the steeper temperature-induced declines in k_{cat}/K_M versus k_{cat} also relate to the notion that k_{cat} reports the properties of the enzyme bound species (the substrate–enzyme complex and the following states), whereas k_{cat}/K_M relates to both the free and the bound enzyme [33]. A protein–ligand complex is by default more stable than the free protein state. The increase in active site floppiness in the face of increased thermal energy is therefore more pronounced in the free compared to the bound enzyme.

That $Q_{10}{}^{k_{cat}}$ is larger than $Q_{10}{}^{k_{cat}/K_M}$ also suggests that factors in addition to activation energy differences affect $Q_{10}{}^{enz}$ and L_{10} values, as argued above. This is because k_{cat} reports the enzyme-bound transitions state to a larger extent than k_{cat}/K_M. Moreover, if $Q_{10}{}^{enz}$ and $Q_{10}{}^{non}$ differ only because of activation energies, then $Q_{10}{}^{enz}$ is expected to correlate with catalytic proficiency; a measure of transition state stabilization. Although data are sparse, such correlation is clearly not observed (Figure S1, Table S6).

Thermophilic enzymes low activity at ambient temperature

The catalytic cycles of enzymes include multiple steps, chemical steps other than the rate-determining one, and physical steps such as substrate binding and product release. Indeed, in certain enzymes, conformational dynamics have an apparent contribution to turnover [34,35]. However, the Q_{10} values of the vast majority of thermophilic enzymes, including those where impaired dynamics at ambient temperature has been claimed (e.g., [10,36,37]) are the same as for mesophilic enzymes (Figure 1).

How does the above claim reconcile with observations of thermophilic enzymes being, at ambient temperature, more conformationally rigid than mesophilic enzymes [38] (see also [1,39])? A stabilized backbone can become rigid with no effect on the mobility of active site residues or on the kinetic parameters of an enzyme [19,40]. Increased scaffold stability also shifts the temperature–activity profile towards higher temperature [19,40]. Thus, the overall rigidity of the fold of an enzyme does not necessarily affect, or report on, the flexibility of its active site [19,40,41], and comparisons of active-site mobility of thermophilic and mesophilic enzymes at different temperatures are not available, to the best of our knowledge. Furthermore, reports of thermophilic enzymes that, at ambient temperature, are as active or even more active than their mesophilic counterparts also exist [42–44].

The argument of excess rigidity is also supported by the effect of denaturing agents, assuming they induce higher conformational flexibility and thereby increase rates of thermophilic enzymes at ambient temperature [1]. The reported activity stimulations are, however, generally low: 1.5–4-fold; that is, on the order of that induced by an ~10°C increase (e.g., [45–47]). Noncontinuous changes in enzymatic rate–temperature dependencies have also been reported, primarily in mutants [48], and these may in fact reflect the trapping of inactive substates at low temperature.

Most importantly, a high operational temperature has manifestations that directly relate to the catalytic machinery. For example, the side chain pK_a of basic residues such as histidine, lysine, and arginine is reduced by up to 2.5 units upon a change from 25 to 100°C [42]. The pK_a affects the charge distribution of the active site and may thus lead to lower rates [22]. Indeed, pK_a shifts may have driven the evolutionary reshaping of active sites: in cysteine desulfurases, the catalytic lysine in mesophilic orthologs (pK_a=6.6) is replaced by an aspartate in the thermophilic ones [49]. Although we are unaware of relevant data, measuring the pH-rate profiles at different temperatures may indicate how relevant temperature–pK_a changes might be. Beyond pK_a values, different interactions have different temperature dependencies: the strength of ionic bonds decreases with temperature [22], whereas hydrophobic interactions may become stronger [4]. Thus, physical changes, including changes in water organization, may account for low ambient temperature rates in enzymes that are evolutionarily optimized for high temperature.

Furthermore, given no pK_a and other bond-strength perturbations, thermophilic enzymes might exhibit higher rates at ambient temperature. Thus, the actual decline in rate acceleration with increased temperature might be even higher than manifested by the observed Q_{10} values.

Temperature adaption of dynamics

Can we identify a clear case of temperature adaptation of structural dynamics? The best example for large conformational changes that directly affect enzymatic rates is allosteric transitions. In this respect, thermophilic enzymes may in fact be too rigid at ambient temperature; namely, the activation energy barriers between the allosterically regulated T and R states might be tuned for high temperature. Indeed, the allosteric triggering of a hyperthermophilic lactate dehydrogenase became operative at ambient temperature by the introduction of destabilizing mutations that relate to the oligomeric interface and away from the active site. These mutations affected the equilibrium between the R and T states of the enzyme, without changing the structures or catalytic properties of these states [50].

The above example is, however, an exception. Only when a relatively large conformational change that occurs via a high activation energy barrier is directly rate determining, might a thermophilic enzyme be considered too rigid to be active at low temperature. In such cases, the $Q_{10}{}^{enz}$ values would be discontinuous and much higher than the average of 2.

Concluding remarks and outstanding questions

The universality of enzymatic rate–temperature dependencies calls for a re-examination of the widely accepted hypothesis of class-specific rate–temperature dependencies. It suggests that adaptation of enzymes to temperature relates primarily to the challenge of maintaining their fold and active site catalytic preorganization (the positioning of catalytic side- and main-chain groups and their optimal electrostatic state) [28] at their operational temperature. It also suggests that excess of rigidity is rarely a limiting factor in enzyme catalysis. If anything, active-site floppiness (i.e., insufficient rigidity) is. In any case, we surmise that enzymatic rate–temperature dependencies of enzymes must be viewed not only with respect to the operational temperature of the enzyme, but also in

comparison to the dependency of the corresponding nonenzymatic reaction. This comparison suggests that the catalytic powers (rate acceleration, and catalytic proficiency) of all enzymes decrease at increased temperature (on average, 16-fold per 25°C; average L_{10}=3.02).

We therefore suggest that a systematic analysis of Q_{10} for nonenzymatic reactions is needed, and a re-evaluation of the rule of thumb of Q_{10}=2. First, the potential biases due to temperature-induced changes in water properties should be examined [18], and particularly for slow reactions that tend to show higher Q_{10}^{non} values. Second, the origin of the universally observed temperature-induced declines of enzymatic rate accelerations requires further exploration. To what degree this decline relates to differences in the enzymatic versus nonenzymatic activation energies, and specifically to enthalpy versus entropy [13], or to increased floppiness as proposed here, remains to be established. Third, at the structural level, measurements of global parameters that report the average mobility of all protein residues need to be complemented by local measurements of mobility of specific active-site residues. Fourth, causes of the low rates of thermophilic enzymes at low temperatures, other than impaired dynamics, need to be considered, including pK_a shifts of catalytic residues and active site hydration changes with temperature. These factors could also be explored by evolving in the laboratory a thermophilic enzyme towards high rate at low temperature (the opposite, evolution of mesophilic enzyme for thermophilicity has been repetitively pursued, e.g., [51–53]). Finally, the concept of active-site floppiness and how rate–temperature dependencies report this factor need to be explored. Floppiness could well be a major limiting factor of enzyme efficiency [54], and thus a key parameter for optimization of engineered and designed enzymes [55].

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tibs.2013.11.001.

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