introduction to Biochemistry

Sheet Slide

Number 25

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Models of metabolic pathway regulations:

1. Feedback regulation: The process by which the product of a metabolic pathway influences its own production by controlling the amount and/or activity of one or more enzymes involved in the pathway, this influence could be inhibitory or stimulatory.

A. Negative feedback regulation: A common type of control occurs when an enzyme present early in a biochemical pathway is inhibited by a late product of pathway.

Example:

B. Positive feedback regulation: A common type of control occurs when an enzyme present early in a biochemical pathway is stimulated by a late product of pathway, this stimulation increase or amplify the first response.

Example: coagulation or blood clotting is an example of positive feedback.
2- **feedforward regulation**: occurs when a metabolite produced early in a pathway activates an enzyme that catalyzes a reaction further down the pathway, this type of reaction usually fasten the reaction.

Examples: blood coagulation and poisoning.

- This is important in poisoning, because the poison will stimulate an enzyme in the pathway leading to quick elimination of the poison.

**Regulation of Metabolic Pathways**

**Feed-forward Activation**

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3- **committed steps**: is an effectively irreversible enzymatic reaction that occurs at a branch point during the biosynthesis of some molecules, these types of reactions are exergonic.

- A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return.

- For example, the committed step for making product E is \((B \rightarrow C)\), not \((A \rightarrow B)\).

- The committed step for making product z is \((X \rightarrow Y)\), not \((B \rightarrow X)\) because it is reversible.

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**Example of a committed steps:**
4-Rate limiting reactions: The slowest step in a metabolic pathway or series of chemical reactions, which determines the overall rate of the other reactions in the pathway.

-These reactions could be slow for the following reasons:
  - Requires high amount of energy, so Enzymes will be highly regulated, because cells are trying to save energy as much as possible.
  - High km values (low affinity) of enzymes to its substrate.

Enzymes in disease diagnosis:

-Most enzymes exist inside the cells and certain enzymes only exist in the cell or in low amounts in the serum, so if these enzymes are found in high amounts in the serum it will be an indication for certain disease, abnormality or damage in certain tissues and organs.

Serum: In blood, the serum is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor; it is the blood plasma not including the fibrinogens. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms).

The distribution of enzymes in the body is known, for example lactate dehydrogenase 1 (LDH 1) is found mainly in the heart, LDH 5 mainly found in the liver.

-some important enzymes for abnormality indications:
  - The amino transferases: alanine transaminase, ALT and aspartate aminotransferase, AST.
  - Lactate dehydrogenase, LDH.
  - Creatine kinase, CK (also called creatine phosphokinas, CPK).

-ALT and AST: an indication of liver abnormality.

-The typical liver enzymes measured are AST (not specific for the liver) and ALT (specific for the liver). ALT is particularly diagnostic of liver involvement as this enzyme is found predominantly in hepatocytes (liver cells).

-AST is found in higher levels in the liver than ALT, so the detection of AST is easier.

-An increase in the level of AST and ALT is an indication of liver damage.

-When assaying for both ALT and AST the ratio of the level of these two enzymes can also be diagnostic. Normally in liver disease or damage that is not of viral origin the ratio of ALT/AST is less than 1. However, with viral hepatitis the ratio will be greater than 1.

-LDH, CPK and myoglobin: an indication of heart abnormality.

-High concentration of myoglobin could be an indication of MI (myocardial infraction), but myoglobin is not only found in the heart, it is also found in the muscle tissue so it is not accurate for MI detection.
-these enzymes and proteins have certain peaks for a certain amount of time.
- Myoglobin level rise in the serum for almost two days and then it go back to normal.
- LDH reach its highest levels after five days and stays in the serum for almost two weeks.
- Troponin reach its highest levels in two days and stays in the serum for 5 days.
- CPK rise in two days and stays in the serum for 4 days.
- This important in diagnosing disease specially for indicating MI.

-LDH in indicating diseases:
We have five types of LDH isoenzymes: LDH1, LDH2, LDH3, LDH4, and LDH5.
Isoenzymes: each of two or more enzymes with identical function but different structure that differ in amino acid sequence but catalyze the same chemical reaction.
-LDH1 is specific for cardiac tissue, and any change in its concentration in the blood is an indication for cardiac damage.
-A comparison of serum levels of LDH-1/LDH-2 ratio is diagnostic for myocardial infarction (heart attacks). Normally, this ratio is less than 1. Following an acute myocardial infarct, the LDH ratio will be more than 1.
-CPK

-CPK is found primarily in heart and skeletal muscle as well as the brain. Therefore, measurement of serum CPK levels is a good diagnostic for injury to these tissues.

-Not necessarily for heart damage.

-CPK is found in three isoenzymes:
  
  - CPK3 (CPK-MM) is the predominant isozyme in muscles, also found in high amounts in cardiac tissue.
  - CPK2 (CPK-MB) accounts for about 35% of the CPK activity in cardiac muscle, but less than 5% in skeletal muscle.
  - CPK1 (CPK-BB) is the characteristic isozyme in brain and is in significant amounts in smooth muscle.

-if CPK3 level increase in the blood this is an indication of abnormality in the skeletal muscle or cardiac muscle, for cardiac muscle also CPK2 levels will increase too.

-Since most of the released CPK after a myocardial infarction is CPK-MB, an increased ratio of CPK-MB to total CPK may help in diagnosis of an acute infarction, but an increase of total CPK in itself may not.

-The CPK-MB is also useful for diagnosis of reinfection because it begins to fall after a day and disappears in 1 to 3 days after the first infraction, so if it rise again after 3 days this an indication for a second myocardial infarction so subsequent elevations are indicative of another event, troponin is not useful in this situation because it stays longer in the blood.

-test for CPK and LDH isoenzymes: 8 samples (CPK1-CPK3) and (LDH1-LDH2).
-each horizontal line describes a test, so we have eight different tests.

-each vertical line represents a certain enzyme at different tests.

-for MI are concerned about LDH1 and LDH2.

-Normal LDH patterns should be: LDH2>LDH1>LDH3>LDH4>LDH5.

-Normal (the ratio LDH1/LDH2 is less than 1), abnormal (the ratio LDH1-

-for LDH patterns:

- Test#8, shows that LDH2 level is more than LDH1, which is normal.
- Test #2, a test after a few hours from myocardial infraction still indicates that LDH2level is higher than LDH1,because it takes a long time till LDH1 become higher than LDH2.
- Test#1, after 24 hours from test 2, LDH1 level become higher than LDH2 level, so it is an indication for MI.
- Test#6, after 1day from test 1 LDH1 is still higher than LDH2.
- Test#5, after 2 days from test 1 LDH1 is still higher than LDH2.
- Test#4, shows that LDH5 level is higher than normal (the highest).
- Test 7, shows that LDH5 is higher than normal and LDH1 level is higher than LDH2, this person has MI and liver abnormality.
- Remember that LDH stays in the blood for a week or two.

- For CPK patterns:
- Test #8, normal condition, notice that we have a low concentration of CPK-MM, which is normal in the blood, this result from cell renewal.
- Test #2, after a few hours from infraction, notice that MM and MB levels is high indicating an infraction, in contrast to LDH which takes a longer time to change its levels.
- Test #1, after a day from infraction, MM and MB levels is still increasing.
- Test #5 and #6, MM and BB levels start to decrease, after 3 days the CPK levels will come back to normal.
- Remember that CPK levels stays in the blood for almost 3 days.
- Test #4, high MM level, an indication of liver dieses.
- Test #7, high MM and MB levels, an indication of liver disease and MI, if we don’t know that the patient does not have a liver disease we cannot be sure from test #7 because it is the same as test #2, so LDH test will be better test for liver disease and MI together.

- Troponin
  - Like all cardiac markers, troponins have a unique diagnostic window.
  - Troponin levels rise within four to six hours after the beginning of chest pain or heart damage, and stay elevated for at least one week.
  - This long elevation allows detection of a myocardial infarction that occurred days earlier, but prevents detection of a second infarction if it occurred only days after the first.
  - Nowadays troponin is mostly used in MI indication.

-Cofactors

Enzymes carry out reactions utilizing different catalytic strategies:

1- Some enzymes, such as chymotrypsin, rely on amino acid residues within the active site. Almost all polar amino acids participate in nucleophilic catalysis. Ser, Cys, Lys, & His can participate in covalent catalysis

- Histidine: pKa, physiological pH & acid–base catalysis

2- Other enzymes increase their repertoire by employing cofactors (non-protein compounds that participate in the catalytic process). These enzymes are called Conjugated enzymes, because they are associated with non-covalent group, so they are holoenzymes.

- There are 3 types of cofactors:
  - Protein based cofactors
  - Metals, if they are associated tightly with the enzyme we call them Metallo-enzymes, if loosely we call them metal-associated enzymes.
  - Small organic molecules, we call them coenzymes, if these coenzymes are attached tightly (covalently) to the enzyme we call them prosthetic groups, if coenzymes are loosely attached to the enzyme we call them co-substrates. These Coenzymes can be regenerated and get back to their normal state.
- an example of prosthetic group is heme group, because it is a small organic molecule bound tightly (covalently) to proteins.
- coenzymes are usually derived from vitamins.
- Coenzymes functions:
  - activation-transfer reactions
  - oxidation-reduction reactions

- Activation-transfer reactions:
  They usually participate directly in catalysis by forming a covalent bond.

Characteristics:

- Two groups in the coenzyme:
  - Forms a covalent bond with substrate (functional group).
  - Binds tightly to the enzyme (binding group).

- Dependence on the enzyme for additional specificity of substrate & additional catalytic power

- Some important coenzymes:

- Thiamin, active form is called thiamin pyrophosphate, TPP.
Thiamin (vitamin B1) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver.

- It is involved in decarboxylation reactions.
- The pyrophosphate provides negatively charged oxygen atoms and chelates Mg2+ that is tightly bound to the enzyme.
- The function of the phosphate group that it is the group which binds to the enzyme and also it binds to metals, so sometimes the phosphate group is attached to a metal without attaching to the enzyme directly.
- TPP is involved decarboxylation reactions (removing CO2 from a substrate).

-TPP mechanism:
  - It has two sites, the binding side which is the phosphate group, and the reactive site which is the carbon between nitrogen and sulfur.
  - The phosphate group attach to the enzyme, and the reactive carbon attach to the carboxyl group of the substrate.
  - It attacks the terminal carboxyl group of the substrate, as in pyruvate, and it extract the CO2 from the substrate and then release it.

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\text{H}_3\text{C} - \text{C} - \text{O}^- + \text{TPP} \xrightarrow{1} \text{H}_3\text{C} - \text{C} - \text{O}^\text{N} + \text{CO}_2 \xrightarrow{2} \text{H}_3\text{C} - \text{C} - \text{O}^\text{N}^+ \]

- α-ketoglutarate dehydrogenase

- Decarboxylation of α-ketoglutarate into succinyl CoA by α-ketoglutarate dehydrogenase.

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\text{-OOC} - \text{C} - \text{O}^- + \text{NAD}^+ + \text{CoA} \xrightarrow{\alpha\text{-ketoglutarate dehydrogenase}} \text{CH}_2 + \text{CO}_2 + \text{NADH}
\]

- Source: pantothenate (B5), which is a precursor of alanine and pantoic acid.

- It is important for the metabolism of carbohydrate, fats and proteins where it attacks carbonyl groups & forms acyl thioesters (the “A”).
A molecule conjugated to CoA is energy-rich.

Conversion of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex.

- Condensation of acetyl CoA and oxaloacetate into citrate by citrate synthase.

- This reaction is driven by the high energy of Acetyl CoA (because it is a high energy molecule.

- It has two groups involved in the reaction:
  - Binding group: adenosine 3',5'- bisphosphate (tight & reversible)
  - Functional group (reactive group): sulfhydryl group (nucleophile)
- **vitamin B6** (active form: Pyridoxal phosphate).

- It has many forms depending on the functional group it contains.

- pyridoxal (aldehyde group), pyridoxamine (amino group) and pyridoxine (hydroxyl group), pyridoxal phosphate (phosphate group).

- The active form is pyridoxal.

- Function: it is related to the Metabolism of amino acids via reversible transamination reactions, so it is a coenzyme for transaminase.

- Mechanism: The reactive aldehyde forms a covalent bond with the amino groups, then the ring nitrogen withdraws electrons from bound amino acid (cleavage of bond).

- The aminogroup is extracted from the amino acid, which goes to the outside in the form of keto acid.

- The keto acid enters the active site.

- The binding group is the phosphate.
- Vitamin B7 (biotin)

- It is required for carboxylation reactions (adding a carboxyl molecule so enlarging the molecule

- this coenzyme needs to bind covalently to lysine to activate.

- active form is called biocytin.

- sources: food & intestinal bacteria Deficiencies are generally seen after long antibiotic therapies or excessive consumption of raw eggs (egg white protein, avidin, has high affinity for biotin).

- Examples of enzymes Pyruvate carboxylase and Acetyl CoA carboxylase (fatty acid synthesis).

- biotin is synthesized by intestinal bacteria, and that’s why it is hard to have biotin deficiency, except for certain people who have biotinides, or taking a lot of antibiotics will kill intestinal bacteria.