Regulatory Fe$^{II/III}$ Heme: The Reconstruction of a Molecule’s Biography$^+$

Toni Kühl$^*$ and Diana Imhof$^{[a]}$
Introduction

Approximately 80% of heme (i.e., heme b: FeII–protoporphyrin IX) in the human body is produced and found in red blood cells, 15% is in the liver, and the remaining 5% is synthesized in other tissues. In contrast to its rather limited sites of production, the role of FeVI heme in proteins is multifunctional. Basically, two aspects of action in heme–protein interactions need to be stressed. Firstly, there is a great variety of hemoproteins containing FeIII heme as an essential component for the protein architecture, as well as for the individual biological functions. Secondly, studies demonstrating the occurrence and impact of FeIII heme regulation of proteins have become an increasingly major focus in FeIII heme research over the last decade. This is supported by the steadily growing number of such proteins.

Recently, comprehensive and excellent reviews on heme sensor proteins and their importance in the development of distinct diseases have been published; these focus on issues such as the correlation between cancer and changes in FeIII heme levels. For example, Girvan and Munro review the role of FeVI heme in the context of distinct proteins and discuss their binding parameters (if available). The central point is the function of FeVI heme-based gas sensor proteins, which represent a rather huge class of FeVI heme-binding proteins. A review by Hooda et al. highlights the importance of FeIV heme in the development of diseases such as anemia, porphyrias, Alzheimer’s disease (AD), and, as the primary focus, the development of diseases that were not always attributed to this small molecule. Diseases such as cancer or Alzheimer’s disease highlight the reawakened interest in heme, whose function was previously believed to be completely understood.

This review outlines the progress and significant results obtained concerning proteins whose biological activity/function is modulated by temporary interaction with free FeVII heme (“regulatory heme”). We describe the current knowledge of FeIII heme-regulated proteins in the context of their cellular/biochemical process, and highlight the consequences of imbalanced hemeostasis, in particular how FeIII heme-regulated proteins are influenced by this and participate in FeIII heme-induced diseases. The fundamental role of FeVIII heme as a prosthetic group in proteins and its impact on important biochemical processes is first summarized, as the availability of free FeVIII heme also depends on the proper functioning of hemoprotein synthesis and degradation.

Heme as an Essential Component in Hemoproteins

The integration of FeVIII heme into hemoproteins is usually essential for their function in the context of specific biochemical processes, such as binding and transport of gases (e.g. oxygen, nitric oxide), catalytic processes, and electron transfer reactions (Scheme 1 A).

Gas transport and storage

The ubiquitous and well-studied globins hemoglobin, myoglobin, neuroglobin, and leghemoglobin are the most common proteins to use FeVIII heme for gas transport. More specifically, they are responsible for the removal, and storage of essential molecules such as oxygen within the cell. A common feature is the incorporation of one to four FeIII heme molecules with oxygen binding to the central FeIII ion. In humans, FeIII heme is predominantly found in the hemoglobin of erythrocytes (80%).

Heme-based gas sensors

Another common function of FeVIII heme is as a “heme-based gas sensor” (Scheme 2 A). These proteins carry FeVIII heme as a permanently bound prosthetic group in a regulatory domain responsible for the recognition and binding of gases (e.g. CO, NO, O3). In response to this, the activity of the catalytic domain is changed. Depending on the structure, four main types of heme-based gas sensor can be distinguished: 1) Per-Amt-Sim (PAS) domain sensors, 2) globin-coupled sensors, 3) CooA-heme binding domain sensors, and 4) heme-NO-bind-
ing proteins. Each can be coupled to various functions of the catalytic domain. Typical heme-based gas sensors are neuronal PAS-domain-containing protein 2 (NPAS2, Mus musculus; involved in circadian rhythm) and CO-sensing transcriptional activator (CooA, Rhodospirillum rubrum; regulation of expression of coo-operons). Both are transcription factors that contain a Fe^{II/III} heme molecule in their characteristic heme-binding pocket. By offering gas molecules such as CO, their function is modulated in a Fe^{II/III} heme-dependent manner.

Human proteins such as soluble guanylate cyclase (sGC) and cystathionine β-synthase (CBS) with their Fe^{II/III} heme can be similarly regulated by gases. Fe^{II/III} heme-binding CBS is a lysase catalyzing the condensation of serine and homocysteine to a cystathionine intermediate in sulfur metabolism. Modified conditions, for example, changed pH or the application of gases (CO, NO) lead to significant changes in enzyme activity. In this context, Fe^{II} heme is less active than oxidized heme. In addition, NO and CO lead to enzyme inhibition exclusively for Fe^{II} heme.

Soluble guanylate cyclase (sGC) is activated by attachment of NO or CO to its Fe^{II/III} heme. The resulting increase in cGMP (second messenger) leads to activation of protein kinase G; subsequent phosphorylation of downstream proteins influences signaling pathways that are involved in the cardiovascular and the nervous systems. In addition to NO, (lower) sensitivity of sGC for CO has been reported; sGC is also interesting as the function of Fe^{II/III} heme is not completely solved. Recently it was reported that this protein can be regulated by Fe^{III} heme, and that it also exists in an apo form in vivo. This is another example of a particular protein for which the link between heme-binding and heme-regulation are not fully understood.

**Heme-mediated enzyme catalysis**

NO (required for the activation of various heme-based gas sensors) is synthesized by the Fe^{II/III} heme-containing enzyme nitric oxide synthase (NOS). Three isoforms can be distinguished: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). These enzymes catalyze the conversion of arginine to citrulline with concomitantly released NO. Within the reaction mechanism, both reduced (Fe^{II}) and oxidized (Fe^{III}) heme appear in complex with NO, whereas the release of NO is induced by Fe^{III} heme only. In contrast, primarily Fe^{III} heme triggers oxidation of NO to nitrate (by using oxygen).

Other enzymes in which Fe^{II/III} heme plays an important catalytic role are peroxidases, cytochrome P450 proteins, nitrate reductase, and heme oxygenase. For these enzymes, (unlike sGC) the binding of Fe^{II/III} heme and the mechanism of action are quite well understood in most cases. Especially for cytochrome P450, a huge set of representatives has allowed in-depth analysis of the structural features of Fe^{II/III} heme binding and substrate conversion. In the liver and certain areas of the brain, the main class of hemoprotein is cytochrome P450. These monooxygenases have a strong influence on oxidative, peroxidative, and reductive metabolism of numerous endogenous substances (steroids, bile acids, fatty acids, etc.). For many of these enzymes, conversion of a broad spectrum of exogenous substances (e.g., drugs, alcohol, environmental toxins) is essential for health. Recruitment of oxygen for substrate oxidation as well as electron transfer is performed by the Fe^{III} heme molecule of the enzyme. Different iron oxidation states (II, III, IV) have been identified during the reaction cycle; Fe^{II} is the “resting” state.

Peroxidases and catalases include a huge number of enzymes that carry Fe^{III} heme at the catalytic site. These convert hydrogen peroxide to less reactive products, whereas catalases release water and oxygen, peroxidases oxidize a further substrate (electron donor) in addition to releasing water. The significance of Fe^{III} heme is its function in recruiting hydrogen peroxide and, in this context, with the formation of high valency iron–oxygen intermediates that lead to oxidation of the substrate.

Additionally, heme oxygenases are responsible for Fe^{III} heme degradation to biliverdin, CO, and ferrous iron.
heme binds to the protein and leads, in a stepwise manner, to the recruitment of oxygen and its degradation.\(^{[18]}\)

Electron transport

Cytochrome \(b_5\) is an essential protein in electron transport and involves oxidation/reduction of its heme group.\(^{[19]}\) Cytochrome \(b_5\) also forms part of other proteins (e.g., nitrate reductase, mitochondrial sulfite oxidase, flavocytochrome \(b_5\)), where the Fe\(^{III}\) heme is also responsible for electron transfer.\(^{[12d, 20]}\) For cytochrome \(b_5\) and others (e.g. flavocytochrome \(b_5\)), regeneration of Fe\(^{III}\) heme and, thus the whole protein, is carried out by electron transfer to Fe\(^{III}\) heme-containing cytochrome \(c\).\(^{[12d, 21]}\) By using the redox activity of the heme iron in cytochrome \(b_5\), cytochrome \(c\) takes up and transports electrons along the membrane to the cytochrome \(c\) oxidase complex (respiratory chain).\(^{[21]}\) However, these cytochromes differ in their binding of Fe\(^{III}\) heme: cytochrome \(b_5\) carries non-covalently bound heme \(b\), whereas heme \(c\) in cytochrome \(c\) is covalently attached at the propionate side chains to cysteine residues of the protein.\(^{[22]}\)

Thus, the character (e.g. binding mode, consensus sequence) and consequences of Fe\(^{III}\) heme binding are obviously influenced by multiple factors, particularly protein architecture and environment. Similarity of hemoprotein biochemical function is not necessarily indicative of a similar construction. This implies an even more complicated situation for Fe\(^{III}\) heme-regulated proteins, because less-distinctive features with respect to binding determinants and characteristics are expected.

Heme as a regulatory effector of protein function

There are several reports of a pool unbound cellular Fe\(^{III}\) heme (~0.1–1.0 \(\mu\)M).\(^{[3]}\) The significance of this free Fe\(^{III}\) heme to the proteins mentioned above has not been take into account. However, the unbound Fe\(^{III}\) heme does not necessarily account solely for the synthesis of hemoproteins; over the past two decades it role as a regulator of numerous proteins has gained importance.\(^{[19]}\) Extracellular Fe\(^{III}\) heme is usually bound to proteins such as hemoglobin, albumin, and hemopexin, but various conditions (e.g., bleedings after injury) can lead to increased levels of free Fe\(^{III}\) heme in tissues. In hematomas, the concentration of (mainly) oxidized heme (Fe\(^{III}\) heme) can exceed 350 \(\mu\)M.\(^{[24]}\) After injury, release of heme from erythrocytes leads to oxidation of heme ferrous iron to form methemoglobin and subsequent liberation from the protein. This oxidation correlates with the formation of reactive oxygen species (ROS). Similar processes are found for other intra- and extracellular hemoproteins after cell lysis. Thus, for the surroundings of heme in several proteins, the binding capacity for Fe\(^{III}\) heme is significantly lower.\(^{[3b]}\) In contrast, Fe\(^{III}\) heme (rather
than Fe II heme) shows increased binding for cysteine in cytochrome P450 proteins and NOS. Assuming no other amino acids significantly increase the binding capacity for Fe II heme, for these proteins an inverse effect with respect to binding of FeII heme or Fe III heme is observed compared to hemoglobin.[25]

The availability of free Fe II and Fe III heme occurs under different conditions, both intracellularly and extracellularly, thus demonstrating that a regulatory impact on proteins is possible. These effects can be observed at different stages of protein synthesis and degradation, in signal transduction, and in ion channel regulation, as well as in immune responses, in the reaction to diatomic gases, and many other processes (Scheme 1B). The modes of action of such heme sensors are shown in Scheme 2B.[6]

### Heme biosynthesis and metabolism

High levels of free Fe III heme (1.0–10.0 μm) are toxic due to the formation of ROS.[3a] Consequently, the synthesis and degradation of Fe III heme need to be strictly controlled. The biosynthesis of Fe III heme is performed in eight steps in mitochondria and the cytoplasm. It is controlled in nearly all cells (Scheme 3) by regulating the levels of the first enzyme in the synthesis (non-specific aminolevulinic acid synthase, ALAS1) in a Fe II/III heme-inducible negative feedback loop. High concentration of Fe III heme prevents transcription, translation, and translocation of ALAS1 into mitochondria.[26] In contrast, the erythrocyte-specific isoform ALAS2 is controlled completely differently with respect to FeII/III heme. Here, an increase in the levels of ALAS2 is observed upon application of Fe II/III heme.[26a] Translocation into mitochondria is also different.[23e, 26b] One reason for the increase in ALAS2 levels is associated with the regulation of iron-regulatory protein 2 (IRP2). In the case of ALAS2, its translation is inhibited by binding of IRP2 to iron-responsive elements (IREs) in the 5'-untranslated region.[2a, 27] Binding of Fe II/III heme to IRP2 induces oxidation of the protein and subsequent ubiquitylation and proteasomal protein degradation,[2a, 23b] which, in turn, allows translation of ALAS2 mRNA.[27]

But degradation of IRP2 can also decrease protein synthesis, as for example in the case of the transferrin receptor.[27] 3' IREs of the receptor mRNA are in the 3'-untranslated region; binding of IRP2 stabilizes the mRNA thereby supporting its translation. Thus, Fe III heme-induced degradation of IRP2 increases the probability of transferrin receptor mRNA degradation thus reducing protein synthesis.[27] IRP2 is obviously involved in the maintenance of iron homeostasis.[27] Another protein that regulates iron metabolism similarly to IRP2 is the iron-response regulator (Irr) protein from *Bradyrhizobium japonicum*. Binding of Fe III heme also induces degradation of Irr.[2a, 28] Additionally, iron deficiency leads to inhibition of aminolevulinic acid dehydratase in this bacterium (essential for Fe III heme biosynthesis).[29]

The binding of regulatory Fe III heme to the Fe III heme-degrading enzyme heme oxygenase 2 (HO-2) has also been de-
Rhythmically oscillating Fe II/III heme levels are reflected in regulatory processes in prokaryotic bacteria, and mammals is regulated by Fe II/III heme, for example, in wound healing. Upregulation of HO-1 is achieved through translation of the protein by Fe II/III heme during circadian rhythm. Therefore, binding of Fe II/III heme to DNA results in gene expression of proteins, for example Bmal1, in a Fe II/III heme-induced manner. Additionally, Rev-erbβ was shown to act as a heme-based gas sensor for NO and CO upon Fe II/III heme application. For this reason this protein and others (e.g. NPAS2) have dual-role characteristics: heme sensor and, after Fe II/III heme recruitment, heme-based gas sensor.

Transcription, circadian rhythm, and heme-sensitive gas sensor

For a broad range of proteins, transcription in yeast, photosynthetic bacteria, and mammals is regulated by Fe II/III heme, for example, heme activator protein 1 (HAP1), Period 2 (Per2), transcriptional repressors Rev-erbα and Rev-erbβ, and transcription factor PpsR (Scheme 4). Bach1 belongs to this list and shows significant reduction in affinity for DNA upon binding of Fe II/III heme; this allows the transcription of genes for proteins that are regulated by Fe II/III heme and, thereby, protein translation is controlled. Phosphorylation at Ser51 decreases its affinity for DNA.

Translation and miRNA processing

Translation is regulated in numerous ways. For example, binding of Fe II/III heme to DGCGR8 (DiGeorge syndrome critical region 8 protein) is essential, to form the complex for synthesizing miRNA. Translation initiation factor 2 (eIF2α) has a significant role in translation initiation. Phosphorylation at Ser51 decreases its activity, and protein synthesis is consequently inhibited. This process has been observed in erythrocytes, and is mainly relevant for the synthesis of α- and β-globins. It can be triggered by eIF2α kinase (HRI, heme-regulated inhibitor), which was downregulated upon Fe II/III heme application. For the latter, an intrinsic phosphorylation event in the HRI molecule was.
ing, and intermolecular disulfide bridge formation between monomers in the HRI homodimer was observed, thereby resulting in loss of kinase activity. This, in turn, allowed the synthesis of globin molecules to bind excess Fe\textsuperscript{III} heme to form hemoglobin.\cite{38}

The influence of Fe\textsuperscript{II} heme on translation has also been reported by affecting aminoacylation of amino acids to their corresponding tRNAs. This process is catalyzed by specific aminocyl tRNA synthetases. For example, arginyl tRNA synthetase (ArgRS) oligomerizes in vitro upon binding to Fe\textsuperscript{III} heme, thereby losing its activity.\cite{40} In contrast, tryptophanyl tRNA synthetase (TrpRS) and its sensitivity for Fe\textsuperscript{III} heme revealed an inhibitory effect.\cite{43} Similarly, the activity of dipeptidyl peptidase 8 (DPP8), which is known to play a role as a mediator of T-cell activation, was inhibited upon application of Fe\textsuperscript{II} heme in vitro.\cite{41} In addition to structural investigations, we reported the effect of Fe\textsuperscript{III} heme on DP8 by using an enzyme assay employing the modified dipeptide Ala-Pro-nitroanilide as the substrate.\cite{44} Inhibition by Fe\textsuperscript{III} heme occurred at the low-micromolar level (IC\textsubscript{50} = 11.5 ± 1.2 μM), which is higher than the concentration of free Fe\textsuperscript{II} heme in the heme pool. This indicates that inhibition of dipeptidyl peptidase 8 is most important under pathophysiological conditions of increased levels of Fe\textsuperscript{II} heme (e.g., hematomas). All these data emphasize the role of Fe\textsuperscript{II} heme in the regulation of pathways and cascades that are closely connected to the immune system.

Concerning inflammatory processes, an increase of interleukin-8 (IL-8), both mRNA and protein, was observed upon Fe\textsuperscript{II} heme application in human neutrophils.\cite{42} This was caused by increased activation of protein kinase C (PKC).\cite{43} However, inhibition of PKC still resulted in increased IL-8 expression following Fe\textsuperscript{II} heme application. This led to the suggestion that further modes of regulation by Fe\textsuperscript{II} heme exist, although these have not been fully explored or described.\cite{42} Regarding PKC, several reports have demonstrated a link to the mitogen-activated protein kinase (MAPK) pathway (Scheme 6).\cite{45} This signal

**Scheme 5.** Translation and proteasomal protein degradation are regulated and cross-linked by Fe\textsuperscript{III} heme at different levels. Inhibition of HRI in erythrocytes increases synthesis of α- and β-globins; these integrate Fe\textsuperscript{II} heme during the formation of hemoglobin. Arginyl-tRNA synthetase cross-links processes of translation and proteasomal protein degradation. Through attachment of Fe\textsuperscript{III} heme to proteins Per2, Bach1, Ir, and IRP2, their own proteasomal degradation is induced. In contrast, degradation processes that are mediated by ATE1, UBR1, UBR2, and arginyl-tRNA synthetase are inhibited by Fe\textsuperscript{II} heme. (HRI-P: phosphorylated HRI; elf2α(P): phosphorylated elf2α).

**Scheme 6.** Influences of Fe\textsuperscript{III} heme on signal transduction pathways, immune responses, and transmembrane ion transport. Fe\textsuperscript{II} heme binds directly to ion channels, which, in the case of H501, leads to increased vasconstriction in the brain.\cite{44} In most reports, changes in signal transduction levels and immune responses are general reactions upon application of Fe\textsuperscript{III} heme to whole cells or from growth of cells on Fe\textsuperscript{III} heme-deficient media. Neudesin and neuferricin are the first extracellular, temporary Fe\textsuperscript{II} heme-binding proteins, and show different effects upon Fe\textsuperscript{III} heme application.\cite{42} Reports on the cross-link of PKC and MAPK-pathway raise the suggestion of further crossovers not mentioned here. (ERK1/2-P: phosphorylated extracellular signal-regulated kinase 1/2; Raf-P: phosphorylated Raf; MEK1/2: phosphorylated MAPK/ERK kinase 1/2; NGF: nerve growth factor).

**Signal transduction, immune response, and transmembrane ion transport**

In addition to these regulatory effects of Fe\textsuperscript{III} heme on metabolism, protein synthesis, and protein degradation, an impact on immune response and inflammatory processes is known (Scheme 6).\cite{42} For example, C1q (the recognition unit of the first compound of the classical complement pathway) plays an important role for activation of the complement system. Upon binding of Fe\textsuperscript{II} heme to C1q the mechanism of recognition between immunoglobulin G and C-reactive protein changes. This leads to reduced binding and thus prevents activation of further complement compounds.\cite{42} In vitro studies of the interaction of recombinant blood coagulation factor VIII and Fe\textsuperscript{III} heme revealed an inhibitory effect.\cite{43} Similarly, the activity of dipeptidyl peptidase 8 (DPP8), which is known to play a role as a mediator of T-cell activation, was inhibited upon application of Fe\textsuperscript{II} heme in vitro.\cite{41} In addition to structural investigations, we reported the effect of Fe\textsuperscript{II} heme on DPP8 by using an enzyme assay employing the modified dipeptide Ala-Pro-para-nitroanilide as the substrate.\cite{44} Inhibition by Fe\textsuperscript{III} heme occurred at the low-micromolar level (IC\textsubscript{50} = 11.5 ± 1.2 μM), which is higher than the concentration of free Fe\textsuperscript{II} heme in the heme pool. This indicates that inhibition of dipeptidyl peptidase 8 is most important under pathophysiological conditions of increased levels of Fe\textsuperscript{II} heme (e.g., hematomas). All these data emphasize the role of Fe\textsuperscript{II} heme in the regulation of pathways and cascades that are closely connected to the immune system.
transduction pathway to the nucleus via ERK1/2, MEK1/2, and Raf can also be activated by Fe^{II/III} heme. Usually, activation of the MAPK pathway is induced by ligand binding to a protein tyrosine kinases (e.g. epidermal growth factor receptor, platelet-derived growth factor receptor). However, in HeLa cells, Fe^{II/III} heme-regulated proteins such as Jak2 and Src were also substantially influenced by Fe^{II/III} heme, as was the MAPK pathway. 

Motivated by studies on cerebral vasospasm, ion channels have gained increased interest concerning protein regulation by Fe^{II} heme in recent years (Scheme 6). One of the most intensively studied proteins is the human large conductance calcium-dependent potassium channel Slo1 (hSlo1). Intracellular binding of Fe^{II} heme to the channel yielded a reduction in K\(^+\) influx by (reversible) inhibition of the ion channel. In addition, a voltage-gated potassium channel (KnC; subtype K\(_n\).1.4 from Rattus norvegicus) showed intracellular interaction with Fe\(^{II}\) heme; this impaired the inactivation process by the “ball-and-chain” N terminus of the channel protein induced by Fe\(^{III}\) heme thus prolonging the opening of the channel pore. This apparently contradictory influence of Fe\(^{III}\) heme on ion channel activity highlights the complex network in which Fe\(^{III}\) heme influences not only ion channels but also many different proteins in a wide variety of cellular processes.

Pathophysiological Consequences of Heme-Associated Dysregulation

The ubiquitous presence and this diversity of function renders Fe\(^{III}\) heme a central anchor in numerous essential processes. Dysregulation and interruptions in the typical Fe\(^{II/III}\) heme-associated pathways can result in severe problems.

Porphyria and anemia

Complete termination of Fe\(^{III}\) heme biosynthesis is lethal for the organism. Several types of Fe\(^{III}\) heme deficiency are caused by reduced biosynthesis, for example, anemia and porphyria. Anemia can develop under various conditions, such as iron deficiency, defective globin synthesis, and dysfunction of ALAS2, and is characterized by dramatically reduced hemoglobin synthesis. Phenotypic changes in erythrocytes are typically observed, with sickle cell anemia (mutation in \(\beta\)-globin) as the most familiar variant. Sideroblastic anemia is closely linked to Fe\(^{III}\) heme biosynthesis, and results from less-active ALAS2. This enzyme catalyzes the rate-limiting step of the covalent attachment of succinyl-coenzyme A to form 5-aminolevulinic acid. Hindrance of this reaction (or its extent) causes an increase in toxic iron. Classical symptoms of anemia are pallor, breathlessness, exhaustion, and general weakness. However, synthesis of Fe\(^{III}\) heme is an eight-step process, and thus malfunctions can also arise in the other seven enzymes (for an overview see Zhang and Sessoms).

Defects and reduced rates of the relevant enzyme activities lead to accumulation of Fe\(^{III}\) heme precursors such as porphyrin (the origin of “porphyria”). Typical symptoms are photosensitivity and neurological disturbance. Accumulation of Fe\(^{III}\) heme precursors can also lead to red teeth and skin lesions upon light exposure, because of the light sensitivity of porphyrin precursors and subsequent ROS formation. Acute attacks have the characteristic symptoms abdominal pain, vomiting, occlusion, hypertension, tachycardia, and dysfunction of the bladder. Mental problems often also appear (depression, anxiety, sleep disorders, hallucination, and paranoia). Porphyrrias are divided into inherited and acquired, as well as hepatic and erythropoietic. For erythropoietic porphyrias, light sensitivity is often the only characteristic symptom, whereas in most hepatic porphyrias acute attacks and neurodegenerative lesions are described (e.g., AD as discussed in the next section).

Neurodegenerative diseases

Alzheimer’s disease (AD), Parkinson’s disease and (age-related) dementia are a selection of severe neurodegenerative disorders. In recent years, an increasing number of studies have been performed to clarify the correlation between altered Fe\(^{III}\) heme levels and the occurrence of Fe\(^{II/III}\) heme-related diseases. In general, in most of the affected cells, increased levels of HO-1 are observed. Degradation of Fe\(^{III}\) heme, which is catalyzed by HO-1, leads to the formation of bilirubin, which exhibits antioxidative properties, and to CO, which has an important role as a neurotransmitter. These positive effects, however, contrast with the release of ferrous iron, which is toxic in high doses. Therefore, a balanced system is essential for Fe\(^{III}\) heme degradation, and this can be exemplified by the classical neurodegenerative diseases AD and Parkinson’s disease. In AD, increased levels of HO-1 cause a decrease in Tau protein expression, and this leads to a reduced activation of the MAPK pathway. In 2004, Fe\(^{III}\) heme interaction with human amyloid-\(\beta\) protein (\(A\beta\)) was shown in vitro for the first time, thus shedding light on how Fe\(^{III}\) heme is involved in the development of AD. In addition, a complex formed between \(A\beta\) and Fe\(^{III}\) heme displayed peroxidase activity; this was suggested to cause additional oxidative stress in AD affected cells. Thus, the disease-related, typically elevated oxidative stress levels are further elevated. In the initial phase, the \(A\beta\)-Fe\(^{III}\) heme complex prevents the formation of cytotoxic \(A\beta\) aggregates. In parallel, reduction in the levels of ALAS1 and porphobilinogen deaminase (and, subsequentely, of Fe\(^{III}\) heme biosynthesis) have also been observed in the early phases of AD. This was suggested to induce Fe\(^{III}\) heme deficiency, which does not prevent the development of the characteristic plaques in contrast to the formed \(A\beta\)-Fe\(^{III}\) heme complexes at non-deficient cells for heme biosynthesis.

In Parkinson’s disease, increased levels of HO-1 are accompanied by the appearance of “Levy bodies” in affected dopaminergic neurons. This suggests a therapeutic approach in which increased expression of HO-1 recovers the cell’s redox homeostasis to achieve neuroprotective and anti-inflammatory effects.
Of relevance for dementia (as well as other neurodegenerative diseases), application of extracellular Fe^{II/III} heme to cortical neurons induced rescue of the affected cells, thus preventing degradation of neurites and apoptosis. A survival-promoting signal is induced by recovering ERK1/2 activity upon Fe^{III} heme application (Scheme 6). Cortical neurons that grow under Fe^{III} heme depletion showed significantly decreased neurite generation. Additionally, a dependence of ERK1/2 activity on activation of membrane-localized N-methyl-D-aspartate (NMDA) receptors was observed. Fe^{III} heme depletion caused a decrease in calcium influx into the cell, and this reduced ERK1/2 activity. There was a simultaneous decrease in expression, phosphorylation level, and functionality of NMDA receptors; these are indispensable for the processes of learning and memory, as well as for synaptic plasticity.

Aging

A decrease in Fe^{II/II} heme synthesis is found with increasing age. The resulting Fe^{II/II} heme deficiency causes problems such as neurodegeneration and reduction of Complex IV (respiratory chain). The latter is accompanied by an increase in oxidative stress and malfunctions in calcium homeostasis. Medication use increases with age, yet the drugs need to be converted by cytochrome P450 proteins. Synthesis of these proteins is indispensable in most cases where increased amounts of pharmaceuticals need to be managed. But, usually decreased Fe^{II/II} heme biosynthesis is caused by decreased levels of ALAS1 and heme oxygenase. Consequently, Fe^{III} heme depletion leads to reduced availability of cytochrome P450. Cytochrome P450 CYP1A1, for example, is reported to require Fe^{III} heme for its transfer from the cytosol to the endoplasmic reticulum to be finally converted into its active form.

All these reports clearly identify Fe^{II/III} heme as a risk factor that has a significant influence on aging processes of cells; however, in most cases the molecular basis leading to these conditions has yet to be elucidated.

Hemolysis and cerebral vasospasm/subarachnoid hemorrhage

In contrast to porphyria and anemia, increased levels of Fe^{III} heme can also cause physiological disturbances. Uncontrolled release of Fe^{III} heme (concentrations above physiological) by lesions in cells and tissues can arise for very different reasons. Hemolytic anemia, sickle-cell anemia, malaria, trauma, and bleeding are typical causes of increased concentration of regulatory Fe^{III} heme. Subsequently, the organism responds by stimulating a proinflammatory signal to initiate an immune response. Degradation of Fe^{III} heme is then an essential step that is realized by a HO-1-induced switching off of the original trigger of inflammation.

Hemolysis is an Fe^{III} heme-releasing processes that induces degradation of erythrocytes in the liver and spleen under controlled conditions, when erythrocytes are approximately 120 days old. If this process is initiated earlier (i.e., pathophysiological conditions), excess cytotoxic Fe^{III} heme is released from hemoglobin, and this urgently needs to be bound and degraded. In the case of defects in Fe^{III} heme degradation, hyperbilirubinemia can develop; this can cause liver dysfunction and jaundice among other disorders. A classical dysfunction of this type is neonatal jaundice, which is induced by insufficient activity of UDP-glucuronyl transferase. It can be treated by application of blue light to convert excess bilirubin to non-toxic products. The consequences of untreated, repeatedly occurring hemolysis are ubiquitous and can result in severe disorders ranging from arteriosclerosis to kidney failure.

Bleeding caused by injuries results in increased concentrations of Fe^{III} heme (> 350 μM) in the resulting hematomas. Typically, the heme oxygenase system and efficient Fe^{III} heme-binding proteins (hemopexin, albumin) serve as scavengers to buffer the action of free Fe^{III} heme. However, Fe^{III} heme levels that exceed the normal have negative consequences due to free regulatory Fe^{III} heme, both intracellularly and extracellularly. For the latter, Fe^{III} heme uptake is a prerequisite to activate the immune system to increased different pathways and the complement system. This contrasts with the reports on inhibition of C1q-mediated initiation of the complement system by Fe^{III} heme (see above). Another proinflammatory signal is the formation of ROS caused by Fe^{III} heme; this leads to activation of transcription factors NF-κB, AP-1, and STAT (independently, there was an increase in the expression of adhesion proteins and selectins in endothelial cells; in turn recruits leucocytes). The combination of these and other factors (for details see ref. [51a]) enables an adequate response by the immune system to increased regulatory Fe^{III} heme after injury.

Hemorrhagic infarct, intracerebral bleeding, and subarachnoid hemorrhage (SAB) are responsible for an increase in free Fe^{III} heme levels and thus toxicity. Typical symptoms of SAB are pathophysiological cerebral vasospasm. After bleeding in the central nervous system, hemoglobin is released from erythrocytes within a few hours. In addition to the formation of cytotoxic ROS by Fe^{III} heme and Fe^{III} heme degradation products such as iron, accumulation of these can cause brain atrophy. In an alternative route, Fe^{III} heme degradation leads to bilirubin oxidation products A and B (BOX A, BOX B) and 4-methyl-3-vinylmaleimide (MMV), which are suggested to be involved in the development of vasocostrictive processes in cerebral vasospasm. Also, Fe^{III} heme might directly affect ion channel proteins, for example, H101 (Scheme 6) in cerebral tissue; these are modulated by Fe^{III} heme binding to an intracellular site on the channel.
Cancer

Growth and apoptosis are significant in a cell’s fate. Growth is regulated by the cell cycle, but apoptotic mechanisms are controlled by proteins that can be regulated in different ways, thus causing events ranging from cell-cycle arrest to specific differentiation and/or cell degradation.[12] The impact of FeII/III heme is cell-type-specific,[32] as was demonstrated with PC12 neurons and K562 erythroid cells, which differentiate upon treatment with FeII heme. In contrast, in HeLa cells increased cell growth and cell-cycle activity was reviewed,[32] Different proteins are responsible for this process, for example, p18, p21, and p53 as negative regulators of cell cycle, and cyclin-dependent kinase 1 (Cdk1) and cyclin-dependent kinase 4 (Cdk4) as positive regulators. In K562 cells, differentiation was initiated after FeIII heme application through induction of p18 and p21, and these was a simultaneous decrease in cyclin D2 expression.[32,46] In contrast, in HeLa cells FeIII heme depletion led to an increase in p21 and p53 and inhibition of Cdk1 and Cdk4, thus forcing the cell into cell-cycle arrest and eventually apoptosis.[32,46]

Detailed analysis of HeLa cells identified FeIII heme as regulator of the phosphorylation activity of various proteins of the MAPK pathway (Scheme 6): activation and phosphorylation of Raf, MEK1/2, and ERK1/2.[46] In addition, protein tyrosine kinases (e.g., Jak2 and Src) showed significantly changed phosphorylation activity upon application of FeIII heme.[46] Jak2, for example, was activated and phosphorylated on tyrosine residues 1007/1008, whereas phosphorylation of tyrosine 530 of Src inhibited its protein tyrosine kinase activity. Src is a well-known oncogene and is thus a promising target for a tumor therapy.[48,68]

Derived from their studies, Ye et al. postulated that inhibition of FeIII heme biosynthesis in cancer cells would be a starting point for blocking cell proliferation and, moreover, for inducing senescence and apoptosis in affected tissue.[46] Yao et al. suggested the use of FeIII heme analogues to mimic or change the reactions of protein tyrosine kinases upon application of FeIII heme or its analogues.[46] Finally, a recent comprehensive overview by Hooda et al. extensively underlines the direct correlation between dietary FeIII heme intake and the risk of different types of cancer, type 2 diabetes, and coronary heart diseases.[26]

Summary

Today FeII/III heme is recognized as a temporary regulator of protein activity and as a key player in a complex network that interacts with a broad spectrum of cellular processes (circadian rhythm, transcription, translation, signal transduction, immune response, etc.). The implications of such processes for the development of diseases such as AD, cerebral vasospasm, and cancer caused by dysregulation of the level of free FeII/III heme are obvious. This emphasizes the necessity for a detailed understanding at the molecular level of processes affected by regulatory FeII/III heme in a cellular context; this is, in our opinion, still missing. The intention of this review is to combine the current knowledge on FeII/III heme-regulated proteins and related diseases in an ambitious attempt to support closing this gap.

Although there is a well-tuned mechanism for discriminating free and bound FeII/III heme in an organism, the level of free FeII/III heme significantly influences the proportion of regulatory free FeII/III heme. Because of the influence of FeII/III heme on a broad repertoire of FeII/III heme-regulated proteins, the disturbance of homeostasis is a clear indicator for the development of the aforementioned disorders.

Outlook

The selection here of FeII/III heme-regulated proteins and pathways is not intended as a complete registration of FeII/III heme-binding and FeII/III heme-regulated proteins; nor do we provide an in-depth view on the molecular (structural) basis of the specific FeII/III heme–protein interactions. Instead, we chose these examples to provide indicators as to where the topic is likely to head in the next few years. FeII/III heme is far from being one of the molecules checked off the list of valuable research topics; its involvement in a broad variety of biochemical processes as a regulator illustrates the new opportunity for researchers to participate in the exploration of its involvement in the development and progression of diseases. Thus, in our opinion, novel diagnostic and therapeutic strategies and pharmaceuticals in the near future can be derived by exploiting research related to regulatory FeII/III heme.

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