

FACTORS AFFECTING **ENZYME ACTIVITY**

B Sc 3rd SEMESTER

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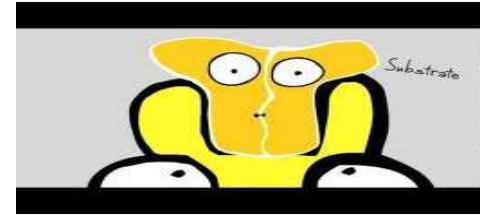
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ENZYME ACTIVITY



Enzyme activity occurs within the confined pocket on the enzyme and this is known as the active site . The molecule that is bound by the active site and acted upon by the enzyme is known as the substrate . Thus formed an intermediate complex known as the enzyme-substrate complex.

contd



This complex serves as the starting point for defining the kinetic behaviour of enzyme activity.

Enzyme being a biological catalysts, thus its main function is to increase the rate of reaction. The study of this rate of enzyme catalyzed reaction is termed as enzyme- kinetics. Thus enzyme-kinetics is that branch of enzymology dealing with factors affecting enzyme activity.

FACTORS

The major factors influencing enzyme activity are , viz ,

- 1.[E]
- 2.[S]
- 3.pH
- 4.Temp

[E]

As is true for any catalysts , the initial rate of enzyme activity depends directly on [E]. This initial rate is the rate measured before sufficient product formation.

Thus the conc of enzyme has a direct role on activity .

[S]

The substrate saturation curve for an enzyme catalyzed reaction show that with a fixed [E] and an increased in [S] will result to a rapid rise in the reaction rate or initial velocity designated as V_o .

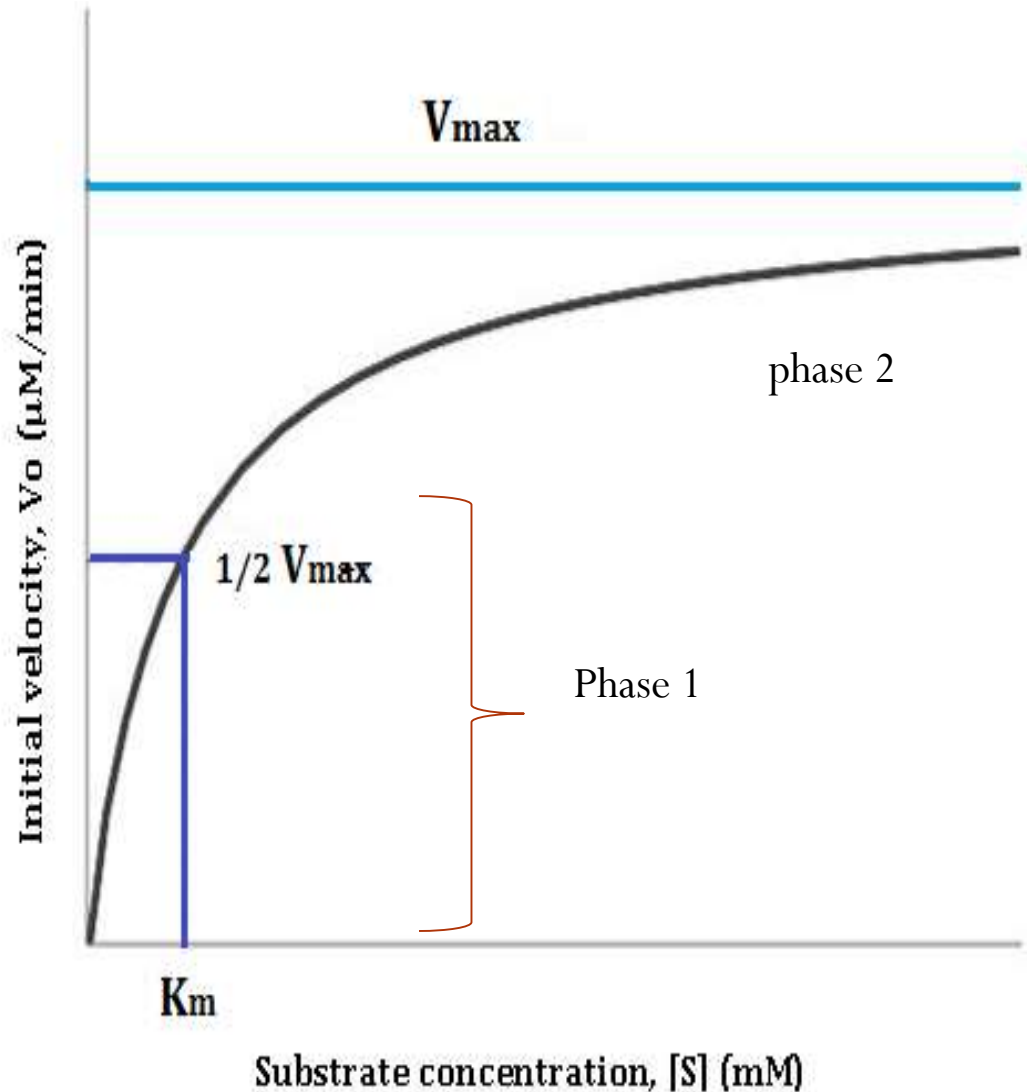
As the [S] continues to increased however the increase in the reaction rate begins to slow down , until no further change in velocity is observed .At this point all the active site of the enzyme becomes saturated with the substrate . But the rate of reaction will further increase if more enzyme is added .The point at which there is no further change in velocity $V_o = V_{max}$

contd

The fig drawn shows the S saturation curve .

As seen from the graph ,the enzyme catalyzed reaction is di-phasic ,ie ,it consists of two phases , viz ,

- 1)Phase I
- 2)Phase II



Phase I

This phase obeys the 1st order kinetics, since the rate of enzyme activity varies with the $[S]$, ie, at low $[S]$, V_o is directly dependent on $[S]$. At this phase the active site of the enzyme molecule is not saturated with the substrate.

Phase 2

This phase obeys the zero order kinetics ,since the rate of enzyme activity does not vary with the $[S]$, V_o does not increased as $[S]$ increases . V_o becomes virtually independent to $[S]$ and approaches the maximal limit . Since rate no longer depend on $[S]$ thus the enzyme activity obeys the zero order kinetics at this point . Here v directly depend on $[S]$.

contd

The curve thus gives an initial clue that an enzyme interact directly with its substrate by binding it .

This curve is termed as the Michaelis – Menten curve.

contd

This kinetic pattern of the graph led Victor Henri to propose in 1903 that enzymes combine with its substrate molecule so as to form the ES complex. This idea was being extended by Michaelis and Menten in 1913 into the general theory of enzyme action or enzyme activity . They postulated that ,

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1) Enzyme first combine reversibly with its substrate thus forming an ES complex.



2) ES complex then break down yielding the free Enzyme and Product.



contd

3) The enzyme exists in two forms at any instant of enzyme-catalyzed reaction, i.e., the free enzyme and the bound enzyme.

At low $[S]$ ----- E remain free as E

At high $[S]$ ----- E remain bound as ES

The state in which the E react with the S so as $[ES]$ is being built up this state is termed as the Pre-Steady state .

contd

The state in which the $[ES]$ remain constant is termed as the Steady state.

Michaelis and Menten concerned their analysis with this Steady state rate known as the Steady state kinetics.

The curve expressed the relationship between the enzymatic-reaction rate and $[S]$ and the curve is always hyperbolic in shape.

contd

The curve can be expressed algebraically and such expression gives the equation termed as the Michaelis Menten equation

$$V_o = \frac{V_{\max} [S]}{K_m + [S]}$$

This is termed as the Michaelis Menten equation.

pH

Enzyme substrate recognition and the catalytic events that takes place are greatly dependent on pH . An enzyme possesses an array of ionizable side chains and prosthetic groups that not only determine its structure but may also be intimately involved in its active site . Also the substrate itself often has the ionizing groups ,and one or more of the ionic form may preferentially interact with the enzyme.

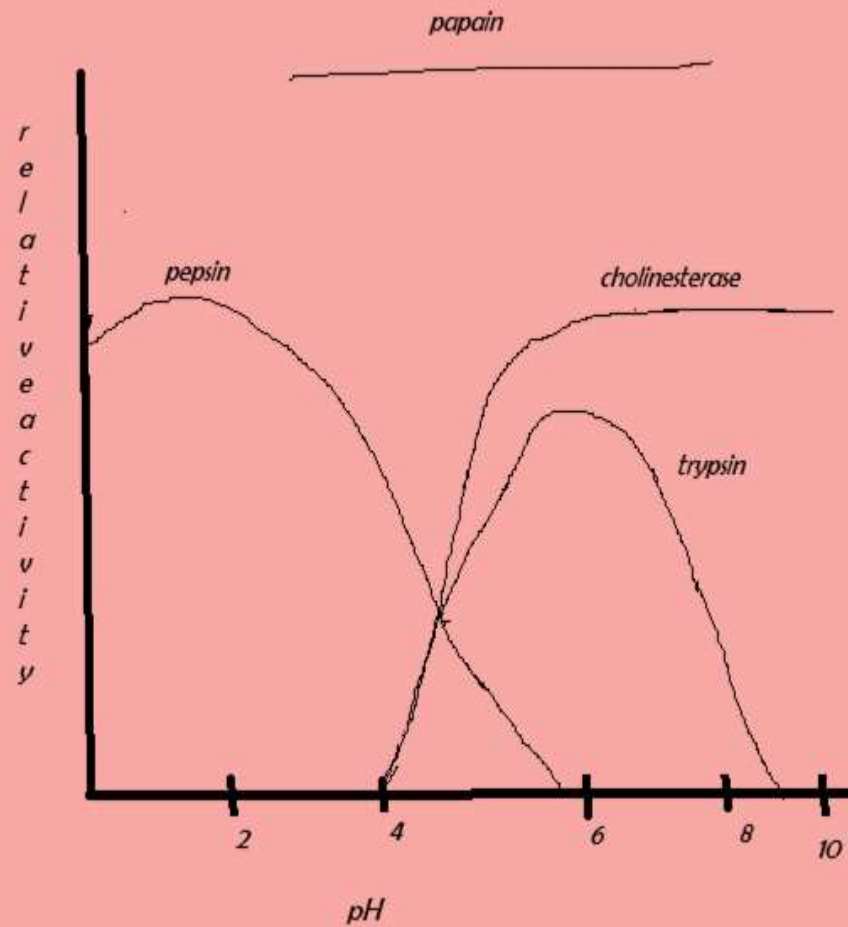
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Thus the active site must be in proper ionic form so as to maintain the conformation of the active site , bind the substrate and catalyzed the reaction .

But enzymes in general are active only over a limited range of pH and most have a particular pH at which their catalytic activity is optimal .

contd

The fig illustrates the relative activity of four enzymes as a function of pH . Although the pH optimum of an enzyme often reflects the pH of its normal environment ,but the optimum may not be the same .This differences suggest that the pH-activity response of an enzyme may be a factor in the intracellular region of its activity.



pH activity profile of four enzymes

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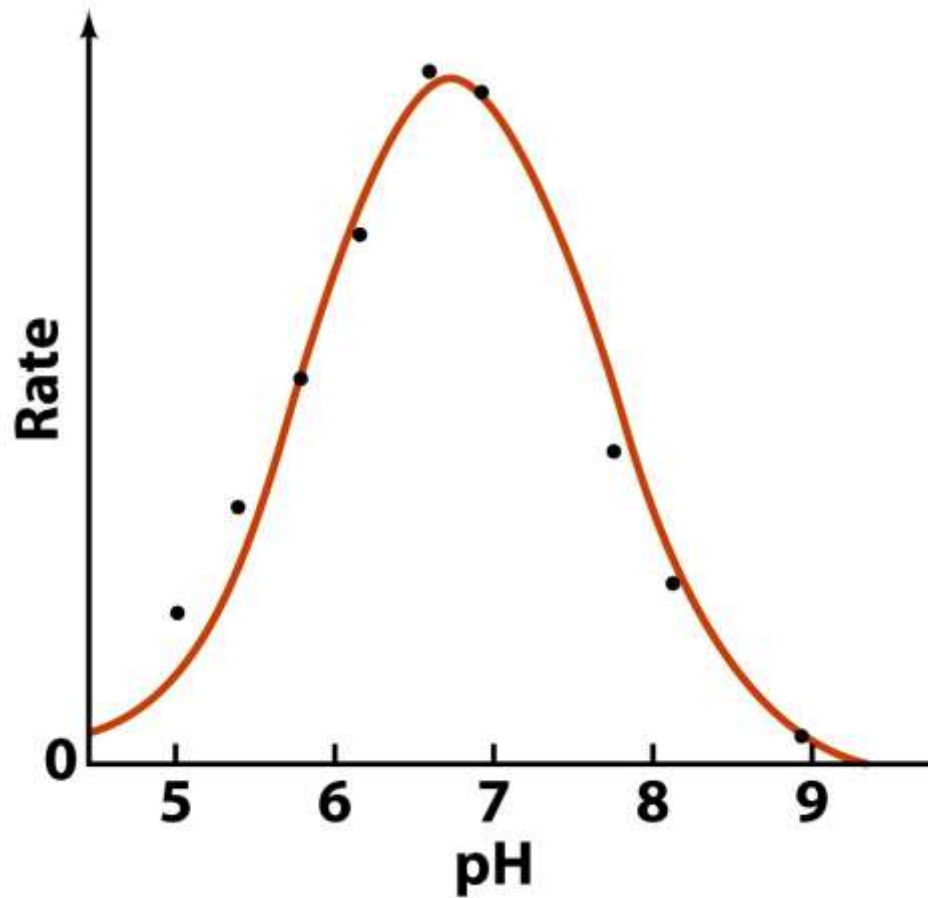
From the fig we see that :

- 1) Trypsin an intestinal protease has a slightly alkaline pH optimum.**
- 2) Pepsin a gastric protease has pH optimum near 2.**
- 3) Papain a protease found in papaya is insensitive to pH between 4-8.**
- 4) Cholinesterase activity is pH sensitive below pH 7.**

contd

- **Effects of pH on Enzyme Activity**
- **Most enzymes are active only within a narrow pH range of 5-9.**
- **Reaction rates exhibit bell-shaped curves in dependence of pH (reflects ionization state of important residues)**
- **pH optimum gives information about catalytically important residues, if 4/5 \rightarrow Glu, Asp; 6 \rightarrow His, 10 \rightarrow Lys**

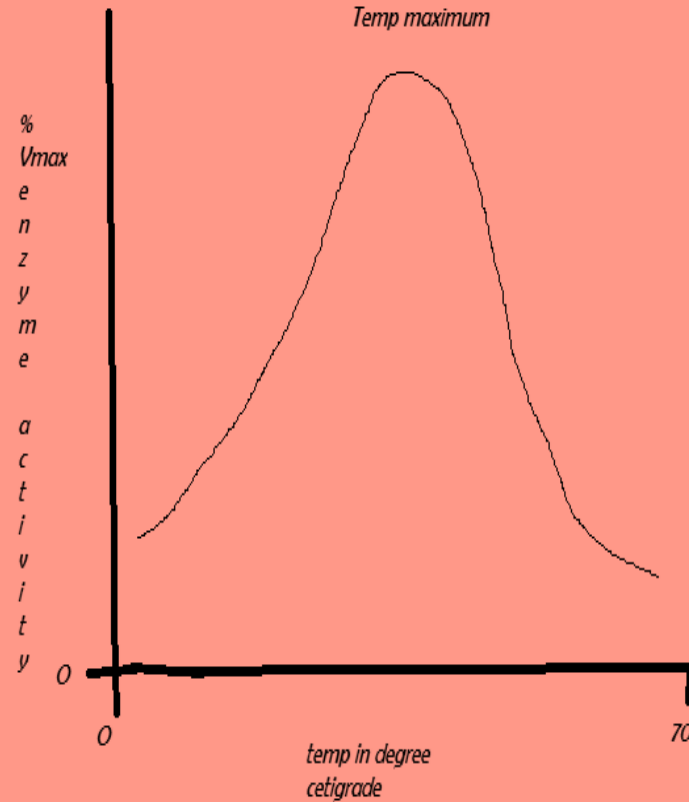
pH Optimum of Fumarase



Temp

Like in most chemical reactions ,the rate of enzyme catalyzed reaction generally increased with increasing temp .But this holds only for a limited range of temp as shown in the fig .

Effect of temp on enzyme activity



THINK POSITIVE

Say to yourself every morning:

- Today is going to be a great day!*
- I can handle more than I think I can!*
- Things don't get better by worrying about them!*
- I can be satisfied if I try to do my best!*
- There is always something to be happy about!*
- I'm going to make someone happy today!*
- It's not good to be down!*
- Life is great, make the most of it!*

BE AN OPTIMIST!

