## 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

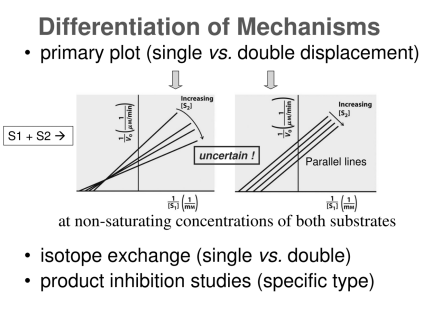
## Determination of enzyme activity

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

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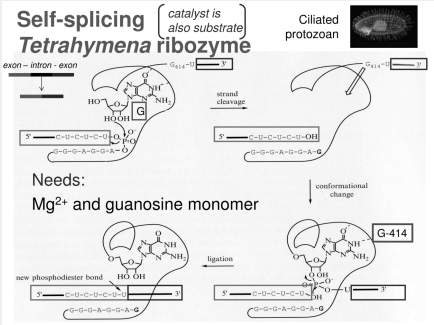
# Cheatography

## Enzymes Cheat Sheet

by iplorip via cheatography.com/45365/cs/13354/

### 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

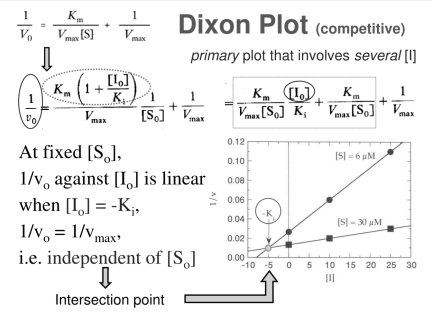
### Radioisotope

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

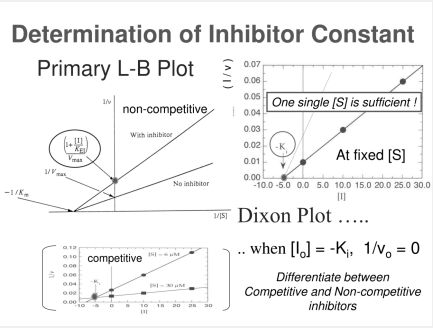
Radioligand stimulate bead to emit light, when in **close proximity**
High affinity capture system: biotinylated substrate & streptavidin-coated beads
NO separation needed. S or P bind to bead

### Competitive inhibitor




Same site, mutually exclusive
Vmax unchanged Km increased

### Non-competitive inhibitor




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

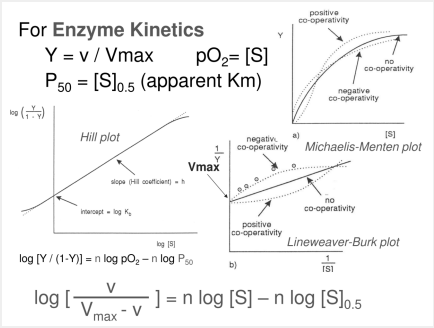
### Pre-steady state kinetics

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

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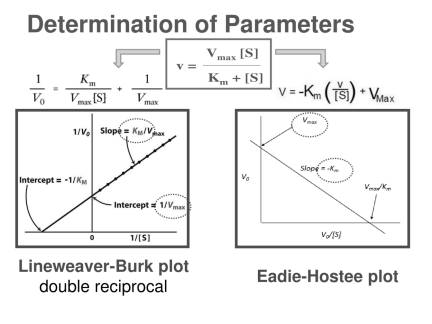
## Cofactor

| | | |
|---|---|---|
| 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 $1^{st}$) |

**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 $1^{st}$ 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 transcarbamoylase(ATCase) | ATP&CTP |
| $2^{nd}$ 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)

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