## 7. Enzymes accelerate chemical reactions.

The figure below shows an Arrhenius plot for the uncatalyzed reaction of 1-methylorotic acid (OMP).

(a) Estimate $\Delta H^{\ddagger}$ from the figure.

(b) Estimate $\Delta S^{\ddagger}$ at $T = 300\,\text{K}$.

(c) At $T = 25°\text{C}$, the enzyme OMP decarboxylase accelerates this reaction 1.4×10^{17}-fold. How fast is the catalyzed reaction at $25°\text{C}$?

(d) What is the binding constant of the enzyme to the transition state of the reaction at $T = 300\,\text{K}$?
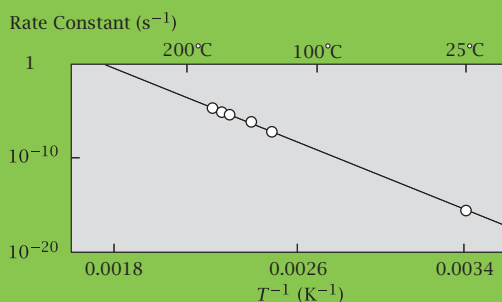
Source: A Radzicka and R Wolfenden, *Science* **267**, 90–93 (1995).

(a) Since

$$\frac{k_2}{k_1} = \left(\frac{kT_2/h}{kT_1/h}\right) \exp\left[-\frac{\Delta H^{\ddagger}}{k}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)\right],$$

you have

$$\ln\frac{k_2}{k_1} = \ln\left(\frac{T_2}{T_1}\right) - \frac{\Delta H^{\ddagger}}{k}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

Take two points, such as $(T_1, k_1) = (560\,\text{K}, 1\,\text{s}^{-1})$ and $(T_2, k_2) = (294\,\text{K}, 1 \times 10^{-16}\,\text{s}^{-1})$. Then

$$\Delta H^{\ddagger} = \frac{k\left[\ln(T_2/T_1) - \ln(k_2/k_1)\right]}{\dfrac{1}{T_2} - \dfrac{1}{T_1}}$$

$$= \frac{(1.987\,\mathrm{cal\,K^{-1}\,mol^{-1}})\left[\ln\left(\frac{294}{560}\right) - \ln\left(1 \times 10^{-16}\right)\right]}{(0.0034 - 0.0018)\,\mathrm{K^{-1}}}$$

$$= 45\,\mathrm{kcal\,mol^{-1}}.$$

(b) To get $\Delta S^{\ddagger}$, choose a point, say $(T, k) = (300\,\mathrm{K}, 1 \times 10^{-16}\,\mathrm{s^{-1}})$, and use the expansion

$$\ln k_2 = \ln\left(\frac{kT}{h}\right) - \frac{\Delta H^{\ddagger}}{kT} + \frac{\Delta S^{\ddagger}}{k}$$

and rearrange to get

$$\begin{aligned}
\Delta S^{\ddagger} &= k\left[\ln\left(\frac{k_2}{kT/h}\right) + \frac{\Delta H^{\ddagger}}{kT}\right] \\
&= (1.987\,\mathrm{cal\,K^{-1}\,mol^{-1}})\left[\ln\left(\frac{1 \times 10^{-16}\,\mathrm{s^{-1}}}{6.25 \times 10^{12}\,\mathrm{s^{-1}}}\right) + \frac{45{,}000\,\mathrm{cal\,mol^{-1}}}{600\,\mathrm{cal\,mol^{-1}}}\right] \\
&= 17\,\mathrm{cal\,K^{-1}\,mol^{-1}}.
\end{aligned}$$

(c) $\quad (1.4 \times 10^{17})(1 \times 10^{-16}\,\mathrm{s^{-1}}) = 14\,\mathrm{s^{-1}}$

(d) The binding constant is simply the rate enhancement, 1.4×10^{17}. This is one of the largest catalytic rate enhancements known.

8. Rate increase with temperature.

A rule of thumb used to be that chemical reaction rates would roughly double for a 10-degree increase in temperature, say from $T_1 = 300\,\mathrm{K}$ to $T_2 = 310\,\mathrm{K}$. For what activation energy E_a would this be exactly correct?

Using $k_2/k_1 = 2$ gives

$$E_a = \frac{R\ln 2}{\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} = \frac{(1.987\,\mathrm{cal\,K^{-1}\,mol^{-1}})\ln 2}{(3.33 \times 10^{-3} - 3.226 \times 10^{-3})\mathrm{K^{-1}}} = 12.8\,\mathrm{kcal\,mol^{-1}} = 53.5\,\mathrm{kJ\,mol^{-1}}.$$

This rule of thumb is not particularly useful in general.

Figure A

k_1 (M^{-1}s^{-1})

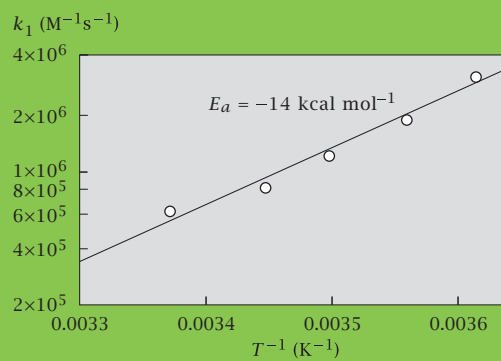E_a = −14 kcal mol^{-1}

T^{-1} (K^{-1})

Figure B

Source: CR Cantor and PR Schimmel, *Biophysical Chemistry*, Part III, WH Freeman, San Francisco, 1980.

Notice that there are three possible thermal signatures: positive activation energy (where rates increase with temperature), negative activation energy (where rates decrease with temperature), and entropy-dominated processes (in which the rate doesn't change much with temperature). All the sequences having only A and U nucleotides appear to have negative activation energy barriers. It's not understood why. None of the cases shown are entropy-dominated.

11. Drug dissociation from DNA.

Drug A is an uncharged molecule containing a three-ring aromatic chromophore linked to three amino acids: valine, N-methylvaline, and proline. This drug binds to double-stranded DNA, with the planar ring system intercalating between base pairs and the peptide portion lying in the minor groove.

(a) The aqueous solubility of drug A decreases with increasing temperature. What is the sign of $\Delta H°$ for the dissolution process?

(b) This drug is generally poorly soluble in water, i.e., $\Delta G° > 0$ for dissolution. What is the sign of $\Delta S°$ for this process?

(c) Give a brief explanation for your answer to (b) in terms of solvent properties.

(d) The figure below shows data for the temperature dependence of the rate constant for dissociation of drug A from duplex DNA. Use this plot to estimate the activation energy E_a and the entropy of activation $\Delta S^{\ddagger}$. Comment on the role of solvent in determining the value of $\Delta S^{\ddagger}$.
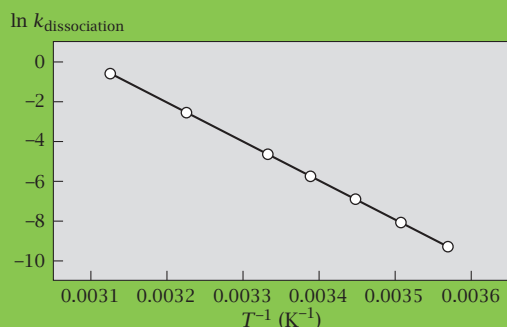
(a) For the process drug(solid) $\rightarrow$ drug(aqueous), the van't Hoff equation gives

$$\frac{\partial \ln K}{\partial T} = \frac{\Delta H°}{RT^2}.$$

And since K goes down when T goes up, $\Delta H°$ must be negative.

(b) $\Delta G° = \Delta H° - T\Delta S°$. Since $\Delta H° < 0$, then $\Delta S°$ must be < 0.

(c) This result is unusual in that dissolution (change from solid to solution phase) usually entails an increase in S. The observed decrease in S must be due to the hydrophobic effect; that is, the drug is lipophilic and thus when dissolved in water, the water undergoes an ordering, which actually lowers S. This decrease in S more than compensates the increase in S due to the change in state of the drug.

(d) The Arrhenius equation for rate constant k is

$$k = Ae^{-E_a/RT} \implies \ln k = \ln A - \frac{E_a}{RT}.$$

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Thus, $E_a = -R \times$ (slope of the given plot)

Determine the slope by picking two points on the line, such as

$$\ln k = -2 \text{ at } 1/T = 0.0032 \quad \text{and} \quad \ln k = -4 \text{ at } 1/T = 0.0033$$

$$\implies \text{slope} = \frac{-2 - (-4)}{0.0032 - 0.0033} = -20{,}000.$$

$$E_a = -8.314(-2000) = 166 \text{ kJ mol}^{-1}.$$

$\Delta S^{\ddagger}$ is related to the pre-exponential factor A by the equation

$$A = \frac{\kappa k T}{h} e^{(1 + \Delta S^{\ddagger})/R} \implies \frac{\Delta S^{\ddagger}}{R} = \ln\left(\frac{hA}{\kappa k T}\right) - 1.$$

Set the transmission factor κ to 1, and determine the intercept $\ln A$ of the plot above.

$$y = mx + b, \text{ use the point } (x, y) = (0.0032, -2), \ m = -20{,}000$$
$$\implies b = y - mx = -2 + 20{,}000 \times 0.0032 = 62 = \ln A,$$

$$A = 8.438 \times 10^{26} \implies \Delta S^{\ddagger} = 262 \text{ J K}^{-1}.$$

This represents an increase in S, which is expected when the complex of the drug with DNA starts to fall apart. Working against this increase in S is a decrease in S due to partial ordering of water around the lipophilic drug as it starts to come off the DNA, and hence becomes exposed to water. However, the net balance appears to be in favor of the increase in S due to the loosening of the complex.

12. Series versus parallel reactions.

(a) You have a reaction in which A converts to B through 2 steps in series:

$$A \xrightarrow{k_1} I \xrightarrow{k_2} B,$$

where the temperature dependences of the steps are given by the Arrhenius law:

$$k_1 = ae^{-E_1/RT},$$
$$k_2 = ae^{-E_2/RT}.$$

Derive an expression for the total rate k_{tot}, from A to B, as a function of a, E_1, E_2, and T. (Hint: the time from A to B is the sum of times from A to I and from I to B).

(b) Now consider instead two steps in parallel:

$$A \xrightarrow{k_1} B, \qquad A \xrightarrow{k_2} B.$$

Using the Arrhenius equations from (a) the individual steps, derive the temperature dependence for k in this case. (Hint: now the rates add.)

(a) Since the times add,

$$t_{\text{tot}} = t_1 + t_2,$$

$$\frac{1}{k_{\text{tot}}} = \frac{1}{k_1} + \frac{1}{k_2},$$

$$k_{\text{tot}} = \frac{k_1 k_2}{k_1 + k_2},$$

$$k_{\text{tot}} = \frac{A e^{-(E_1+E_2)/RT}}{e^{-E_1/RT} + e^{-E_2/RT}}.$$

(b) Since the rates add,

$$k_{\text{tot}} = k_1 + k_2 = A(e^{-E_1/RT} + e^{-E_2/RT}).$$

13. A cricket thermometer.

The frequency that crickets chirp increase with temperature. You can calculate the temperature in degrees Celsius by adding 4 to the number of chirps you hear from a cricket in 8 s. In this way, you can use a cricket as a thermometer.

(a) What four temperatures would you have measured if the number of chirps in 8 s were 16, 21, 26, and 31?

(b) From this data, make an Arrhenius plot and calculate the activation energy in kcal mol^{-1}. This will tell you about a rate-limiting biochemical reaction that underlies cricket chirping.

(c) You observe that crickets outside a fast-food restaurant chirp faster than their cousins in the wild. The fast-food crickets chirp 30 times in 8 s at 25°C and 40 times in 8 s at 35°C. By how much did the fast-food additive reduce the activation barrier for cricket chirping?

(d) If the equilibrium enthalpy for the chirping reaction, Δh°, is $10\,\text{kcal mol}^{-1}$, what is the activation energy for the reverse chirp reaction for the normal crickets?