UNIT-II Part-2

Enzymes as Biocatalysts

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Q1. What is a catalyst? Explain the mechanism of enzyme action.

Ans. -A catalyst is a chemical species which enhances the rate of a reaction without undergoing any change itself.

-It decreases the E_{at} (Activation energy)

-It decreases the E_{at} for both the forward and backward reaction. So a catalyst decreases the time taken to reach the equilibrium condition.

- It alter the path is one which has lower E_{at}.

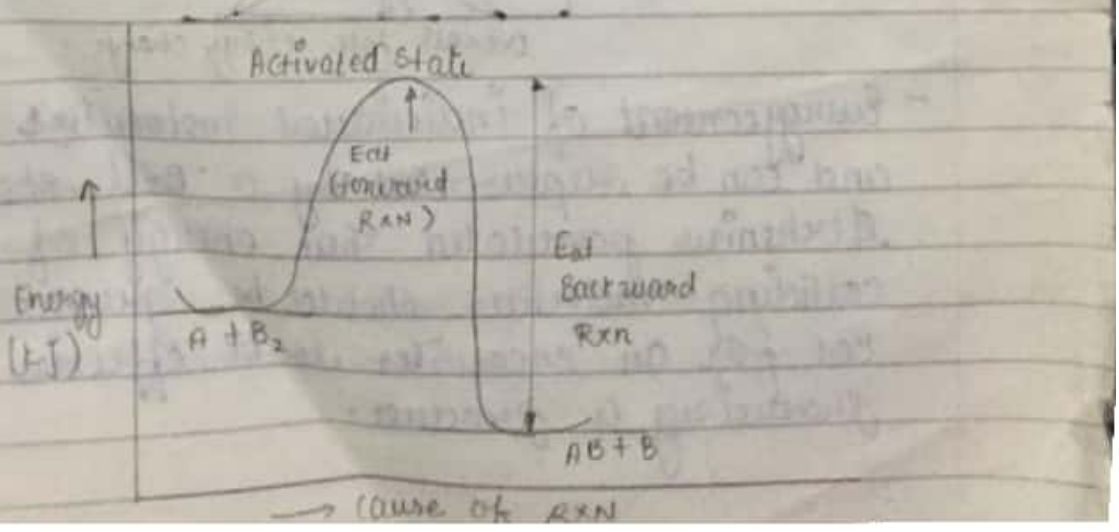
- A catalyst speeds up the attainment of the equilibrium

eg:- $N_{2(g)} + 3H_{2(g)} \xrightleftharpoons[\uparrow T, \uparrow P]{Fe^{2+}} 2NH_{3(g)}$ {Haber's process}

eg: $\underbrace{A_{(g)} + B_{2(g)}}_{300KJ} \xrightleftharpoons{50KJ} \underbrace{AB + B}_{250\ KJ} + \underset{50KJ}{\text{Energy}} \uparrow$

$A - - - - - + B - B \longrightarrow A - - - B - - - B$

$A - B - B \longrightarrow A - B + B + E \uparrow$



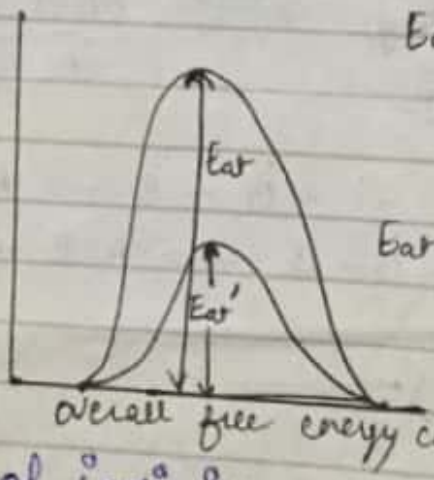
E_{at} (Activation Energy) :- It is the amount of energy in calories, required & being molecules in 1 mole of substance (substate) at a given temperature is the activated state / Transch ionstate

It is the barrier in energy, that has to be overcome before the reactants convert into products

Rate of Reaction $\propto$ conc of Transistion state / Activated state species.

(i.e How many molecules have enough E is cross the barrier)

Transition temperature: It is the temperature sufficient to bring the reactants to the top of the energy hill to reactive state



E_a → activation energy of uncatalyzed reaction.

E_a' → activation energy of enzyme catalysed reaction

- Energy content of individual molecules vary and can be represented by a Bell shaped curve Arrhenius postulated that energy of the colliding molecules should be greater than the Ea for an encounter is be effective in producing a product.

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NOMENCLATURE & CLASSIFICATION OF ENZYMES

Enzymes were given trivial names

- consist of suffies (-ase) added is substrate OR
- implied something about the reaction catalyzed
- Proteolytic enzymes ended with (-in) eg- trypsin, pepsin

European Commission

E C gives a ~~Synth~~ systematic name than a trivial names now enzymes are given International names, ending in ase and a classification numbers.

The classification no. has 4 digits. The nomenclature is recommended by E.C set up in (1954) by IUBMB (International Union of Biochemistry & mol. Biology).

		a	b	c	d
eg	E.C	2.	7.	1.	1

(a)- Type of reaction catalysed.
2. stands for transferases

(b)- Indicates subclass. (type of substrate / bond cleaved)
7. stands for subclass phosphotransferase.

c- Indicates sub-subclass (type of e^- acceptor or group removed)

1. Stands for phosphotransferase with $-OH$ group as acceptor.

d- Serial no. of enzymes in sub-subclass

1. Stands for D-glucose as PO_4^{3-} group acceptor.

Classification no. — Class — function

1. $CH_3-CH(OH)-COO^-$ (Lactate) $\rightleftharpoons$ $CH_3-C(=O)-COO^-$ (Pyruvate)

OXIDOREDUCTASES: $NAD^+ \curvearrowright NADH + H^+$ ⇌ $NAD^+ \curvearrowleft NADH + H^+$

- catalyzes redox reactions.

2. $(dNHP)_n + dNTP$ ⇌ $(dNMP)_{n+1} + PP_i$

Transferases: DNA polymerase

Transfers functional group from (molecule) to another.

3. Acetyl choline + $H-OH$ → acetate + choline

Hydrolases

Function: cleavage of bonds by hydrolysis

Lyases

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4. $CO_2 + H_2O \rightleftharpoons H_2CO_3$ (carbonate hydrolase)
carbonic acid

function :- formation of double bonds by removal of groups or addition of group to double bonds.
(Removal of a group from substrate (not by hydrolysis) often leaving = bonds.

ISOMERASE

5. $CH_2-O-PO_3^{2-}$
$C=O$
CH_2OH
DHAP

$\rightleftharpoons$ Triose Phosphate Isomerase

$CH_2-O-PO_3^{2-}$
$CH-OH$
$C=O$
H
GAP

function : Transfer of groups within the molecule to yield isomeric forms.

LIGASES

6. $CH_3-\underset{\underset{O}{\|}}{C}-COO^- + CO_2 \rightleftharpoons$ (Pyruvate carboxylase, ATP) $^-OOC-CH_2-\underset{\underset{O}{\|}}{C}-COO^-$
oxaloacetate

- Synthetic joining of 2 molecules coupled with breakdown of pyrophosphate bond in NTP/ATP
- Formation of C-C, C-S, C=O, & C-N bonds by condensation of ATP.

The EC divided enzymes into 6 main classes, on the basis of total reaction catalyzed. Each enzymes was assigned code no. consisting of 4 elements, separated by dots.

Imp° Enzyme Kinetics

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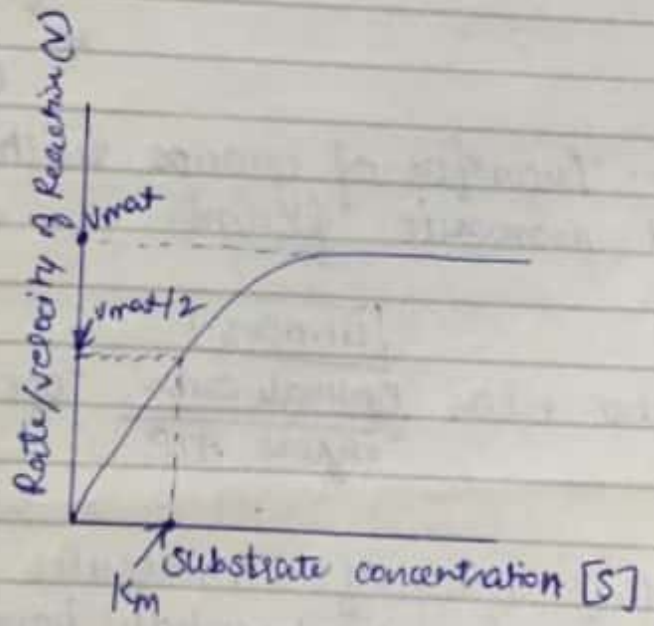
Michaelis - Menten equation

In 1913, a general theory of enzymes action & was developed by michaelis & menten. (The Michaelis - menten theory assumes that the enzymes E just combine with substrate S to form an ES complex which later breakes down in the the second step is free enzyme & product P)

The equation expressess the mathemetical relationship between the initial rate of the enzyme catalyzed reaction, the conc. of substrate and certain characteristic of the enzymes.

Michaelis, menten equation is the rate equation for reaction catalyzed by enzymes having a single substrate.



① Km or Michaelis Menten constant: Substrate concentration at which rate of the reaction is half of its maximum value.

② Vmax: Maxium velocity or Max. rate of reaction: which is achieved when whole enzyme is saturated with substrate i.e. when $[E_0] = [ES]$.

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Enzyme Kinetics

$$E + S \underset{K-1}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$$

Equilibrium between E, S & ES complex, at steady state. Rate of formation of ES = Rate of breakdown of ES.

$$K_1[E][S] = K_{-1}[ES]$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1}}{K_1} = K_s \quad [\text{Diss. const. of ES complex}]$$

$$\frac{[E][S]}{[ES]} = K_s$$

$$E_0 = E + ES$$

$$\frac{([E_0][S] - [ES][S]}{[ES]} = K_s$$

$$[E_0][S] - [ES][S] = K_s[ES]$$
$$[E_0][S] = K_s[ES] + [ES][S]$$
$$[E_0][S] = [ES](K_s + [S])$$
$$\frac{[E_0][S]}{K_s + [S]} = [ES]$$

But rate of formation of product;

$$V_0 = K_2[ES]$$
$$V_0 = K_2 \frac{[E_0][S]}{K_s + [S]}$$

But when substrate is ↑, $[ES] = [E_0]$ [∵ All enzymes is bound]

$$V_{max} = K_2[E_0]$$

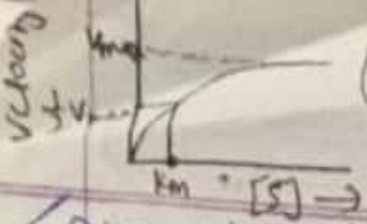
$$\Longrightarrow \boxed{V_0 = \frac{V_{max}[S]}{K_s + [S]}}$$

Michaelis - Menten Equation

When $V_0 = \frac{1}{2}V_{max}$

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}, \text{ or } 2[S] = [S] + K_m$$

or $[S] = K_m$



(8) K_m = Michaelis Menten constant : substrate concentration at which velocity of the reaction is half of its max value.

Imp multiple factors affect the Rate of Enzymes catalyzed Reaction.

1- Temperature :- The rate of enzymes catalyzed reaction generally increase with temp, within the temp range in which enzyme is stable & retain full activity while raising temperature increases the rate of an enzyme-catalyzed reaction; this holds only over a strictly limited range of temperatures.

The reaction rate initially increases as temperature rises leading its increased function energies (E_K) of the reacting molecular.

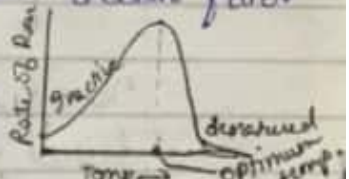
Eventually however, the kinetic energy of the enzyme exceeds the energy barrier for breaching the weak hydrogen & hydrophobic bonds that maintain its 2° & 3° structure.

At this temperature, denaturation with an accompanying loss of catalytic activity predominate. Enzyms from human with body temp 37°. generally exhibit stability upto 45-55°C.

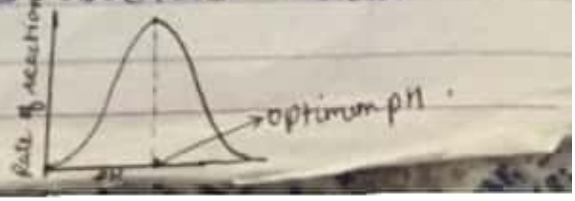
Enzymes from microbes that inhabit natural hot springs or hypothermal vents on ocean floor may be stable upto / above 100°C

$Q_{10} = 2$

Imp.



2. PH : Most enzymes have a characteristic PH at which their activity is maximal; above or below this pH the activity declines. when an enzymes activity is measured at several PH values, optimal activity typically is observed between



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PH values of 5 and 1 few enzymes (eg-pepsin) are active at ph value outside this range.

The PH determines the:

1. Enzymes denature at high or low PH

2. Alteration in charged state of enzyme or substrate.

PH can change structure of enzymes or change charge on residue that binds substrate

At low PH,

$Enz^- + Sn^+ \longrightarrow En\,SH$

Enzymes lose its charge. $E^- + H^+ \longrightarrow EH$

At high PH

SH^+ ionizes and loses its '+ ve' charge

$SH^+ \longrightarrow S^* + H^+$

3. Enzyme conc: Initial rate is proportional to Enzyme ~~and~~ concentration.

Note: This holds only for initial rates.

4. Substrate concentration: If substrate conc. [S] is increased while all other conditions are kept constant, the initial velocity (V_0) increase is a maximum value of Vmax & no further.

The velocity increases upto a point where the enzymes is said to be "saturated" with substrate is Es and is later unaffected by further increases in substrate conc.

perties / Characteristics of Enzymes: 10

1. All enzymes are proteins (except Ribozymes)
2. They retain their identity at the end of the reaction.
3. Extremely small concentration of enzymes are capable of bringing about measurable changes.
4. Enzymes' activity is maximum at a particular temperature called optimum temperature.
5. They are sensitive to change in pH of reaction medium and their activity is max at optimum pH.
6. They are specific in their action.
7. Many enzymes require non-protein compores called cofactors for activation.
8. Most of the enzymes catalyze both forward & backward reaction.
9. They accelerate the rate of the reaction by reducing the activation energy.
10. They do not affect the amount of energy released or absorbed during the reaction.

Coenzymes and cofactors:

- Many ~~prot~~ enzymes require a non-protein component called a co-factor for their activity. In this case, the inactive component of an enzyme (lacking co-factor) is called apoenzyme whereas the active component of enzyme, containing the cofactor is called holoenzyme.

Cofactor is categorised into following types:

(a) Prosthetic group: When a cofactor is so tightly bound that it is difficult to remove without damaging the enzyme it is called prosthetic group.

for eg. Porphyrin moiety of peroxidase.

(b) Metal ions: Generally, they participate in catalytic mechanism but some are necessary for maintaining the proper conformation of enzyme. They may be either loosely / tightly bound.

for eg. Carboxypeptide requires Zn^{+2}

DNA polymerase requires Mg^{++}

(c) Coenzyme: When a cofactor is an organic molecule it is called coenzyme. They are less tightly bound.

eg FAD (Flavin adenine dinucleotide)