**HEME METABOLISM**

Biochemistry-1(PHL-284)

Mahmoud N. Nagi, Ph.D.
Professor

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**Structure and nomenclature**

**Heme Synthesis**
- Site
- Reactions
- Regulation
- Diseases of heme synthesis (porphyrias)

**Heme degradation**
- Conversion of heme to bilirubin
- Conversion of bilirubin to bilirubin diglucuronide
- Metabolism of bilirubin diglucuronide by intestinal bacteria
- Differences between bilirubin and bilirubin diglucoronide
- Hyperbilirubinemia (jaundice)
Heme Metabolism

Heme is a member of a family of compounds called **porphyrins**. Many important proteins contain heme as a prosthetic group.

**Heme proteins**
- Hemoglobin (oxygen transport)
- Myoglobin (oxygen transport)
- Cytochromes (electron transport)
- Catalase (H₂O₂ utilization)

**Structure of Porphyrins**
- The base structure is porphin
- Made up of 4 pyrrole rings
- Linked by 4 methyne (=CH-) groups
- Porphyrins are substituted at positions 1-8

The common substituents are often abbreviated as follows:

A = acetic acid (-CH₂COOH)  P = propionic acid (-CH₂CH₂COOH)
M = methyl (-CH₃)  V = vinyl (-CH=CH₂)

**Porphyrins chelate metals**
- Iron --> heme

**Properties of porphyrins**
- Color: dark red/purple
- Fluorescent

**Porphyrinogens differ from porphyrins:**
- Number of hydrogens
- Pattern of double bonds

**Properties of porphyrinogens**
- Colorless
- Not fluorescent
- Easily auto-oxidized to porphyrins
Heme

Pyrole  Abbreviated version of pyrole

Porphin, showing the four pyrrole rings and the Roman numerals which designate them. Arabic numbers indicate positions at which substituents may be attached. Greek letters denote the methene bridges.

Schematic representation of porphin.
Names of Porphyrins:

The names of the porphyrins of interest consist of a word and a number, e.g., uroporphyrin III. The word denotes the kinds of substituents found on the ring, and the number denotes how they are arranged.

**There are three important words:**

- **uroporphyrin** contains A and P only
- **coproporphyrin** contains M and P only (A has been changed to M)
- **protoporphyrin** contains M and P and V (some P has been changed to V)

**There are two important numbered series, I and III.**

Series II and IV do not occur in natural systems.

- In series I the substituents repeat in a regular manner, e.g., APAPAPAP (starting with ring I).
- In series III the order of substituents in ring IV is reversed: APAPAPPA.

If three kinds of groups are present, as in the protoporphyrins, its immediate precursor is variously referred to as protoporphyrin III or protoporphyrin IX.

**Solubility**

Depends on number of carboxylate groups, -COO-

- uroporphyrins, 8 carboxylates (more soluble)
- coproporphyrins, 4 carboxylates
- protoporphyrins, 2 carboxylates (less soluble)

This determines routes of excretion
-CH$_2$COOH (A) $\rightarrow$ -CH$_3$ (M)

acetic acid               methyl

-CH$_2$-CH$_2$COOH (P) $\rightarrow$ -CH$_2$-CH$_3$ (E) $\rightarrow$ -CH=CH$_2$ (V)

propionic acid                 vinyl
Heme Synthesis

Site: partly in the mitochondria and partly in the cytoplasm.

Reactions:

1) Delta-aminolevulinic acid synthase (ALA synthase)
   The substrates are succinyl-CoA and glycine
   The product is delta-aminolevulinic acid (ALA).
   An essential cofactor is pyridoxal phosphate (vit B-6).
   This is the rate-limiting reaction of heme synthesis in all tissues, and it is therefore tightly regulated.

2) ALA dehydratase
   The substrates are two molecules of ALA.
   The product is porphobilinogen, the first pyrrole.
   ALA dehydratase is a -SH containing enzyme.
   It is very susceptible to inhibition by lead.

3) Uroporphyrinogen I synthase and uroporphyrinogen III cosynthase
   Production of uroporphyrin III requires two enzymes. The substrates are four molecules of porphobilinogen.

4) Uroporphyrinogen decarboxylase
   Decarboxylates the acetic acid groups, converting them to methyl groups.

5) Coproporphyrinogen III oxidase
   Catalyzes the conversion of two propionic acid groups to vinyl groups

6) Protoporphyrinogen IX oxidase
   Protoporphyrinogen IX oxidase converts the methylene bridges between the pyrrole rings to methenyl bridges.
7) **Ferrochelatase**

Ferrochelatase adds Fe\(^{++}\) to protoporphyrin IX, forming heme.
- The enzyme requires Fe\(^{++}\), ascorbic acid and cysteine (reducing agents).
- Ferrochelatase is inhibited by lead.

**Regulation of heme synthesis**

**Substrate availability:** Fe\(^{++}\) must be available for ferrochelatase.

**Feedback regulation:** heme is a feedback inhibitor of ALA synthase.

**Effects of drugs and steroids:** Certain drugs and steroids can increase heme synthesis via increased production of the rate-limiting enzyme, ALA synthase.
Site and reactions of heme synthesis
Reaction Catalyzed by Coproporphyrinogen III Oxidase (Mitochondrial)

\[
\begin{align*}
\text{Coproporphyrinogen III} & \quad \rightarrow \quad \text{Protoporphyrinogen IX} \\
\end{align*}
\]

Reaction Catalyzed by Protoporphyrinogen IX Oxidase (Mitochondrial)

\[
\begin{align*}
\text{Protoporphyrinogen IX} & \quad \rightarrow \quad \text{Protoporphyrin IX} \\
\end{align*}
\]

Reaction Catalyzed by Ferrochelatase (Mitochondrial)

\[
\begin{align*}
\text{Protoporphyrin IX} & \quad \rightarrow \quad \text{Heme} \\
\end{align*}
\]
Porphyrias:

Porphyrias may be divided into two major types.

**Erythropoietic porphyria** is a defect of porphyrin metabolism of blood-producing tissues.

**Hepatic porphyria** is a defect in porphyrin metabolism of the liver.

Either type may be **hereditary** (caused by a gene defect) or **acquired** (due, say, to poisoning).

Examples of porphyria:

Two of the several types of porphyria will serve to illustrate some of the biochemical issues involved.

**Acute intermittent porphyria** (defect of hepatic uroporphyrinogen I synthase activity).

- porphobilinogen (the substrate) accumulates, and is excreted in the urine.
- Heme synthesis is reduced. ALA synthase activity therefore increases.
- There are neurological symptoms, which cannot be explained.

**Congenital erythropoietic porphyria** (defect of uroporphyrinogen cosynthase).

- Large amounts of type I porphyrins
- Skin photosensitivity
Heme Degradation

Most of the heme which is degraded comes from hemoglobin in red blood cells, which have a life span of about 120 days. There is thus a turnover of about 6 g/day of hemoglobin. Normally, senescent red blood cells and heme from other sources are engulfed by cells of the reticuloendothelial system. The globin is recycled or converted into amino acids, which in turn are recycled or catabolized as required. Heme is oxidized.

1) Conversion of heme to bilirubin (cells of the reticuloendothelial system in spleen, liver and bone marrow)

Heme ring is cleaved by a microsomal heme oxygenase between the I and II pyrrole rings.

Biliverdin reductase reduces the central methene bridge of biliverdin, producing bilirubin.

The high lipid solubility of bilirubin determines its behavior and its further metabolism.

- that it must be transported in the blood by a carrier; the physiological carrier is serum albumin.
- that it is soluble in the lipid bilayers of cell membranes.

2) Conjugation of bilirubin with glucuronic acid: (hepatocytes)

This increased its water solubility, decreases its lipid solubility and eases its excretion. Conjugation is accomplished by attaching two molecules of glucuronic acid to it in a two step process by UDP glucuronyl transferase. The reaction is a transfer of two glucuronic acid groups sequentially to the propionic acid groups of the bilirubin. The major product is bilirubin diglucuronide.

Bilirubin diglucuronide is excreted in the bile. It is subject to subsequent transformations to other species by the intestinal bacteria.
Conjugation of Bilirubin

Two separate steps

Two UDP-glucuronic acid

Two UDP

Bilirubin diglucuronide
3) Metabolism of bilirubin diglucuronide by intestinal bacteria.

In normal individuals, intestinal bilirubin is acted on by bacteria to produce the final porphyrin products, urobilinogens and urobilins, that are found in the feces. A small fraction of urobilinogen is reabsorbed into the blood, extracted by the kidney, and excreted in the urine. Bilirubin and its catabolic products are collectively known as the **bile pigments**.

The clinical determination of plasma bilirubin distinguishes between conjugated (direct) and unconjugated (indirect) bilirubin.

The reaction, called the **van den Bergh reaction**, is a coupling of bilirubin with a diazonium salt to form a colored complex.

**Conjugated** bilirubin is water soluble and reacts directly. This is called the **DIRECT bilirubin**.

**Unconjugated** bilirubin bound to albumin, alcohol is added to release it into solution, where it can now react. This is called the **INDIRECT bilirubin**.
Hyperbilirubinemia (jaundice)

1. Pre-hepatic (hemolytic jaundice):
   - results in increased production of bilirubin.
   - more bilirubin is conjugated and excreted than normally, but
     the conjugation mechanism is overwhelmed, and an
     abnormally large amount of **unconjugated** bilirubin is found
     in the blood.

2. Hepatic:
   2.1 Gilbert's disease
   - may be caused by an inability of the hepatocytes to **uptake**
     bilirubin from the blood
   - As a result, **unconjugated** bilirubin accumulates.
   2.2 Physiological jaundice and Crigler-Najjar syndrome
   - **Conjugation** is impaired.
   - **Unconjugated** bilirubin is retained by the body.
   2.3 Dubin-Johnson syndrome
   - Inability of the hepatocytes to **secrete** conjugated bilirubin
     after it has been formed.
   - **Conjugated** bilirubin returns to the blood.

3. Post-hepatic (biliary obstruction)
   - by (for example) biliary calculi causes backup (interference
     with the secretion) and reabsorption of conjugated bilirubin.
   - Blood levels of **conjugated** bilirubin increase.
Defective Secretion of Conjugated Bilirubin from Liver Cells

Obstruction Somewhere in the Biliary Network (Intrahepatic or Extrahepatic)