Hematology

BIOCHEMISTRY

☑ Sheet ☐ Slide ☐ Handout

Number: **4**

Subject: **Heterogeneity of Hemoglobin**

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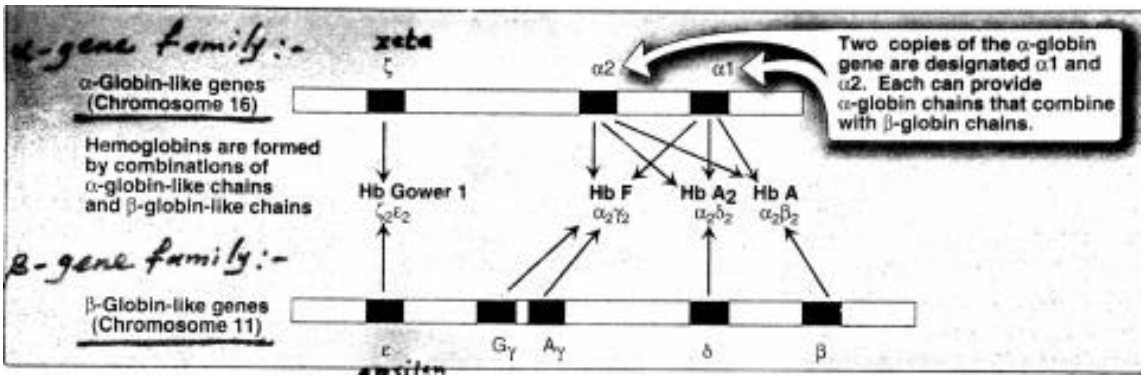
Heterogeneity of Hemoglobin

- Similar to other tetrameric proteins, hemoglobin (Hb), can have different subunits (other than the alpha and beta subunits).
- What are the other possible subunits?
  1. Beta like chain (Epsilon – ε): replaces beta chain in certain types of Hb.
  2. Alpha like chain (Zeta – ζ): replaces the alpha chain in certain types of Hb.
  3. Delta chain (δ).
- Normal hemoglobin:
  1. Composed of 2 alpha and 2 beta chains.
  2. The alpha chain gene is found on chromosome 16 as pairs on each homologous chromosome accompanied by the zeta chain gene.
  3. The beta chain gene is found on chromosome 11 as a single copy on each homologous chromosome accompanied by the genes for the delta, gamma, and epsilon chains.



- Why do these heterogeneous types of hemoglobin even exist?
  1. They appear during development. The hemoglobin during early development is known as developmental hemoglobin.
  2. They appear as a result of mutations. These mutations result in up to 700 variants. Some of these mutations end up forming abnormal hemoglobin.
  3. Some heterogeneous types of hemoglobin (such as HbA_2) are synthesized in minute quantities and may persist for a lifetime.

- During embryonic development, there are many variations in the hemoglobin structure which may result in different types including:
  1. Hemoglobin Gower
     - Synthesized in the first month after conception
     - Synthesized in the embryonic yolk sac
     - Composed of 2 zeta (alpha-like) chains, and 2 epsilon (beta-like) chains
  2. Fetal Hemoglobin (HbF)
     - Formed during the 5^{th} week of gestation
     - The site of synthesis shifts from the yolk sac to the live and then to the bone marrow
     - The epsilon subunit is replaced with an alpha subunit and the zeta subunit is replaced with a gamma subunit.
     - Shortly before birth, the gamma chain synthesis declines and the beta chain synthesis starts to make the adult hemoglobin (HbA).
     - Its structure differs from that of the adult hemoglobin by 37 amino acids.
  3. HbA_2
     - Shortly before birth, the delta chain is activated to a certain extent
     - This type of hemoglobin makes about 2% of the total hemoglobin (minor component).

Form	Chain Composition	Fraction of Total Hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2%-5%
$HbA1_c$	$\alpha_2\beta_2$-glucose	3%-6%

Clinical Correlate

- $HBA1_c$
  1. Is produced by non-emzymatic glycosylation of the normal HbA
  2. Glycosylation occurs on the N-terminal valine residue of the beta chain
  3. Glucose is the main sugar that binds to hemoglobin, but other sugar phosphates, like glucose-6-phosphate, may bind in negligible amounts
  4. Has a high diagnostic value
  5. Normally it should range between 3%-5%
  6. In prediabetic and diabetic patients, it may rise above 6%
  7. It is considered more accurate than measuring blood glucose because blood glucose can fluctuate according to certain physiologic conditions. In fact, some prediabetic patients as well as diabetic patients might have low blood glucose, but their $HbA1_c$ is high.
  8. It shows the concentration of circulating glycosylated hemoglobin in a long period of time (120 days; which is the life span of erythrocytes). Therefore, it reflects glucose accumulation in blood over a long period of time.

Hemoglobinopathies

- Hemoglobinopathies are divided into:
  1. Structural abnormalities
     - Any change in the genes responsible for the globin proteins lead to changes in structure.
     - Many studies were carried on the structure of hemoglobin, because:
       a. It's one of the of the very first proteins discovered
       b. Easily available (anyone can give many blood samples)
       c. Many variants were described during screening of the population
  2. Quantitative abnormalities
     - Amount of alpha or beta chains produced might be affected
- Fortunately, most of the structural variants are not associated with any symptoms. However, those associated with symptoms were studied extensively (because the purpose of any study is to know the relationship between structure and function).
- When the changes in structure were studied, certain physiologic changes were investigated, including:
  1. Hb becoming less soluble
  2. Hb with higher tendency to oxidize Fe^{2+} (ferric) to Fe^{3+} (ferrous).
  3. Unstable Hb
  4. Hb with increased or decreased affinities for O_2 binding
  5. These abnormalities were found to be highly variant among different regions (each region encompasses unique variants).

Mutant Hemoglobin[a]	Position Nun
α Chain	
$G_{Honolulu}$	30
$G_{Philadelphia}$	68
I	16
M_{Boston}	58
Norfolk	57
$O_{Indonesia}$	116
β Chain	
C	6
D_{Punjab}	121
$G_{San\ Jose}$	7
E	26
S	6
Zurich	63

- Changes in amino acids:
  1. Surface amino acids:
     - Despite the fact that changes in surface amino acids are mostly not associated with any symptoms, some are in fact associated with symptoms due to the fact that changes in the structure of Hb made the whole protein less soluble in the cytosol.
     - It forms polymers such as: HbS and HbC (common in Africa)
     - HbE is rare (common in Ceylon and Malaysia)
     - Hb Punjab (common in India and Pakistan)

2. Active site substitution:
    - The heme pocket in the active site is formed mainly from hydrophobic amino acids. They prevent the conversion of the ferrous form of iron to the ferric form. (Remember that oxidation of iron is rare.)
    - Certain substitution in the active site allow ferrous to become ferric, producing a form of hemoglobin called methemoglobin.
    - Hydrophobic amino acids are substituted with hydrophilic ones. Hydrophilic residues allow water to get in and aid in the oxidation of ferrous iron to ferric iron (oxidized by the hydra electric constant of water).
    - Proximal histidine might be substituted with tyrosine, thus producing methemoglobin through the previously mentioned mechanism.
    - Unstable Hb; gene mutation affects the conformational changes of the protein structure, disrupting the 3D shape, hence, making Hb less soluble.
    - The new protein will precipitate at 45 degrees.
    - Some of these changes involve breaking certain helices by proline. Remember that helices NEVER contain proline. Therefore, proline disrupts the helix.
    - Substitution with large amino acids that are normally not found at contact sites. Subunits are linked together by interactions between small amino acids. If these were substituted by other large amino acids, the interaction would be weakened. Thus, cooperativity will be affected and oxygen affinity as well.
    - When serine is substituted with phenylalanine, the protein is disrupted.
    - E helices and B helices are connected by a B6 glycine residue. If this residue is substituted with arginine, the interaction between the helices will be disrupted.
    - Substitution of His146 (responsible for 50% of the Bohr effect) will result in more hemoglobin in the R state.
    - Substitution in the sites of allosteric effectors binding affects affinity of oxygen binding. Any increase or decrease in oxygen affinity is considered pathological.

HbS & HbC

- HbS is the result of substitution of the negatively charged glutamic acid on the beta chain with the hydrophobic valine residue. This is found in sickle cell disease. ($\alpha_2{}^{A}\beta_2{}^{6Glu\rightarrow Val}$)
- HbC is the result of substitution of the negatively charged glutamic acid with the positively charged lysine. ($\alpha_2{}^{A}\beta_2{}^{6Glu\rightarrow Lys}$)

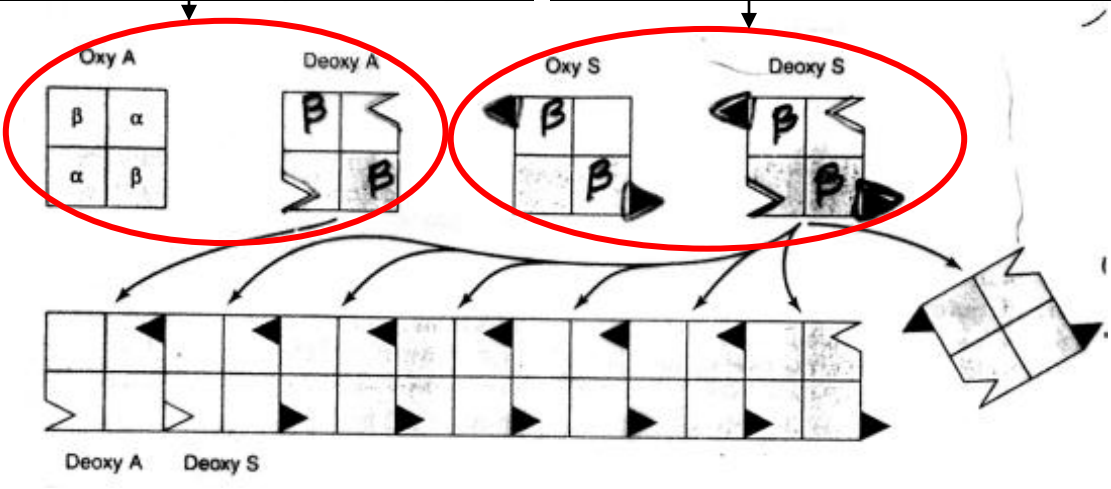
Sickle Cell Disease

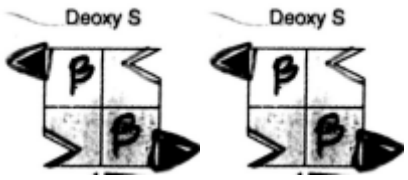
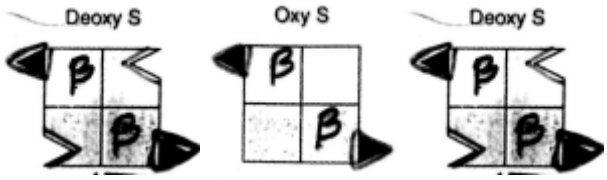
- Patients could be heterozygous (carriers) or homozygous.
- Homozygous patients (have two copies of the affected gene):
  1. 100% of their Hb is affected
  2. The disease was studied among African Americans (because they make up a significant portion of the American population).
  3. 1 out of every 500 people were homozygous.

- The disease can be detected easily by electrophoresis:
  1. It is done in agarose gel.
  2. The sample is placed in the pores (or wells).
  3. Normal Hb containing negatively charged glutamic acid will migrate from the cathode (negative charge) to the anode (positive charge) at a faster rate than HbS containing hydrophobic valine instead of glutamic acid.
- HbS polymerizes and forms long aggregates that are insoluble, hence distort the shape of the erythrocytes converting them from flexible biconcave cells to rigid sickle shape cells.
- These new erythrocytes will hardly pass through small capillaries; this will occlude small vessels and cause anoxia (which causes severe pain sometimes manifested in the form of severe headaches due to the small size of capillaries in the head).
- The aggregate formation shortens the erythrocytes' life span from 120 days to 17 days after which hemolysis occurs.
- Sickle Cell Trait:
  1. Heterozygous individuals (carriers)
  2. Have mild symptoms
  3. They have 50% normal Hb and 50% HbS
  4. These patients can live normally as long as they avoid hypoxic conditions (high alititudes, strenuous exercise, anesthesia, etc..)

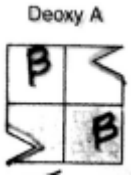
❖ Tendency for polymerization:

Normal Hb	**HbS**
Even if there is a condition favoring an increase in the deoxy state, normal Hb will not polymerize because the ends are complementary to each other.	Due to the high amount of hydrophobic amino acids, protrusions on the Hb are present. These protrusions are complementary to neighboring pockets.

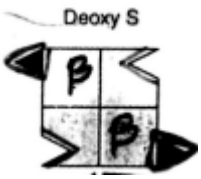


	
Conditions favoring the deoxy state, aggregation will be high and symptoms are more severe.	Conditions favoring the oxy state will disrupt the aggregation and make symptoms of anoxia milder

- In sickle cell trait:



50% normal



50% abnormal

1. There are no symptoms unless the patient is hypoxic.
2. The presence of the normal Hb and the oxy Hb will disrupt the polymer.
3. HbS trait (heterozygous) prevails in areas where malaria is endemic.
4. Sickle cell gene mutations provide natural protections against deadly *Plasmodium falciparum* (malaria).
5. Looking at the map of Africa, an overlap between sickle cell trait and malaria prevalence is evident.
6. Protection is due to the decrease in the life span of erythrocytes. It will not be 17 days as in sickle cell anemia but it is definitely going to be less than 120 days. This will prevent the malaria parasite from completing its life cycle. Other abnormalities that provide protection against malaria include: G6PD deficiency, Thalassemia and PK deficiency.

HbC VS HbSC

- Erythrocytes are not sickle shaped.
- HbC patients have mild chronic anemia.
- HbC patients have no infarctive crisis.
- Hydration with no other special therapy is required.
- Double heterozygous (HbSC) patients have sickle cells, anemia (which is milder than HbS) and less frequent painful crises.
- Investigational studies showed that the presence of higher extent of HbF will prevent sickle cell crisis.
- A drug called hydroxyurea was found to increase HbF, reducing symptoms of sickle cell crisis.
- Management for sickle cell disease: hydration, antibiotics, blood transfustions, and analgesics.
- Sickle cell crisis is more serious in small capillaries by blocking them, less blood will be delivered to the organ.
- All negative allosteric effectors mentioned above will favor the deoxy state worsening the crisis.