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Structural and biochemical studies on novel bacterial heme proteins

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Abstract

Heme, an iron-porphyrin prosthetic group, is essential for multiple biochemical functions including respiration, electron transfer, oxidative stress mitigation, and energy metabolism. In bacteria, hemoproteins support diverse physiological processes and act as key virulence determinants in pathogenic interactions with hosts. Bacteria have evolved sophisticated systems for acquiring, trafficking, degrading, and synthesizing heme, notably under iron-limited conditions imposed by host nutritional immunity. Recent structural and biochemical studies reveal alternative heme biosynthesis pathways, non-canonical degradation systems, intricate regulatory networks (Including Fur-dependent and post-transcriptional controls), and emerging roles of heme in cell signaling and environmental sensing. This review synthesizes current understanding of bacterial heme structure, function, uptake and trafficking, degradation and biosynthesis, regulation, and potential antimicrobial targets, with an emphasis on advances from 2022 to 2025.

Keywords: Heme, bacterial heme proteins, heme biosynthesis, heme uptake, iron acquisition, regulatory networks, pathogenesis

Introduction

Heme is an iron-containing tetrapyrrole prosthetic group found in a wide range of hemoproteins where it functions as a redox cofactor, electron carrier, oxygen binder, or catalytic center. Structurally, heme is composed of a protoporphyrin IX macrocycle coordinated to a central iron atom and is essential across all biological life forms (Everse, 1998) [7]. In bacteria, hemoproteins have been implicated in respiratory electron transport, hydrogen peroxide detoxification, oxygen activation, and anaerobic metabolism such as denitrification. Pathogenic bacteria exploit host heme as a rich iron source during infection, countering host nutritional immunity by developing specialized heme acquisition and utilization systems that are regulated by complex genetic networks. Recent work emphasizes the importance of heme properties in pathogenesis and the host-pathogen interface, highlighting heme's dual roles as both cofactor and signaling molecule during bacterial infection (Chen & Skaar, 2025) [2].

Structure and Biochemical Properties of Heme Porphyrin Macrocycle and Iron Coordination

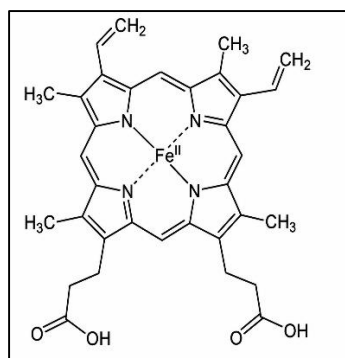


Fig 1: Structure of heme protein

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Heme's fundamental structure consists of four pyrrole rings (Designated I–IV) linked by methenyl (–CH=) bridges to form a highly conjugated macrocycle. The iron atom in heme typically forms six coordination bonds: four equatorial bonds with pyrrole nitrogens and up to two axial ligands, one provided by a proximal amino acid such as histidine or tyrosine and the other by substrate or gas molecules (Everse, 1998) ^[7]. The electronic structure of heme enables diverse redox activities essential for electron transfer and catalysis. Distinct heme types (*e.g.*, heme a, b, c, d) differ in substituent side chains and protein coordination environments, which in turn influence their functions in different biological contexts.

Spectroscopic Properties and Redox States

Heme proteins display characteristic absorbance spectra due to their conjugated porphyrin ring, prominently the Soret band around ~400 nm. The central iron can reversibly cycle between ferrous (Fe²⁺) and ferric (Fe³⁺) states, enabling its participation in redox-dependent reactions critical for respiration and oxidative stress responses.

Functional Roles of Bacterial Hemoproteins

Electron Transfer and Respiratory Complexes

Cytochromes are heme-containing electron carriers that mediate single-electron transfers within bacterial respiratory chains. The oscillation of heme iron between Fe²⁺ and Fe³⁺ states facilitates efficient electron flow from donors to acceptors. Respiratory heme A-containing oxidases have been traced to ancestral iron-oxidizing bacteria, suggesting deep evolutionary roots for these complexes (Degli Esposti *et al.*, 2021) ^[6]. Anaerobic bacteria also utilize heme in electron transport processes such as denitrification, where cytochrome cd1 nitrite reductases and other hemoproteins are key components.

Defense against Oxidative Stress

Catalases and peroxidases degrade hydrogen peroxide to water and oxygen, serving as crucial antioxidant defenses. Catalase enzymes exhibit some of the highest known turnover rates and rely on ferric heme as a central catalytic cofactor (Everse, 1998) ^[7].

Oxygenases and Oxygen Activation: Heme-dependent monooxygenases and dioxygenases catalyze substrate oxidation reactions, incorporating oxygen atoms into a range of substrates and facilitating metabolic transformations.

Bacterial Heme Uptake and Trafficking Systems

Because free iron is limited within the host due to sequestration by host proteins (*e.g.*, hemoglobin, hemopexin), pathogenic bacteria have evolved highly efficient heme acquisition systems.

Gram-Negative Uptake Mechanisms

In Gram-negative bacteria such as *Pseudomonas aeruginosa*, secreted hemophores (*e.g.*, HasA) capture extracellular heme and deliver it to outer membrane receptors (HasR), where TonB-dependent transport facilitates its periplasmic translocation. Similar systems are found in clades like enteropathogens (*e.g.*, *Shigella*

dysenteriae) that employ ABC transporters (*e.g.*, ShuUV) to move heme into the cytoplasm.

Gram-Positive Uptake Systems

Gram-positive bacteria such as *Staphylococcus aureus* utilize surface-anchored proteins (IsdH, IsdA, IsdC) that bind heme and relay it to membrane permeases through NEAT domains, an elegant solution to heme acquisition across thick peptidoglycan layers.

Recent studies also highlight alternative uptake strategies, including de-chelataze enzyme systems in commensal anaerobes like *Bacteroides thetaiotaomicron*, which liberate iron from heme while leaving the porphyrin intact, expanding our understanding of heme utilization beyond oxidative breakdown.

Bacterial Heme Degradation Pathways

Canonical Heme Oxygenases

Bacteria commonly utilize heme oxygenases to degrade heme into biliverdin, free iron, and carbon monoxide. This not only provides essential iron but also mitigates heme's cytotoxicity.

Non-Canonical Heme Degradation Enzymes

Non-canonical enzymes such as IsdG/I in *Staphylococcus aureus* degrade heme to unusual chromophores like staphylobilin, demonstrating distinct structural and mechanistic features compared to classical HOs. Furthermore, systems like MhuD in *Mycobacterium tuberculosis* reveal diheme binding and distinct catalytic strategies, broadening the landscape of bacterial heme metabolism. Emerging evidence suggests additional anaerobic pathways and de-chelataze mechanisms may contribute to heme breakdown in diverse bacteria.

Bacterial Heme Biosynthesis Pathways

Bacterial heme biosynthesis begins with 5-aminolevulinic acid (ALA), synthesized via either the C4 (succinyl-CoA + glycine) or C5 (glutamyl-tRNA) pathway, depending on the species (Layer *et al.*, 2010) ^[8].

Protoporphyrin-Dependent (PPD) Pathway

The classical route converts uroporphyrinogen III to protoporphyrin IX, followed by iron insertion via ferrochelatase (PpfC).

Coproporphyrin-Dependent (CPD) Pathway: A recent review highlights regulation of bacterial CPD heme biosynthesis, especially in Gram-positive organisms, and discusses regulatory mechanisms responsive to metabolic cues such as iron and oxygen levels.

Siroheme-Dependent (SHD) Pathway

The most ancient route, the SHD pathway, proceeds through precorrin-2 and siroheme intermediates, differing markedly from PPD and CPD routes.

Efficient engineering of heme biosynthesis in bacteria, including exploiting the SHD pathway in industrial strains like *Corynebacterium glutamicum* for enhanced heme production, underscores both the biochemical diversity and biotechnological potential of these pathways.

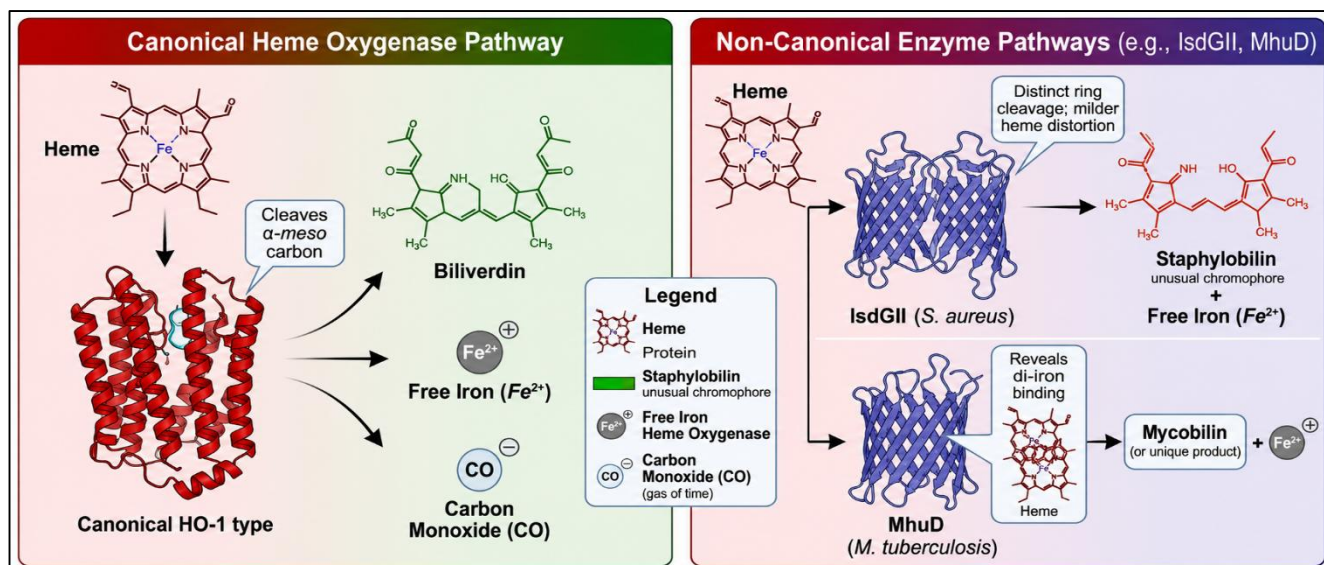


Fig 2: Mechanisms of Bacterial Heme Degradation. The diagram compares canonical and non-canonical pathways for bacterial heme catabolism. The Canonical HO-1 Pathway (left) uses alpha-helical heme oxygenases to cleave the alpha-meso carbon, releasing biliverdin, free iron (Fe^{2+}), and carbon monoxide (CO). Conversely, Non-Canonical Enzymes (right), such as IsdG/I and MhuD, utilize a beta-barrel fold and "ruffled" heme distortion to produce unusual chromophores like staphylobilin or mycobilin. Notably, the MhuD system from *M. tuberculosis* exhibits unique di-heme binding.

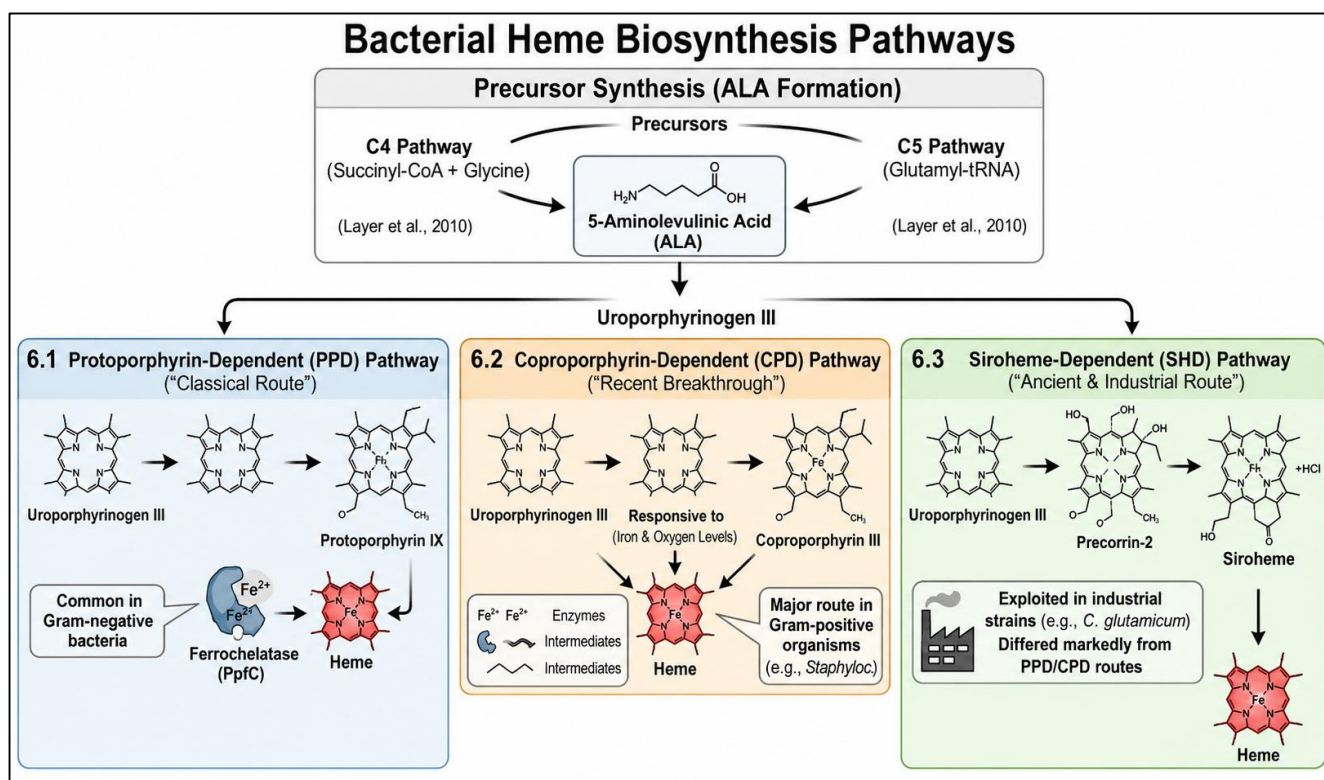


Fig 3: Bacterial Heme Biosynthesis Pathways. This figure delineates the three primary routes of bacterial heme production, beginning with the synthesis of 5-aminolevulinic acid (ALA) via the C4 or C5 pathways. From the common intermediate uroporphyrinogen III, the process branches into: (6.1) the Protoporphyrin-Dependent (PPD) "classical" route where ferrochelatase (Ppfc) inserts iron into protoporphyrin IX; (6.2) the Coproporphyrin-Dependent (CPD) route, prevalent in Gram-positive bacteria and regulated by iron and oxygen levels; and (6.3) the ancient Siroheme-Dependent (SHD) pathway, which utilizes precorrin-2 and siroheme. The SHD route is particularly notable for its use in metabolic engineering within industrial strains like *C. glutamicum*.

Regulation of Heme Metabolism

Fur-Dependent Transcriptional Control

The Ferric uptake regulator (Fur) protein acts as a global repressor of iron and heme acquisition genes in Gram-negative bacteria. It controls expression of TonB-dependent hemoprotein receptors, siderophore systems, and other iron homeostasis elements in organisms such as *Escherichia coli* and *Vibrio cholerae* using intracellular iron as a cofactor.

Post-Transcriptional and Post-Translational Controls

RNA thermometers and heme-responsive regulatory motifs modulate heme receptor expression based on environmental signals such as temperature and intracellular heme levels, a mechanism seen in pathogens like *Shigella dysenteriae* and pathogenic *Escherichia coli*.

Recent research indicates that heme itself can regulate transcription factors and catalyze signaling responses,

revealing its role in redox sensing and environmental adaptation. For example, heme binding to transcription factors involved in hydrogen sulfide sensing underscores a previously unrecognized regulatory axis with implications for stress tolerance and antibiotic strategies.

Emerging Roles of Heme in Bacterial Physiology

Beyond classical metabolic roles, heme acts as a regulatory signal influencing processes such as biofilm formation, oxidative stress adaptation, and interspecies interactions. Heme utilization studies in opportunistic bacteria like enterococci reveal its significance in energy metabolism and stress resilience, even in organisms that cannot synthesize heme *de novo*. Studies in engineered microbial systems further illustrate how differential pathway compartmentalization and metabolic control can influence heme yield, with implications extending from basic bacterial physiology to industrial biotechnology.

Clinical and Therapeutic Implications

Because pathogenic bacteria depend on heme acquisition for invasion and survival, targeting heme uptake systems (*e.g.*, TonB transporters, Isd surface proteins, and regulatory networks) is a promising antimicrobial strategy. Mechanistic elucidation of heme uptake and signaling pathways could inspire novel therapeutics aimed at disrupting bacterial iron metabolism in an era of rising antibiotic resistance.

Conclusion

Heme is a multifunctional molecule central to bacterial metabolism, envelope physiology, and pathogenesis. Recent advances from 2022–2025 have significantly expanded our understanding of bacterial heme uptake systems, alternative biosynthetic routes, non-canonical degradation mechanisms, and regulatory controls. Furthermore, emerging evidence for heme's role in signaling and adaptation suggests new research frontiers. Continuing integration of structural, genomic, and biochemical approaches will deepen insights into bacterial heme biology and facilitate the development of novel antimicrobial strategies.

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