Selected Problems of Molecular Biophysics

(1100-5BM15)

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LECTURE 17

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Problem 3: Physics of enzymes (part 3)

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On the Temperature Dependence of Enzyme-Catalyzed Rates

V. L. Arcus, E. J. Prentice, J. K. Hobbs, A. J. Mulholland, M W. Van der Kamp, C. R. Pudney, E. J. Parker, and L. A. Schipper

Biochemistry, 55:1681-1688 (2016)

One of the critical variables that determine the rate of any reaction is temperature. For biological systems, the effects of temperature are convoluted with myriad (and often opposing) contributions from enzyme catalysis, protein stability, and temperature-dependent regulation, for example.

The rate of any chemical reaction is a function of the temperature (T) and the energy difference between the reactants and the transition state, the so-called activation energy (E_a). Arrhenius was the first to formalize this relationship in the 19th century (based on empirical observations) with his famous eponymous equation (Arrhenius equation)

$$k(T) = A \exp\left(-E_a / RT\right)$$

where k is the rate constant and R is the universal gas constant. Early in the 20th century, the development of transition state theory (TST) by Eyring, Polanyi, and others led to the Eyring equation for a first order rate constants,

$$k = \frac{\kappa k_B T}{h} \exp\left(-\Delta G^{\ddagger} / RT\right)$$

where $\Delta G^{\ddagger}$ is the change in Gibbs free energy between reactants and the transition state, k_B and h are Boltzmann's and Planck's constants respectively, and κ is the transmission coefficient, usually assumed 1 for simplicity. This led to an understanding of, and statistical mechanical justification of, the terms in the Arrhenius expression. The Arrhenius and Eyring equations are found in most modern (bio)chemistry textbooks and provide an excellent description of the temperature dependence of a wide array of chemical processes.

The Origin and Status of the Arrhenius Equation

S. R. Logan

Journal of Chemical Education, 59:279-281 (1982); 179 cittions, 2023-11-28

Thus, it is now widely accepted that although, over a limited range of temperature, plots of ln k(T) against T^{-1} are acceptably linear and may quite reasonably be used for purposes of interpolation, Equation

$$k(T) = A \exp(-E_a / RT)$$

is not in general obeyed, even by elementary chemical reactions, in the sense that unique constants A and E_a do not exist for each reaction. Thus, it is now widely recognized that even when the Arrhenius plot is apparently linear, E_a is best regarded as an empirical or a phenomenological quantity, defined as in Equation

$$E_a = -R \frac{d \ln k(T)}{d(1/T)}$$

and, to minimize misunderstandings, termed "the Arrhenius activation energy". In many instances, such as the cases cited in the previous paragraph, it is a quantity with no (not even approximate) physical significance. Thus, although at the end of his paper, Arrhenius attempted to justify his equation theoretically, it has now been re-located, in the spirit of the opening section of his paper, to that part of the framework of reaction kinetics which deals with the empirical treatment of reaction data.

In 1903, Svante Arrhenius was awarded the third Nobel Prize for Chemistry. The citation did not mention his contributions to reaction kinetics, nor did his Nobel Lecture. Both concentrated on his formidable achievements in electrochemistry. But there can be no doubt, that this distinguished scientist was also responsible for a most significant advance in reaction kinetics, for which he is justly commemorated.

The development of the Arrhenius equation

Keith J. Laidler

Journal of Chemical Education, 61:494-498 (1984); 944 citations, 2023-11-28

Today the influence of temperature on the rates of chemical reactions is almost always interpreted in terms of what is now known as the Arrhenius equation. … The apparent activation energy E_a is now defined in terms of this equation.

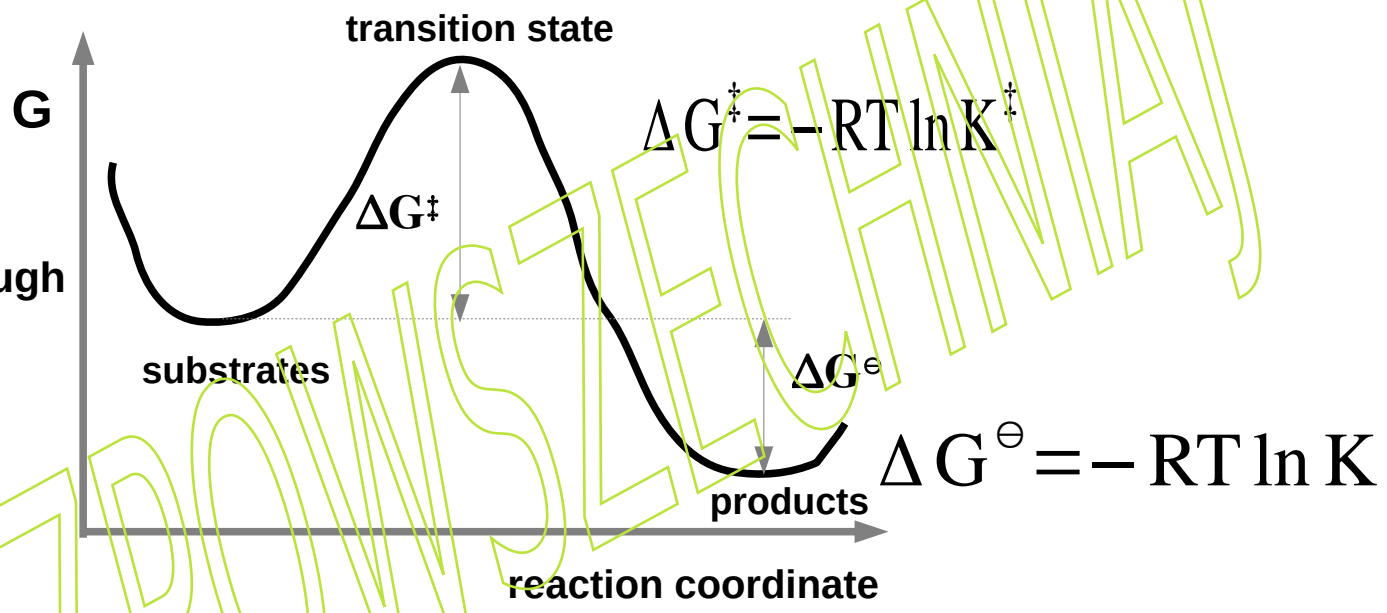
(Unimolecular) Reaction rate according to the transition state theory

Reaction

$$A \underset{k_-}{\overset{k_+}{\Leftrightarrow}} B$$

is considered as going through an intermediate state I‡:

$$A \underset{k^{\ddagger}_-}{\overset{k^{\ddagger}_+}{\Leftrightarrow}} I^{\ddagger} \overset{k^{\ddagger}}{\rightarrow} B$$



$$\Delta G^{\ddagger} = -RT \ln K^{\ddagger}$$

$$\Delta G^{\ominus} = -RT \ln K$$

where k‡ is the frequency of vibration in the transient state. Equating $k^{\ddagger}h = k_B T$, we have

$$k^{\ddagger} = \kappa \frac{k_B T}{h}$$

where κ is the transmission coefficient.

Velocity of reaction is defined by:

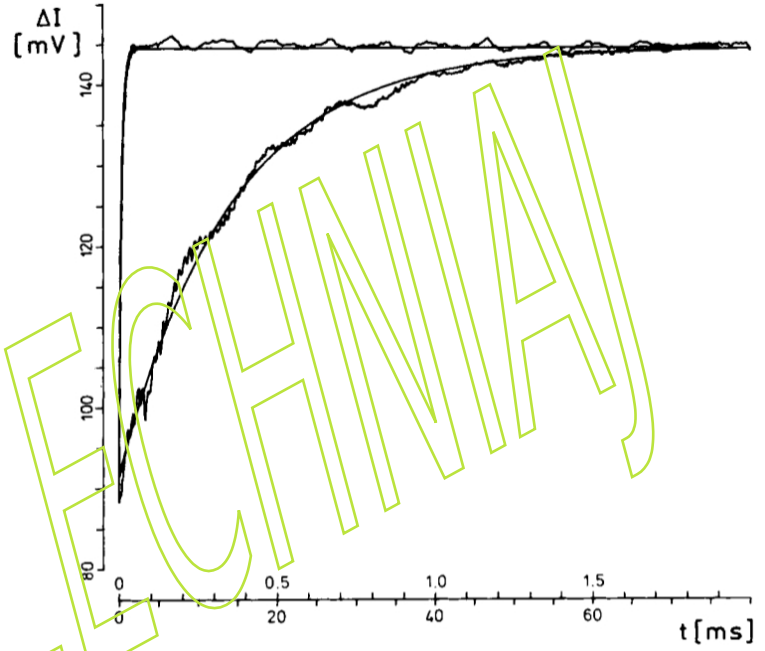
$$v = k^{\ddagger}[I^{\ddagger}] = k^{\ddagger} K^{\ddagger}[A] = k_+[A]$$

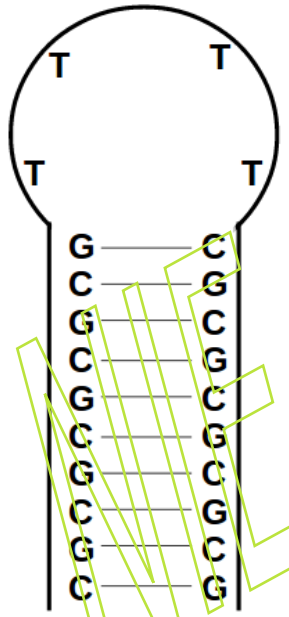
$$[I^{\ddagger}] = K^{\ddagger}[A]$$

$$k_+ = \kappa \frac{kT}{h} \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right)$$

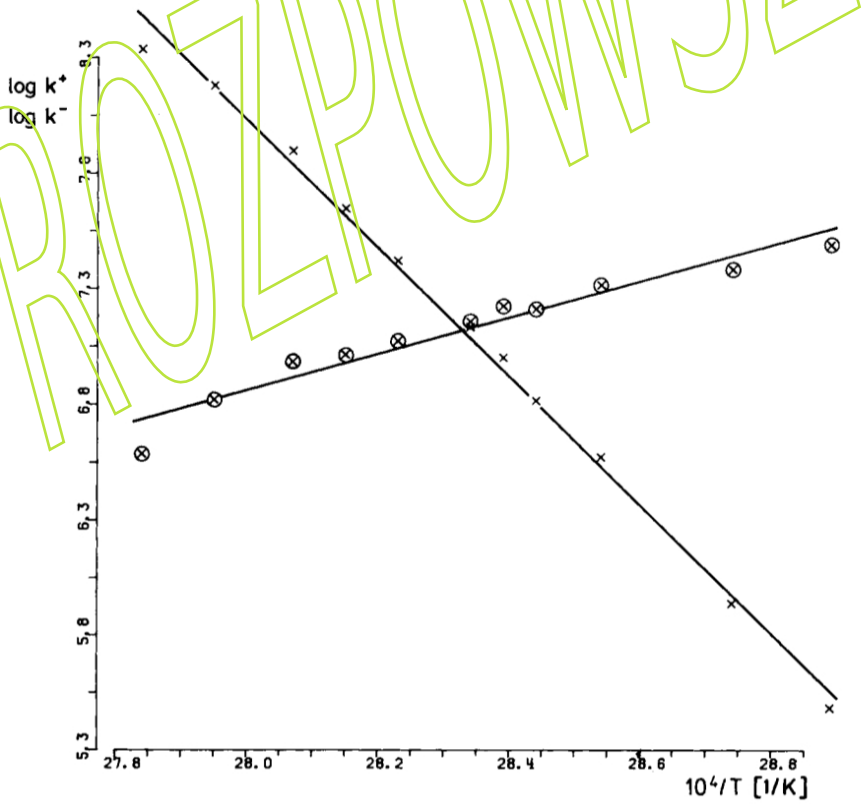
Helix-Coil dynamics of a Z-Helix Hairpin

The helix-coil transition of a Z-helix hairpin formed from $d(C\text{-}G)_5T_4(C\text{-}G)_5$ has been characterized by equilibrium melting and temperature jump experiments in 5 M $NaClO_4$ and 10 mM Na_2HPO_4, pH 7.0. The temperature jump relaxation can be described by single exponentials at a reasonable accuracy. The rate constant of helix formation is in the range of 1300 s^{-1} with a relatively small temperature dependence, whereas the rate constant of helix dissociation is found in the range between 200-4500 s^{-1} with a very strong temperature dependence. Arrhenius plots are linear within experimental accuracy and show a strong positive activation enthalpy of 235 kJ/mole for helix dissociation, whereas helix association is connected with a negative activation enthalpy of approximately −50 kJ/mole. The negative activation enthalpy clearly demonstrates that helix formation cannot be described as a simple elementary reaction step.





$$\text{hairpin} \underset{k^+}{\overset{k^-}{\Leftrightarrow}} \text{coil}$$



$$\log\left(\frac{k^{\mp}}{k_B T/h}\right) = -\frac{E_a^{\mp}}{RT}$$

$$E_a^- = +230\,kJ/mole$$
$$E_a^+ = -65\,kJ/mole$$

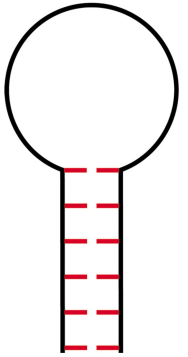
× = k^-
⊗ = k^+

Biopolymers, 27:1319-1327 (1988)

$$\text{hairpin} \underset{k^+}{\overset{k^-}{\Leftrightarrow}} \text{coil} \qquad \frac{1}{\tau} = k^- + k^+ \qquad K = \frac{k^+}{k^-}$$

$$k^+ = A^+ e^{-E_a^+/RT} \qquad k^- = A^- e^{E_a^-/RT} \qquad \ln K = -\frac{\Delta H^\ominus}{RT} + \frac{\Delta S^\ominus}{R}$$

The most remarkable feature of these results is the negative sign of E_a^+. This tells us that the helix-forming reaction is not an elementary reaction following the equation above.



$$\text{hairpin} \underset{k_{+2}}{\overset{k_{-2}}{\Leftrightarrow}} \text{nucleus} \underset{k_{+1}}{\overset{k_{-1}}{\Leftrightarrow}} \text{coil} \qquad [nucleus] = \frac{k_{+1}}{k_{-1}}[coil]$$

$$\frac{d[hairpin]}{dt} = k^+[coil] - k^-[hairpin] = k_{+2}[nucleus] - k_{-2}[hairpin]$$

$$k^+ = \frac{k_{+1}}{k_{-1}} k_{+2} \qquad k^- = k_{-2}$$

$$E_a^+ = E_a^{nucleus \to hairpin} + \Delta G^\ominus_{coil \to nucleus} \qquad E_a^- = E_a^{nucleus \to coil}$$

Using the relationship between the Gibbs free energy, enthalpy and entropy, we can write the Eyring equation in the following form

$$k = \frac{\kappa k_B T}{h} e^{(-\Delta G^{\ddagger}/RT)} = \frac{\kappa k_B T}{h} e^{(\Delta S^{\ddagger}/R)} e^{(-\Delta H^{\ddagger}/RT)}$$

Time-resolved temperature-jump/drop infrared spectroscopy is used to measure the melting and refolding dynamics of a 12-nucleotide RNA sequence comprising a UACG tetraloop and a four-base-pair double-stranded GC stem, comparing them to an equivalent DNA (TACG) sequence.

Table 1. Thermodynamic Parameters Obtained via Van't Hoff, Arrhenius, and Eyring Analyses

		RNA	DNA	
	T_m	81 °C	76 °C	
Van't Hoff	ΔH	118.3	124.2	kJ mol^{-1}
	ΔS	334	356	J K^{-1} mol^{-1}
	ΔG^{a}	14.8	13.9	kJ mol^{-1}
Arrhenius	$E_{a,m}$	83.3	64.0	kJ mol^{-1}
	$E_{a,r}$	−48.2	−45.2	kJ mol^{-1}
Eyring	$\Delta H_m^{\ddagger}$	80.4	61.2	kJ mol^{-1}
	$\Delta S_m^{\ddagger}$	86	46	J K^{-1} mol^{-1}
	$\Delta G_m^{\ddagger a}$	53.8	46.8	kJ mol^{-1}
	$\Delta H_r^{\ddagger}$	−51.2	−48.1	kJ mol^{-1}
	$\Delta S_r^{\ddagger}$	−324	−315	J K^{-1} mol^{-1}
	$\Delta G_r^{\ddagger a}$	49.1	49.5	kJ mol^{-1}

[a] All ΔG calculated at 37 °C.

J. Phys. Chem. Lett., 13:9171-9176 (2022)

Ploting ln(k) versus 1/T (Arrhenius plot) often results in a straight line, and fitting data to equation

$$\ln k = \ln A - \frac{E_a}{RT}$$

yields the activation energy. Plotting ln(k/T) versus 1/T (Eyring plot) often results in a straight line, and fitting data to equation

$$\ln \frac{k}{T} = \ln \frac{k_B}{h} - \frac{\Delta H^{\ddagger}}{RT} + \frac{\Delta S^{\ddagger}}{R} \quad \textbf{or} \quad \ln k = \ln \frac{k_B T}{h} - \frac{\Delta H^{\ddagger}}{RT} + \frac{\Delta S^{\ddagger}}{R}$$

yields the activation enthalpy and entropy, assuming they are constant over the temperature range.

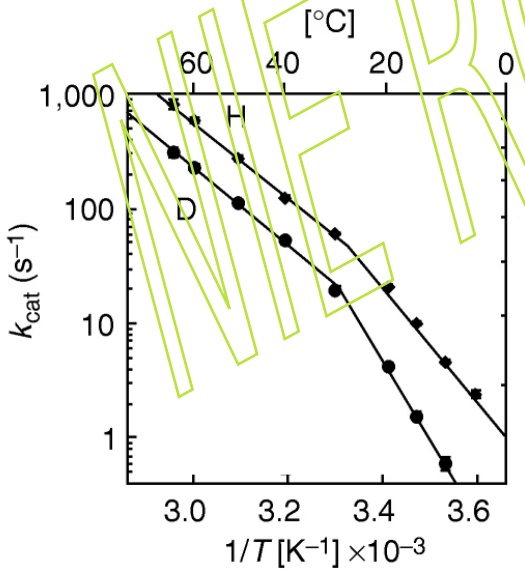
Origin of the Non-Arrhenius Behavior of the Rates of Enzymatic Reactions

Subhendu Roy, Patrick Schopf, and Arieh Warshel

J. Phys. Chem. B, 121:6520-6526 (2017)

Our analysis has advanced the idea that the reason for the non-Arrhenius trend reflects the temperature dependence of the rearrangements of the protein polar groups in response to the change in the charge distribution of the reacting system during the transition from the ground state (GS) to the transition state (TS). Here we examine the validity of our early proposal by simulating the catalytic reaction of alcohol dehydrogenase (ADH) and determine the microscopic origin of the entropic and enthalpic contributions to the activation barrier.

Our finding strongly suggests that the nonlinear trend reflects the entropic contributions of the protein environment.



Arrhenius plots of k_{cat} for protonated (diamonds) and deuterated (circles) substrates of a thermophilic alcohol dehydrogenase.
Nature, 399:496-499 (1999)

The Nobel Prize in Chemistry 2013

© Nobel Media AB. Photo: A. Mahmoud
Martin Karplus
Prize share: 1/3

© Nobel Media AB. Photo: A. Mahmoud
Michael Levitt
Prize share: 1/3

© Nobel Media AB. Photo: A. Mahmoud
Arieh Warshel
Prize share: 1/3

The Nobel Prize in Chemistry 2013 was awarded jointly to Martin Karplus, Michael Levitt and Arieh Warshel "for the development of multiscale models for complex chemical systems."

Alcohol dehydrogenases (ADH) are a group of dehydrogenase enzymes that occur in many organisms and facilitate the interconversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.

A dehydrogenase is an enzyme belonging to the group of oxidoreductases that oxidizes a substrate by reducing an electron acceptor, usually NAD^+/$NADP^+$ or a flavin coenzyme such as FAD or FMN.

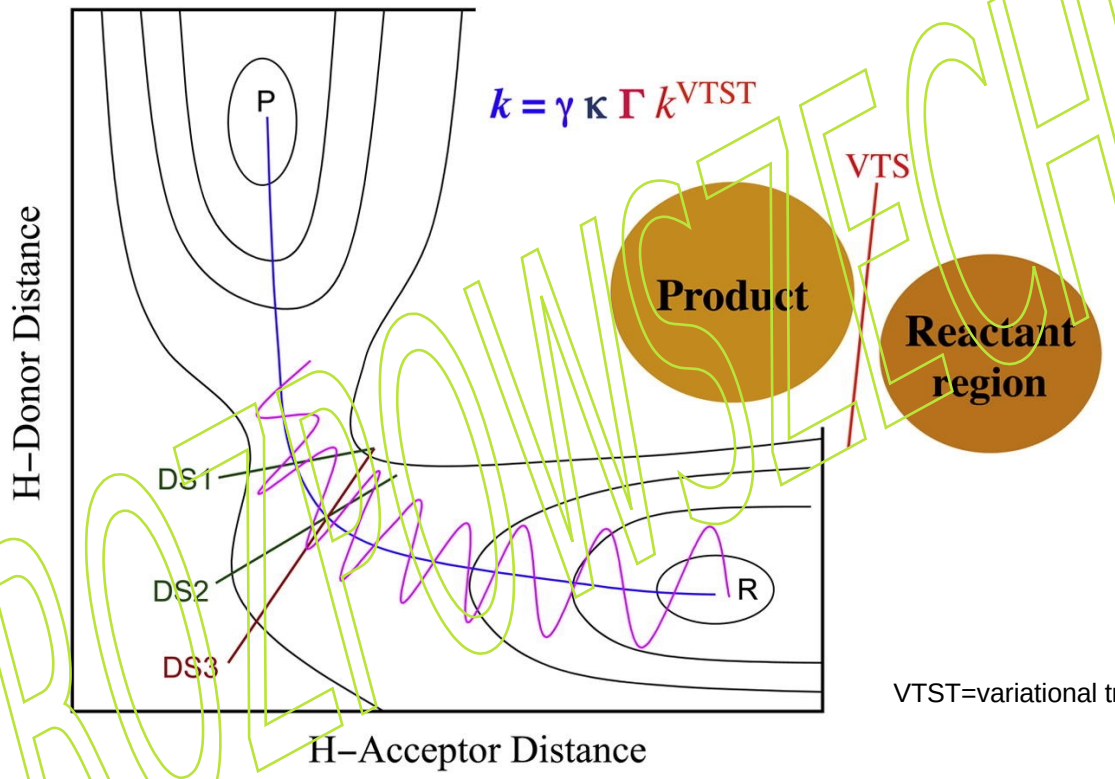
In biochemistry, an oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule, the reductant, also called the electron donor, to another, the oxidant, also called the electron acceptor.

wikipedia

Transition state theory for enzyme kinetics

Donald G. Truhlar

Archives of Biochemistry and Biophysics, 582:10-17 (2015)



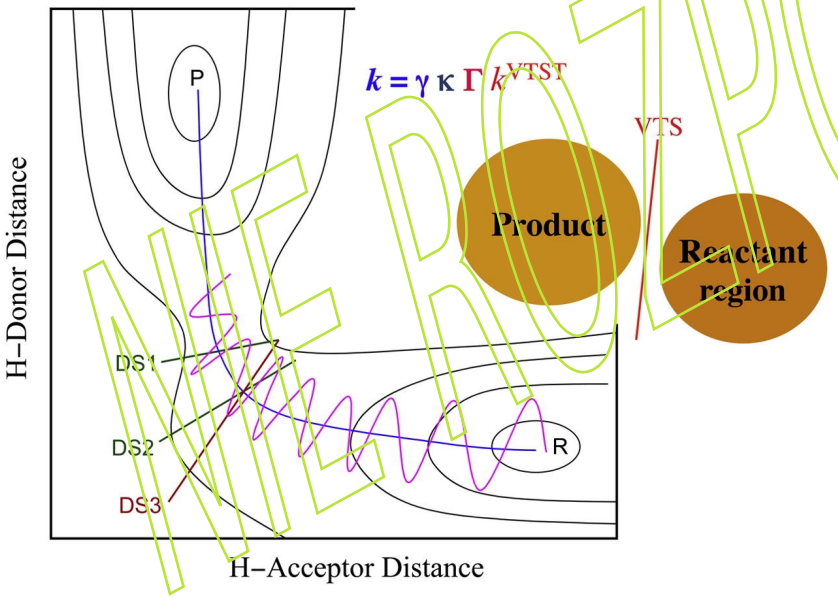
DS=dividing surface

VTST=variational transition state theory

Enzyme catalysis occurs by a variety of mechanisms, and enzyme kineticists use a variety of levels of theory to calculate reaction rates catalyzed by enzymes. Most of these methods, in one way or another, attempt to calculate the free energy of activation, a quantity whose meaning is defined by transition state theory.

Transition state theory was originally developed in the context of gas-phase reactions, but it was extended to condensed reactions shortly thereafter.

The original formulation for condensed-phase reactions was in terms of quasithermodynamic concepts, in particular quasiequilibrium between the transition state and the reactants. The term quasiequilibrium is used for two reasons:
(i) the transition state needs to be in equilibrium with the reactants, but the products states may be unpopulated;
(ii) the transition state is missing one degree of freedom. For example, Evans and Polanyi defined the transition state as "an infinitesimally thin layer of phase space" extending to infinity in all directions except the reaction coordinate. Thus a transition state is a mathematical entity that is like a real molecule but is missing one degree of freedom, namely the reaction coordinate.



Since the transition state theory rate is proportional to a the quasiequilibrium constant between the transition state (sometimes called the activated complex), the transition state rate is interpreted in terms of a generalization of the concept of free energy of reaction, namely the free energy of activation, which is the difference in free energy between the mathematically defined transition state and the reactants, and the temperature dependence allows us to separate this into an enthalpy of activation and an term involving the entropy of activation. These functions are quasithermodynamic because they refer to a transition state rather than a real chemical species.

Archives of Biochemistry and Biophysics, 582:10-17 (2015)

Basic concepts of transition state theory in a classical world:

Phase space is the 6N-dimensional space consisting of the 3N-dimensional coordinate space and the 3N-dimensional space of conjugate momenta. Points in phase space are called phase points; they are the "states" of a classical system. A region of phase space is said to be in local equilibrium if the relative population of states in that region satisfies a Boltzmann distribution. Note that since most phase points have nonzero momentum they are constantly moving from one position in phase space to another (from one state to another); the motion of a phase point in phase space is called a trajectory.

(i) assume all phase points are populated according to a Boltzmann distribution.

(ii) Define a (6N−1)-dimensional surface (to be called the dividing surface) that separates reactant regions of phase space from product regions. If reactants and products are well defined there will be a relatively sparsely populated region of phase space between them; the dividing surface should be in this region. Furthermore, since we usually define this surface to be independent of the 3N momentum components and the 6 overall translations and rotations, it has only (3N−7) degrees of freedom.

(iii) Calculate the one-way flux of phase points across the dividing surface. Note the net flux will be zero because the system is assumed to be at equilibrium, but the one-way flux is not zero. Because we are doing this calculation in a classical world, the one-way flux counts all trajectories passing through the transition state in the direction of products. In a classical world, systems with an energy below the lowest energy in the dividing surface cannot reach the transition state; if the dividing surface passes through the saddle point, the saddle point will be the lowest-energy point in the transition state. The difference between the saddle point energy and the equilibrium energy of reactants is called the classical barrier height.

When one calculates the one-way flux in step (iii), one finds that the one-way flux equals k_BT/h times $\exp(\Delta G_{C,act}/k_BT)$, where k_B is Boltzmann's constant, T is temperature, h is Planck's constant, and $\Delta G_{C,act}$ is a quantity that is exactly the same as one would get if one calculated the free energy of the dividing surface minus the free energy or reactants (the free energy of activation).

It is a purely classical derivation and yet the result involves h. This would never happen in real thermodynamics; it happens here because translating the partition function for the missing degree of freedom from quantum mechanics to classical mechanics involves h. The second aspect is the relation of rate constants to one-way flux coefficients. In order to measure a rate constant that is independent of time, i.e., independent of the concentrations (that is why they are called rate constants), the system must have reached a steady state. At that point, for a noninfinitesimal rate, local equilibrium is perturbed, and k_1 and k_{-1} are not equal to equilibrium one-way fluxes.

$$rate = -d[A]/dt = k_1[A] - k_{-1}[B]$$

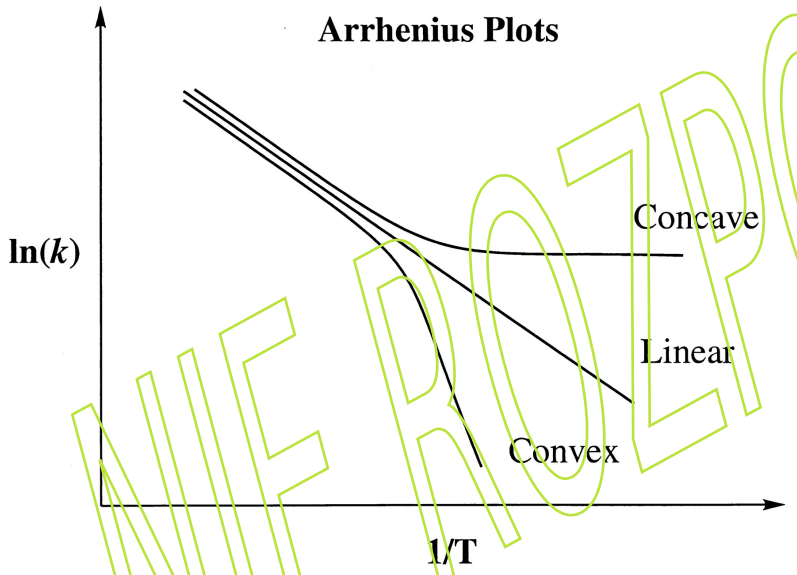
Archives of Biochemistry and Biophysics, 582:10-17 (2015)

Convex Arrhenius plots and their interpretation

Donald G. Truhlar and Amnon Kohen

Proc. Natl. Acad. Sci., USA, 98:848-851 (2001)

This paper draws attention to selected experiments on enzyme-catalyzed reactions that show convex Arrhenius plots, which are very rare. The analysis presented here shows that in such systems, the rate coefficient as a function of energy is not just increasing more slowly than expected, it is actually decreasing.



Experiments on reactions catalyzed by a variety of thermostable and mesostable dehydrogenase and oxidase enzymes have shown that in some cases the Arrhenius plot for the chemical step is convex. The striking feature of these reactions is the positive convexity* C of their Arrhenius plots, where

$$C \equiv -\frac{d^2 \ln k(T)}{d^2(1/T)}$$

We write

$$C \equiv -\frac{T^2}{R}\frac{dE_a}{dT}$$

where

$$E_a = -R\frac{d\ln k(T)}{d(1/T)}$$

E_a is the temperature-dependent phenomenological energy of activation, and R is the gas constant.

Thus a positive convexity means that E a decreases with increasing temperature.

*wypukłość, Jeśli funkcja f(x) jest dwukrotnie różniczkowalna na (a, b), to aby była ona wklęsła (wypukła ku górze) (w przedziale (a,b)), wystarczy żeby druga pochodna w tym przedziale była niedodatnia; convex=wypukły

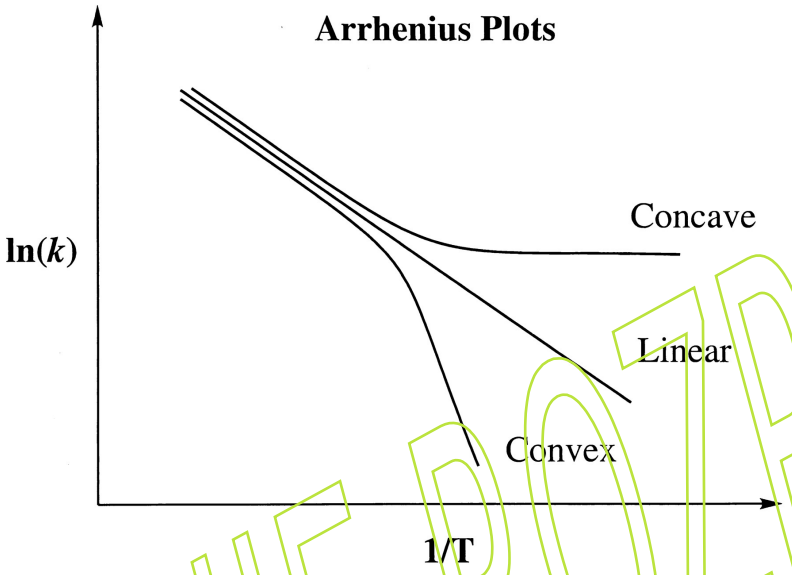
$$\frac{d}{dT} = \frac{d}{d(1/T)} \cdot \frac{d(1/T)}{dT} = -T^{-2} \frac{d}{d(1/T)}$$

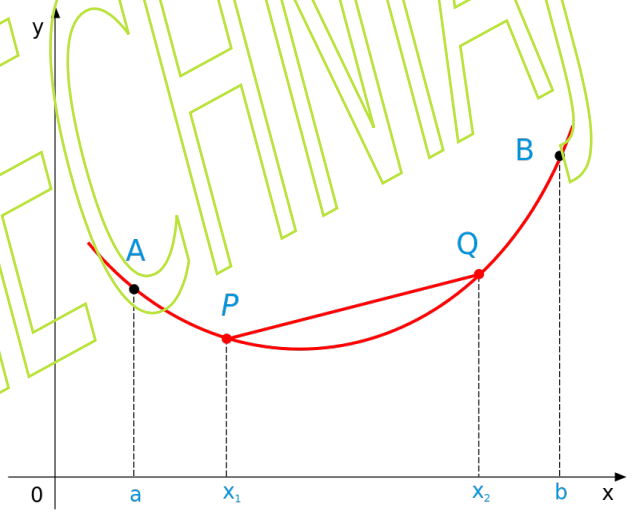
$$C \equiv -\frac{d^2 \ln k(T)}{d^2(1/T)} = -\frac{d}{d(1/T)} \left(\frac{d \ln k(T)}{d(1/T)} \right) = -\frac{d}{d(1/T)} \left(\frac{d\left(\ln A - \frac{E_a}{RT} \right)}{d(1/T)} \right) = \frac{d(E_a/R)}{d(1/T)} = -T^2 \frac{d(E_a/R)}{dT}$$

compare with $C \equiv -\frac{T^2}{R} \frac{dE_a}{dT}$

Convex functions

In mathematics, a real-valued function is called convex if the line segment between any two points on the graph of the function lies above the graph between the two points (wikipedia-en). Convexity and concavity of a function - properties of a function that describe its position relative to its tangent at a given point. If the curve is above the tangent we say it is convex, if the curve is below the tangent we say it is concave (wikipedia-pl). A small number of authors call convex functions in the sense of the above definition concave and vice versa.





Thus, Truhlar's convex function is concave in the Wikipedia definition. Positive convexity C means

$$C \equiv -\frac{d^2 \ln k(T)}{d^2(1/T)} = -\frac{d}{d(1/T)}\left(\frac{d \ln k(T)}{d(1/T)}\right) = -\frac{d}{d(1/T)}\left(-\frac{E_a}{R}\right) = \frac{d}{d(1/T)}\left(\frac{E_a}{R}\right) > 0$$

This condition is met when an increase in the value of 1/T is accompanied by an increase in the value of E_a.

Truhlar's statement from the previous slide: "Thus a positive convexity means that E_a decreases with increasing temperature" is valid, which was good to check due to Truhlar's reversal of convex/concave.

wikipedia

$$k = \frac{\kappa k_B T}{h} e^{(-\Delta G^{\ddagger}/RT)} = \frac{\kappa k_B T}{h} e^{(\Delta S^{\ddagger}/R)} e^{(-\Delta H^{\ddagger}/RT)}$$

The Eyring equation in this simplest form is sufficient many purposes. An assumption often made with respect to the above Equation is that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are independent of temperature (and hence that $\Delta G^{\ddagger}$ varies with temperature according to the Gibbs equation: $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$). Indeed, this assumption holds well for many reactions involving small molecules in standard solvents. However, a number of investigators have noted deviations from this Equation when plotting temperature versus enzyme-catalyzed rates, suggesting that the above assumption is not valid and that there is a more complex temperature dependence for these systems.

When considering introducing modifications to the Eyring equation that could improve agreement with experiments, it was noticed that enzymes are flexible macromolecules of high molecular weight and with correspondingly high heat capacities (C_p). The C_p of a system is a fundamental thermodynamic parameter that quantifies the temperature dependence of the enthalpy (H) and entropy (S) according to

$$\Delta G^{\ddagger} = \Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p \left(T - T_0\right) - T\left[\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p \left(\ln T - \ln T_0\right)\right]$$

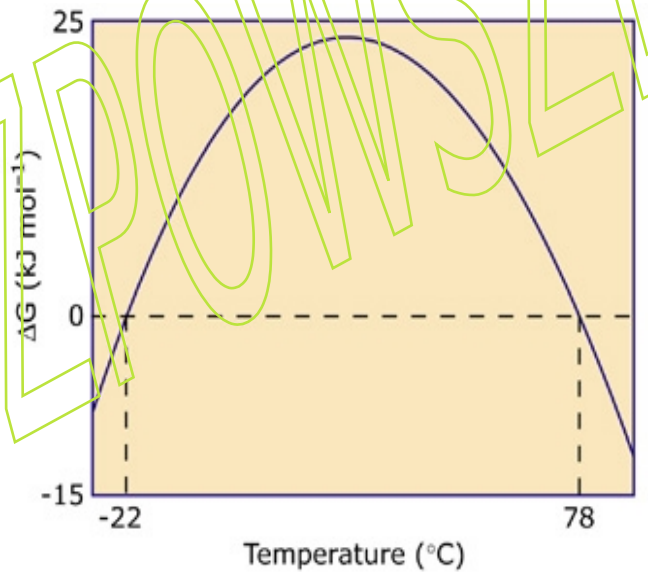
Biochemistry, 55:1681-1688 (2016)

Heat capacity or thermal capacity is a physical property of matter, defined as the amount of heat to be supplied to an object to produce a unit change in its temperature. The SI unit of heat capacity is joule per kelvin (J/K).

$$C = \lim_{\Delta T \to 0} \frac{\Delta Q}{\Delta T}$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p \left(T - T_0\right) - T\left[\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p \left(\ln T - \ln T_0\right)\right]$$

We came across the above equation in Lecture 10 when discussing cold denaturation of proteins.



$$\Delta G(T) = \Delta H(T_{ref}) - T\,\Delta S(T_{ref}) + \Delta c_p \left(T - T_{ref} - T \ln\left(T/T_{ref}\right)\right)$$

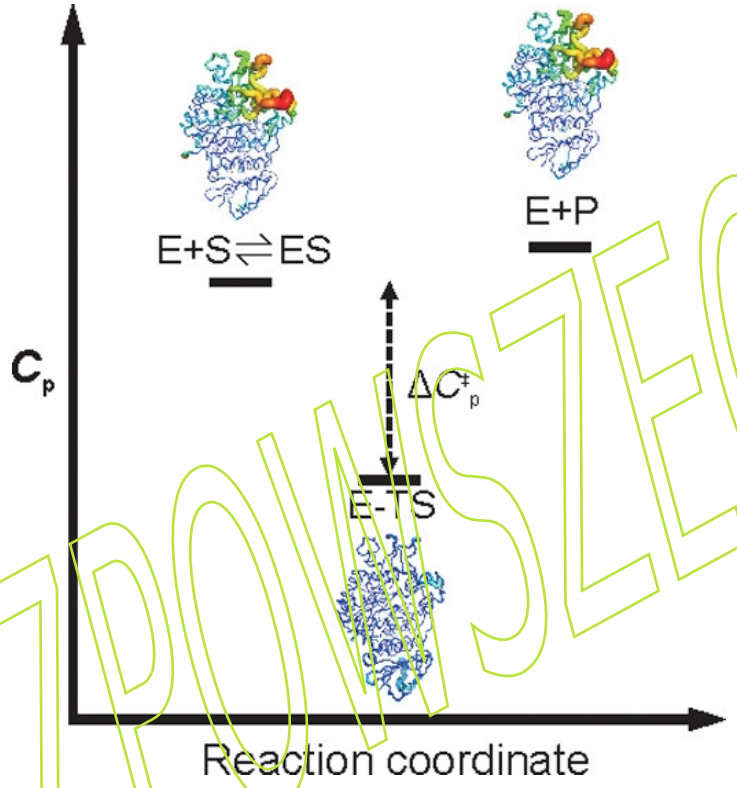
Incorporation of a $\Delta C_p^{\ddagger}$ term into the Eyring equation gives equations for the rate constant k:

$$k = \frac{\kappa k_B T}{h} e^{\frac{\Delta S^{\ddagger}_{T_0} + \Delta C_p^{\ddagger}\left(\ln T - \ln T_0\right)}{R}} e^{-\frac{\Delta H^{\ddagger}_{T_0} + \Delta C_p^{\ddagger}\left(T - T_0\right)}{RT}}$$

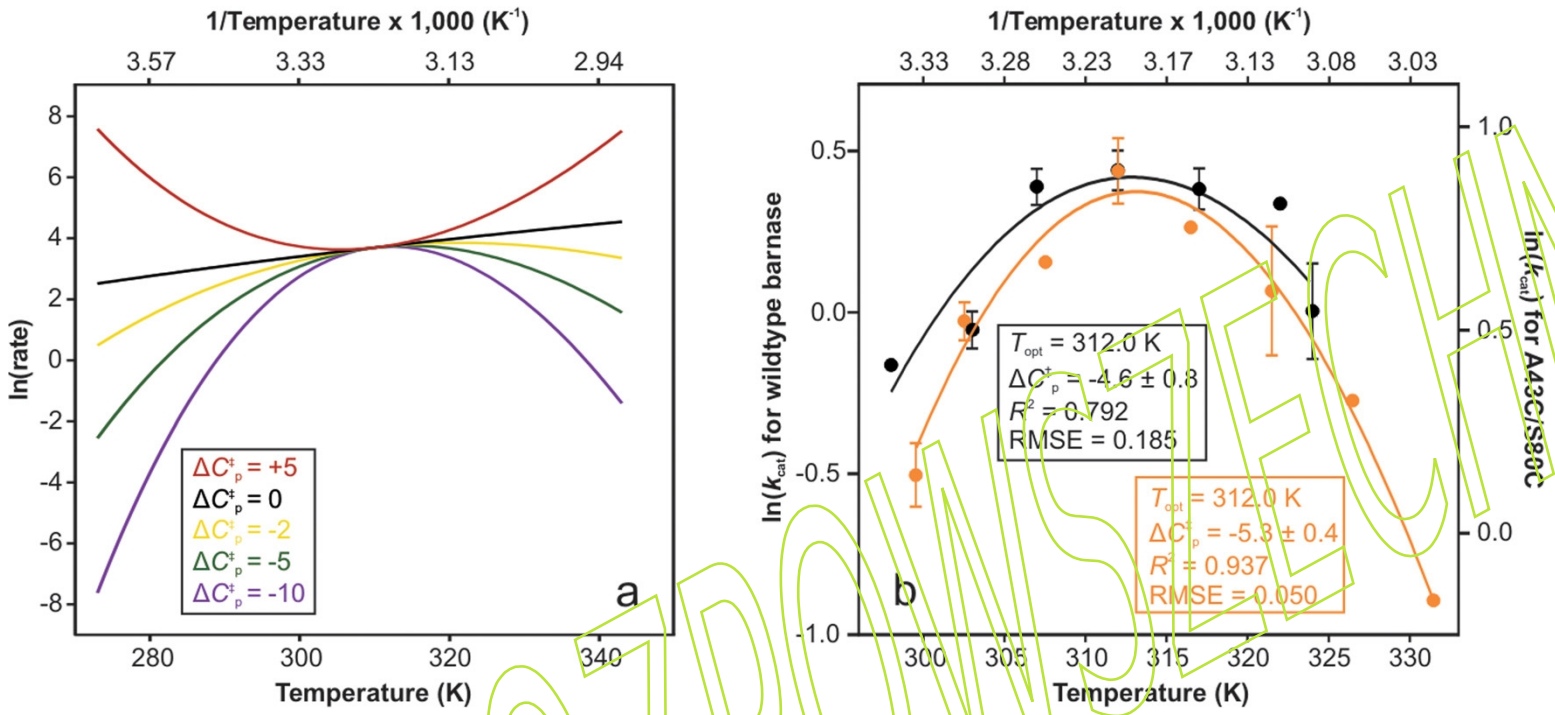
$$\ln k = \ln \frac{\kappa k_B T}{h} - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C_p^{\ddagger}\left(T - T_0\right)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C_p^{\ddagger}\left(\ln T - \ln T_0\right)}{R}$$

If $\Delta C_p^{\ddagger} = 0$, the above equations take the form of classical Eyring equations. However, for reactions catalyzed by enzymes with high heat capacities, the $\Delta C_p^{\ddagger}$ term may be nonzero, and the above equations should be implemented. Is there a difference in heat capacity between the enzyme−substrate and enzyme−transition state species for enzyme-catalyzed reactions (i.e., is $\Delta C_p^{\ddagger}$ nonzero for enzyme-catalyzed reactions)? If so, what are the consequences for the temperature dependence of enzyme catalyzed rates?

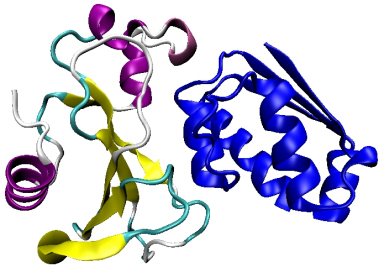
Biochemistry, 55:1681-1688 (2016)



The increase in enzymatic rates with temperature up to an optimum temperature (T_{opt}) is widely attributed to classical Arrhenius behavior, with the decrease in enzymatic rates above T_{opt} ascribed to protein denaturation and/or aggregation. This account persists despite many investigators noting that denaturation is insufficient to explain the decline in enzymatic rates above T_{opt}. Here we show that it is the change in heat capacity associated with enzyme catalysis ($\Delta C^{\ddagger}_p$) and its effect on the temperature dependence of $\Delta G^{\ddagger}$ that determines the temperature dependence of enzyme activity (ACS Chem. Biol., 8:2388-2393 (2013)).



Barnase and barstar are the extracellular ribonuclease and its intracellular inhibitor produced by *Bacillus amyloliquefaciens.*



$$\ln k = \ln \frac{\kappa k_B T}{h} - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (\ln T - \ln T_0)}{R} \quad (*)$$

Effect of $\Delta C^{\ddagger}_p$ on the temperature dependence of reaction rates.
(a) Schematic representation of the relationship between temperature and rate as modeled by eq (*) when different values of $\Delta C^{\ddagger}_p$ are applied.
(b) Fit of eq (*) to temperature-rate data for barnase (black ●) and A43C/S80C (orange ●). Data shown are the initial rate of enzyme activity at different temperatures as a function of the enzyme concentration (k_{cat}) and the mean of at least two replicates. Error bars, where visible, represent the SD; $\Delta C^{\ddagger}_p$ values are in kJ mol^{-1} K^{-1} (±SE). T_{opt} is defined as the highest temperature at which the maximum rate of activity was observed.

ACS Chem. Biol., 8:2388-2393 (2013)

On the Temperature Dependence of Enzyme-Catalyzed Rates

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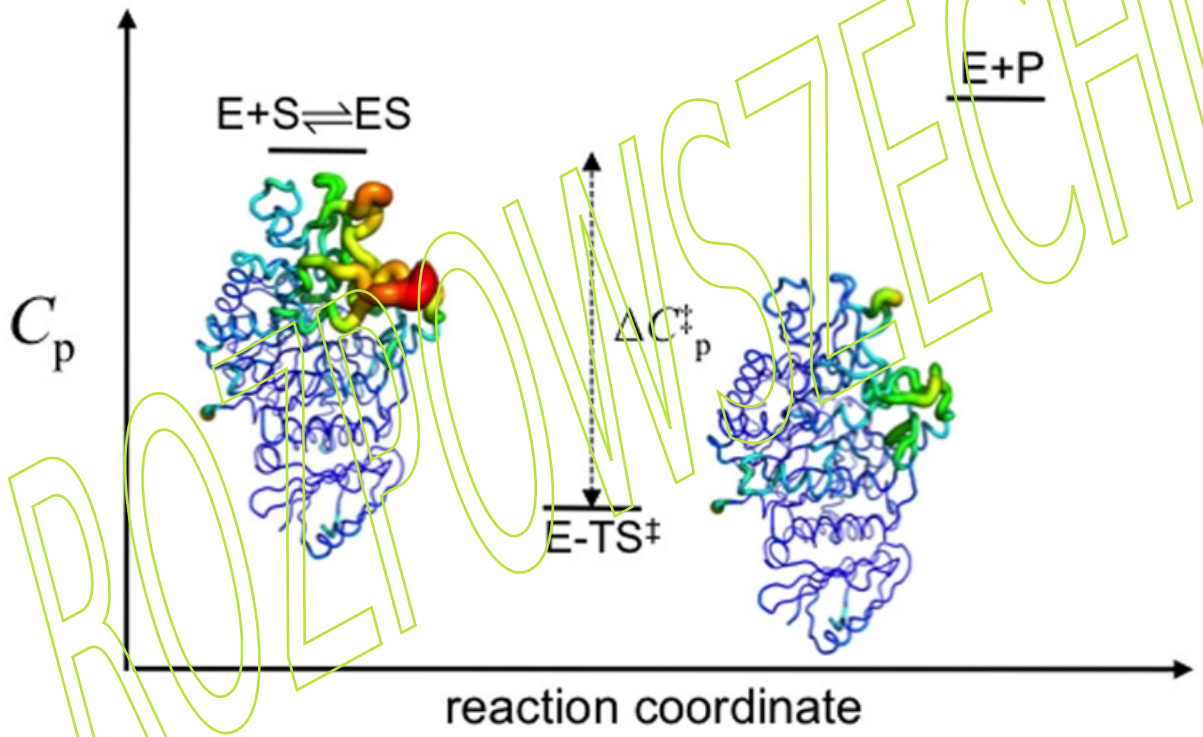
Biochemistry, 55:1681-1688 (2016)

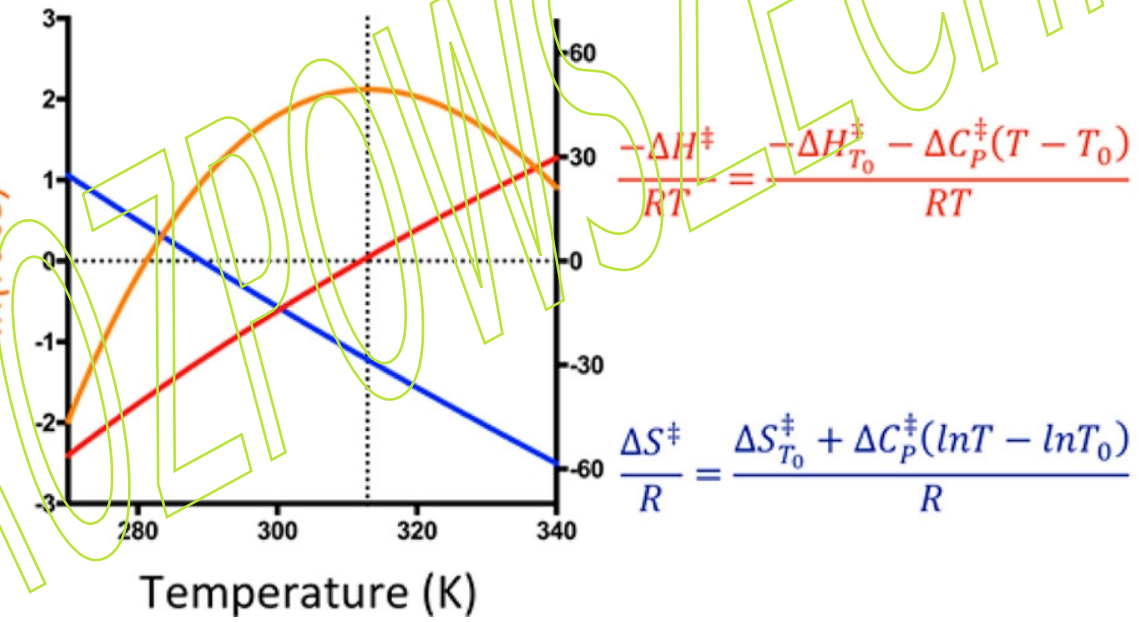


We have coined the phrase "macromolecular rate theory (MMRT)" to describe the temperature dependence of enzyme-catalyzed rates independent of stability or regulatory processes. Central to MMRT is the observation that enzyme-catalyzed reactions occur with significant values of $\Delta C_p^{\ddagger}$ that are in general negative. That is, the heat capacity (C_p) for the enzyme-substrate complex is generally larger than the C_p for the enzyme-transition state complex. Consistent with a classical description of enzyme catalysis, a negative value for $\Delta C_p^{\ddagger}$ is the result of the enzyme binding relatively weakly to the substrate and very tightly to the transition state.

Incorporation of a $\Delta C_p^{\ddagger}$ term into the Eyring equation gives equations for the rate constant k:

$$k = \frac{\kappa k_B T}{h} e^{\frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (\ln T - \ln T_0)}{R}} e^{-\frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (T - T_0)}{RT}}$$

$$\ln k = \ln \frac{\kappa k_B T}{h} - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (\ln T - \ln T_0)}{R} \quad (*)$$

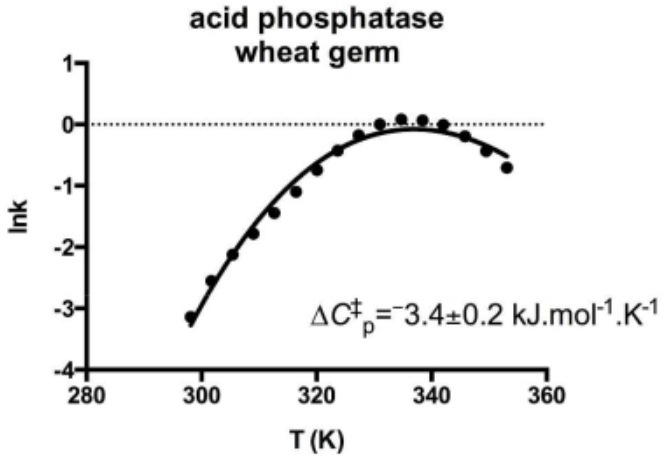


The temperature dependence of ln(rate) showing MMRT (orange, left-hand y-axis) and the individual contributions to ln(rate) from the enthalpy term ($-\Delta H^{\ddagger}/RT$) and the entropy term ($\Delta S^{\ddagger}/R$), red and blue lines, respectively (right-hand y-axis). The vertical dotted line shows T_{opt} and $-\Delta H^{\ddagger} = RT_{opt}$.

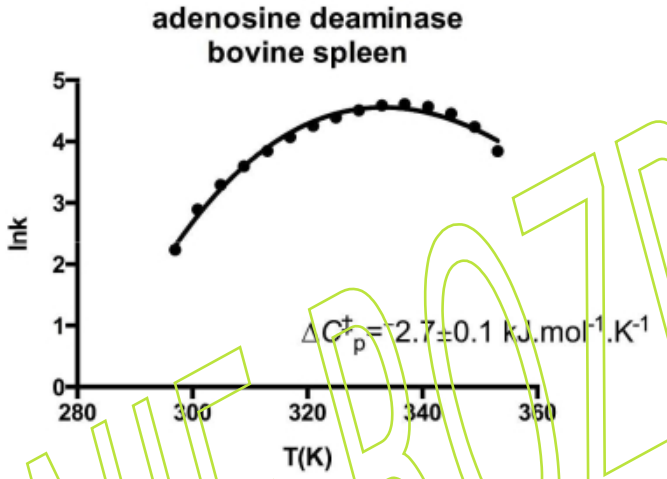
It follows that for $\Delta C_p^{\ddagger} < 0$, the rate of an enzyme catalyzed reaction initially rises with temperature and then reaches an optimum temperature (T_{opt}) after which the rate falls again, in contrast to simple Arrhenius and Eyring kinetics.

The increase in rate at temperatures up to T_{opt} is driven by the enthalpic term ($-\Delta H^{\ddagger}/RT$). However, this term is slowly overcome by the entropic term ($\Delta S^{\ddagger}/R$) at temperatures above T_{opt}, leading to a reduction in the reaction rate.

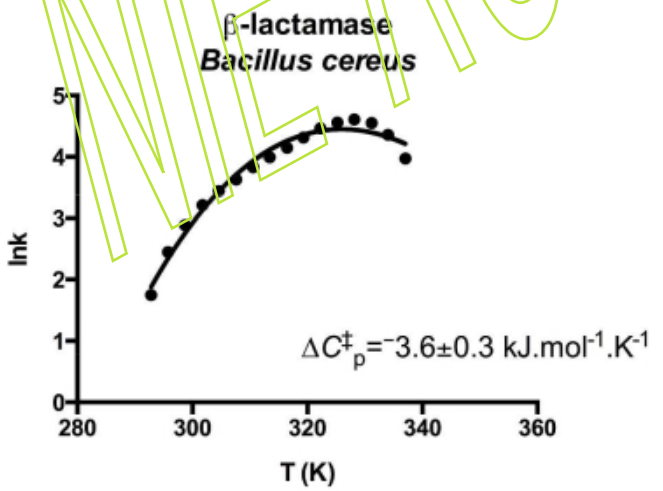
Biochemistry, 55:1681-1688 (2016)



Equation (*) fitted to data taken from:
(1) Peterson, M. E., Daniel, R. M., Danson, M. J., and Eisenthal, R. (2007) The dependence of enzyme activity on temperature: determination and validation of parameters. Biochem J 402, 331.
(2) Peterson, M. E., Eisenthal, R., Danson, M. J., Spence, A., and Daniel, R. M. (2004) A new intrinsic thermal parameter for enzymes reveals true temperature optima. J. Biol. Chem. 279, 20717–20722.



Acid phosphatases (APase) are a family of enzymes that non-specifically catalyze the hydrolysis of monoesters and anhydrides of phosphoric acid to produce inorganic phosphate at an optimum pH of 4 to 7.



Adenosine deaminase is an enzyme involved in purine metabolism. It is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues.

Beta-lactamases, (β-lactamases) are enzymes produced by bacteria that provide multi-resistance to beta-lactam antibiotics such as penicillins, cephalosporins, cephamycins, monobactams and carbapenems (ertapenem).

Biochemistry, 55:1681-1688 (2016)