ENZYMOLOGY

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- Unit-2 Enzyme Inhibition
- Lecture-2
- Content- Reversible and Irreversible enzyme Inhibition

Inhibition

- Inhibitor : An Enzyme inhibitor is a Enzyme compound that decreases or diminish the rate or velocity of an enzyme-catalyzed reaction by influencing the binding of S and /or its turnover number.
- The inhibitor may be organic or inorganic in nature Inhibitors - drugs, antibiotics ,toxins and antimetabolite or natural products of enzyme reaction.

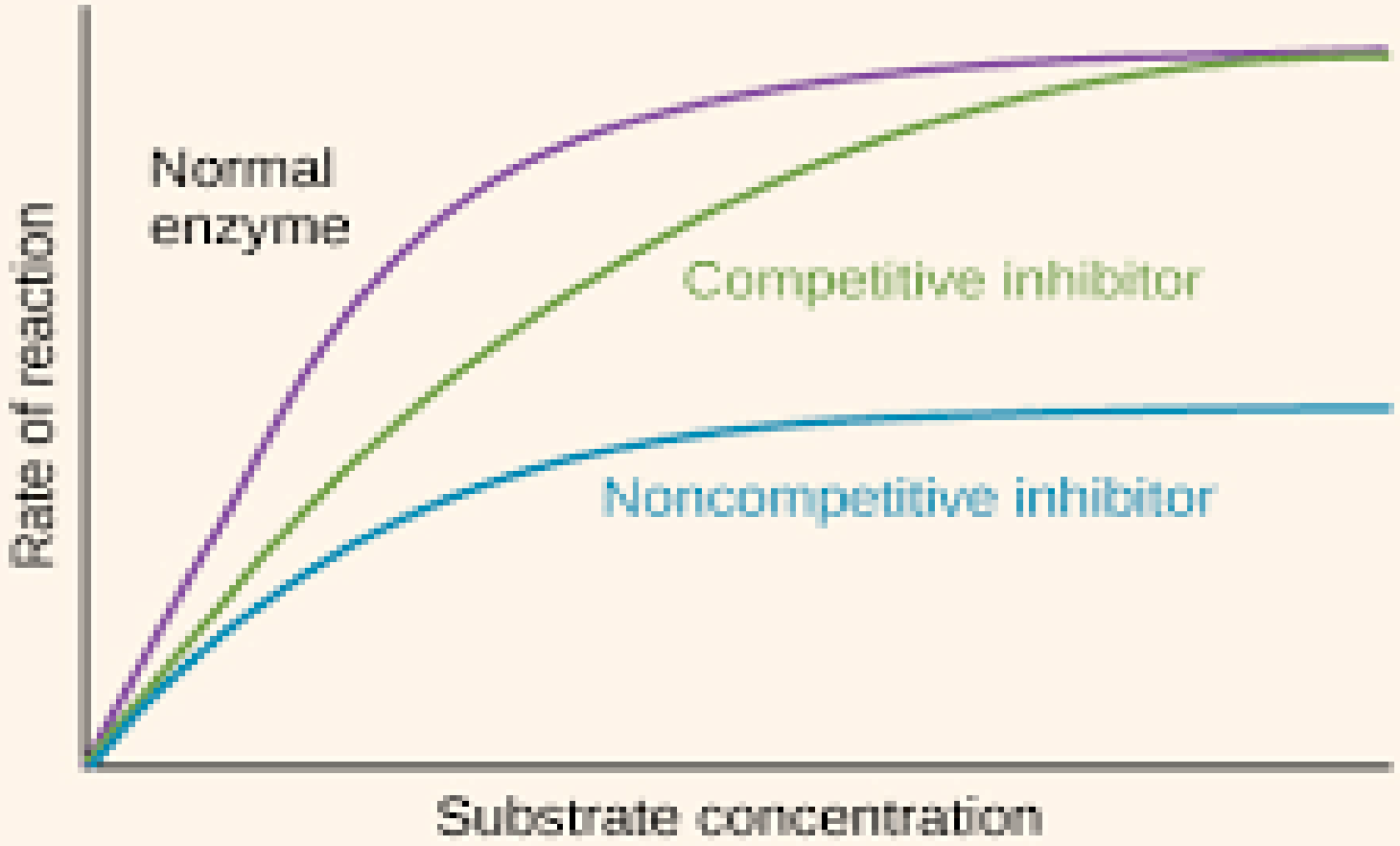
- Type of enzyme Inhibitors
- Competitive Allosteric Suicide Inhibitors
- Non- Competitive
- Uncompetitive
- Reversible Inhibition Inhibitor binds non-covalently (weak interaction) with Enzyme If inhibitor is removed – action of E fully restored - Reversible
- An Equilibrium is established between the free inhibitor & EI Complex and is defined by an equilibrium constant (Ki) E + I E I The activity of Enzyme is fully restored on removing the Inhibitor by dialysis

Reversible Irreversible

Competitive Inhibition

- Inhibitor binds reversibly to the same site that the substrate binds - competes with the S for binding. ☐ Substrate analogue – I closely resembles the S
- I can be reversed by increasing the conc. of S – reversible
- Degree of inhibition - depend on the conc. of S & I and on the relative affinities of the enzyme for S & I

Enzyme Inhibition



- Competitive Inhibition Apo enzyme Velocity is decreased - effective concentration of enzyme is reduced Km is increased -affinity of the enzyme towards substrate is apparently decreased in presence of the inhibitor
- Vmax is not changed No inhibitor Inhibitor Vmax ½ Vmax Km New Km [s] v 1/Vm -1/Km 1/S 1/V No Inhibitor -1/Km Inhibitor
- Malonate is a competitive inhibitor of SDH COOH CH2 CH2 COOH Succinate dehydrogenase FAD FADH2 Succinate COOH H -C C-H COOH COOH CH2 COOH Malonate Fumarate Similarity in three dimensional structure b/w S and I

- Antibacterial action of sulpha drugs (sulfonamide) - structural analog of PABA COOH H2N PABA- para amino benzoic acid NH2
- Bacteria Pteroid synthetase Sulfonamide inhibits the bacterial enzyme O H2N S O Sulfanilamide PABA + 7,8-dihydropteroic acid Folic acid Non toxic to human –human cannot synthesize Folic acid
- Clinically useful Competitive Inhibition Drugs Target Enzyme Therapeutic Use STATINS - Atorvastatin , simvastatin HMG CoA reductase Decrease plasma Cholesterol level
- Antihyperlipidemic agents Allopurinol Xanthine oxidase Gout Methotrexate Dihydrofolate reductase Cancer Captopril & Enalapril Angiotensin converting enzyme High blood pressure Dicoumarol Vit.K-epoxide-reductase Anti-coagulan

- Non-competitive Inhibition Inhibitor binds at a site other than the active site of the enzyme □ I has no structural resemblance to the S – No competition for binding □ Increase in the S conc. does not relieve this I □ I & S binds at different site – formation of both EI and EIS complexes is possible. □ EIS – forms product at a slower rate than ES □ Reaction is slowed down but not halted.
- Non-competitive Inhibition
- Product is formed at slower rate but not E halted I
- Km value is unchanged - I do not interfere with the binding of S to E Vmax decreases - I cannot be overcome by increasing the conc. of S No inhibitor Inhibitor Vmax Vmax i ½ Vmax Km [s]□ v ½ Vmax i Inhibitor No Inhibitor 1 Vmax 1 Km 1/s 1/v -
- Non competitive inhibitor Inhibitor Enzyme inhibited Heavy metals – Ag2+ ,Hg2+ , Pb2+ Binding with cysteinyl SH gr of E Pepstatin Pepsin Soyabean trypsin inhibitor Trypsin Ethanol or narcotic drugs Acid phosphatase

- Uncompetitive Inhibition : binds only to the ES complex , not to free E cause structural distortion of the active site - E catalytically inactive can’t be reversed by increasing the [S] since I doesn't compete with S for the same binding site □Inhibition of placental alkaline phosphatase (Regan iso-enzyme) by phenylalanine . Enzyme Enzyme S Enzyme I S I
- E + S E S E + P + I ESI Inhibitor Vmax Vmax i ½ Vmax Km [s]□ ½ Vmax i Vmax = Decreases Km = Decreases Kmi v I has no affinity for free E No Inhibitor I No I -1/Km 1/v -1/Km 1/Vmax 1/Vmaxi 1/s

- Irreversible Inhibition Inhibitor binds covalently (strong) with the enzyme irreversibly so it can't dissociate from the enzyme Inhibitor cause conformation change at active site of the E- destroying their capacity to function as catalysts. Enzyme activity is not regained on dialysis / by increasing the conc. of S □ A variety of poisons, such as iodoacetate, OP poisoning and oxidizing agents act as irreversible inhibition.
- Irreversible Inhibition In terms of kinetics – irreversible is similar to non competitive inhibition Vmax – Decreased Km – No change Vmax Vmax i ½ Vmax Km [s]□ v ½ Vmax i No inhibitor Inhibitor

Thanks