

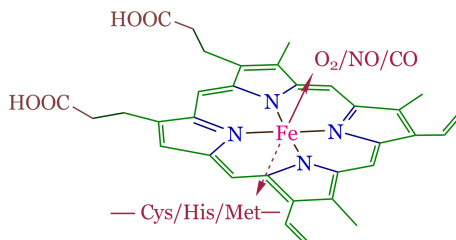
# INTRODUCTION

Li Zhang

## Overview

Heme, iron protoporphyrin IX (Fig. 1), is arguably one of life's most central molecules. Most of us know about heme because of hemoglobin — the molecule that transports oxygen from the lung to all other organs and tissues in the human body. It is heme that gives hemoglobin the unique oxygen-binding property. The distribution of heme is not even throughout the human body. Roughly 80% of heme in humans is made and present in red blood cells; fifteen percent is made and present in the liver and the rest is distributed in other tissues. All human cells presumably make a basal level of heme for the synthesis and proper functioning of certain proteins and enzymes that use heme as a cofactor or a prosthetic group.

Many living organisms ranging from bacteria to humans can synthesize heme *de novo* (1). Those that do not synthesize heme *de novo*, like *Caenorhabditis elegans*, still require heme for survival and acquire heme via dietary intake (2). Heme and porphyrins have been the subject of fascination and intense studies for scientists for over a century. Porphyrins are compounds composed of a macrocycle of four pyrrole rings linked by four methene bridges. They include metalloporphyrins, such as hemes and chlorophylls. In the early 20th century, scientists started to investigate heme and its porphyrin precursors, due to their association with a class of interesting diseases called porphyrias. Porphyrias are inherited (mostly autosomal dominant) and acquired disorders associated with *partially* defective enzymatic activities of the heme biosynthetic pathway and increased levels of heme precursors (3).



**Fig. 1.** The structure of heme. Heme is composed of a macrocycle of four pyrrole rings, with four methyl groups, two vinyl groups and two propionate groups attached. The hydrophobic parts of heme are indicated in green. The four nitrogen atoms of pyrrole rings coordinate the heme iron ion. Iron ion can coordinate two axial ligands, which may be Cys, His or Met residue in proteins or small molecules, including oxygen, nitric oxide and carbon monoxide.

It is not confirmed, but is a popular belief that the first documented case of any porphyria disorder dates back to the time of Hippocrates. The term porphyria is derived from the Greek term *porphura* which means “purple pigment” in reference to the color of body fluids in people suffering from a porphyria. Urine that contains porphyrins or porphyrin precursors turns different colors when exposed to the air; black urine became a tell-tale sign of Acute Intermittent Porphyria (AIP). Porphyrias are classified as hepatic or erythropoietic in type, depending on the primary organ in which excess production of porphyrins or precursors takes place (3). Two types of clinical symptoms are associated with porphyrias: cutaneous photosensitivity and acute attacks involving abdominal pains, psychiatric manifestations such as anxiety, depression, and confusion, and neurological manifestations. Erythropoietic porphyrias are associated with only cutaneous photosensitivity; hepatic porphyrias can be associated with both cutaneous photosensitivity and acute attacks. Those associated with acute attacks are also called acute porphyrias.

It has been suggested that persons with congenital erythropoietic porphyrias were the werewolves or vampires of legend. Due to the accumulation of high levels of heme precursors in such subjects, they can have reddish teeth and strong cutaneous photosensitivity. As such, subjects may have skin mutilation, hypertrichosis, and desire to eschew light exposure. This may have led to the superstition of werewolves. Medical records also suggest that many members of the European royal families, including James, IV and I, George III, Frederic the Great of Prussia, and Kaiser

Wilhelm, suffered from acute porphyrias (4). The neurological and psychiatric manifestations associated with acute porphyrias provide a logical explanation for King George's illness. In 1993, Alan Bennett wrote a play, "The Madness of King George" in which he loosely based the King's ailments on porphyria rather than a psychological basis (5).

The latter part of the 20th century has seen a spurt of research on heme and heme proteins due to the importance of heme as a prosthetic group or a cofactor in key proteins and enzymes that support life. These include the following: Hemoglobin and myoglobin that transport and store oxygen; cytochromes and oxidoreductases that support cellular energy generation and biosynthesis; cytochromes P450s that are important for drug metabolism and the synthesis of endogenous substances such as lipids and steroids; cytochrome peroxidases that synthesize molecules critical for innate immune reactions; and oxygenases that synthesize important neuromodulators nitric oxide and carbon monoxide.

More recently, scientists have discovered that heme can serve as a signaling molecule, and thereby regulate a wide array of molecular and cellular processes in living organisms. For example, heme can impact the growth, differentiation and survival of many mammalian cells (6). Heme also controls fundamental molecular and cellular processes, such as protein synthesis, gene transcription, protein localization and assembly. This book is designed to provide you with a complete and up-to-date view of the versatile and fascinating roles of heme in controlling many fundamental biological processes in living organisms, particularly in humans.

To fully understand and appreciate the versatile roles of heme in living processes, it is necessary to have a clear understanding of the structure and chemistry of the heme molecule. Figure 1 illustrates the structure and key chemical features of heme. Heme is composed of a macrocycle of four pyrrole rings. The four nitrogen atoms chelate one iron ion. Iron ion can be in the ferrous ( $\text{Fe}^{+2}$ ) or ferric state ( $\text{Fe}^{+3}$ ). The word heme is usually used as a generic term to identify both ferrous and ferric forms of iron protoporphyrin IX. Properly, however, heme refers only to ferrous protoporphyrin IX, whereas ferric protoporphyrin is called hemin. In air, hemin is more stable than heme. Hemin has a positive charge and is usually isolated with a counterion like chloride.

Heme is a small molecule by molecular mass, and by comparison with macromolecules, such as proteins and nucleic acids in living cells. Yet, it is full of complexity and chemical intricacies. The heme molecule (Fig. 1) contains parts that are highly hydrophobic, including the porphyrin ring

and the methyl and vinyl groups, and parts that are hydrophilic, including the iron ion and propionates. Such chemical attributes allow heme to fit in hydrophobic environments as well as hydrophilic ones, and to form hydrophobic interactions as well as salt bridges. Furthermore, the iron ion can adopt and oscillate among several oxidation and electron spin states, enabling heme to freely transfer electrons and to interact with a wide array of molecules, inorganic as well as organic, large and small. Heme is arguably the most chemically and biologically versatile molecule in living organisms.

The properties of the iron ion in heme account for its many biological functions. First, the iron ion can be coordinated by six ligands, but the four pyrroles of heme provide only four ligands. Thus, the heme iron ion can be coordinated by an additional two axial ligands (Fig. 1). This allows heme to associate with proteins and to bind to small molecules such as oxygen, nitric oxide and carbon monoxide. The amino acid residues that chelate the heme iron can be histidine and methionine, as in globins, cytochromes, cytochrome *c* oxidases and other oxidoreductases. The heme iron can also be coordinated by cysteine, as in cytochrome P450 enzymes. Occasionally, tyrosine can be an axial ligand for the heme iron in proteins. Heme in proteins can be five- or six-coordinated. If the heme iron is five-coordinated in proteins, then the heme iron often binds to small molecules, including oxygen, nitric oxide and carbon monoxide. This allows heme in proteins and enzymes to bind, transport, sense or use these small molecules. The second property of the heme iron is that it can adopt and oscillate among multiple oxidation states, the more stable +2 and +3 states, and the less stable +4 state in certain catalytic intermediates (7). This enables heme enzymes to perform electron transport and oxidation/reduction.

The porphyrin part of the heme molecule also contributes to the biological functions of heme. Four methyl groups, two vinyl groups and two propionate groups are linked to the porphyrin ring in heme (Fig. 1). The methyl groups and vinyl groups, along with the macrocycle, are hydrophobic. Thus, heme is often situated in a hydrophobic pocket in certain enzymes, such as in hemoglobin and *c*-type cytochromes. The propionate groups often form hydrogen bonds or salt bridges with amino acid residues in enzymes or with solvent molecules. These groups can also allow heme to be covalently attached to proteins and enzymes. For example, in *c*-type cytochromes, two cysteine residues in the Cys-X-X-Cys-His motif are attached to two vinyl groups in heme. This type of modified

heme is referred to as heme C in the literature. The methyl and propionate groups can be modified in various enzymes. Modified forms of heme include heme A and heme D. The detailed structures of these hemes can be found elsewhere (8). The role and structural environment of heme in globins and cytochrome enzymes have been extensively studied and well documented (7).

What is absent, however, from the existing literature is a broad, cohesive, and in-depth analysis of the fascinating roles of heme in diverse biological processes and the underlying molecular bases. This book aims to fill this void. It provides the readers a clear idea about the origin, breadth, and depth of heme biology. In Chapter 2, we explain how heme is synthesized in humans, how its synthesis is regulated in various tissues and organs, and what kinds of diseases can arise when heme biosynthesis becomes defective. In Chapter 3, we describe how heme can control the first step in gene expression, transcription. We show the in-depth molecular mechanisms by which heme controls two master regulators, Hap1 and Bach1. In Chapter 4, we provide a complete view of the role of heme in controlling protein synthesis in red blood cells, and the diseases associated with heme regulation. In Chapter 5, we explain the diverse roles, good and bad, of heme in brain functions. This should allow the readers to appreciate how heme acts to promote neuronal functions and how defective heme function can contribute to various neurological problems in humans. Chapters 6 and 7 describe several recently discovered cases showing the critical roles of heme in regulating key molecules affecting many physiological and diseases processes. Specifically, an essential miRNA processing factor, DiGeorge Critical Region 8 (DGCR8), is regulated by heme. Additionally, two protein tyrosine kinases, Jak2 and Src, are regulated by heme. Defective functioning of these proteins is known to cause major health issues, such as cancer and hematological diseases.

To those readers who want to know more about the chemical and structural characteristics that underlie the diverse roles of heme, Chapter 8 provides an in-depth review of the current literature about heme–protein interactions. Finally, in Chapter 9, we show how the properties of heme biosynthetic pathway and heme precursors can be used in clinical applications. It is amazing that we can take advantage of the photosensitivity of heme precursors to treat serious diseases like cancer, while this property contributes in part to the problems associated with porphyrias. Covering such broad areas associated with heme and also providing in-depth analyses of heme signaling and heme-protein

interactions, this book can be informative and useful for both general and expert readers.

## References

1. Ponka P. 1999. Cell biology of heme. *Am J Med Sci* 318: 241–256.
2. Rajagopal A, Rao AU, Amigo J, Tian M, Upadhyay SK, Hall C, Uhm S, Mathew MK, Fleming MD, Paw BH, Krause M, Hamza I. 2008. Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. *Nature* 453: 1127–1131.
3. Anderson KE, Sassa S, Bishop DF, Desnick RJ. 2009. Disorders of heme biosynthesis: X-linked sideroblastic anemia and the porphyrias. In *The Metabolic and Molecular Bases of Inherited Disease*, eds. CR Scriver, AL Beaudt, WS Sly, D Valle, C Barton, KW Kinzler, B Vogelstein, Chapter 124, pp. 1–53. New York: The McGraw-Hill Companies, Inc.
4. Moore MR. 1990. Historical introduction to porphyrins and porphyrias. In *Biosynthesis of Heme and Chlorophylls*, ed. HA Dailey, pp. 1–54. New York: Green Pub. Associates and Wiley-Interscience.
5. Rich F. 1993. Review/Theater: The Madness of King George; Creating a Lovable George III. In *The New York Times*, pp. 1. New York.
6. Mense SM, Zhang L. 2006. Heme: A versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases. *Cell Res* 16: 681–692.
7. Messerschmidt A, Huber R, Poulos T, Wieghardt K. 2001. *Handbook of Metalloproteins*. West Sussex: John Wiley & Sons Ltd.
8. Ortiz de Montellano PR. 2009. Hemes in Biology. In *Wiley Encyclopedia of Chemical Biology*, pp. 240–249. West Sussex: John Wiley & Sons Ltd.