BCH-202 ENZYMOLOGY (BCH-2002)

Unit II

Enzyme Kinetics

Lecture material (28.04.22)

pH Dependency of Enzyme Activity

Most enzymes are active within only a narrow pH range, typically 5 to 9. This is a result of the effects of pH on a combination of factors: (1) the binding of substrate to enzyme, (2) the ionization states of the amino acid residues involved in the catalytic activity of the enzyme, (3) the ionization of the substrate, and (4) the variation of protein structure (usually significant only at extremes of pH).

The rates of many enzymatic reactions exhibit bell-shaped curves as a function of pH. For example, the pH dependence of the rate of the reaction catalyzed by fumarase produces the following curve:

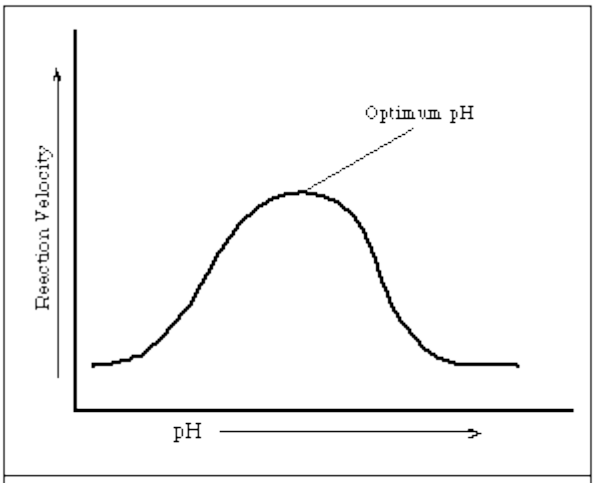


FIGURE 14
Effect of pH on reaction rate

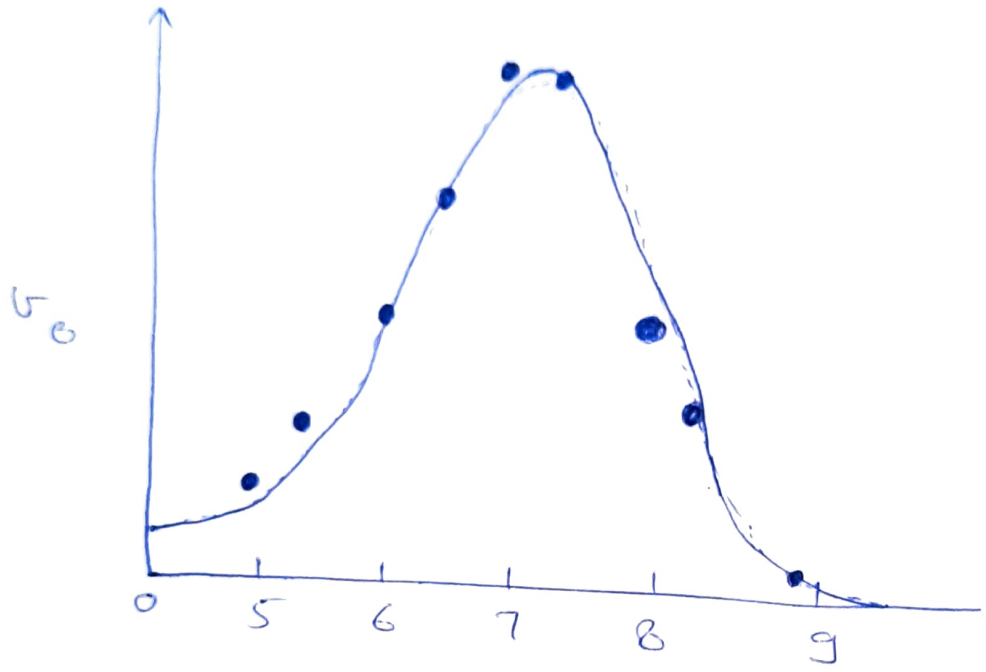
Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

The observed pK's (the inflection points of the curve) often provide valuable clues to the identities of the amino acid residues essential for enzymatic activity. For example, an observed p K of ~4 suggests that an Asp or Glu residue is essential to the enzyme. Similarly, pK's of ~6 or —10 suggest the participation of a His or a Lys residue, respectively. However, the pK of a given acid-base group may vary by as much as several pH units from its expected value, depending on its microenvironment (e.g., an Asp residue in a nonpolar environment or in close proximity to another Asp residue would attract protons more strongly than otherwise and hence have a higher p K). Furthermore, pH effects on an enzymatic rate may reflect denaturation of the enzyme rather than protonation or deprotonation of specific catalytic residues. The replacement of a particular residue by site-directed mutagenesis or comparisons of enzyme variants generated by evolution is a more reliable approach to identifying residues that are required for substrate binding or catalysis.

In addition to pH, other environmental factors, such as temper¬ ature and salt concentration, affect enzyme catalysis, typically producing bell-shaped activity curves as shown here for pH. The optimal conditions (the peak of the activity curve) frequently corre¬ spond to the prevailing environmental conditions of the cell or organism, indicating that evolution has fine-tuned enzymes for maximum efficiency.

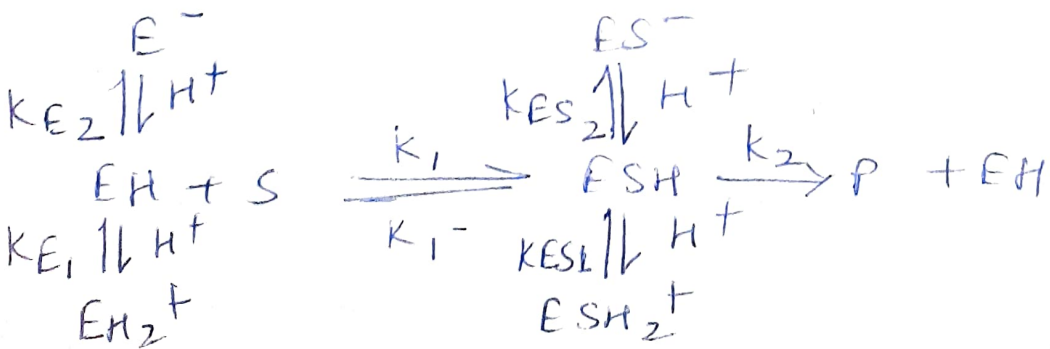
pH dependence of Simple Michaelis Menten Enzyme

The initial rates for many enzymatic reactions exhibit bell-shaped curves as a function of pH



The effect of pH on the initial rate of the reaction catalyzed by the enzyme fumarase

These curves reflect the ionizations of certain amino acid residues that must be in a specific ionization state for enzyme activity. The following model can account for such pH effects.



In this expansion of the simple one-substrate - no back reaction mechanism, it is assumed that only EH and ESH are catalytically active.

The Michaelis - Menten equation for this model, which is

$$v_0 = \frac{V'_{max}[S]}{K'_M + [S]}$$

Here the apparent Michaelis - Menten parameters are defined

$V'_{max} = V_{max}/f_2$ and $K'_M = K_M(f_1/f_2)$

where

$$f_1 = \frac{H^+}{K_{E_1}} + 1 + \frac{K_{E_2}}{[H^+]}$$

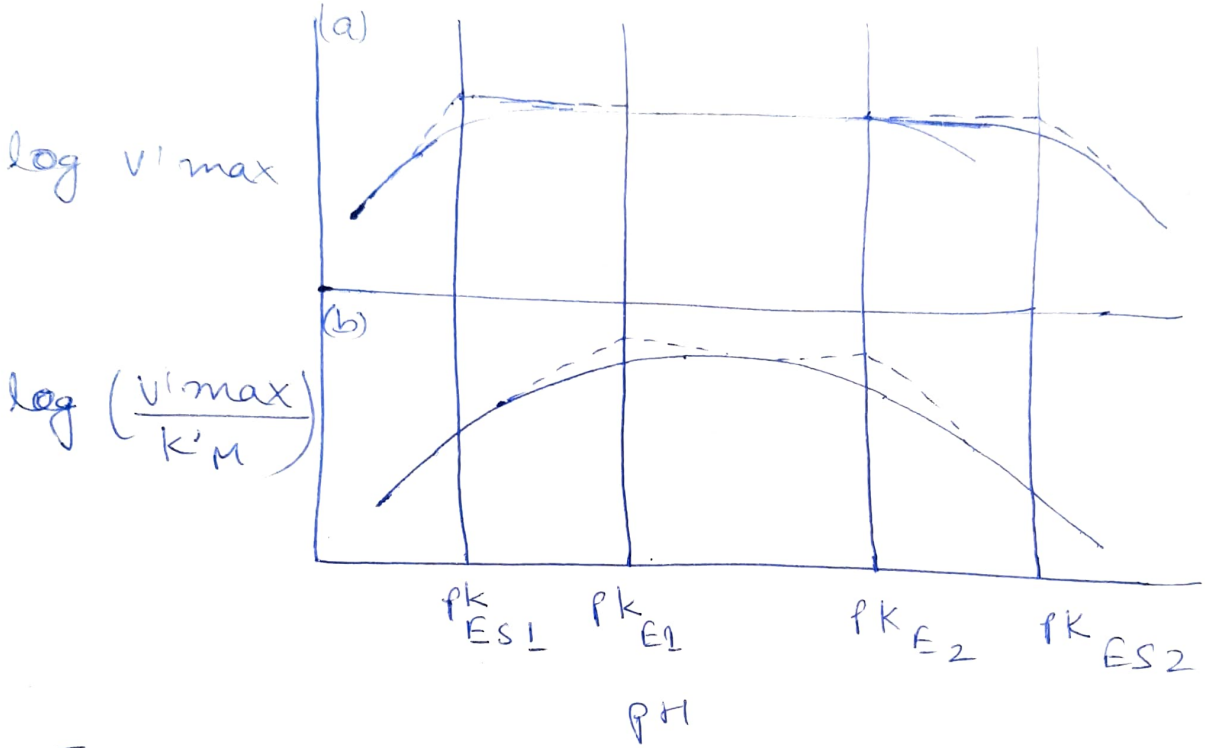
$$f_2 = \frac{[H^+]}{K_{ES_1}} + 1 + \frac{K_{ES_2}}{[H^+]}$$

and V_{max} and K_M refer to the active forms of the enzyme, EH & ESH. At any given pH the eq. $\left[v_0 = \frac{V'_{max}[S]}{K'_M + [S]}\right]$ behaves as a simple Michaelis - Menten eq., but because of the pH dependence of f_1 and f_2, V_0 varies with pH in a bell-shaped manner.

Evaluation of Ionization constants:

The ionization constants of enzyme that

of the curves of log V'max versus pH, which provides values of K_{ES1} & K_{ES2} and of log (V'_{max}/K'_M) versus pH which yields K_{E1} and K_{E2}.



The pH dependence of (a) log V'max and (b) log V'_{max}/K'_M illustrating how the values of the molecular ionization constants can be determined by graphical extrapolation.

This of course, entails the determination of the enzyme's Michaelis - Menten parameters at each of a series of different pH's.

The measured pk's often provide valuable clues as to the identities of the amino acid residues essential for enzymatic activity. For example, a measured pK of ca 4 suggests (a)

Similarly, pK's of ~6 or ~10 suggest the participation of a His or a Lys residue, respectively. However, a given acid - base group may vary by as much as several pH units from its expected value as a consequence of the electrostatic influence of nearby charged groups, as well as of the proximity of regions of low polarity. e.g. the carboxylate groups of a Glu residue forming a salt bridge with a Lys residue is stabilized by the nearby positive charge and therefore has a lower pK than it would otherwise have that is, it is more difficult to protonate. Conversely, a carboxylate group immersed in a region of low polarity is less acidic than normal because it attracts protons more strongly than if it were in a region of higher polarity. The identification of a kinetically characterized pK' with a particular amino acid residue must therefore be verified by other types of measurements such as the use of group-specific reagents to inactivate a putative essential residue.

Intramolecular reactions