Enzyme regulation

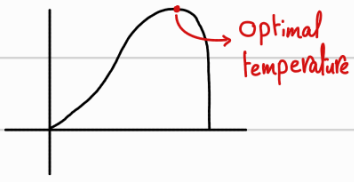
non specific regulation
Regulation of enzymatic activity

Non specific Regulation

① Temperature

↑ Temperature, ↑ collisions so the rate increases

But, at very high temperature enzymes denature



② PH

Affects protonation (ionization) of proteins

It is enzyme-dependent

optimal pH of:

Chymotrypsin (8), Pepsin (2)

Cholinesterase (>7), Papain not affected

③ Regulate enzyme amount

1) Regulation at gene level
2) Regulate enzyme degradation
3) Synthesis of isozymes

They are slow mechanisms and affect half-life

④ Compartmentalization

Reduces area of diffusion

So, more possible colloids

Enzyme Complexing

- Multiple enzymes together
- such as Pyruvate dehydrogenase

⑤ Isozymes ⇝ different enzymes produced by different genes in different tissues, and can perform the same Reaction

(Same Substrate, Same product)

Examples A) Lactate dehydrogenase (LDH)

- It is a tetramer of H and M subunits
- has 5 isozymes (LDH 1-5)

LDH 1 (H_4) in the heart (only aerobic)

- Convert lactate (NAD^+) → Pyruvate ($NADH$)
  - higher affinity
- Inhibited by high concentration of pyruvate

LDH 5 (M_4) in the Liver and muscles ⇝ can be anaerobic

- Converts pyruvate ($NADH$) → Lactate (NAD^+)
  - higher affinity
- Not inhibited by pyruvate

B) Glucokinase & Hexokinase

- phosphorylates Glucose

Glucokinase (Hexokinase IV) in Liver (store glucose) and pancreas

- It has a low efficiency to provide (release) glucose from stores into the tissues
- Not inhibited by high concentration of Glucose-6-phosphate
- Activated by insulin, Inhibited by Glucagon

Hexokinase in the muscles and RBC

- High efficiency to trap Glucose in the cell
- Inhibited by high concentration of Glucose-6-phosphate

Regulation of enzymatic activity

1) Inhibitors → Reversible / Irreversible

☆ Reversible inhibitors ⇝ bind the enzyme non-covalently

- Competitive (↑ K_m)
  - Compete substrate to bind the active site, and can Overcome by increasing substrate
- Non-Competitive (↓ V_{max})
  - bind other site, which affects the catalytic site
- Un-Competitive (↓ K_m, ↓ V_{max})
  - bind the ES complex

☆ Irreversible inhibitors ⇝ bind the enzyme tightly (covalently)

Covalent inhibitors
- Examples: ① Sarin nerve gas
- ② Diisopropyl fluorophosphate (DFP)
- ③ Insecticides (malathion, Parathion)
- ④ Aspirin ⇝ inhibit (COX 1,2)

Substrate, transition state analogs
Suicide Inhibitors
Examples:
① Penicillin (has β-lactam Ring) ⇝ inhibits Glycopeptidyl transpeptidase (Bacterial cell wall)
② Methotrexate ⇝ inhibit dihydrofolate reductase and so inhibit nucleotide base synthesis

Heavy metals
⇒ Non-specific: high dose of Mercury (Hg) which bind sulfidryl groups
⇒ Specific: Lead (Pb) replaces normal functional metals such as Calcium, Zinc and iron

2) Regulation through Conformational changes

A) Interaction between Protein subunits
such as: PKA which is activated by dissociation of (2 R) Subunits from the (2C) subunits
☆ activated by cAMP

B) Reversible Covalent modifications
1) Phosphorylation, dephosphorylation
- add Ⓟ to serine, threonine, tyrosine
- Glycogen Synthase ⇝ inhibition
- Glycogen phosphorylase ⇝ activation

2) Adenylylation ⇝ add AMP to tyrosine
3) Uridylylation ⇝ add UMP
4) ADP-Ribosylation
5) Methylation ⇝ add methyl to carboxyl
6) Acetylation ⇝ add acetyl to Lysine

Glycogen Phosphorylase (GP):
☆ GP (a) ⇝ phosphorylated ⇝ active (R)
☆ GP (b) ⇝ dephosphorylated ⇝ inactive (T)
- activated by add AMP
- Inhibited by ATP, Glucose 6-Phosphate

☆ G-proteins
- GEF
- Activated by GTP
- Inhibited by GDP
  - GAP, GDI

C) Irreversible Covalent modifications
irreversible removal of Pro-region from N-terminus

Zymogen (Proenzyme)
- Inactive enzymes such as: trypsinogen, Chymotrypsinogen, Pepsinogen

D) Allostery ⇝ binding of regulatory molecule to a site and affecting another site
- Positive = activation, Negative = inhibition
- Homo = Regulatory molecule same as substrate, Hetero = different molecules
- Cooperativity: Homoallostery, where binding active site affects other active sites

☆ Allostery doesn't follow Michaelis equation
- X K_m, $K_{0.5}$ ↙
- Sigmoidal curve

K system ⇝ change $K_{0.5}$, same V_{max}
V system ⇝ change V_{max}, same K_m

Example:
Aspartate transcarbamoylase (ATCase)
- inhibited by CTP (follows K system)
- activated by ATP, GTP, Purines

Metabolic regulation

- Negative feed back
  - late product inhibits an early enzyme
  - Such as Hexokinase
- Positive feedback
  - late product activates an early enzyme
  - Clotting (thrombin)
- Feed-forward activation
  - Substrate activate a downward enzyme
  - such as Glycolysis, Poison elemination

☆ Committed step ⇝ first irreversible step without point of return (PFK in Glycolysis)

☆ Rate limiting step ⇝ Slowest step ($\uparrow K_m$, $\uparrow \Delta G^{\ddagger}$) and can be committed step

Diagnosis

ALT, AST

- liver enzyme
- ALT/AST > 1 ⇝ Viral hepatitis
- ALT/AST < ⇝ Non viral disease

LDH

- ↑ LDH 5 ⇝ Liver disease
- LDH 1 / LDH 2 < 1 ⇝ Normal
- LDH 1 / LDH 2 > 1 ⇝ Myocardial infarction (MI)

CPK

↑ CPK-MB ⇝ MI

disappears 1-3 days after MI

can be used to diagnose a second infarct

Troponin

↑ Troponin ⇝ MI

- last for a week
- Can't detect second infarction

Cofactors

- organic ⟶ Coenzymes (vitamins)
  - Covalent ⇝ Prosthetic group
  - Noncovalent ⇝ Co-Substrate
- Inorganic ⟶ metals

☆ Activation transfer Coenzymes

Covalent catalysis

① Thiamin PyroPhosphate (TPP)

Vitamin B1 (Thiamin)

Functional group: Reactive carbon

Binding group: Pyrophosphate (Decarboxylation) bind Mg^{+2}

⇒ Pyruvate dehydrogenase

⇒ α-keto glutarate dehydrogenase

② Coenzyme A (CoA)

B5 (Pantothenate)

Functional group: Sulfydryl

Binding group: adenosine bisphosphate (Metabolism of fat, sugr, protein)

⇒ Pyruvate dehydrogenase

⇒ Citrate dehydrogenase

③ Pyridoxal phosphate

B6 (Pyridoxal)

Functional group: Reactive aldehyde

Binding group: Reactive aldehyde (Transamination, Metabolism of amino acids)

④ Biocytin ⇝ Functional group: N of Biotin ring
B7 (Biotin) binding group: Lysine (carboxylation)

used by:
→ Pyruvate Carboxylase
→ Acetyl CoA carboxylase

high consumption of antibiotics and raw egg (avidin) cause Vitamin B7 deficiency

☆ Oxidatio Reduction coenzymes

① FAD, FMN
B2 (riboflavin)
Functional group: 2 N of riboflavin ring
Binding group: adenosine nucleotide
(transfer e^- as H atoms)
⇒ Succinate dehydrogenase

NAD^+, $NADP^+$ (co-substrate)
B3 (Niacin)
Functional group: C opposite to N
Binding group: Nucleotide part
(transfer e^- as H^- ion)
⇒ dehydrogenases

Ascorbic acid
Vitamin C
⇒ Prolyl hydroxylase
Vitamin C act as anti-oxidant

☆ Metals → bind substrate; accept and donate electrons

1) Mg^{+2} ⇝ chelate TPP and ATP
⇒ Hexokinase
⇒ pyruvate dehydrogenase
⇒ α-ketoglutarate dehydrogenase

2) Se
⇒ Glutathion peroxidase

3) Mn^{+2}
⇒ superoxide dismutase

4) Zn^{+2}
⇒ Carboxypeptidase
⇒ Carbonicanhydrase → Zinc participates directly in the reaction
⇒ Alcohol dehydrogenase → Zinc stabilizes the charge after the reaction

usually binds to (His)