Lactate dehydrogenase

lactate + NAD+ --> pyruvate + NADH + H+
tetrameric
H-form: aerobic , heart L-->P
M-form: anaerobic , muscle/liver P-->L
Reagents: Lactate,NAD+,Oxidized PMS, Oxidized NBT
Specific Activity stain
LDH-1: pyruvate inhibition
LDH-1/2: 2-hydroxybutyrate as S
LDH-4/5: greater heat stability

Creatine Kinase

Creatine + ATP <--> creatine phosphate + ADP + H+
Dimeric
Cardiac: MM+ **MB** (Myocardial infarction)
Skeletal: MM
Brain: BB

Chymosin (Rennin)

Aspartic protease
Cleave single peptide bond,
release acidic C-terminal peptide
Ca induced aggregation of modified casein micelle--> precipitate as curd

Affinity label

Specific & Irreversible inhibitor
Specificity group & reactive group resembles substrate
TPCK on His-57 of Chymotrypsin

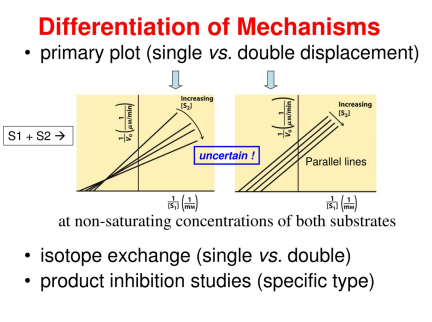
Determination of enzyme activity

NAD+ : absorbance change at 340nm
FAD: absorbance change at 440 nm X

Deter

Follow INITIAL rate, rate drops
1. substrate depletion
2. reverse reaction
3. product inhibition
4. enzyme stability

Differentiation



Homeostatsis(regulation)

1. [S] control, M-M vs Cooperativity
2. Allosteric effect
3. [S]cycle, 2-way, 6-Phosphofructokinase & Fructose bisphosphatase
4. Zymogen activation
5. Covalent modification (phosphorylation,adenylylation,myristoylation,ADP-ribosylation,methylation,acetylation,ubiquitination)
6. Enzyme cascade
7. Cascade amp
8. Enzyme induction/degradation

Modification of amino acids

Active site residues more susceptible
Ser-195 on chymotrypsin by DIPF -->Activity
Modify 1st with [S]/[I] to protect active site, then modify again in absence
ADH inactivated by iodoacetate more than iodoacetamide
2AA involved: pKa >2 units apart:Good
Close--> tVmax never achieved

Lite Beer

Barley:α-amylase cannot break down α-1,6 bond + dextrin --> Yeast
Glucoamylase from Aspergillus niger: break α-1,6 bond, less dextrin

Aspartame

Thermolysin
L-phenylalanine + N-protected L-aspartate
N: benzyloxycarbonyl

Catalysis

1. Strain/Distortion: entropy reduction
2. Acid-Base : carbonic anhydrase
3. Covalent catalysis: serine protease
4. Lower Ea: Zn&Arg127 in carboxypeptidase A stabilize TS

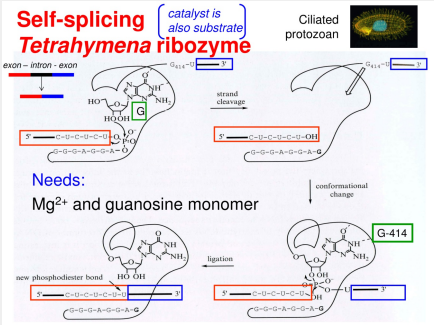
Histidine can be both e^- donor/acceptor
TS analogue: **pyrrole-2-carboxylate** on *proline racemase* as inhibitor
Abzyme: mimic Ferrochelatase

By **iplorip**
cheatography.com/iplorip/

Published 31st October, 2017.
Last updated 31st October, 2017.

Sponsored by **Readability-Score.com**
Measure your website readability!
https://readability-score.com

Ribozyme



RNA less versatile (4 building blocks AUCG)
unable to form large non-polar region
nucleic acid preferred as substrate
RNA susceptible to hydrolysis

Organic Cofactors

Organic Cofactors

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin **Vitamin B_7**
Coenzyme A	Acyl groups **Vit B_5**	Pantothenic acid and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin) **Vit B_3**
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate **Vit B_9**
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Catalytic cofactor: e.g. TPP/FAD
Stoichiometric cofactor: cosubstrate

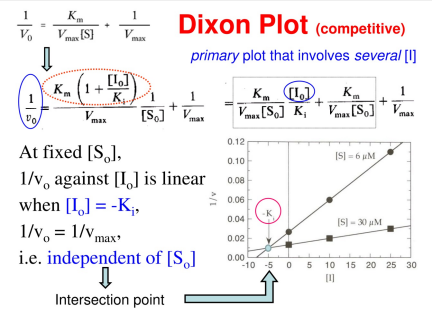
Radioisotope

Glutamate decarboxylase
CO2 : trap gas in alkali
Monoamine oxidase
R-CHO:extracted by ether after acidification (acidified R-NH2 will remain in aq phase)
Cholinesterase
COOH: ion exchange, importance of **label position**

Scintillation Proximity Assay

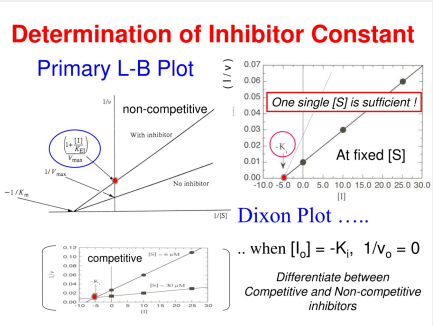
Radioligand stimulate bead to emit light, when in **close proximity**
High affinity capture system: biotinylated substrate & streptavidin-coated beads
NO separation needed. S or P bind to bead

Competitive inhibitor



Same site, mutually exclusive
Vmax unchanged Km increased

Non-competitive inhibitor



ESI present, Km unchanged, Vmax decrease, equal Ki,same % inhib.

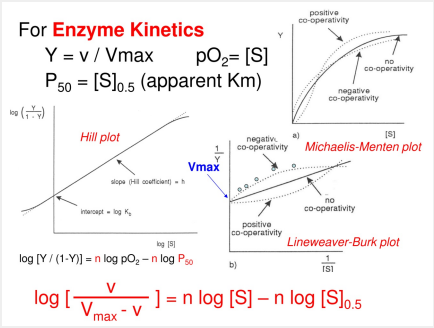
Pre-steady state kinetics

E+S--> ES(flurorescence)
Stopped flow technique, follow time course of fluorescence change

Irreversible inhibitor

Diisopropyl phosphofluoridate (**DIPF**) modifies *serine* on AchE

Hill Coefficient



Important: Choice of [S]
Cooperativity: Same **site&ligand**

Chymotrypsin

Active site **Ser195,His57&Asp102** form charge relay system--> high reactivity of Ser195
Selective for carboxyl side of aromatic or large hydrophobic residue(Met)
Biphasic kinetics:
1. Burst phase: covalent complex
2. SS Phase: hydrolysis+ recovery
Double displacement, p-nitrophenolate

Sponsored by **Readability-Score.com**
Measure your website readability!
https://readability-score.com

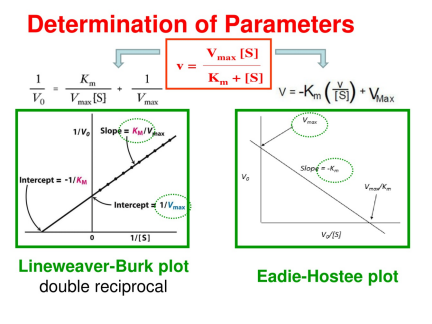
Cofactor

Apoenzyme	inactive form	
Cofactor	non-protein	
Holoenzyme	active	
Metalloenzyme	participate in reaction(lewis acid)	Zn in carbonic anhydrase
Metalloenzyme	stabilize transition state	Zn in carboxypeptidase A
Metal activated enzyme	maintain active conformation	K+ in pyruvate kinase
Metal activated enzyme	form substrate complex bridge	Mg in kinase
Prosthetic group	tightly bound	NH2 on pyridoxal phosphate of aspartate transaminase
Coenzyme	loosly bound	
Cosubstrate(coenzyme)	convert to product after Rx	NAD+

Coenzyme analogue as drug

Drug	Analogue of	enzyme inhibited	MOA & Use
Sulfonamide	PABA	dihydropteroate synthase	folic acid synthesis,Abx
Methotrexate	Folate	dihydrofolate reductase	THF synthesis,childhood leukemia

Plot



Uncompetitive inhib.

S binding to E--> expose site for I binding
both Km Vmax decrease to same extent, ESI present, same slope

Mixed inhibition

Binding affinity(Ki) not the same
Vmax decrease, Km can in/decrease

Suicide substrate

P irreversibly bind to E
Deprenyl on MAO on Flavin prosthetic group

Substrate inhibition

High [S] favour ESS(nonproductive binding)
e.g. succinate dehydrogenase (select points for drawing)

Single displacement Rx

Random sequential	creatine kinase
Compulsory order	ADH(NAD^+ bind 1st)

Ternary complex present

Double displacement Rx (Ping-Pong)

aspartate transaminase
aspartate+ α-ketoglutarate -->oxaloacetate+ glutamate (NH2 displaced)

Isotope exchange

Occurs only in double displacement; exception: maltose phosphorylase
isotope from 1st P back to 1st S in absence of 2nd S e.g. sucrose phosphorylase
Glu-Fru + Fru* <--> Glu-Fru* + Fru

Diff. subunits of multimeric enzyme

Catalytic & Regulatory	Aspartate transcarbamoylase(ATCase)	ATP&CTP
2nd unit modify specificity	Lactose synthase	α-lactalbumin
2 diff. cat. units	tryptophan synthase(α2β2)	Tunnel connect active sites

Lysozyme

hydrolyze glycosidic bond bet C-1 of NAM and C-4 of NAG, **Non-identical site**
Site of cleavagea: bet D&E, distorted Ring D
Glu-35 as acid, H^+ to O of glycosidic bond
Carbonium cation stabilized by
1. -ve charge on Asp-52
2. half-chair formation of sugar D (strain)
(resonance stabilize +charge on C-1 with O)

Sponsored by **Readability-Score.com**
Measure your website readability!
https://readability-score.com