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

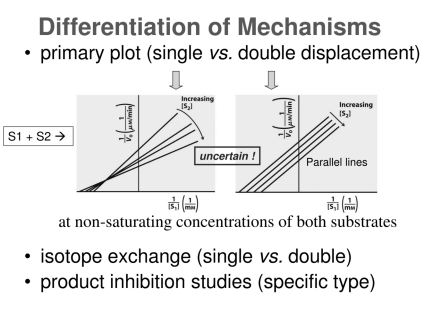
Determination of enzyme activity

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/

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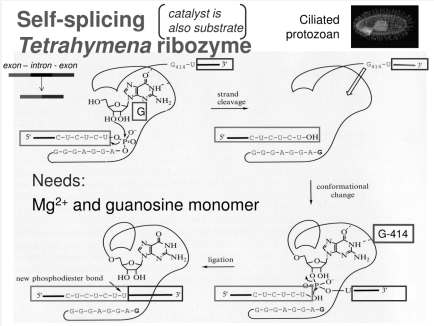
Ribozyme



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

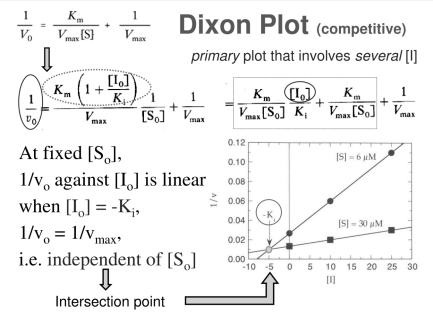
Radioisotope

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

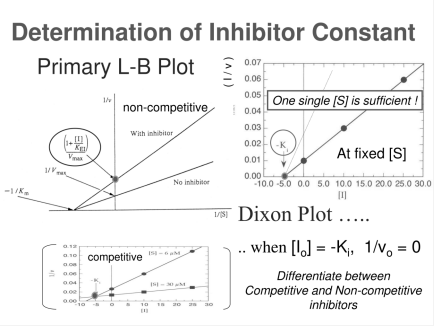
Radioligand stimulate bead to emit light, when in **close proximity**
High affinity capture system: biotinylated substrate & streptavidin-coated beads
NO separation needed. S or P bind to bead

Competitive inhibitor



Same site, mutually exclusive
Vmax unchanged Km increased

Non-competitive inhibitor



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

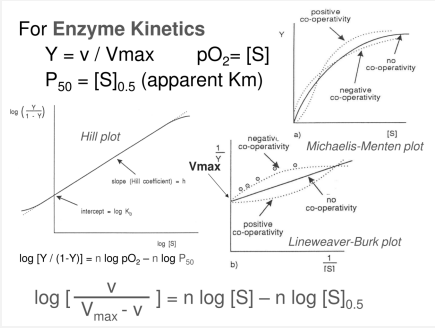
Pre-steady state kinetics

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

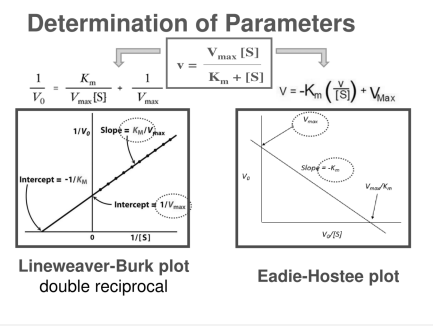
Cofactor

Apoenzyme	inactive form	
Cofactor	non-protein	
Holoenzyme	active	
Metall-oenzyme	partic-ipate in reacti-on(lewis acid)	Zn in carbonic anhydrase
Metall-oenzyme	stabilize transition state	Zn in carbox-ypeptidase A
Metal activated enzyme	maintain active confor-mation	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	
Cosubstra-te(coe-nzyme)	convert to product after Rx	NAD+

Coenzyme analogue as drug

Drug	Analogue of	enzyme inhibited	MOA & Use
Sulfon amide	PABA	dihydr-opteroate synthase	folic acid synthe-sis,Abx
Methot rexate	Folate	dihydr-ofolate reductase	THF synthe-sis,ch-ildhood leukemia

Plot



Lineweaver-Burk plot
double reciprocal

Eadie-Hostee 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 1^{st})

Ternary complex present

Double displacement Rx (Ping-Pong)

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

Isotope exchange

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

Diff. subunits of multimeric enzyme

Catalytic & Regulatory	Aspartate transcarbamo-ylase(ATCase)	ATP&CTP
2^{nd} unit modify specificity	Lactose synthase	α-lact-albumin
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)

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

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