G. General Acid-Base Catalysis

Towards a Better Chemical Mechanism via Catalysis

There are two types of mechanisms we'll be discussing this semester. **Kinetic mechanisms** are concerned with rate constants and specified intermediates. The Michaelis-Menten mechanism is one example, the competitive inhibition mechanism is another. In this lecture, and those that follow, we'll be talking about **chemical** (or **reaction**) **mechanisms**, which are concerned with the structures of reactants, intermediates and products, and the pathway taken from beginning to end. Since we'll be looking largely at the transformation of organic compounds, these chemical mechanisms will involve electron pushing.

Consider a simple reaction from organic chemistry - the hydrolysis of an ester to generate a carboxylic acid and an alcohol (Eq. G.1).

$$R\text{-}CO_2R' + H_2O \Leftrightarrow R\text{-}CO_2H + R'\text{-}OH \qquad \text{(Eq. G.1)}$$

The reaction is generally described by the nucleophilic attack of a water molecule on the carbonyl carbon of the ester to generate a tetrahedral intermediate with both an oxyanion and an oxonium ion (Figure G.1). Following proton shuffling between the ex-nucleophile and the soon-to-be leaving group, the tetrahedral intermediate collapses, releasing the alcohol from the tetrahedral center. As you may recall, this is not a particularly rapid reaction because of the presence of two intermediate species that possess two relatively unstable functional groups - the oxyanion and the oxonium ion. Neither is as stable as an alcohol. Therefore there is a substantial free energy barrier associated with this pathway.

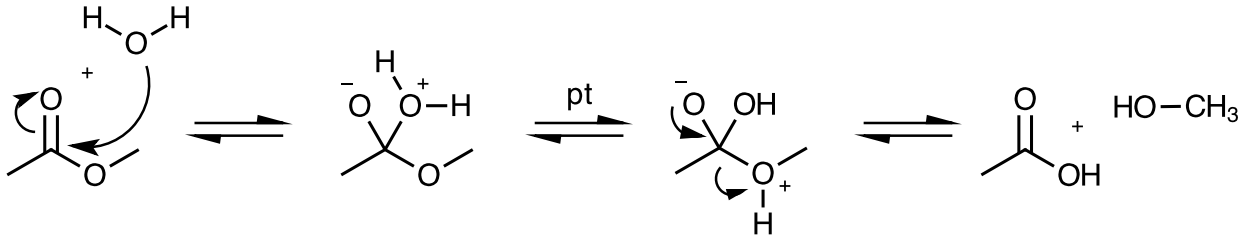

Figure G.1. Hydrolysis of an ester through two unstable intermediates.

In organic chemistry, you learned that there are two ways to accelerate this reaction. One improves the quality of the nucleophile by applying **specific base** catalysis ("specific base" refers to hydroxide). Under basic conditions, a high concentration of hydroxide allows the mechanism in Figure G.2 to proceed. While it still involves generation of an oxyanion intermediate, there is no additional insult of an oxonium ion to that injury, and of course, you started with an oxyanion (hydroxide), so the starting material is not significantly more stable than the intermediate. As for the production of the alkoxide, that gets quenched by the carboxylic acid, leading to a much more stable anion, a carboxylate, than was present initially.

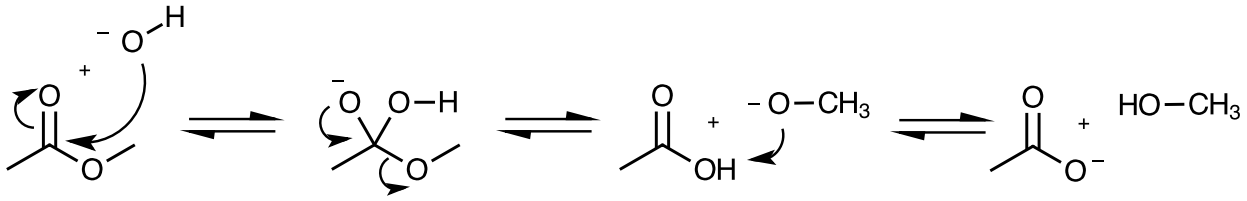

Figure G.2. Base catalyzed hydrolysis of an ester. Note that base provides a more potent nucleophile. It is a less stable molecule than water, which means that you're starting at a higher free energy, so that the intermediates (also containing oxyanions), are of comparably high free energy.

The second approach to catalyzing hydrolysis is by activating the carbonyl electrophile via **specific acid** hydrolysis (where "specific acid" refers to hydronium ion). By running the reaction in concentrated aqueous acid, it is possible to generate a sufficient concentration of the protonated ester to promote rapid hydrolysis (Figure G.3). Again, the starting oxonium ion is not significantly more stable than the intermediate oxonium groups (at least not compared to the starting materials in Figure G.1), so the free energy barrier to this mechanism is reduced.

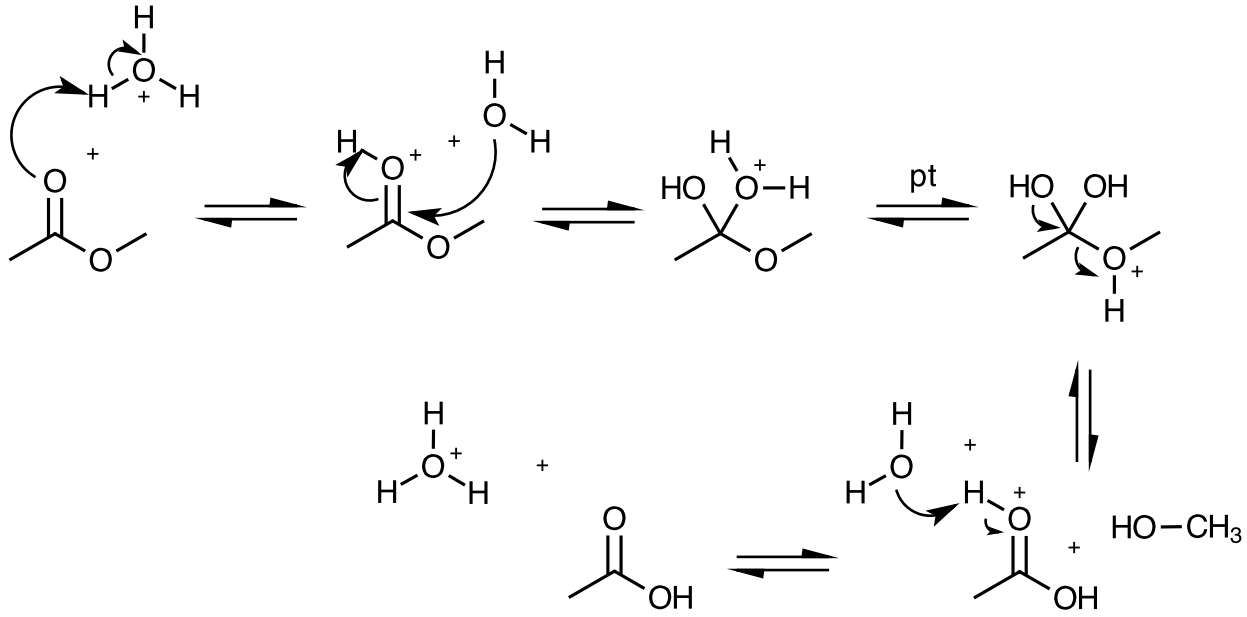

Figure G.3. Specific acid-catalyzed hydrolysis of an ester. Note that the first step represents activation of the electrophile so that the weak nucleophile can attack. Although there are unstable intermediates, relatively speaking, the starting catalyst is likewise higher in free energy, so that the barriers are significantly reduced.

Specific acid and base catalysis of ester hydrolysis depend on relatively unstable molecules to promote formation of relatively unstable intermediates without a huge jump upwards in free energy. While a great strategy for the chemistry lab, it works less well for the cell, where 1 M hydroxide or hydronium ion would be lethal. Another approach is needed.

General acid/base catalysis takes advantage of solutes more acidic than water (pK_a < 14) and more basic that water (conjugate acid pK_a's of greater than 0) to protonate and deprotonate atom groups that are ill-prepared to carry additional charge along a reaction pathway. Returning to Figure G.1, the water nucleophile is a poor nucleophile in part because it becomes an oxonium group in the tetrahedral intermediate. The ester is a poor electrophile because it generates an oxyanion in the intermediate. What if an acid (HA-) were present to protonate the carbonyl oxygen and a base (B-) were present to deprotonate the nucleophilic water? No unstable intermediate need form (Figure G.4). So long as the conjugate base of HA is more stable than the oxyanion and BH^+ is more stable than the oxonium group in Figure G.1, the barrier to catalysis is considerably lower.

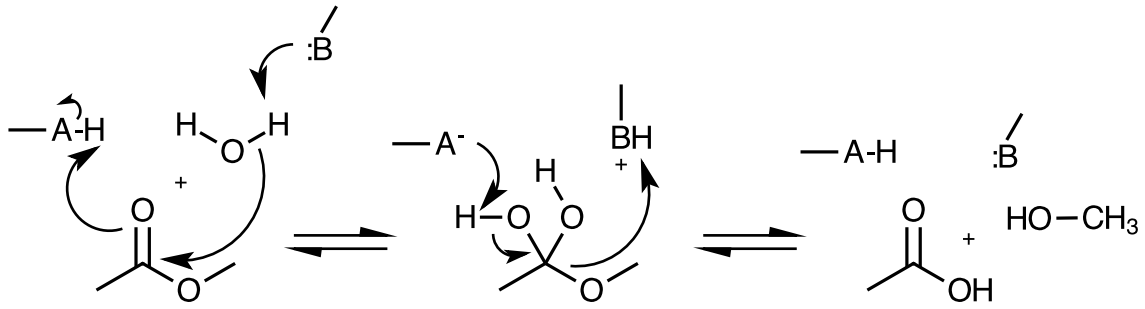

Figure G.4. Catalysis of ester hydrolysis with a general acid (HA) to protonate the carbonyl oxygen and a general base (B) to deprotonate the nucleophilic water. Note that protonation and deprotonation are simultaneous with the addition reaction so that no charge builds up on the transition state/intermediate.

A Caution on Mechanism Drawing

As noted in Figure G.4, an important part of general acid-base catalysis is coupling proton transfer to the reaction of interest, so that no unstable intermediate is presented in the mechanism. Let's say that the proton transfer steps proceed after the nucleophilic attack (Figure G.5). In that instance the unstable intermediate in Figure G.1 is produced, creating a significant free energy barrier to catalysis. Since that is exactly what we want to avoid, the chemical mechanism in G.5 provides no advantage over the uncatalyzed process. (A similar argument could be made for executing proton transfer *before* the nucleophilic attack.)

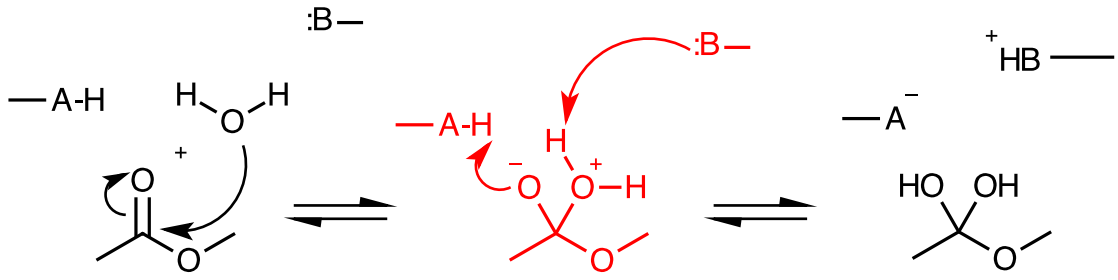

Figure G.5. The bad things that happen when proton transfer doesn't happen concurrently with nucleophilic attack. You end up with a nasty looking intermediate (in **red**).

So, here's a challenge to be met in your electron-pushing diagrams – see if you can avoid implicating oxonium and alkoxide ions in intermediates, unless they are plausibly stabilized by resonance or by other structural features of the enzyme active site/substrate.

General Acid-Base Catalysis in Enzymes

Proteins are happily endowed with several amino acid residues capable of serving as general acids and general bases in catalysis. In general, there are two important rules to follow (also summarized in Figure G.6):

- The amino acid serving as a general acid should have a pK_a greater than physiological pH, so that it is protonated in the enzyme active site. Common amino acid residues capable of performing that function include His, Cys, Tyr, Lys and Arg (all have pK_a's near or above 7). It is not impossible for other amino acids to play the role, but an acidic residue like Asp would need to be in an unusual environment to be deprotonated at pH 7.4.

- The general base should be conjugate to an acid with a pK_a lower than physiological pH. so that it is deprotonated in the enzyme active site. Common general bases will therefore be Asp, Glu and His (all of which have conjugate acid forms with pK_a's near or below 7). Other amino acids may play this role (for example, Lys), but would need to be in an unusual chemical environment.

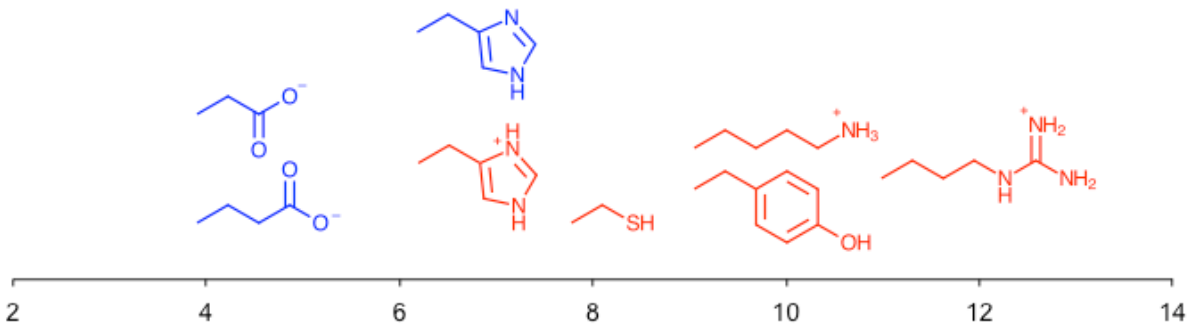

Figure G.6. Structures of amino acid side chains commonly involved in general acid base catalysis, organized by pK_a (horizontal axis). Note that histidine can readily perform both roles since its pK_a is near neutrality.

The identification of acid/base active residues in a catalytic mechanism is often achieved by performing pH-rate profiles, where k_{cat} is measured over a range of pH values. Note that these rate "constants" are only constant at a given pH (and temperature), and as active site residues are protonated and deprotonated, those constants change accordingly. The simplest graphical display of the data is shown in Figure G.7. As some indicator of rate is plotted vs. pH, one sees an increase in the rate from low pH to moderate pH as the active site base becomes deprotonated an ready for business. The pK_a of the base's conjugate acid can be determined visually via the inflection point at low pH, or analytically by curve fitting. At moderate pH (above the pK_a of the general base's conjugate and below the pK_a of the general

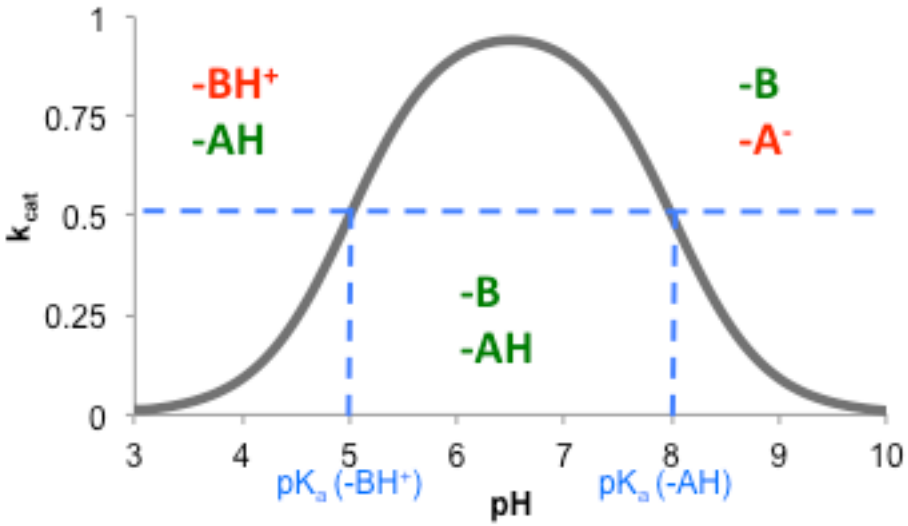

Figure G.7. Titration curve of enzyme activity vs. pH where general acid-base catalysis is involved. Note that, at low pH, the enzyme is relatively inactive due to the protonated state of the general base ($-BH^+$) which renders it in active. Likewise at high pH, the general acid becomes deprotonated ($-A^-$), which inactivates the protein. The pK_a's can be visualized by looking for the pH that gives the inflection points at ½ maximal activity (dashed blue lines for emphasis).

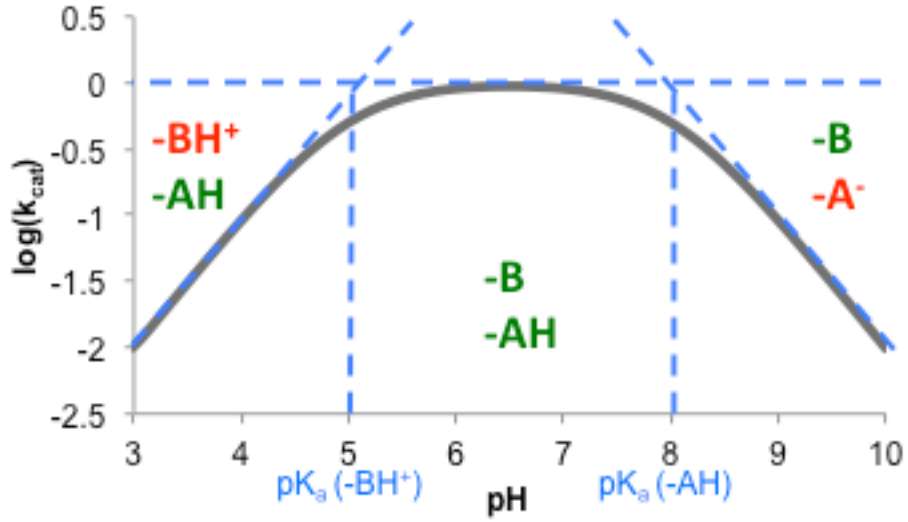

Figure G.8. Titration curve of the logarithm of enzyme activity vs. pH where general acid-base catalysis is involved. Note that the pK_a's can be visualized by looking for the pH at which lines modeling the different regimes of the titration intersect (see Appendix G.1).

acid), the enzyme reaches maximum activity. But, activity diminishes again at higher pH as the general acid is deprotonated, leaving the enzyme without one of its key catalytic groups.

An alternate visualization of the data employs a logarithmic axis for the rate data, and the pK_a's of the conjugate acid of the general base and of the general acid can be identified as intersections of lines marking different regions of the plot (Figure G.8). The algebra that validates that graphical interpretation is provided in Appendix G.1.

It should be noted that an explicit assumption is being made in these interpretations - namely that the differences in activities are due to the protonation state of general acids and bases in the active site. In point of fact, a protonation or deprotonation event far from the active site can have dramatic effects on enzyme activity. While these curves are good provisional evidence for general acid base catalysis, *other experiments are needed to confirm the assignment of the amino acid side chains responsible for the shape of the titration curve.*

On a similar note, it is not uncommon to show plots of k_{cat}/K_m vs. pH. Like k_{cat}, k_{cat}/K_m is a rate constant and can capture the pK_a values of amino acids involved in general acid base catalysis. However, the second order rate constant, k_{cat}/K_m, also captures substrate binding. Thus an inflection point could be due to the protonation or deprotonation of a residue that interacts with the bound substrate. A comparison of the plot obtained with k_{cat}/K_m with a plot obtained using k_{cat} will make that clear. If both inflection points persist in both plots, then they are not likely due to substrate binding residues.

Brønsted Analysis

In Figure G.5, I cautioned you not to draw mechanisms in which proton transfer precedes the rate determining step, since that implies the formation of an unstable intermediate prior to catalysis. As with all rules, that one is not universal (though it is a superior initial assumption). In fact, proton transfer can preceed and follow a rate-determining step. Brønsted analysis is used to measure the degree of proton transfer that has taken place in the transition state.

Might get this added by Tuesday...

Appendix G.1

In Figure G.8, the pK_a of the general base can be identified as the pH at which an upward sloping line at low pH intersects a flat line that models the data at moderate pH. The reason this is appropriate can be demonstrated in the following algebra. The fraction of general base in its activated, deprotonated form can be determined as follows (Eq AG.1)

$$\frac{[B]}{[B]_{tot}} = \frac{[B]}{[B]+[BH^+]} = \frac{K_a[B]/[H^+]}{[B]+K_a[B]/[H^+]} = \frac{K_a}{[H^+]+K_a} \qquad \text{(Eq AG.1)}$$

Where [B] is the concentration of activated general base and $[B]_{tot}$ refers to the total concentration of the species in its conjugate acid and base forms. K_a is the acid dissociation constant for the conjugate acid of BH^+.

At low pH, it could be argued that the concentration of H^+ is considerably greater than the K_a of the base's conjugate acid (pH is below pK_a). That yields the following approximation (Eq AG.2).

$$\log_{10}\left(\frac{[B]}{[B]_{tot}}\right) = \log_{10}\left(\frac{K_a}{[H^+]+K_a}\right) \approx \log_{10}\left(\frac{K_a}{[H^+]}\right) = pH - pK_a \qquad \text{(Eq AG.2)}$$

That is, the logarithm of the fraction of deprotonated base will increase linearly with pH (slope of one). Since rate is proportional to the fraction of deprotonated base, it is predicted that the logarithm of the rate will co-vary similarly with pH.

However, above the pK_a, one might assume that the concentration of hydrogen ion is much less than the K_a (pH is greater than pK_a), in which case the fraction of base in its deprotonated form is unity (Eq AG.3), leaving a flat unchanging rate profile at higher pH.

$$\log_{10}\left(\frac{[B]}{[B]_{tot}}\right) = \log_{10}\left(\frac{K_a}{[H^+]+K_a}\right) \approx \log_{10}\left(\frac{K_a}{K_a}\right) = 0 \qquad \text{(Eq AG.3)}$$

Since a line is predicted at pH values below the pK_a and a different line is predicted above the pK_a, where they intersect should indicate the pK_a.

I leave it as an exercise to show that the same will be true for the deprotonation of a general acid.