Hemoglobin synthesis

8 steps

- 4 in the mitochondria
- 4 in the cytosol

مش شرط ع الترتيب

- 1 in mitochondria
- 4 in cytosol
- 3 in mitochondria

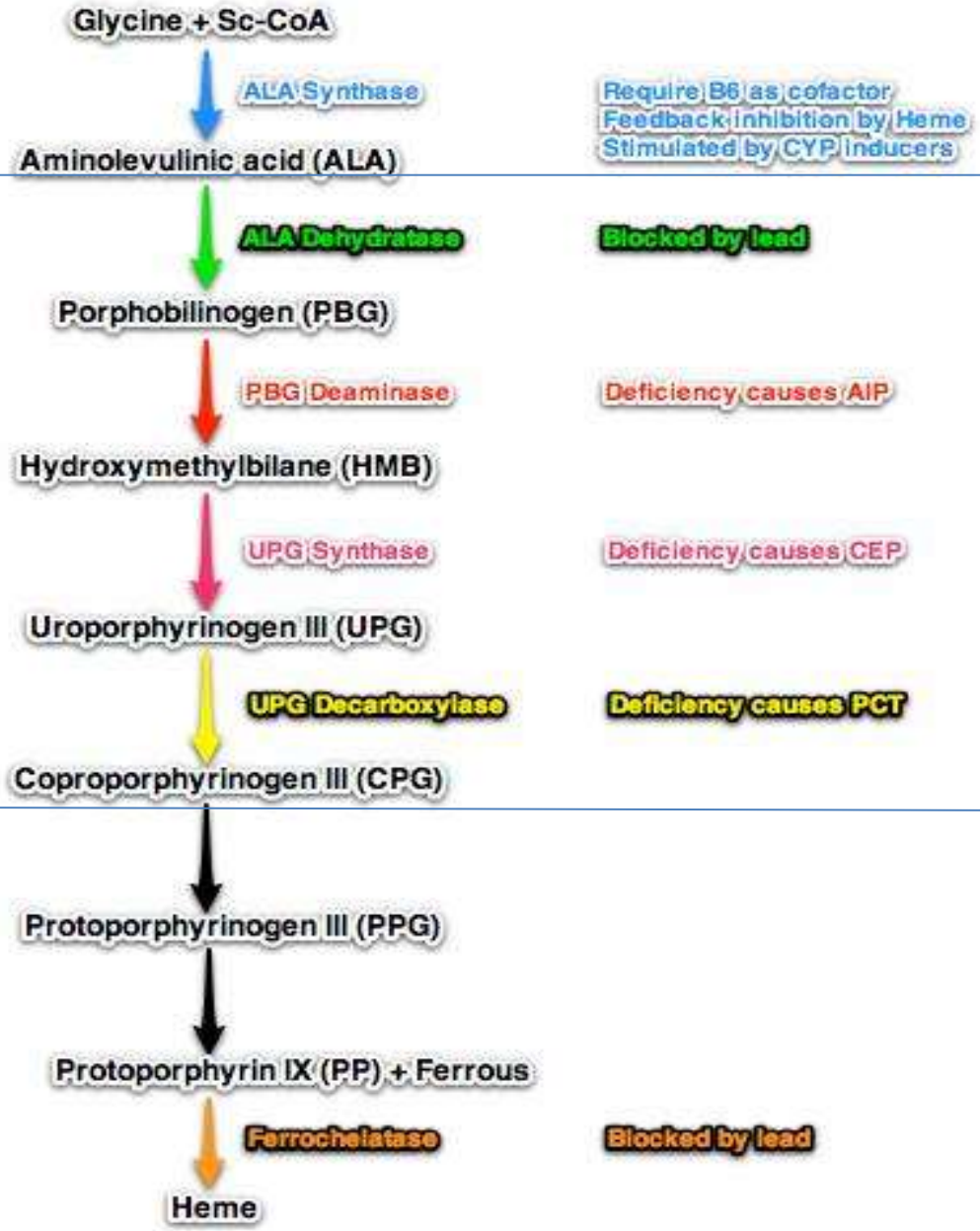
Why don't you react to what happened in one place?

It‘s a way of enzyme activity regulation, called Compartmentation or Compartemetalization
Which means that not all reactions happen at the same site inside the cell, so adding more regulatory factors. → double layer mitochondrial membrane

*if all heme synthesis step are done in the cytoplasm ➡ NO control ➡ over production of Heme ➡ increase the production of globin ➡ more hemoglobin ➡ more erythrocytes (polycythemia)

- Increase the blood viscosity
- Slow blood flow
- Thrombi → stagnation of blood
- Adding more load on the cardiac muscle & may be heart failure

*All cells that have mitochondria are able to synthesize heme



1 in mitochondria

4 in cytosol

3 in mitochondria

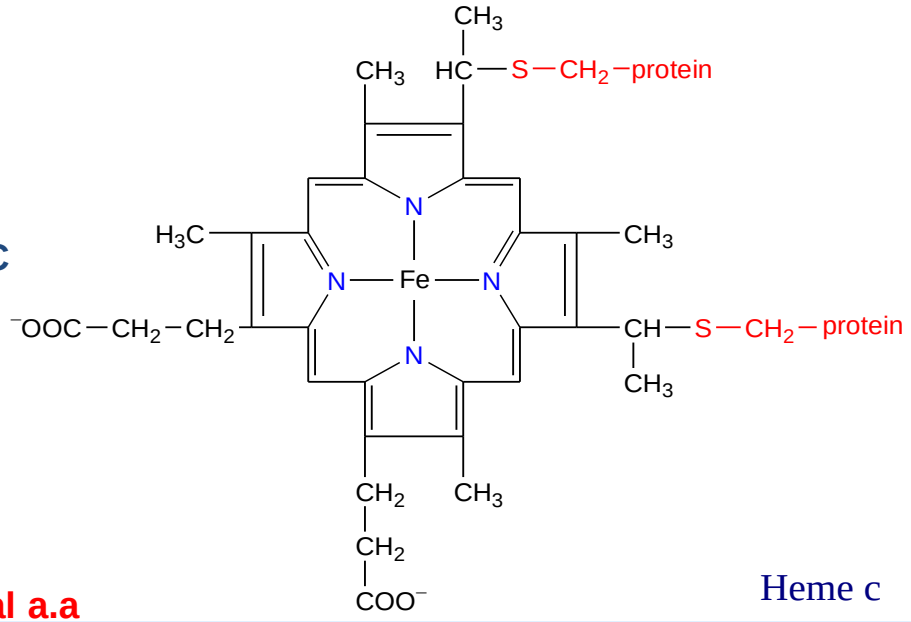
Mnemonic for steps in heme synthesis

- S – SOME - Succinyl CoA
- G – GOOD -Glycine
- D – DOCTORS -Delta-Amino Levulinic Acid
- P – PALPATE -Porphobilinogen
- H – HEART - Hydroxymethylbelane
- U – UNDER -Uroporphobilinogen 3
- C – COVER -Coproporphyrinogen 3
- P- PRODUCES -Protoporphyrinogen
- P- PURE -Protoporphyrin
- H – HEME -Heme

- Heme is the prosthetic group of : hemoglobin(RBCs), myoglobin (muscles) & cytochromes (mitochondria).

Heme B

Heme A ,C



- Heme is an asymmetric molecule.

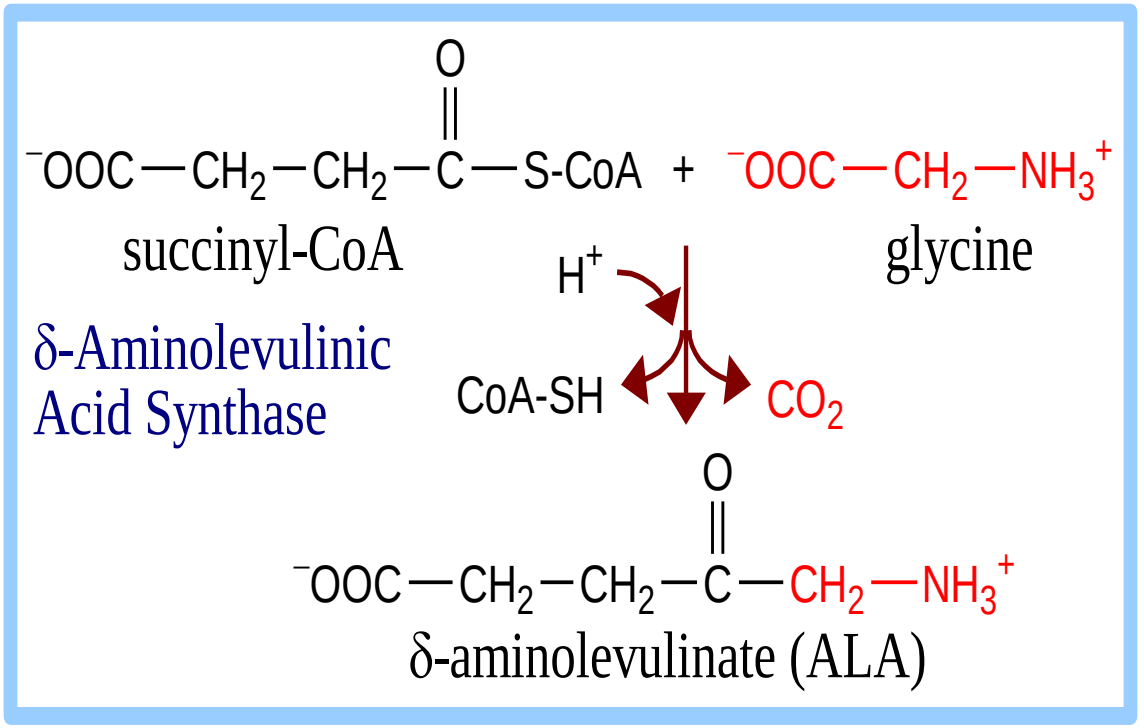
Heme synthesis

- Heme synthesis begins with condensation of glycine & succinyl-CoA, with decarboxylation, to form δ-aminolevulinic acid (ALA).

Non essential a.a

- Pyridoxal phosphate (PLP) serves as coenzyme for δ- aminolevulinate synthase (ALA synthase), an enzyme related to transaminases. WHY?

Because it uses the same cofactor of transomiases



- CoA~SH & the glycine carboxyl are **lost** following the condensation.

Vit. B6 act as co-factor (PYRODISEM)

- ALA synthase is catalyzing the committed step of the heme synthesis pathway, & is usually **rate-limiting for the overall pathway**.

Feedback regulation

- Regulation occurs through control of **gene expression**.

- Heme functions as a feedback inhibitor, repressing the transcription of ALA synthase gene in most cell لانه هاي هي ال rate limiting step فلو منعتها رح يقل انتاج الهيم

- A variant of ALA synthase expressed only in developing erythrocytes is regulated instead by availability of iron in the form of iron-sulfur clusters.

There are two forms of ALAS:

1-ALAS1 is considered a house-keeping gene and is expressed in all cells (located on chromosome **3**). Controlled by the heme (feedback inhibition)

2-ALAS2 is an erythroid-specific form of the enzyme, expressed only in fetal liver and adult bone marrow (located on the X chromosome).

controlled under the effect of availability of iron

ALAS1	ALAS2
expressed in all cells	expressed only in fetal liver and adult bone marrow
Controlled by the heme (feedback inhibition) علاقة عكسية	controlled under the effect of availability of iron علاقة طردية
located on chromosome **3**	located on the **X** chromosome

X → Sideroblastic anemia

In fetus → liverداخل الRBCsيكون تصنيع ال ALAS2

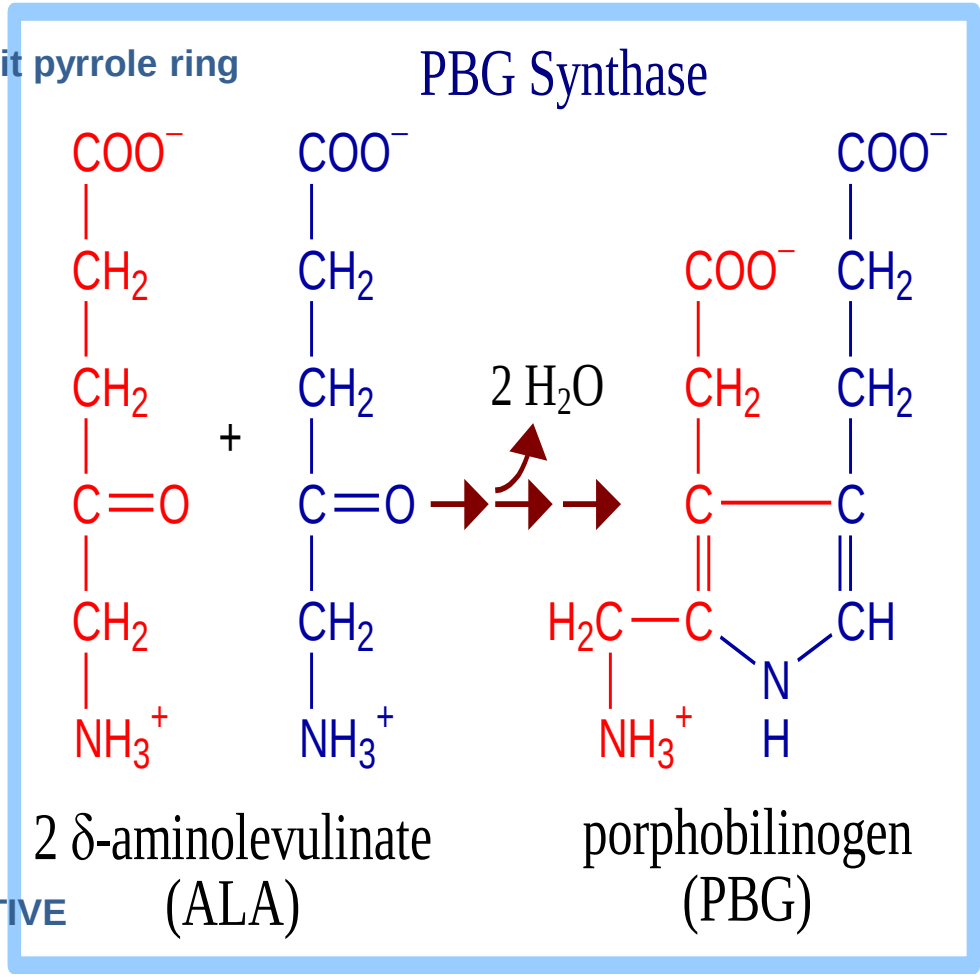
بعد الولادة يصبح التصنيع بالBM

- PBG synthase (porphobilinogen synthase), also called ALA dehydratase, catalyzes condensation of two molecules of δ- aminolevulinate to form the pyrrole ring of porphobilinogen (PBG).

-The Zn^{++} in the active of site mammalian porphobilinogen synthase, acting as binding sites for ligands including cysteine S , it can also bind Pb^{++} (lead).

- Inhibition of porphobilinogen synthase by Pb^{++} results in elevated blood ALA, as impaired heme synthesis leads to de-repression of the transcription of ALA synthase gene.

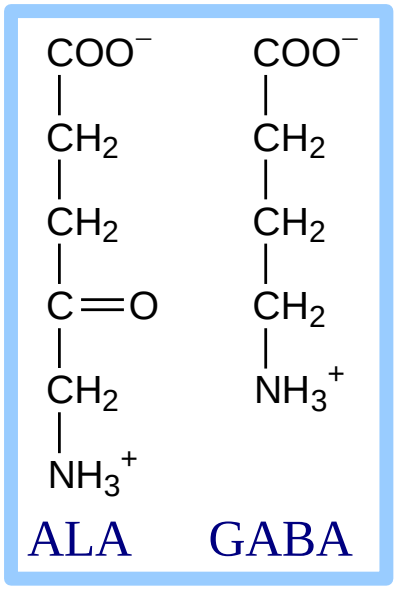
First compound we find in it pyrrole ring



COMPETITIVE

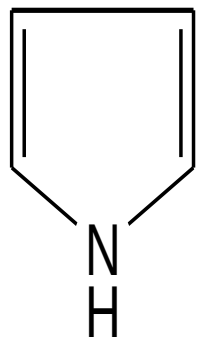
Lost NH2

- High ALA is thought to cause some of the neurological effects of lead poisoning, although Pb^{++} also may directly affect the nervous system. Brain toxicity
- ALA is toxic to the brain, perhaps due to:
  1- Similarity in the structures between ALA and GABA (γ- aminobutyric acid). Inhibit the action of GABA result in convulsions
  2- ALA autoxidation generates reactive oxygen species (oxygen radicals).



في الأطفال مش دائما ممكن بسبب B9 ↓

- **Porphobilinogen (PBG) is the first pathway intermediate that includes a pyrrole ring.**
- The porphyrin ring is formed by condensation of **4** molecules of porphobilinogen. **Acute intermittent porphyria**
- Porphobilinogen deaminase (hydroxymethylbilane synthase) catalyzes successive PBG condensations, initiated in each case by elimination of an amino group. it leads to the formation of the tetrapyrrole hydroxymethylbilane. **Open ring**

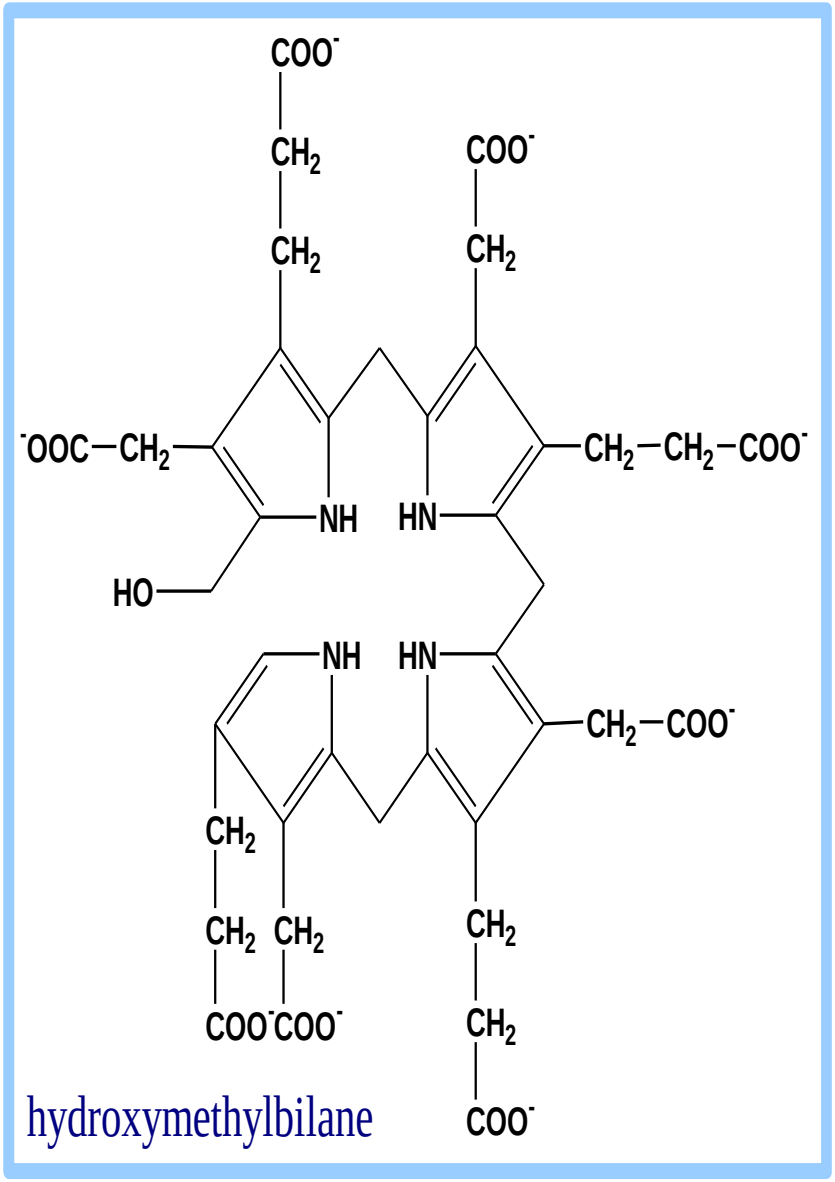


pyrrole

Hydroxymethylbilane has two fates:

1- The most important is regulated, enzymatic conversion to uroporphyrinogen III, the next intermediate on the path to heme which is mediated by a holoenzyme comprised of uroporphyrinogen synthase plus a protein known as **uroporphyrinogen III cosynthase**.

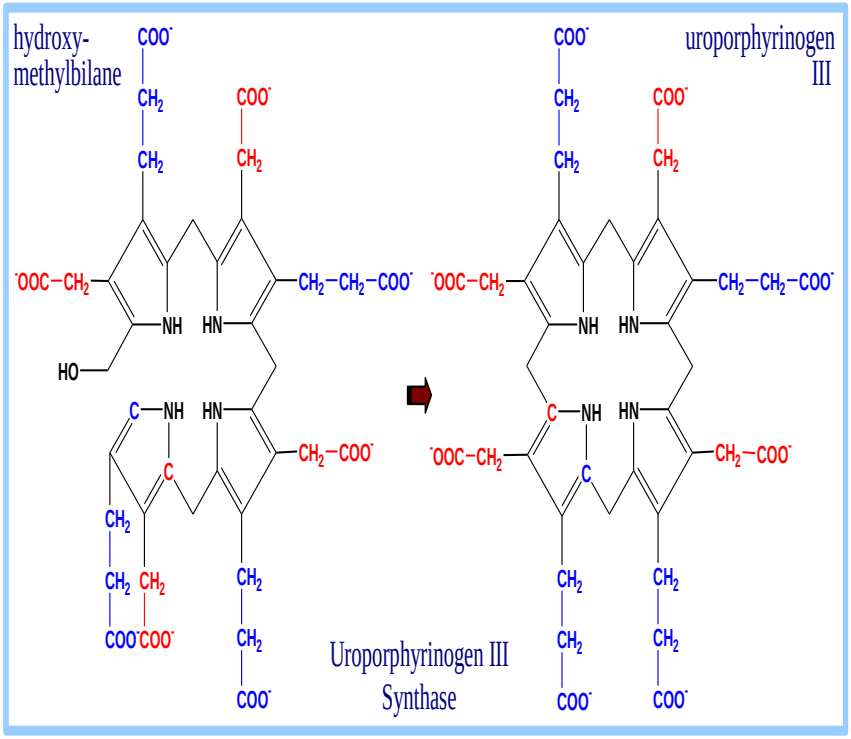
2- Hydroxymethylbilane can also non – enzymatically cyclize forming **uroporphyrinogen I**. تلقائيا



- Uroporphyrinogen III synthase converts the linear tetrapyrrole hydroxymethylbilane to the macrocyclic uroporphyrinogen III.
- Uroporphyrinogen III synthase catalyzes ring closure & flipping over of one pyrrole to yield an asymmetric tetrapyrrole.

-The distribution of **acetyl & propionyl** side chains, as flipping over of one pyrrole yields an **asymmetric tetrapyrrole.**

- Uroporphyrinogen III is the precursor for synthesis of vitamin B12, chlorophyll, and heme, in organisms that produce these compounds.

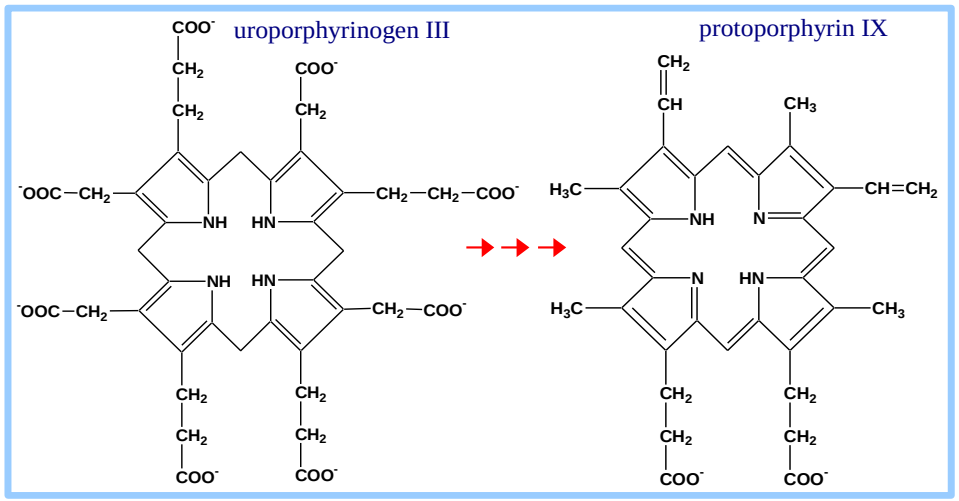


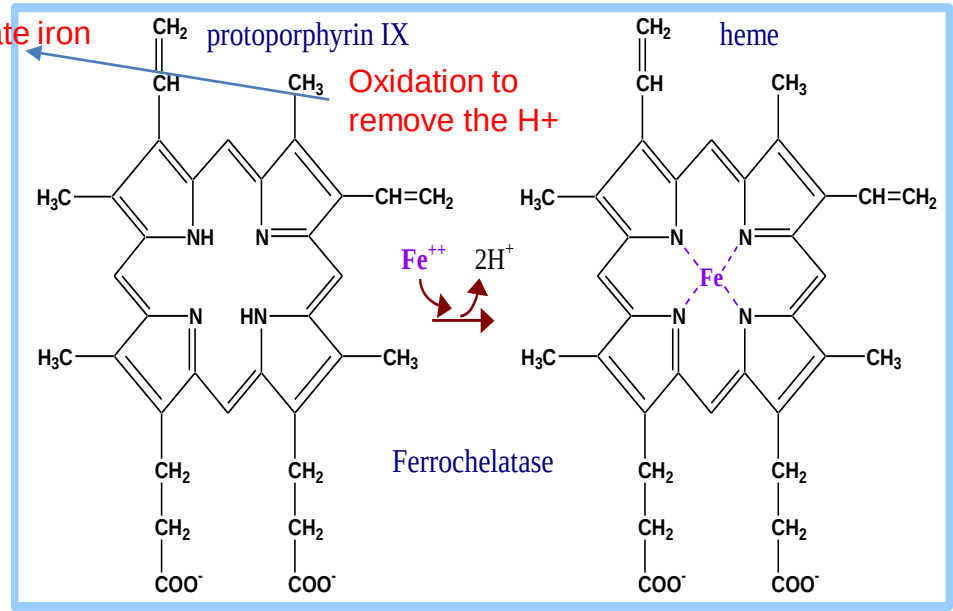
*why iron atom make 6 bonds in the hemoglobin?

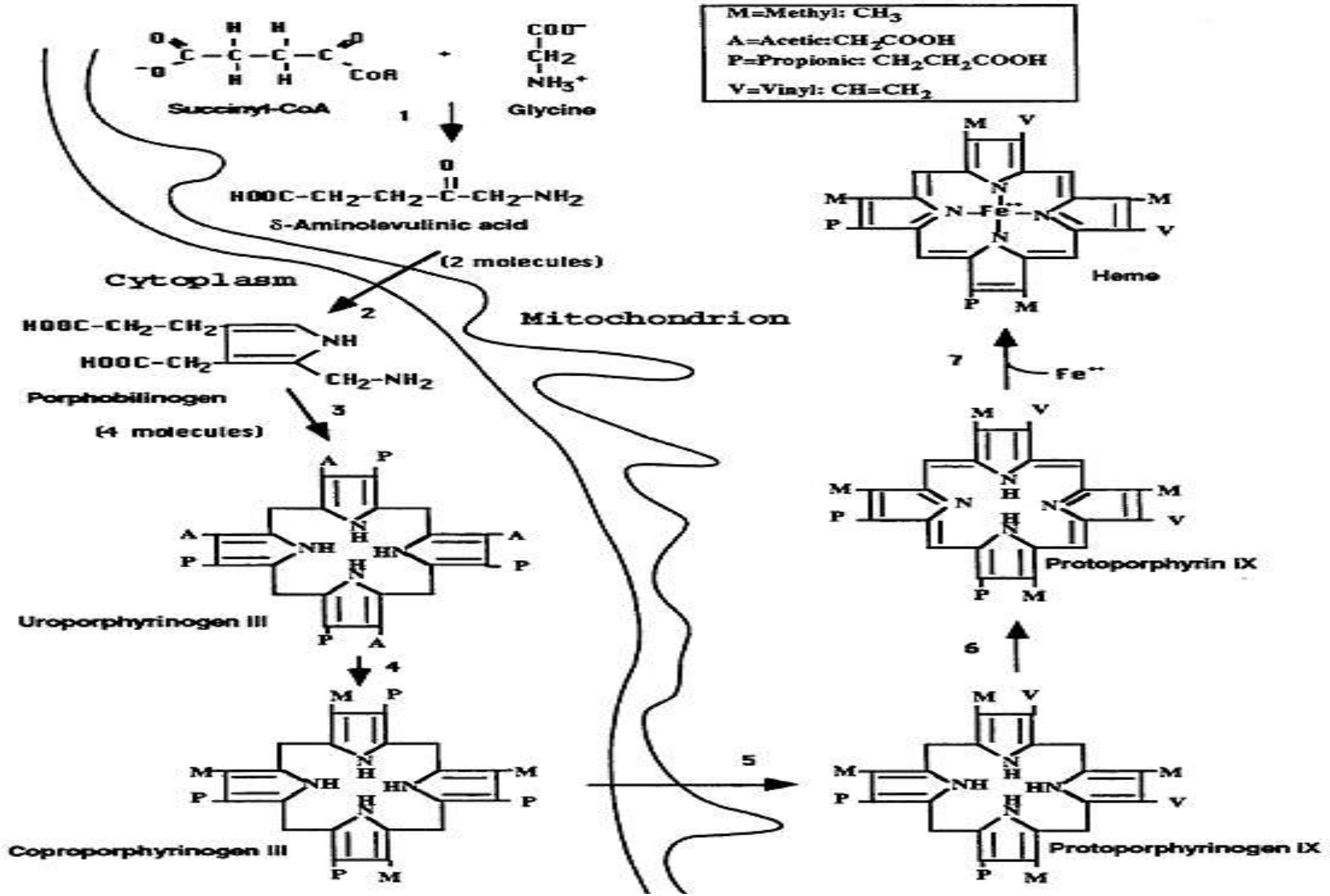
To avoid the oxidation & transformation into the ferric form

*even though it can be oxidized & thus there is an enzyme “hemoglobin reductase”

- Conversion of uroporphyrinogen III to protoporphyrin IX occurs in several steps.
- All 4 acetyl side chains are decarboxylated to methyl groups (catalyzed by uroporphyrinogen decarboxylase) **4CO2 → A , M** **في السلايد اللي بعده**
- Oxidative decarboxylation converts 2 of 4 propionyl side chains to vinyl groups (catalyzed by Coproporphyrinogen oxidase) **2CO2 → P , V**
- Oxidation adds double bonds (Protoporphyrinogen oxidase).
- Fe++ is added to protoporphyrin IX via Ferrochelatase, a homodimeric enzyme containing 2 iron-sulfur clusters.
- A conserved active site His, along with a chain of anionic residues, may conduct released protons away, as Fe++ binds from the other side of the porphyrin ring, to yield heme.



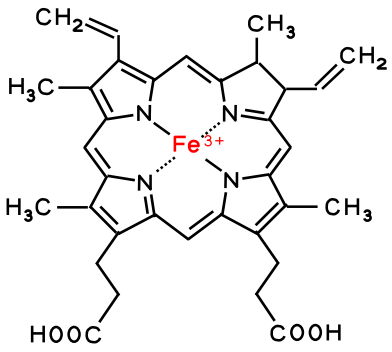




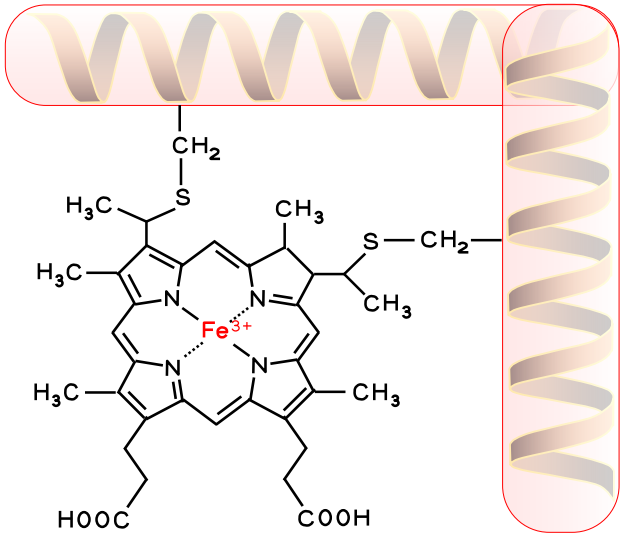
Pathway of Heme Biosynthesis

- In addition to the heme *b* found in hemoglobin, there are two different forms of heme found in cytochromes such as those involved in the process of oxidative phosphorylation.
- Cytochromes of the *c* type contain a modified iron protoporphyrin IX known as heme *c*.
- In heme *c* the 2 vinyl (C=C) side chains are covalently bonded to cysteine sulfhydryl residues of the apoprotein.
- Only cytochromes of the *c* type contain covalently bound heme.
- Heme *a* is also a modified iron protoporphyrin IX.
- Heme *a* is found in cytochromes of the *a* type and in the chlorophyll of green plants.

Heme *b*

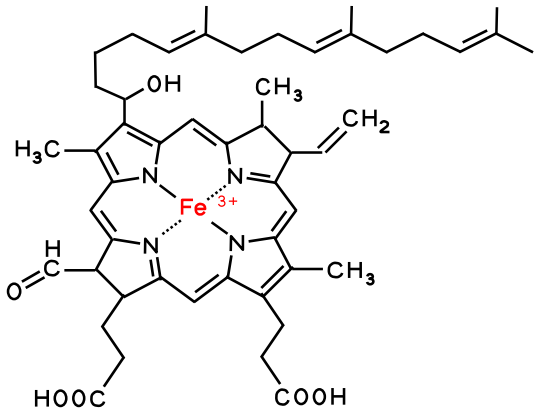


Heme *c*



Unsaturated F.A. chain bound to vinyl group of the 1st ring of heme

Heme *a*



- Regulation of transcription or post-translational processing of enzymes of the heme synthesis pathways differs between erythrocyte forming cells & other tissues.

- In erythrocyte-forming cells there is steady production of pathway enzymes, limited only by iron availability.

- In other tissues expression of pathway enzymes is more variable & subject to feedback inhibition by heme.

-The rate-limiting step in hepatic heme biosynthesis occurs at the ALA synthase catalyzed step, which is the committed step in heme synthesis.

-The Fe^{3+} oxidation product of heme is termed hemin which acts as a feed-back inhibitor on ALA synthase.

- Hemin also inhibits transport of ALA synthase from the cytosol into the mitochondria as well as represses synthesis of the enzyme.

- In erythroid cells all of the heme is synthesized for incorporation into hemoglobin and occurs only upon differentiation when synthesis of hemoglobin proceeds.

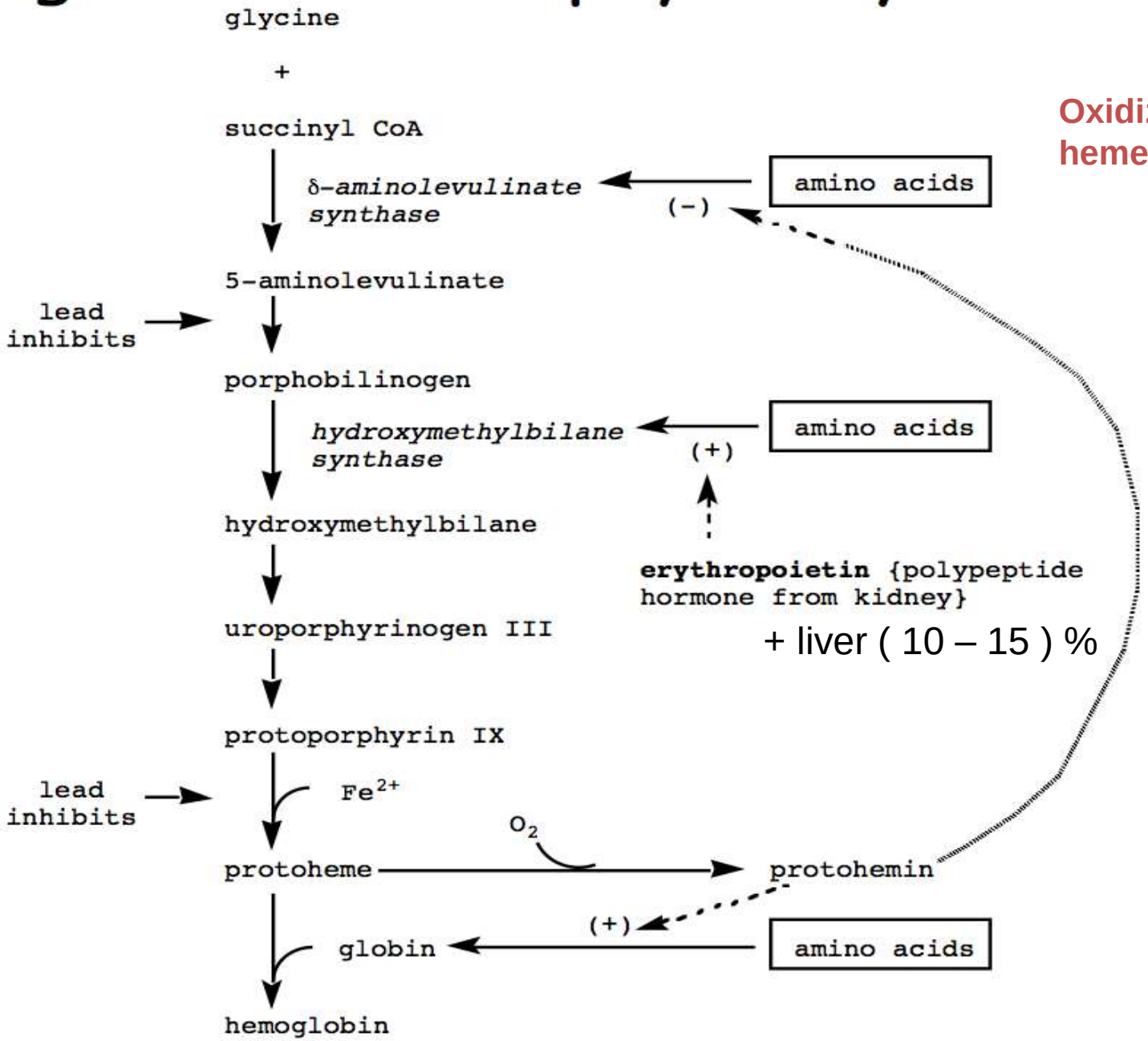
-When red cells mature both heme and hemoglobin synthesis ceases.
-The hemoglobin must, therefore, survive for the life of the erythrocyte.

- In reticulocytes (immature erythrocytes) heme stimulates protein synthesis.

- Additionally, control of heme biosynthesis in erythrocytes occurs at numerous sites other than at the level of ALA synthase.

- Control has been shown to be exerted on ferrochelatase, the enzyme responsible for iron insertion into protoporphyrin IX, and on porphobilinogen deaminase.

Regulation of Porphyrin Synthesis



* ALA synthase 1 is controlled by the final product [protoheme]

- If there is ↑ in heme production it will be oxidized to produce [protohemin] which has 3 effects # 1 positive & 2 Negative

1. Negative effect on the ALA Synthase enzyme
2. Positive effect to allow globin Synthesis to bind with protoheme & produce heme globin [globin + protoheme ⟶ hemoglobin]
3. Prevent the transportation of ALA synthase enzyme from the cytosol to mitochondria

* erythropoietin Stimulate the synthesis of the [Hydroxymethylbilane] enzyme which participate in heme synthesis

- exogenous inhibition of heme synthesis by lead

- lead inhibit & ① ALA dehydratase or porphobilinogen
② ferro chelatase

تبييض السلايد السابق

Archive ;

1. in heme synthetic pathway, one of the following sets of enzymes is starting and finalizing the asymmetrical substitutions of the four pyrrole rings of heme molecule?
Select one:
a. Coproporphyrinogen oxidase and protoporphyrinogen oxidase.
b. Porphobilinogendeaminase and uroporphyrinogen decarboxylase.
c. Porphobilinogen synthase and protoporphyrinogen Oxidase.
d. ALA synthase and hydroxymelliylbllane synthase.
e. Uroporphyrinogen synthase Ill and coproporphyrinogen oxidase.
Ans : e

2. Which one of the following sets of enzymes in heme synthetic pathway can be inhibited by lead?
a. ALA synthase and ALA dehydratase.
b. PBG synthase and PEG deaminase.
c. Uroporphyrinogen synthase Ill and ALA synthase.
d. Uroporphyrinogen decarboxylase and Coproporphyrinogen oxidase.
e. Ferrochelatase and ALA dehydratase.
Ans : e

3. A eight years old girl with abdominal pain and motor neuropathy, she was diagnosed of having congenital erythropoietic porphyria, which catabolite of the following can be detected in her urine?

a. ALA.
b. PEG.
c. 7- carboxylate porphyrin.
d. Uroporphyrinogen I.
e. Protoporphyrin.

Ans : a ?

4. Which of the following enzymes in heme synthetic pathway is requiring a cosynthase molecule for the asymmetric substitution of heme tetrapyrrole ring?

a. ALA synthase.
b. Uroporphyrinogen synthase III.
C. PBG deaminase.
d. Protoporphyrinogen oxidase.
e. Uroporphyrinogen decarboxylase.

Ans : b

5. A 4 years old boy came to the hospital suffering from burning sensation in the exposed areas of the skin to sun light, his blood analysis reveals the presence of porphyrin in the erythrocytes, which one of the following genes is suspected to have a mutation responsible for this disease?
a. Coproporphyrinogen oxidase.
b. Uroporphyrinogen decarboxylase.
c. Ferrochelatase.
d. ALA synthase.
e. Uroporphyrinogen synthase lll.
Ans : e

6. The asymmetric substitution of the tetrapyrrole ring of heme starts with the activity of the following enzyme?
a. ALA synthase.
b. PBG synthase.
c. Uroporphyrinogen synthase lll.
d. Coproporphyrinogen oxidase.
e. Coproporphyrinogen decarboxylase.
Ans : c