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Bilirubin

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Abbreviations:
AMVN: 2,2'-azobis(2,4-dimethylvaleronitrile)
GSH: Glutathione
HPLC: High performance liquid chromatography

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Abstract

Bilirubin is the main pigment component in both jaundice and bruises. Heme oxygenase cleaves heme to form biliverdin, which is then reduced by biliverdin reductase, to produce bilirubin as the final product. Bilirubin is insoluble in water and is bound tightly to albumin to prevent uptake into extrahepatic tissues. Bilirubin can be conjugated and then excreted in the bile. When deposited in the skin, bilirubin is the pigment responsible for neonatal jaundice. Bilirubin was also found to be a powerful scavenger of peroxyl radicals to keep albumin from being oxidized. It is able to remove newly formed radicals before they can initiate a chain reaction. Bilirubin is now known to be more than a waste product found in bile or pigment found in jaundice or bruises, it can serve several functions including as an anitoxidant.

Introduction

Georg Stadeler, in 1864, originally defined bilirubin as the dark red pigment in bile [2]. He also discovered the accumulation of bilirubin was the cause of the yellow color in jaundice patients. It was speculated that bilirubin was structural similar to both biliverdin and haematoidin [2]. Bilirubin was also found to be in two forms; direct reacting bilirubin found in the bile, and indirect found in blood [2]. Bilirubin when in the blood is bound tightly to albumin in a 1:1 stoichiometry, and is insoluble in water at physiological pH [1]. Bilirubin was also found to be a powerful scavenger of peroxyl radicals to keep albumin from being oxidized [1]. Phototherapy has been key in the treatment of accumulation of bilirubin in neonatal jaundice. Bilirubin was also found to be a powerful scavenger of peroxyl radicals.

Characteristics of Bilirubin

The crystal structure of bilirubin was determined to be composed of a carbon backbone with four pyrrole rings containing nitrogen. The unconjugated form contained internal hydrogen oxygen bonds that cause it to be convoluted and infolded in a ridge-tile conformation and therefore insoluble in water (Figure 1) [3]. The unconjugated or indirect form binds to albumin and is soluble in the lipid bilayers of the cell membrane.

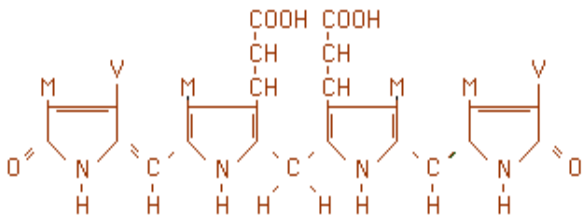

Figure 1. Molecular structure of the unconjugated form of bilirubin. The form is insoluble in water due to the internal hydrogen oxygen bonds [3]. M= $-CH_3$ V= $-CH=CH_2$.

To increase the water solubility of bilirubin conjugation occurs. The process includes the addition of two molecules of glucuronic acid groups to the propionic acid groups of bilirubin producing bilirubin diglucuronide or the conjugated form of bilirubin (Figure 2) [3]. Bilirubin diglucuronide can then be excreted into the bile [3].

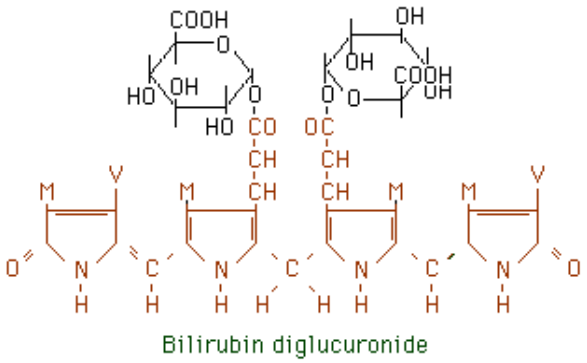

Figure 2. The molecular structure of the conjugated bilirubin. The propionic acid groups have two glucuronic acid groups added to produce the water-soluble form [3].

The molecular extinction coefficient was determined by dissolving bilirubin in chloroform. The result was a coefficient of 5.0-6.0 x 10^4 M^{-1} cm^{-1} at 450 nm (figure 3) [4]. The molecular coefficient is the reason that bilirubin is has a yellow pigment.

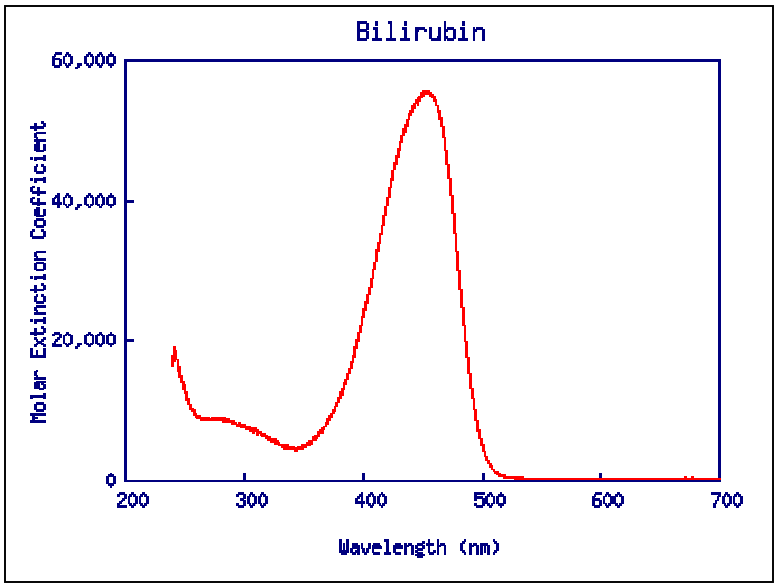

Figure 3. The absorption spectrum of bilirubin when dissolved in chloroform. The x-axis is wavelength in nm, and the y-axis is molar extinction coefficient [4].

Bilirubin can have an absorption spectra ranging from 390-460 nm and a molar coefficient at λ_{max} of 5.0 x10^4 M^{-1} cm^{-1} in aqueous solution and up to 7.0 x 10^4 M^{-1} cm^{-1} in certain organic solvents [5]. The shift to 460 nm was thought to be associated to indirect form of bilirubin bound to albumin [5]. The production of the two forms of bilirubin will be discussed in the next section.

Production of Bilirubin

Bilirubin is the end product of heme degradation. The heme ring is cleaved by heme oxygenase to produce biliverdin, CO, iron (II), and $NADP^+$. Biliverdin is then cleaved between the I and II pyrrole rings by biliverdin reductase to produce bilirubin (or bilirubin monoglucuronide) [3].

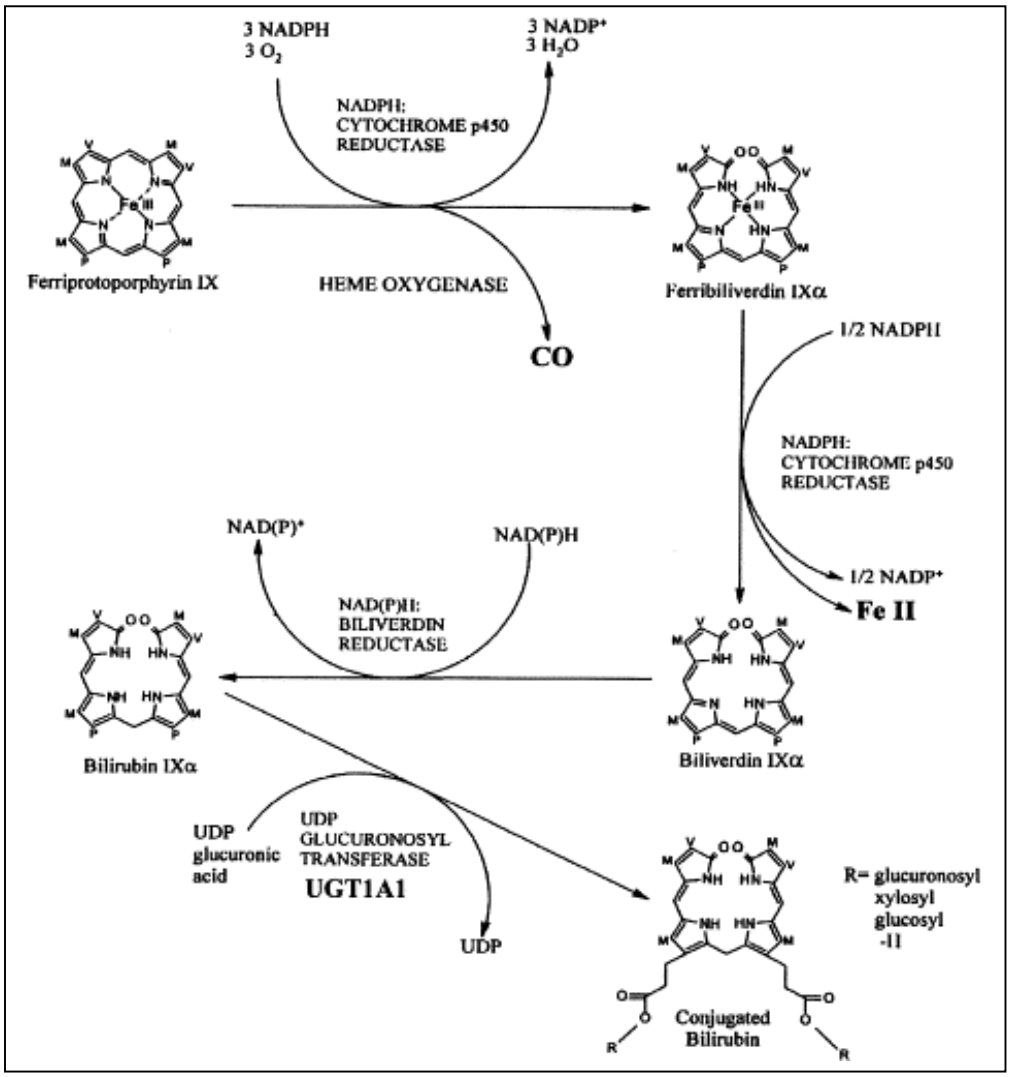

Figure 4. Schematic drawing of the bilirubin metabolism [2]. The degradation by heme oxygenase produces CO, which is thought to be toxic to the cell. CO is known to saturate hemoglobin and decrease the transport of O_2. It was speculated that CO could increase nitric oxide in the cell and affect toxicity indirectly.

Bilivirdin reductase reduces the central methane bridge of biliverdin in the production of bilirubin (Figure 4) [2][3]. The conjugated form is produced in two steps, the transfer of two glucuronic acid groups sequentially to the propionic acid groups of the bilirubin. The transfer is performed by UDP glucuronosyl transferase to produce the conjugated bilirubin, which will be excreted in the bile. The detection method used is high performance liquid chromatography (HPLC) in determining the levels of conjugated bilirubin.

Phototherapy of Neonatal Jaundice

Neonatal jaundice can cause serious problems for babies in respect to brain damage, if untreated kernicterus can ensue. Bilirubin is able to penatrate the blood brain barrier of neonates. Neonatal jaundice is caused by unconjugated bilirubin being deposited in the skin of the newborn. The therapy for neonatal jaundice depends on the amount deposited in the skin. Low levels ranging from 222-325 μM are treated with phototherapy or exposure to light, but if the levels reach 342-428 μM exchange transfusion is employed [6]. It was noted that with the addition of light the color changed from yellow to green. This was described as the bleaching of bilirubin or dehydrogenation to biliverdin [5]. The absorption of light is in the blue range of 420-500 nm [5]. This range will cover the maximum absorption of both bound and free bilirubin. Special blue lamps with specific radiant flux have a spectral range between 420 and 480 nm are employed in the therapy. The resulting reactions of the bilirubin molecule include photoxidation, photodegradation, and photoisomerization. It was originally thought that the oxidation of bilirubin was the main contributor to the beneficial affects of the therapy (Figure 5) [7].

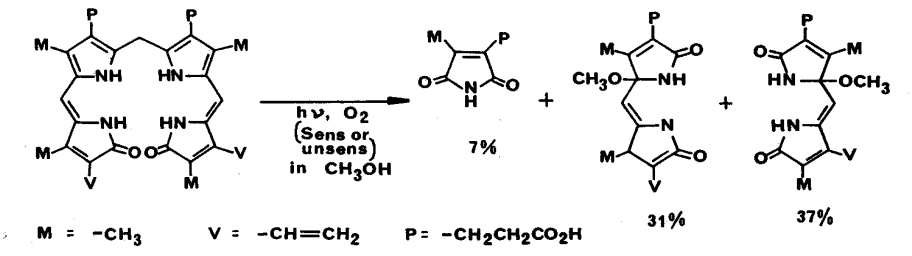

Figure 5. Products of bilirubin oxidation [7].

It was recently discovered that the major mechanism for elimination of bilirubin in tissue and blood is photoisomerization [5]. Spectroscopy and thin-layer chromatography supported the idea of photoisomerization. It was discovered that a cis-trans double bond intercoversion (Z → E) occurred, which disrupted internal hydrogen bonding freeing the carboxyl groups. The transformation produced the water-soluble form 4Z, 15E bilirubin, which could be excreted in the bile [5].

Antioxidant functions

Bilirubin was found to prevent peroxynitrite-mediated protein oxidation, superoxide formation. Bilirubin is a powerful scavenger of peroxyl radicals and $^1O_2$; it can help protect albumin protein and fatty acid against free-radical damage at low physiological concentration [1]. It was thought bilirubin acted on the newly formed free radicals to remove them before they initiated chain reactions that are found in lipid peroxidation. Linoleic acid is involved in a classic chain propagation reaction. The following reactions are the initiation (reaction 1) and propagation (reaction 2 and 3) reactions of linoleic acid. In the experiments, linoleic acid reaction is initiated by radical initiator 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) [8].

$$\mathbf{AMNV} \xrightarrow[\mathbf{LH}]{\mathbf{O_2}} \mathbf{L^\bullet} \qquad \textbf{(1)}$$

$$\mathbf{L^\bullet + O_2 \rightarrow LOO^\bullet} \qquad \textbf{(2)}$$

$$\mathbf{LOO^\bullet + LH \rightarrow LOOH + L^\bullet} \qquad \textbf{(3)}$$

The mechanism proposed was developed by observing a decrease in the absorbance at 450 nm to due to the oxidation of bilirubin. Bilirubin scavenged the chain-carrying peroxyl radical through donation of a hydrogen atom from the C-10 bridge of the tetrapyrrole molecule.

The donation of the hydrogen atom creates a carbon-centered radical ($BR^{\bullet}$), the radical could then react with the second peroxyl radical to produce another nonradical or oxygen (refer to reactions 4, 5, & 6) [8]. The final reaction produced biliverdin, which is quickly recycled back to bilirubin.

$$LOO^{\bullet} + BR \rightarrow LOOH + BR^{\bullet} \quad (4)$$

$$BR^{\bullet} + LOO^{\bullet} \rightarrow BR\text{-}OOL \quad (5)$$

$$BR^{\bullet} + O_2 \leftrightarrow BR\text{-}OO^{\bullet} \quad (6)$$

Bilirubin contains numerous conjugated double bonds that could contribute to the nonreactive of the bilirubin free radical ($BR^{\bullet}$) (Figure 6). The arrow denotes one of the few bis-allelic bonds of bilirubin, the weak attributes of this bond makes it a perfect canidate for donating the hydrogen atom [9].

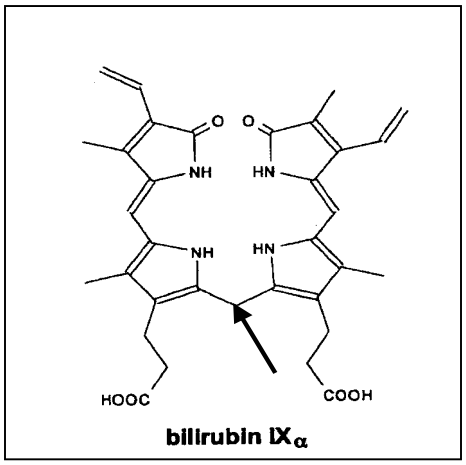

Figure 6. Schmatic of bilirubin the arrow denotes the weak bis-allelic bond found in bilirubin which could donate a hydrogen atom [2, 9].

Another report stated that as little as 10 nM of bilirubin would protect against 10,000 fold higher concentrations of H_2O_2 [10]. This finding suggested that bilirubin was acting in a way other than as a stoichiometric antioxidant. The group then went on to hypothesize that the reaction of bilirubin with two peroxyl radicals to form biliverdin was then rapidly reduced back

to bilirubin. It was this redox cycle that accounted for the 10,000-fold amplification [10]. They were able to show in experiments when bilirubin was reduced or inhibited that a greater production of reactive oxygen species was observed [10]. Bilirubin acts much like glutathione (GSH) in the respect that it exists in two forms, an oxidized form and a reduced form. The greatest difference is the fact that bilirubin exists in less amounts [10]. Another report showed bilirubin-albumin complexes could react with peroxyl radicals 3.1 times faster than uric acid but not as efficiently as vitamin C [11]. It most be noted that most of the experiments that showed bilirubin as a potent antioxidant was performed *in vitro*.

Conclusions

Bilirubin was discovered to be the end product of heme degradation. The unconjugated form of bilirubin is tightly bound to albumin and is insoluble. This form is found deposited in the skin of neonates where it can lead to jaundice. The esterification of the proprionic acid side chains leads to the formation of conjugated form. Conjugated bilirubin is soluble and is excreted in the bile. The absorption of light in the blue range of 420-500 nm lead to the photoisomerization of bilirubin into the 4Z, 15E bilirubin that can be easily excreted. The antioxidant functions of bilirubin showed a greater ability than glutathione *in vitro* and in recent *in vivo* experiments due to the fact little was needed. It can react with two peroxyl radicals to form biliverdin and then be rapidly reduced back to bilirubin in a classic redox cycle. The mechanism was suggested to be the scavenging of newly formed free radicals before they could initiate a chain propagation reaction. This recent finding could prove bilirubin as a better antioxidant then most known today.

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