The Temperature Dependence of Enzyme Rate Enhancements

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After the initial binding of a substrate by an enzyme, their mutual affinity in water increases by a factor commensurate with the rate enhancement that the enzyme produces in water.[1] During the substrate's transformation, their K_d sometimes attains values as low as 10^{-23} M in the transition state.[2] In seeking to analyze the sources of that attraction, it seems reasonable to inquire whether the rate enhancement produced by an enzyme, and hence the corresponding increase in affinity, tends to be mainly enthalpic or entropic in its origins. An apparent, but misleading, answer to that question is suggested by two familiar generalizations:

(1) The rates of chemical reactions increase with increasing temperature, tending to double in rate ("$Q_{10} = 2$") as the temperature is adjusted from 20 to 30 °C.[3]

(2) Products are formed by many enzyme−substrate complexes at rates that roughly double when the temperature is increased by 10 °C.[4]

If both these generalizations were true, then heats of activation would be similar for enzymatic and uncatalyzed reactions, implying that the catalytic effect of enzymes arises from their ability to increase a reaction's entropy of activation. Recent experiments in this laboratory have uncovered a remarkably large range of rate constants of biological reactions proceeding spontaneously in the absence of enzymes, furnishing a broadly distributed set of benchmarks against which that hypothesis can be tested. Here, we show that very slow reactions tend to be very much more sensitive to temperature than the first of these generalizations would imply, so that rate enhancements by many enzymes increase sharply with decreasing temperature. That behavior has a significant bearing on the thermodynamic properties expected of transition state analogue inhibitors, and is also of interest in considering the forces of attraction that are chiefly responsible for catalysis.

In the work summarized in Figure 1, reactions were conducted at elevated temperatures in sealed quartz tubes (2 mm Internal diameter, 1 mm wall thickness) in solutions containing buffers (typically 0.1 M) at constant ionic strength (typically 0.3, adjusted with KCl) under conditions in which reaction rates were found not to vary with changing pH. To avoid explosions at temperatures above 260 °C, quartz tubes were sealed inside stainless steel pipes to which water had been added, equalizing the pressure across the walls of the quartz tube. After each tube had cooled, the concentrations of reactants and products were analyzed by comparing the integrated intensities of proton magnetic resonances with that of pyrazine, added as an integration standard. Each of these reactions followed simple first order kinetics to completion,

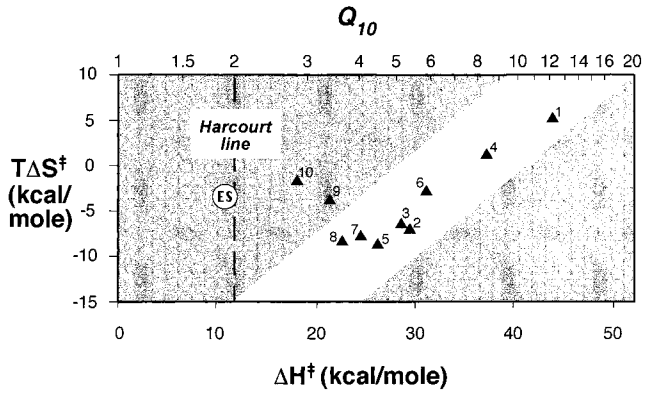

Figure 1. Entropies (vertical axis) and enthalpies (lower horizontal axis) of activation of spontaneous uncatalyzed reactions (▲), compared with ES (circled), an average[4] of the values that have been reported for enzyme−substrate complexes. All values for uncatalyzed reactions fall to the right of the "Harcourt line",[3] representing $Q_{10} = 2$. Diagonal reference lines bounding the clear area represent half-times of 1 year (left diagonal) and 4.5 billion years (right diagonal) at 25 °C. Reactions shown include (1) orotidine 5′-phosphate decarboxylation,[2] (2) β-methyl glucoside hydrolysis,[6] (3) fumarate hydration,[7] (4) methyl phosphate hydrolysis,[8] (5) dimethyl phosphate hydrolysis,[8] (6) mandelate racemization,[9] (7) peptide hydrolysis,[10] (8) cytidine deamination,[11] (9) chorismate mutation,[12] and (10) carbon dioxide hydration.[13]

and yielded a linear Arrhenius plot. The energy of activation (E_{act}) was obtained by plotting the logarithm of observed rate constants as a function of the reciprocal of absolute temperature, and $T\Delta S^{\ddagger}_{25}$ was obtained by subtracting $\Delta G^{\ddagger}_{25}$ from $\Delta H^{\ddagger}$ (equivalent to $E_{act} - RT$). For these uncatalyzed reactions in water, enthalpies of activation ($\Delta H^{\ddagger}$) are seen to span a range (+18 to +45 kcal/mol) that exceeds the variation (−9 to +7 kcal/mol) of the entropy term $T\Delta S^{\ddagger}$ (18 kcal), shown on the vertical scale. Q_{10} values, shown on the upper horizontal axis, corresponding to this dominant variation in $\Delta H^{\ddagger}$, range from 2.9 for CO_2 hydration to 12 for OMP decarboxylation. In contrast, values of k_{cat} for the corresponding enzyme reactions show little variation, with $\Delta H^{\ddagger}$ typically ∼10 kcal/mol and $T\Delta S^{\ddagger}$ typically ∼−5 kcal/mol as shown by the symbol "ES" in Figure 1. Table 1 shows direct comparisons of $\Delta H^{\ddagger}$ for several spontaneous and enzyme-catalyzed reactions.

Variations along the horizontal axis of Figure 1 show that these enzymes invariably lower the reaction's enthalpy of activation substantially, by an amount that tends to determine the effectiveness of the enzyme as a catalyst. Variations along the vertical axis are relatively minor, showing that the entropy of activation of a reaction is sometimes raised or lowered to a modest extent.[18] As a result of this tendency, the corresponding rate enhancements (k_{cat}/k_{non}) increase acutely as the temperature is lowered, as illustrated in Figure 2 by the example of α-glucosidase. The decarboxylation of OMP, another example, exhibits $\Delta H^{\ddagger} = +43.8$

(1) Wolfenden, R. *Nature* **1969**, *223*, 704−705.

(2) Most variations in rate enhancement (>10^{14}-fold) arise from variations in the rate of the uncatalyzed reactions, the rates of most enzyme reactions remaining relatively invariant (Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93).

(3) First noticed in a careful study of the oxidation of HI by H_2O_2 (Harcourt, A. V. *J. Chem. Soc.* **1867**, *20*, 460−495), that tendency has since been observed for so many reactions that it is offered as a rule of thumb by most textbooks that have anything to say about the temperature dependence of reaction rates in water (see, for example: Pauling, L. *College Chemistry*; Freeman: New York, 1950; p 410).

(4) Laidler, K. J.; Peterman, B. K. *Methods Enzymol.***1979**, *63*, 234−257.

(5) This represents an average of k_{cat} values reported for 20 enzymes chosen at random from Chemical Abstracts. Values of $T\Delta S^{\ddagger}_{25}$ and $\Delta H^{\ddagger}$ vary within a relatively narrow range.

(6) Wolfenden, R.; Lu, X.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 6814−6815.

(7) Bearne, S. L.; Wolfenden, R. *J. Am. Chem. Soc.* **1998**, *120*, 833−834.

(8) Wolfenden, R.; Ridgway, C.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 6814−6815.

(9) Bearne, S. L.; Wolfenden, R. *Biochemistry* **1997**, *36*, 1646−1656.

(10) Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105−6109

(11) Frick, L.; MacNeela, J. P.; Wolfenden, R. *Bioorg. Chem.* **1987**, *15*, 100−108

(12) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492−3497.

(13) Roughton, J. F. W. *J. Am. Chem. Soc.* **1941**, *63*, 2930−2940.

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Table 1. Enthalpies of Activation for Enzyme-Catalyzed (k_{cat}) and Nonenzymatic (k_{non}) Reactions

reaction	$\Delta H^{\ddagger}(k_{cat})$	$\Delta H^{\ddagger}(k_{non})$
yeast OMP decarboxylase	11.0[14]	44.4[2]
urease	9.9[15]	32.1[16]
bacterial α-glucosidase	10.5[14]	29.7[6]
staphylococcal nuclease	10.8[14]	25.9[8]
chymotrypsin	8.6[17]	24.4[10]
chorismate mutase	12.7[12]	20.7[12]

kcal/mol for k_{non}, and $\Delta H^{\ddagger} = +11.0$ for k_{cat}, so that the rate enhancement produced by this enzyme increases 6.5-fold as the temperature is lowered from 30 to 20 °C. As a result of this general tendency, enzyme affinities for transition state analogue inhibitors are expected to increase sharply with decreasing temperature, relative to their affinities for conventional substrates or substrate analogues. That behavior is exemplified by the much more negative enthalpy of binding of 1,6-dihydroinosine (−18 kcal/mol) by adenosine deaminsase than for binding of the substrate adenosine (−7.7 kcal/mol) or 1,6-dihydronebularine, a substrate analogue (−8.3 kcal/mol). Correspondingly, $\Delta H^{\ddagger}$ is roughly 8 kcal/mol more favorable for the enzyme-catalyzed than for the uncatalyzed reaction.[19] This sharp temperature dependence of K_i furnishes a new criterion for testing potential transition state analogue inhibitors, and may have a significant bearing on the practical uses of transition state analogues as enzyme antagonists in medicine and agriculture.

What is the source of the misleading generalization that rates of spontaneous chemical reactions in water tend to double with a 10 °C rise of temperature. Rates of reaction are relatively easy to measure if their half-times fall in the range between 1 min and 1 day, with corresponding $\Delta G^{\ddagger}_{25}$ values that range between 20 and 24 kcal/mol at 25 °C. If $T\Delta S^{\ddagger}$ falls in the range between 0 and −10 kcal/mol, as is the case for most spontaneous reactions in water (Figure 1), then $\Delta H^{\ddagger}$ must fall in the range between 10 and 24 kcal, corresponding to Q_{10} values of 2 to 4, in the low range observed by Harcourt[3] and later investigators. When these unrecognized constraints of experimental convenience are abandoned, it becomes evident (Figure 1) that values of $\Delta H^{\ddagger}$ for spontaneous reactions seldom or never fall near the "Harcourt line" ($Q_{10} = 2$), but are distributed over a much wider range. Thus, enthalpies of activation, not entropies of activation, tend to govern the variation in the rates of spontaneous reactions.

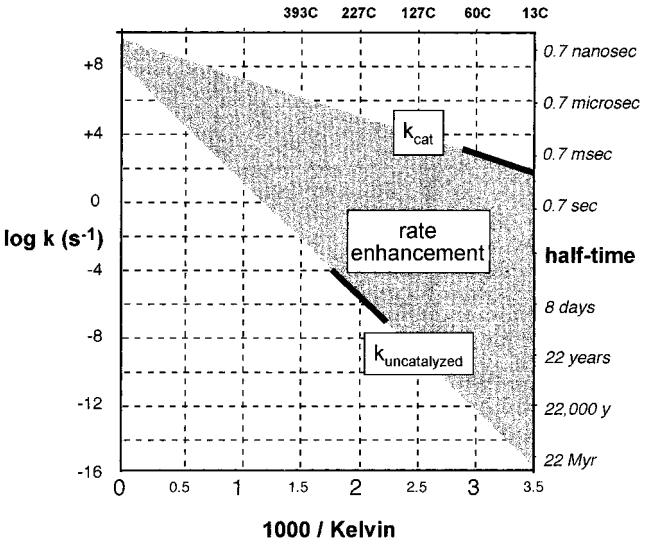

Figure 2. Effect of temperature on the rate enhancement by a $\Delta H^{\ddagger}$-reducing enzyme, illustrated by the rates of hydrolysis of glycosides in the presence (this work) and absence (ref 5) of bacterial α-glucosidase. Dark lines show the actual ranges of temperature over which enzymatic and uncatalyzed reaction rate constants were collected.

The findings presented in Figure 1 suggest that the rate enhancement, and hence the corresponding increase in affinity as the ES complex proceeds from the ground state to the transition state, is largely enthalpic in origin. Thus, the increase in substrate affinity tends to be accompanied by a substantial release of enthalpy. That would be consistent with the formation of new electrostatic and hydrogen bonds that can act synergistically,[9,19] for whose existence much evidence exists in the structures of enzymes crystallized with transition state analogue inhibitors.[20] Figure 2 suggests a possible evolutionary advantage of that release of enthalpy. At the elevated temperatures at which the earliest organisms may have arisen, even a weak catalyst would have allowed the reaction to occur at a substantial rate.[2] Such a catalyst, if it chanced to share the $\Delta H^{\ddagger}$-reducing character of modern enzymes, might have conferred an ever-increasing advantage on the host organism as the surroundings cooled, by enhancing the rate of the desired reaction relative to the rates of other reactions.[21]

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(14) This work.
(15) Wall, M. C.; Laidler, K. J. *Arch. Biochem. Biophys.* **1958**, 5337−5345.
(16) Shaw, W. H. R.; Bordeaux, J. J. *J. Am. Chem. Soc.* **1955**, *77*, 4729−4734.
(17) Kaufmann, S.; Neurath, H.; Schwert, G. W. *J. Biol. Chem.* **1949**, *177*, 792−804.
(18) Each of these reactions involves a single substrate, or water is the second substrate and its concentration is very high. In reactions that involve multiple substrates, or in which ground states and transition states differ markedly in their free energies of interaction with solvent water, entropic contributions might be expected to be more pronounced (Westheimer, F. H. *Adv. Enzymol.* **1962**, *24*, 441−482). That possibility remains to be examined experimentally.
(19) Kati, W. M.; Wolfenden, R. *Biochemistry* **1989**, *28*, 7919−7927.
(20) Nonpolar interactions also play a role in some reactions that involve charge delocalization, but their use in enzyme catalysts appears to be relatively uncommon (for a review, see: Radzicka, A.; Wolfenden, R. *Methods Enzymol.* **1995**, *249*, 284−312).
(21) Among those undesirable side reactions would be reactions leading to destruction of the catalyst itself. For example, the hydrolysis of proteins[10] and nucleic acids is known to be extremely temperature dependent.[8]