

Active site of an enzyme.

The active site of an enzyme is that region of the enzyme where catalysis takes place. It is also the region that binds the substrate and contributes the residues that directly participate in the making and breaking of bonds.

Some features of active site are:

- (i) it is a relatively small portion of the total enzyme volume
- (ii) it is a three dimensional entity
- (iii) substrate binds with relatively weak forces
- (iv) it is a cleft or crevice
- (v) the specificity of binding depends on the precisely defined arrangement of atoms in an active site

Note that: the interaction of substrate and enzyme could be expressed in terms of two models named:

- (i) lock and key model
- (ii) induced fit model.

Enzyme Kinetics

Michaelis and Menten derived equation for enzyme catalyzed reaction involving a single substrate and single product thus:



as

$$v = V_{\max} \times [S] / K_m + [S]$$

where v = initial velocity

V_{\max} = maximum velocity

$[S]$ = substrate concentration

K_m = Michaelis-Menten constant.

Note that: any enzyme that obeys M-M equation will give a hyperbolic curve when the plot of v vs $[S]$ is made.

Significance of K_m and V_{\max} .

K_m is the substrate concentration at half the maximum velocity. It is a measure of affinity of an enzyme for substrate i.e the higher the K_m the lower the affinity and vice versa.

V_{max} is used to express the efficiency of an enzyme operation i.e often used to compare the catalytic efficiency of different enzyme.

ALLOSTERIC ENZYMES

These are regulatory enzymes that functions through reversible non-covalent binding of a modulatory molecule. They usually determine the rate of overall sequence of reaction because they catalyze the committed/slowest step. Such enzyme is usually the first in the sequence of a multienzyme reaction system. They are known to have the following properties:

- (i) They have both catalytic and regulatory sites for binding of substrate
- (ii) Generally larger and more complex than the simple enzyme
- (iii) Shows deviation from classical M-M behaviour in that they give sigmoidal curve for the plot of v vs $[S]$.
- (iv) They undergo conformational changes in binding of modulatory molecule
- (v) They may be inhibited by their modulator (-ve modulator) or stimulated by modulator (+ve modulator)

CO-ENZYMES.

These are additional non-protein part of an enzyme that is required for enzymatic activities. Inorganic forms of coenzyme are called cofactors. Tightly forms of coenzyme are called prosthetic group.

The role of a cofactor is either:

- (i) to alter the three-dimensional structure of the protein and/or the bound substrate in order to activate the interaction of the enzyme with its substrate
- (ii) to actually participate in overall reaction as another substrate.

Different types of coenzymes, type of reaction and group transfer are given below

COENZYMES	TYPE OF REACTION	GROUP TRANSFER
NAD ⁺ /NADP ⁺	oxidation-reduction	hydrogen (electron)
FAD, FMN	oxidation-reduction	hydrogen (electron)

Coenzyme A	activation and transfer of acyl group	acyl group
Lipoic acid	acyl group transfer	acyl group
Thiamine pyrophosphate	acyl group transfer	acyl group
Biotin	carbon (iv) oxide fixation	carbon (iv) oxide
Pyridoxal phosphate	transamination	amide (-NH ₂)
Tetrahydrofolic acid	metabolism of one carbon fragment	-CH ₃ , -CH ₂