Sheet Slides

Subject:	**Biochemistry**
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Number:	**3**

- We will start talking about globulins which appear as 4 bands in electrophoresis (α1/α2/β/ɣ). Each band contains a group of proteins.
- ***α1 band proteins:***

  ****α1 antitrypsin**

- We talked about it previously. Generally, it antagonises the action of trypsin.

- it has many alleles(M/S/Z/F). the problem with ZZ **phenotype** is that it doesn't bind to elastase enzyme efficiently which results in elastase damaging the lung tissue resulting in emphysema.

- Smoking makes it worse, because it makes it even harder for α1antitrypsin to inhibit elastase leading to more disastrous effects to emphysema patients.

- The MM type is the most common and most effective type, whereas ZZ phenotype is a bad, low efficient type (compared to MM, its efficiency is10%).But what is the difference between these polymorphic proteins? In other words, in order to have a polymorphic protein (more than one copy) what do we need to have? What differs to make these different polymorphic proteins? The answer is simple… we need to change **the sequence of amino acids**.

Side note: polymorphism is having more than one isoform of the same molecule. For proteins, it's caused by *genetic variations,* leading to different amino acid sequence.

- As a result of that, the following occurs (in the liver): α1antitrypsin has a β sheet and a loop which normally don't have any affinity towards each other. Now, due to the change in amino acids (to produce ZZ phenotype), affinity arises between the two structures and they would tend come close to each other and attach/bind to each other. When the interaction between the β sheet and the loop is in one protein

molecule it's not a problem, but the problem is that many protein molecules will bind each other due to this, because a β sheet will bind a loop in the next molecule and so on. This affinity between a1antitrypsin molecules results in a1 antitrypsin aggregates in the liver which are not secreted out. These aggregates will continue to assemble and stay in liver cells until they damage them and their normal function. That is why 10% of ZZ phenotype-holding individuals suffer from Cirrhosis.

- From the previous point, note that a1 antitrypsin is made in the **liver**.

****a1 fetoprotein:**

- It is produced from the yolk sac of the fetus, then by liver parenchymal cells once they are mature.
- Normally it is not secreted in adults or in low amounts. Its amount can be normally high only in the case of **pregnancy**.
- All proteins must be in the normal range, and they are regulated in order to keep them in that range. An abnormal increase in their level is a disease sign. Normally the amount of proteins in a tissue cannot increase above normal except in muscle tissue. That's why people increase their protein intake in order to build up their muscle tissue (protein is stored). Other than the muscle tissue, there is **no storage** of proteins and protein level is highly regulated. If a tissue has excess protein it will be lost. Another case where protein level can be increased is the **inflammation process**, where some proteins increase their level and are collectively called acute-phase proteins.
- Acute-phase proteins increase their level dramatically in the cases of acute inflammation, chronic inflammation and also cancer. If the case is liver cancer, we'll have liver cells increasing their number, and their expression and secretion is increased, so liver proteins (plasma proteins are made in the liver) level will increase. (this is a **pathological** increase in protein level).
- Note that in down syndrome foetuses , a1fetoprotein level is **lower** than normal so there is actually a correlation between the two but

it's not really fully understood, like which one caused the other or why? These are all questions that are not answered until now.

- ***a2 band proteins :***
- ****Haptoglobin :**

Note: would you expect to find free hemoglobin(Hb) in blood? We know it's in RBCs. Now, because of RBCs flowing inside blood vessels in a relatively high speed, we would expect that normally they would collide with vessels walls and somehow rupture. This results in lysis of cells and their components become free in blood. Actually 5-10% of our Hb is free in the circulation (outside of blood cells).

- Haptoglobin (Hp) is polymorphic protein which is a tetramer. It consists of 2α and 2β chains. Its function is to bind free hemoglobin into prevent its loss, thus preserving the body's iron, which is the only component of Hb that can't be made by the body. So iron is essential to the body and its loss must be prevented. If no haptoglobin, hemoglobin is lost in the kidneys or in the GI.
- Haptoglobin binds to hemoglobin to form a hemoglobin-haptoglobin (Hb-Hp) complex, which is metabolised later in order to free the iron and use it again.
- Haptoglobin a2 protein half-life is 5 days. When it binds to hemoglobin and forms Hb-Hp complex, it's decreased to 90 minutes. This is good because it means that these complexes will go fast towards the liver, in order to break them and utilize the iron.
- In hemolytic anemia (anemia caused by hemolysis of cells-break down-), when cells break and more hemoglobin is free, so more Hb-Hp complexes will be formed, and they will go to the liver to unbind the two,(so most Hp is in the complex form and in the liver) so in blood, Hp level will be low.
- ****Ceruloplasmin :**
- a2 band protein which binds copper (Cu) which functions as cofactor for enzyme activity (Amine oxidase/copper-dependent superoxide

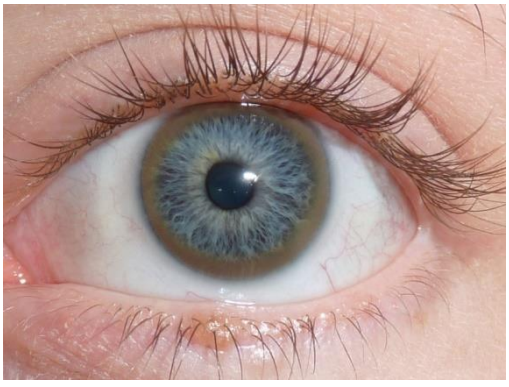
dismutase/ cytochrome oxidase/ Tyrosinase). These are all enzymes that need copper to function properly.

- Copper level in our body is regulated. In blood, ceruloplasmin regulates copper level, whereas in tissues, it's regulated by a group of proteins called Metallothioneins.
- Ceruloplasmin regulates copper level in blood because it can bind to 6 copper atoms. The ceruloplasmin-copper affinity is very high, which means that ceruloplasmin is excellent for **storage** of copper.
- If copper is to be transferred from one tissue to another, ceruloplasmin would be a bad choice for transporting copper, due to the high affinity between the two, so we would expect that ceruloplasmin will never release the copper. So a better choice would be **ALBUMIN**.
- 90% of copper in blood is bound to ceruloplasmin while 10% is being transported through albumin (copper transporting molecule).
- Only when copper level is low that ceruloplasmin releases copper. If it's high, it will bind with more affinity.
- Ceruloplasmin also functions as a ferroxidase: it oxidises iron(ferrous-→ferric). Note that the reduced (ferrous) form is the one capable of binding to oxygen and found in hemoglobin. The ferric form is transported by transferrin.

- *Diseases related to copper metabolism :*
- *Menke's disease :*
- After copper absorption by tissues, it needs to be pumped out in order to function as cofactor for enzymes/ stored with ceruloplasmin/transported…
- The pump which forces copper out is called ATPase (breaks ATP to function) and it's a protein. This pump has two types: a type found in body tissues but not in the liver called ATP7A, and a type found in the liver-and some tissues- but not all tissues called ATP7B. Since it's a protein, mutations can occur resulting in loss (the protein is not there). So we're going to study 2 diseases: one caused by defect

in the pump in **body tissues** (*Menke's disease*), while the other is caused by defective **liver** pump (*Wilson's disease*).

- If the problem is in the ATP7A pump that's normally present in body tissues but not in liver (Menke's), copper will accumulate in body tissues' cells because it's not pumped out so we'll notice some tissues having bronze colour (colour of copper). One example of these tissues is hair, so each hair shaft will have high amount of copper and that's why this disease's other name is kinky hair disease, which causes the hair to be weak, coarse and brittle (easily broken). Microscopically each hair shaft will look flattened. Other tissues will suffer from failure due to the accumulation, and it will even cause **growth failure** because copper will also be in the brain.
- *Wilson's disease:*
- Its cause is a defect in ATP7B pump resulting in no copper pumping outside of liver –and some tissues- cells. The liver which is where ceruloplasmin is synthesized will now start to decrease ceruloplasmin level (less production) in blood because it would be a waste (copper is not pumped out to the circulation, rather it stays inside, so no high level of Cu→no need for ceruloplasmin because of decreased coupling with Cu) .
- One of the tissues that suffer from copper accumulation is the **eye**. A ring is formed around the iris of the eye.



- ***Beta band proteins:***
- ****C- reactive protein (CRP):** Its name is due to its ability to bind to a specific fraction called fraction C of the polysaccharide

present on the cell wall of the bacterium *Pneumococci*, which is a pathogen causing pneumonia.

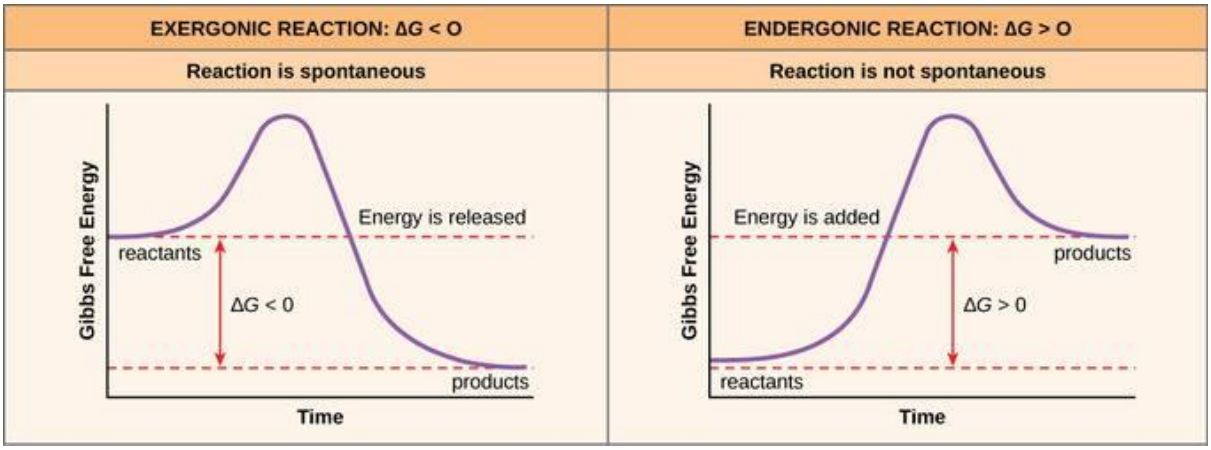
- It is one of the acute-phase proteins (dramatically increased level when acute inflammation/chronic inflammation/cancer) so a blood test for CRP level is very useful in everyday medicine (for testing for presence of diseases).
- Normally, the amount of CRP in blood is very low (1 or 2 mg/L) or may be zero but if it exceeds 10 mg/L it means something is dangerously wrong (we may not know what's wrong but it could be fatal). So anyone with CRP level higher than 10 mg/ L, they must be admitted to the hospital and he is to stay there until the disease is diagnosed.
- CRP level reaches its peak after **2 days** of the tissue damage/inflammation.

BIOENERGETICS

- Energy: the ability to perform work.
- Types of energy in reference to reactions: 1-Kinetic energy 2-Potential energy.
- Biochemistry is the science of studying reactions within biological systems.
- If we look to these reactions from a kinetic point of view, we'll be concerned about the rate, mechanism and steps of the reaction. Another point of view is the potential, where we are concerned in wither the reaction occurs or not (from a thermodynamic view).
- Thermodynamics: science which studies potential energy within matter.
- Bioenergetics: science which studies thermodynamics in biological systems (living organisms).
- Potential energy which is stored in molecules refers to the energy stored in chemical bonds. So if we have A→B as a reaction, the difference in energy stored in chemical bonds of B

and the energy stored in A is called the **free energy difference or ΔG**.

- ΔG = G (final) – G (initial). If negative, this means that we are (products) at a lower energy scale so this reaction is exergonic, spontaneous and favourable. If positive, the reaction is endergonic, nonspontaneous and unfavourable.
- Why do chemical reactions occur? The answer is to reach a more stable state. So why do endergonic reactions occur(by definition, they are reactions that need energy, so the products are at higher energy level-less stable- than the reactants)? It's because we give the reaction the energy needed to reach a state higher in its energy content than the products then the reaction actually occurs.



- For any reaction, there's the energy barrier which is called the **Activation Energy**. For any reaction to occur (spontaneous/nonspontaneous) this barrier must be overcome. Enzymes make reactions happen faster because they lower this barrier so that its easier for molecules to overcome it and proceed the reaction.
- ΔG = ΔH - TΔS
  where ΔG: bond energy difference between products and reactants.
  ΔH : enthalpy difference (heat content of the reaction)
  T: temperature in Kelvin
  ΔS : entropy difference

- So what do we really mean by that? First, entropy is the **randomness** of the molecules. It's an indication of how the molecules are arranged with respect to each other. We must know that any spontaneous system tends to **increase** its entropy (become messy and unorganised). For example, water H2O can exist in 3 forms (solid-liquid-gas) but what actually differs between these forms? Is it bond energy? **NO**, because in each case we have 2 hydrogens attached to an oxygen. But the arrangement of molecules is different, so in ice they are very close to each other, in liquid they are more relaxed and in gas state they are far away from each other. Note that in order to increase entropy (increase randomness/decrease arrangement and order of molecules/move from solid→liquid→gas) it doesn't actually need any effort, but on the opposite, arrangement and organizing molecules always requires effort.
- Now what is ΔH? Put the equation like this: $\Delta H=\Delta G + T\Delta S$. so it's actually the bond energy of a matter plus its entropy. But it's not the determinant wither the reaction is spontaneous or not, only ΔG can serve this role, because ΔH includes another factor which is the entropy so it doesn't give any indication. For example, if we put ice in a room it will melt **by itself**, it does this by taking heat from the surrounding air so we'll notice the air cooling…what we see here is an **endothermic** reaction (ΔH is positive) but yet it's **spontaneous**. It's spontaneous because of *ΔG value rather than ΔH*.
- *Effects of temperature, concentration and catalysts on spontaneity of reactions*:
  *temperature increase-→ increase kinetic energy within molecules-→increase probability of molecules hitting each other and binding to each other. (increase reaction rate)
  *concentration of materials: if increased-→increased hitting and binding.

BUT the important idea is: in a reaction A→B if we change the concentration of either one, will ΔG differ? Put another way; if the concentration changes can a nonspontaneous reaction become spontaneous? The answer is **yes**. *ΔG is **dependent** on the concentration of both A and B.* so if we take B and incorporate it in another reaction as soon as it's being formed, the reaction A→B will not stop so it becomes more spontaneous and more A converts to B. This is seen in our body, which manipulates the concentrations of many reactants and products to change spontaneity of reactions, increasing rate of important reactions and decreasing unnecessary ones.

***Changing concentrations changes ΔG and may result in an endergonic reaction becoming exergonic or the opposite.

*catalysts: they do not affect ΔG. They increase the reaction rate by lowering the energy of activation. (Enzymes interfere with the kinetics of the reaction but not the potential) so they can't make a nonspontaneous reaction become spontaneous or the opposite. So enzyme activity does not interfere with the starting and finish points so the difference stays the same.

- ΔG^0: ΔG under standard conditions (T= 25 C^0/ pressure = 1atm/ concentration of reactants and products = 1 M/ pH= 7). But this is not real actually (not in biological systems) because T= 37 C^0 and the concentration is much lower than 1 M. But we make these conditions in the lab to compare between different reactions.
- Most biological reactions are reversible (reactants convert to products and products can convert to reactants). An important notion is **equilibrium**, which is the point at which the rate of reactants converting to products equals the rate at which products convert to reactants. It *doesn't necessarily mean we have the same concentration of reactants and products.*

  *An example is that if we have 2 rooms: room A contains 200 people and room B contains 50 people. Every second, 3 people go from room A to room B and 3 people are going from room B to

room A, then we say the two rooms are in equilibrium so it doesn't necessarily mean the number of people in the two rooms must be equal (in other cases it might be equal but that doesn't mean equilibrium=equal concentrations).

- What governs the relationship between products and reactants at equilibrium is **the equilibrium constant (Keq),** which indicates the concentration of products per concentration of reactants at equilibrium. If equal at equilibrium→Keq= 1.

THE END