Lecture 30

Chapt 27 (sort of) Catalysis/enzymes

Announcements:
remember seminar today (still some room for lunch)
Let me know about any exam problems with the Tues before T-giving

Outline:
Catalysis
Enzymes
  catalase
  Michaelis-Menten

Review
Nobel stuff – important to pay attention to these things

Arrhenius 1889 – \( k = A e^{-\frac{E_a}{RT}} \) sketch diagram

Transition state theory (also Eyring theory – 1930s by the way)
3 assumptions
1. transition state in thermal equil. with reactants
2. no recrossing of barrier
3. motion along rxn coord is independent of other motions and can be treated as classical translation

If we say \( E_a \) is \( \Delta H^\ddagger \) (really it is \( \Delta U^\ddagger \)) and \( c^0 = 1 \)

\[
K = \frac{k_B T}{h} e^{\frac{\Delta S^\ddagger}{R}} e^{-\frac{E_a}{RT}}
\]

Note similarity to Arrenhius – so Arrenhius is not replaced by TST, just supplemented.

Finally, finished up by saying that elementary rxns do allow one to just write down a rate law directly from stoichiometry.
Catalysis

I think everyone knows that catalysts increase reaction rates by offering up an alternative reaction mechanism – generally one with a lower activation energy – but does not affect equilibrium. Show free energy plot – reactant and product are the same, so equilibrium constant (-RTlnΔG) must not be affected.

Because enzymes introduce a new reaction pathway the pre-exponential factor is also usually different and can be larger or smaller. What is more important, A or Eₐ? But, because there is linear dependence on A and exponential dependence on Eₐ, changes in activation energy are much more important.  

\[ k = A e^{-\frac{E_a}{RT}} \]

Note also that an enzyme does not prevent the reaction from occurring via the normal pathway. Thus:

A → B with rate k₁ (normal slow path)

A + catalyst → B with rate k₂

means that overall kinetics are given by dB/dt = k₁[A] + k₂[A][catalyst]

Of course, normally k₁ is so tiny compared to k₂ that we just ignore it.
Enzymes

Enzymes are simply biological catalysts. I think I can safely say that it is obvious that enzymes are crucial to life as we know it. It may not be obvious that we are still learning a lot about enzymes.

For instance, it wasn’t that long ago (1986) that some researchers had an enzyme that they could not isolate from their RNA. They spent an extraordinary amount of work trying to figure out why the enzyme kept appearing with the RNA. It turns out that the ridiculous thought that RNA might act as an enzyme was correct. They (Cech and also Altman who did some other stuff with RNA enzymes) got the Nobel Prize in Chemistry in 1989.

We now know that is RNA in the ribosome that catalyzes the formation of the peptide bond – perhaps the most important enzymatic reaction of all. (Show 11 August 2000 Science cover.)

Let’s look at one enzymatic reaction as an example:

\[ \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \frac{1}{2}\text{O}_2 \]

This reaction is very slow in aqueous solution: \(-d[\text{H}_2\text{O}_2]/dt = 10^{-8} \text{ M/s}\)
(This corresponds to about 1% of \(\text{H}_2\text{O}_2\) reacting after 3 days (25°C).)

Simple inorganic catalysts such as HBr (or iron salts) speed up reaction 4-5 orders of magnitude.

\(-d[\text{H}_2\text{O}_2]/dt = 10^{-4} \text{ M/s}\)

The enzyme catalase (occurs in blood and some tissues) makes the reaction go 15 orders of magnitude faster:

\(-d[\text{H}_2\text{O}_2]/dt = 10^7 \text{ M/s}\)

**Anyone put **H**2O**2 **on plain skin, on wound?** Very different effects. Catalase makes hydrogen peroxide bubble when you put it on a wound, but there is no catalase on the outside of unbroken skin.

Note that the reaction above is exergonic, \( \Delta G = -103.1 \text{ kJ/mol} \)
mainly from enthalpy changes \( \Delta H = -94.64 \text{ kJ/mol} \)

But, activation energy is really high 71 kJ/mol, implying \( A \sim 1 \times 10^5 \text{ s}^{-1} \)
Catalase lowers activation energy to 8 kJ/mol (enthalpy reduction) and also raises \( A \) to \( \sim 2 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \). The enthalpy effect is most important – typical for modest temperatures like 298 K – but the change in \( A \) (Entropy) still contributes 3 orders of magnitude speedup.
As is typical for many enzyme-catalyzed reactions, this rxn becomes zero order in substrate at high concentrations. i.e. when \([\text{H}_2\text{O}_2]\) gets really big, the rate law changes from

\[ k[\text{H}_2\text{O}_2][\text{catalase}] \]

to

\[ k[\text{catalase}] \]

This means that a plot of reaction rate versus substrate concentration hits a plateau. This maximum rate per mole of enzyme **active sites** is called the catalytic constant or the turnover.

\[ \text{catalytic constant} = k_{\text{cat}} = \frac{V_{\text{max}}}{[\text{E}]_0} \]

Catalase has an extraordinary turnover of \(9 \times 10^6 \text{ s}^{-1}!!\) Most enzymes are
The BIG question:
Why does saturation occur?

This is a crucial link to typical enzyme mechanism. There are actually 3 important experimental observations about enzyme kinetics:

1) Rate of reaction usually linear with enzyme concentration. \( \text{see plot at right} \)

2) At fixed [E], rate is linear in [S] for low [S]. \( \text{prev. plot – low [S]} \)

3) but plateaus/saturates at high [S]. \( \text{prev. plot – high [S] & saturation} \)

Michaelis and Menten put these together (in 1913) and came up with a mechanism that involves a binding event preceding the reaction. This is why there is saturation – if all the [E] is already bound to [S], then more [S] won’t speed up the reaction.

\[
\begin{align*}
[E] + [S] & \xrightleftharpoons[k_-1]{k_1} [ES] \xrightleftharpoons[k_2]{k_-2} [E] + [P]
\end{align*}
\]

This mechanism leads to what rates for [E], [P], and [ES]?\[
\begin{align*}
- \frac{d[S]}{dt} &= k_1 [E][S] - k_{-1} [ES] \\
- \frac{d[ES]}{dt} &= (k_2 + k_{-1}) [ES] - k_1 [E][S] - k_{-2} [E][P] \\
\frac{d[P]}{dt} &= k_2 [ES] - k_{-2} [E][P]
\end{align*}
\]

Note that since no enzyme is ever used up: \( [E]_0 = [E] + [ES] \)
We can rewrite the second equation using this \([E]_0\) and also by using the steady-state approximation on \([ES]\). This is reasonable if we have excess \([S]\) because then we expect a basically fixed amount of the \([E]\) will be bound.

So, setting \(d[ES]/dt = 0\) and plugging in that \([E]_0\) relation to get rid of \([E]\):

\[
[ES] = \frac{k_1[S] + k_2[P]}{k_1[S] + k_-2[P] + k_-1 + k_2}[E]_0
\]

Plugging this back into the \(d[S]/dt\) equation gives us something real complicated. We can make a second approximation by saying that we will only care about the early stages of the reaction. So, the rxn velocity is \(v_0\) and \([S] \approx [S]_0\) and \([P] = 0\).

\[
v_0 = \frac{-d[S]}{dt} = \frac{k_2[S]_0[E]_0}{K_m + [S]_0}
\]

** Key equation!

where

\[
K_m = \frac{k_-1 + k_2}{k_1}
\]

represents the apparent dissociation constant of \([E]\) and \([S]\) (for the reaction written \(ES \rightarrow E + S\).) ** When is \(K_m\) the actual dissociation constant? ** When \(k_-1 \gg k_2\) then \(K_m\) approaches \(k_-1/k_1\) – the dissociation constant.

Notice that \(k_-2\) is gone from the \(v_0\) equation. ** Why? ** \(P = 0\)

** Remember, this important equation applies to the initial velocity when there is excess substrate.**

Notice that when \([S]\) is small... \(K_m \gg [S]_0\), and we get?

\[
v_0 = \frac{k_2[S]_0[E]_0}{K_m}
\]

and we get something linear in \([S]\) just like we should (for fixed \([E]\)).

When \([S]\) is big... \([S]_0 \gg K_m\), and we get?
\[ v_{\text{Max}} = k_2 [E]_0 \]

we get something linear in \([E]_0\) and independent of \([S]\) like we should

Because this corresponds to the saturated region, we call it \(v_{\text{max}}\). \(k_2\) then is the turnover or \(k_{\text{cat}}\) we talked about before. Note again, \([E]_0\) is the concentration of active sites not just of enzymes.

Finally, when \(K_m = [S]_0\), we get:
\[ v_0 = \frac{k_2 [S]_0 [E]_0}{2[S]_0} = \frac{1}{2} v_{\text{Max}} \]

So, the Michaelis constant is the substrate concentration that gives half \(v_{\text{max}}\).

Note that we can rearrange the important equation above:
(substitute \(v_{\text{max}} = k_2 [E]_0\) then invert the rest)
\[ v_0 = \frac{v_{\text{Max}}}{1 + \frac{K_m}{[S]}} \]

and then invert to give
\[
\frac{1}{v_0} = \frac{1}{v_{\text{Max}}} + \frac{K_M}{v_{\text{Max}}}[S]
\]

So, plotting \(1/v_0\) vs. \(1/[S]\) gives \(K_M\) and \(v_{\text{Max}}\). This is called a Lineweaver-Burke plot. Several other plotting techniques exist.

Finally, note that \(k_{\text{cat}}/K_M\) is the specificity constant. If two substrates are competing for one enzyme...

\[
\frac{v_A}{v_B} = \left(\frac{k_{\text{cat}}/K_M}{k_{\text{cat}}/K_M}\right)_A \frac{[A]}{[B]}
\]

So the specificity constant tells you the selectivity of an enzyme for a particular substrate. If \(A\) has a large \(k_{\text{cat}}/K_M\) then its velocity is really fast and \(A\) outcompetes \(B\) for enzyme.