Published in final edited form as:

J Porphyr Phthalocyanines. 2011 ; 15(5-6): 350–356. doi:10.1142/S108842461100332X.

FERROCHELATASE: THE CONVERGENCE OF THE PORPHYRIN BIOSYNTHESIS AND IRON TRANSPORT PATHWAYS*

Gregory A. Hunter^a, Salam Al-Karadaghi^b, and Gloria C. Ferreira^{*,a,c,d}

^aDepartment of Molecular Medicine, College of Medicine, University of South Florida, Tampa, Florida, 33620

^bDepartment of Molecular Biophysics, Centre for Molecular Protein Science, Lund University, Lund, Sweden

^cDepartment of Chemistry, University of South Florida, Tampa, Florida, 33620

^dH. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, 33612

Abstract

Ferrochelatase (also known as PPIX ferrochelatase; Enzyme Commission number 4.9.9.1.1) catalyzes the insertion of ferrous iron into PPIX to form heme. This reaction unites the biochemically synchronized pathways of porphyrin synthesis and iron transport in nearly all living organisms. The ferrochelatases are an evolutionarily diverse family of enzymes with no more than six active site residues known to be perfectly conserved. The availability of over thirty different crystal structures, including many with bound metal ions or porphyrins, has added tremendously to our understanding of ferrochelatase structure and function. It is generally believed that ferrous iron is directly channeled to ferrochelatase *in vivo*, but the identity of the suspected chaperone remains uncertain despite much recent progress in this area. Identification of a conserved metal ion binding site at the base of the active site cleft may be an important clue as to how ferrochelatases acquire iron, and catalyze desolvation during transport to the catalytic site to complete heme synthesis.

Keywords

Ferrochelatase; iron; protoporphyrin IX; chelatase; porphyrin; tetrapyrrole; enzyme

INTRODUCTION

Insertion of metal ions into tetrapyrroles is a relatively facile process, and occurs non-enzymatically in organic solvents under basic conditions [1–5]. In biological systems a class of enzymes called chelatases catalyzes the reaction. Among the metal-containing tetrapyrroles in biology are heme, chlorophyll, cobalamin and coenzyme F₄₃₀. Ferrochelatase, the most extensively studied enzyme within the chelatase class, catalyzes the terminal reaction in heme synthesis in nearly every living organism (Scheme I) [6,7], while magnesium chelatase catalyzes the first committed step in chlorophyll and bacteriochlorophyll synthesis [8]. In both cases the chelation reaction involves insertion of a metal ion, ferrous iron (Fe²⁺) or Mg²⁺, into protoporphyrin IX (PPIX) with expulsion of two protons from the tetrapyrrole, as diagrammed in Scheme I. Although, the reactions are

*This work was supported by grants from the National Institutes of Health (#GM080270) to G.C.F.

*Address correspondence to: Gloria C. Ferreira, Department of Molecular Medicine, College of Medicine, MDC 7, University of South Florida, Tampa, FL 33612-4799, Tel. 813-974-5797; Fax. 813-974-0504; gferreir@health.usf.edu.

seemingly similar, the enzymatic mechanisms behind the metalation reactions appear to be very different. Thus, while ferrochelatases, as they are observed in nature, are structurally conserved and range in monomeric size from 38–51 kDa, with no more than six residues known to be perfectly conserved across evolution [6], magnesium chelatases (and the homologous cobaltochelatase) are ATP-dependent enzymes of the AAA+ class of ATPases. They consist of 3 different subunits (molecular weight ~40 kDa, ~70 kDa and ~140 kDa), the first two of which form a characteristic two-tiered ring complex with six subunits in each ring, while the third is the actual catalytic subunit that binds the porphyrin substrate [8–16]. Twelve to fifteen ATP molecules are required for each reaction cycle, which involves elaborated reaction kinetics [16,17].

Information on the magnesium chelatase reaction is somewhat limited due to the absence of a crystal structure, although the overall structural and conformational dynamics of the complexed subunits of the enzyme have been well characterized [8–16]. In contrast, the metalation reaction of ferrochelatase has been studied in great detail, even down to an atomic level. Researchers have capitalized on this by utilizing the ferrochelatase reaction as a template to construct non-natural metalloporphyrin chelatases from antibodies, RNA, and even DNA [18–21]. No attempts have been made to reconstruct the magnesium chelatase reaction. Thus, the reason Nature devised such an elaborate three sub-unit enzyme to facilitate this reaction remains poorly understood.

Diversity of ferrochelatases

In eucaryotes, ferrochelatase is peripherally associated with the inner mitochondrial membrane, with the active site facing into the membrane [22], while in procaryotes the enzyme is associated with the plasma membrane in some species and cytosoluble in others [23,24]. Ferric iron is not a substrate, and although the enzyme is highly specific for ferrous iron *in vivo*, *in vitro* ferrochelatase catalyzes insertion of a remarkable variety of divalent metal ions into protoporphyrin and other closely related porphyrins, some at rates comparable to iron [25–27]. The inability to incorporate Fe²⁺ into the porphyrin macrocycle due to losses in ferrochelatase activity in humans can lead to accumulation of PPIX, erythropoietic protoporphyria, liver damage, and possibly even death [28].

Ferrochelatases may be monomeric or homodimeric, membrane-associated or soluble, either contain a [2Fe-2S] cluster or no cluster, and for those containing a [2Fe-2S] cluster, it may be primarily localized in the C-terminal region of the enzyme [7], or within a short internal amino acid insert not present in other ferrochelatases [29]. Ironically, the energetic simplicity of the reaction appears to have resulted in a complexity of evolutionary divergences. This diversity often makes it difficult to construct simplifying generalizations about the family of ferrochelatases as a whole.

Nevertheless, the ferrochelatase reaction cycle as it occurs *in vivo* is far more elaborate than the reaction chemistry alone might suggest. As discussed in more detail below, this can be primarily attributed to the extreme instabilities, toxicities, and widely differential solubilities of the two substrates. The necessity for specific molecular recognition of ferrous iron, with exclusion of other transition metal ions such as zinc, is also likely to be an important factor in defining the structural and functional aspects of ferrochelatases. These limitations have driven ferrochelatase biochemistry to evolve in ways that are still being unraveled.

Structure

Crystal structures are available for ferrochelatase from *Bacillus subtilis* [30], *Bacillus anthracis*, *Saccharomyces cerevisiae* [31], and *Homo sapiens* [24]. The *B. subtilis* and human crystal structures are particularly informative as they include numerous structures

with different metal ions and porphyrins bound. Alignment of the *B. subtilis* (PDB 2HK6; iron bound) and human (PDB 2QD1; PPIX bound) ferrochelatases, as depicted in Figure 1, reveals a root mean square deviation of only 2.4 Å between the three-dimensional structures, even though the amino acid sequence identity is less than 10% [24,32,33]. In this way the tertiary structure of the enzyme is conserved, but the primary structures and electrostatic surface potential distributions diverge widely [6].

Further comparison of the human and *B. subtilis* crystal structures has delineated several important differences between the two enzymes. The bacterial enzyme is clearly monomeric, whereas the human enzyme is a homodimer. Additionally, the human enzyme contains an internal hydrophobic region near the N-terminus that is absent in the cytosoluble bacterial enzyme. This region is important for mitochondrial membrane association in eucaryotic ferrochelatases [23,24], and can be seen in Figure 1 as the blue α -helix towards the bottom of the figure, oriented towards the viewer. Another role for this helix would be to prevent the porphyrin from binding to eukaryotic ferrochelatase in the same orientation as in the *B. subtilis* enzyme [34]. Thus, in the soluble *B. subtilis* enzyme the porphyrin is bound such that the propionate side chains point outward into the solvent. This orientation would create a steric clash with the above mentioned helix, if realized, *e.g.*, in the human or yeast enzymes. Instead, in the membrane-associated human enzyme the porphyrin is turned approximately 100° and the propionate side chains are bound within the active site [35]. These differing orientations also make sense from the standpoint of solubility, especially in the case of the membrane-associated ferrochelatases, where the negatively charged propionates are removed from the hydrophobic membrane by being bound within the active site. Finally, a π -helix in the active site unwinds and adopts an extended conformation in some human enzyme metalloporphyrin-bound structures [33], while no such conformational heterogeneity about this helix has been observed in any of the bacterial structures.

The significant biochemical differences in the bacterial and mammalian ferrochelatases suggest that the evolutionarily intermediate yeast ferrochelatase might prove useful for future structural and mechanistic studies. The *B. subtilis* enzyme has been crystallized with iron [32], zinc [35], and cadmium [35], but has proven difficult to co-crystallize with many of the porphyrins observable in human structures. The human enzyme, on the other hand, has not been co-crystallized with any metal ion, but has been crystallized with a variety of porphyrins and metalloporphyrins [27,33,36]. The yeast enzyme has been crystallized with cobalt and cadmium, and the possibility of co-crystallization with porphyrins is worthy of further examination, as it could provide a more enlightened context for understanding the differences between the human and bacterial ferrochelatases.

The biological significance of a [2Fe-2S] iron-sulfur cluster present in animal ferrochelatases has remained elusive despite intense characterization, although it is essential for the catalytic reaction [7,29,37–45]. Possible functions include nitric oxide sensing or iron recruitment.

Ferrochelatase: catalysis and chemistry

Porphyrin metalation, whether it be enzymatic or non-enzymatic, involves the following elementary events: approximation of the metal ion and porphyrin; desolvation of the metal ion; distortion of one or more porphyrin pyrrole rings out of the macrocyclic plane towards the metal ion; formation of metal-nitrogen bonds with two pyrrole nitrogen atoms; and removal and expulsion of the protons bound to the remaining protonated pyrrole nitrogen atoms. Ferrochelatase has been shown or proposed to catalyze each of these events [6].

Clearly, ferrochelatase accelerates heme formation via binding the substrates in optimized positions within the active site. This is evident from Fig. 1, where the iron-bound *B. subtilis*

structure has been aligned to the human PPIX-bound structure. The alignment shows that despite the different orientation of the porphyrins, the position of the iron atom is very close to the central cavity of both PPIX. The iron atom is held in place by perfectly conserved histidine and glutamate residues, while structural data suggest that PPIX binding is primarily driven by hydrophobic interactions that are partially conserved [24]. It is interesting to note that in this alignment the iron atom is not directly adjacent to the center of bound PPIX, and instead is nearest the nitrogen atom of pyrrole ring D. If such an alignment occurs during a normal catalytic cycle it would likely indicate that the bonding steps consistently initiate at this ring, possibly even following a precise reaction sequence around the four pyrrole rings in the order D, C, B, A, or D, A, B, C, similar to what has been determined in computational studies [46].

The process whereby the substrates arrive into the active site is an area that has been increasingly focused on in recent years. Ferrochelatase activity assays designed to study the reaction mechanism allow the substrates to diffuse freely in solution, but this is not believed to occur *in vivo*. *In vivo*, at least with membrane-associated ferrochelatases, PPIX is thought to be directly channeled into the active site of ferrochelatase by protoporphyrinogen oxidase, which produces PPIX in the previous step of the porphyrin biosynthetic pathway [24,47–49]. Eucaryotic protoporphyrinogen oxidase is located at the outer side of the inner mitochondrial membrane and forms what appears to be a highly functional complex with ferrochelatase wherein the active site channels overlap such that PPIX could freely diffuse directly between the enzymes [48,49]. However, there is no direct experimental evidence for this proposal. Such a mechanism makes sense from a toxicological perspective as it would limit accumulation of PPIX, which is highly photoreactive, and causes severe photosensitivity at elevated levels [50].

Direct channeling of iron, although unproven, is also considered to be highly likely, particularly given the toxic instability of ferrous iron towards oxidation in aqueous solution [51,52]. The remarkable selectivity for iron, which is not observed outside the cell, would also be readily explained by a specific channeling chaperone. Finally, the orientation of the active site of the eucaryotic enzyme into the membrane is a further consideration limiting how ferrous iron reaches the porphyrin, since a divalent ion would not readily diffuse through a lipid bilayer. Significantly however, the identity of the suspected channeling protein or enzyme remains a mystery. Possible mechanisms for iron binding that are currently being explored are summarized in Figure 2.

Recently it was shown that mitoferrin, a mitochondrial membrane iron transporter, can form an immunoprecipitable complex with ferrochelatase, suggesting the interesting possibility that it might be the long sought after channeling chaperone [53]. Another possibility that has been proposed is derived from kinetic studies and the observation that the π -helix at the active site unwinds upon metalloporphyrin formation [27] such that the iron binding glutamate residue is oriented into the mitochondrial matrix where it could accept an iron atom from a matrix iron transporter such as frataxin (Figure 3) [6,54]. Upon reformation of the π -helix the iron atom could be delivered directly to the active site from the matrix side of the enzyme. An attractive feature of this somewhat radical proposal is that it would explain the mechanistic function of a highly conserved histidine-phenylalanine pair at the base of the active site [54]. These residues combine to form a second metal ion-binding site, in addition to the catalytic site, which enhances enzyme activity at low micromolar metal ion concentrations, but inhibits activity at higher, non-physiological concentrations. The histidine-phenylalanine pair is ideally positioned to assist in catalyzing desolvation of the iron atom as it approaches the catalytic site while the π -helix re-forms. This would be very important mechanistically because experimental and theoretical studies have identified metal ion desolvation, which is necessary to expose a coordination sphere on the metal ion for

complexation by the porphyrin, as a key step in the overall metalation process [3,5,55–57]. Another key feature of this mechanism is that it predicts a mitochondrial matrix iron transporter such as frataxin would bind to the unwound π -helix structure to channel the metal atom to the active site of ferrochelatase [58–61].

Multiple lines of evidence support the concept of porphyrin strain or distortion as a central feature of the ferrochelatase catalytic mechanism [35,59,62–65]. *N*-alkylated porphyrins have distorted structures and are non-enzymatically metallated at rates 10^3 – 10^5 times faster than undistorted porphyrins [1]. Distorted porphyrins are also strong inhibitors of ferrochelatase with binding affinities extending into the sub-nanomolar range, indicating they are transition state analogs [66–71]. Furthermore, antibodies, RNAs, and DNAs isolated on the basis of binding affinity for the distorted tetrapyrrole *N*-methylmesoporphyrin all catalyze metalation of mesoporphyrin [18–21].

Perhaps the most convincing evidence comes from crystallographic data. The structure *B. subtilis* ferrochelatase with *N*-methyl mesoporphyrin bound revealed an enhanced distortion of ring A to 36° . PPIX bound to the E343K variant of human ferrochelatase is distorted by $\sim 11.5^\circ$ in a domed fashion towards the metal ion binding site, but the extent of distortion might be even greater in the wild-type enzyme [36]. This is true because mutation of the active site iron binding glutamate residue (human number E343) increases the binding affinity of the enzyme for PPIX substantially, to the extent that it co-purifies with the enzyme from cell lysates [32,36,72]. This is consistent with the side chain of this glutamate being involved in porphyrin strain or distortion that is relieved upon mutation, and manifested as tighter binding [73]. It is also consistent, obviously, with this glutamate being essential to iron insertion into PPIX *in vivo*. In summary, there is little doubt that porphyrin distortion occurs during catalysis and currently it is only the extent, nature, and precise timing of the distortion that are still uncertain.

The extent or type of distortion could potentially have an effect on metal ion specificity [59]. Distorted porphyrins can take on a variety of structural variations [74], and chelataes might optimize the transient stability of one or more of these distorted structures, as well as the absolute extent of distortion, in order to best accommodate the chemistry of the metal ion targeted for insertion [65].

One important question that remains to be conclusively answered is whether or not there is an ordered binding of substrates to ferrochelatase. Recent kinetic studies with the recombinant human enzyme suggest that porphyrin binds prior to ferrous iron [25]. The order of binding is highly significant in terms of porphyrin distortion, because the presence of a metal ion at the catalytic site could by itself have a marked effect on porphyrin distortion. The effect of metal ion on distortion might be investigated by looking at the effects of various inhibitory metal ions on Raman spectra of PPIX bound to ferrochelatase. It must be noted however, that many metal ions previously thought to be inhibitors, including cadmium, mercury, and manganese are in fact extremely poor substrates, at least for the human ferrochelatase [27]. The extremely slow rate at which these metal ions are inserted should make them useful in future studies focused on trying to identify any possible reaction intermediates, or the identity and roles of active site residues in catalyzing discrete steps of the metalation reaction.

Once the substrates are bound the bond rearrangements occur. Studies of non-catalytic porphyrin metalation reactions have resulted in the discovery and characterization of a reaction intermediate known as a “sitting-atop”, or SAT, complex [75]. In the SAT complex the metal atom is weakly chelated by the two pyrroline ring nitrogen atoms, and the protons at the other two ring nitrogens are left unaltered. SAT complex formation is

accompanied by large changes in absorbance and fluorescence spectroscopic signals [76–78]. It is not known, however, if the SAT complex that would be formed in the ferrochelatase active site is sufficiently stable kinetically to be treated as a true reaction intermediate that might be used to study the initial chemical steps of the reaction in more detail.

Two protons from PPIX are removed during the catalytic cycle, and various protein residues on both sides of the plane of the bound porphyrin have been proposed to act as catalytic bases for these reactions [33,46]. It is not clear if there is any strict requirement for catalysis of the deprotonation steps however, as *N*-alkyl-porphyrins have pK_a values for loss of an initial ring proton of 8–10 [79]. This would presumably mean that once the first iron-PPIX bond was formed the acidity of the intermediate would be sufficient for proton dissociation to occur without the need for a catalytic base, and water could be a suitable proton acceptor [32].

The rate-limiting step of the overall reaction is, at least *in vitro*, product release [80]. Crystallographic data suggest that in the human enzyme product release is accompanied by unwinding of the π -helix [27]. The possibility of conformational dynamics about the π -helix, and their implications for multiple steps in the reaction cycle, are sure to be a focus of future research.

Summary

We now summarize the eucaryotic ferrochelatase reaction mechanism as we currently envision it. This mechanism is in some areas highly speculative, and in these parts the proposed events are chosen because they do not disagree with the available data, and they provide testable hypotheses for future experiments. Other valid mechanisms for many of these steps have been proposed [7,27,33,36,81–83], and interested readers should refer to these and related articles to gain an even better perspective on this remarkable enzyme.

PPIX is directly channeled to ferrochelatase by protoporphyrinogen oxidase to form a ferrochelatase-PPIX complex. Binding interactions between ferrochelatase and PPIX are used to induce a degree of porphyrin distortion suitable to catalyze formation of at least an initial bond to the incoming metal ion. Ferrous iron is bound secondly, and is directly channeled to ferrochelatase by an iron transporter, the identity of which is still uncertain. If the transporter is membrane embedded or membrane spanning, protoporphyrinogen oxidase must be displaced from binding over the ferrochelatase active site channel, to permit the iron transporter to dock into the same region of the enzyme for channeling of iron into the active site. If the transporter is in the matrix we propose that iron acquisition is dependent upon conformational heterogeneity of the π -helix. Specifically, the π -helix unwinds, projecting the catalytic glutamate out of the matrix side of the enzyme as in Figure 3, where it picks up an iron atom from the transporter. The π -helix then reforms to bring the metal ion into the active site in position for insertion into PPIX. During this structural transition the conserved inhibitory metal ion binding site occupies one or more coordination sites on the metal atom and stabilizes it to oxidation. The conserved phenylalanine within the inhibitory binding site could modulate binding affinity for iron during the structural rearrangement, ensuring iron is passed on to the catalytic site upon π -helix re-formation, and it could also be involved in desolvating the iron atom immediately prior to arrival at the catalytic insertion site. These π -helix dynamics might not be necessary for the *B. subtilis* enzyme, as the active site is exposed to the cytosol, but the conservation of the inhibitory binding site at the base of the active site in all ferrochelatases, including the *B. subtilis* enzyme, does suggest a commonality of function.

The distorted porphyrin rapidly forms a SAT-type complex with the iron atom and bonds are formed between the substrates in a characteristic succession of reaction steps analogous to that proposed on the basis of computational studies of the *B. subtilis* ferrochelatase active site [46], resulting in the formation of bound heme.

Dissociation of heme from the enzyme determines the overall rate of reaction [80], and in the eucaryotic enzyme involves unwinding of the π -helix [27]. Direct interaction with a heme transporter is also possible.

REFERENCES

- Bain-Ackerman MJ, Lavalley DK. *Inorg. Chem.* 1979; 18:3358–3364.
- Fleischer EB, Choi EI, Hambright P, Stone A. *Inorg. Chem.* 1964; 3:1284–1287.
- Funahashi S, Inada Y, Inamo M. *Anal. Sci.* 2001; 17:917–927. [PubMed: 11708094]
- Lavalley DK. *Coordination Chem. Reviews.* 1985; 61:55–96.
- Shen Y, Ryde U. *Chemistry.* 2005; 11:1549–1564. [PubMed: 15662683]
- Ferreira, GC.; Hunter, GA. *The Handbook of Porphyrin Science.* Kadish, KM.; Smith, KM.; Guilard, R., editors. Vol. Vol. 15. World Scientific Publishing Company; 2010.
- Dailey HA, Dailey TA, Wu CK, Medlock AE, Wang KF, Rose JP, Wang BC. *Cell Mol Life Sci.* 2000; 57:1909–1926. [PubMed: 11215517]
- Lundqvist J, Elmlund H, Wulff RP, Berglund L, Elmlund D, Emanuelsson C, Hebert H, Willows RD, Hansson M, Lindahl M, Al-Karadaghi S. *Structure.* 2010; 18:354–365. [PubMed: 20223218]
- Jensen PE, Gibson LC, Henningsen KW, Hunter CN. *J Biol Chem.* 1996; 271:16662–16667. [PubMed: 8663186]
- Willows RD, Hansson A, Birch D, Al-Karadaghi S, Hansson M. *J Struct Biol.* 2004; 146:227–233. [PubMed: 15037253]
- Elmlund H, Lundqvist J, Al-Karadaghi S, Hansson M, Hebert H, Lindahl M. *J Mol Biol.* 2008; 375:934–947. [PubMed: 18068723]
- Lundqvist J, Elmlund D, Heldt D, Deery E, Soderberg CA, Hansson M, Warren M, Al-Karadaghi S. *J Struct Biol.* 2009; 167:227–234. [PubMed: 19545636]
- Sirijovski N, Lundqvist J, Rosenback M, Elmlund H, Al-Karadaghi S, Willows RD, Hansson M. *J Biol Chem.* 2008; 283:11652–11660. [PubMed: 18263581]
- Karger GA, Reid JD, Hunter CN. *Biochemistry.* 2001; 40:9291–9299. [PubMed: 11478896]
- Reid JD, Siebert CA, Bullough PA, Hunter CN. *Biochemistry.* 2003; 42:6912–6920. [PubMed: 12779346]
- Viney J, Davison PA, Hunter CN, Reid JD. *Biochemistry.* 2007; 46:12788–12794. [PubMed: 17929947]
- Sawicki A, Willows RD. *J Biol Chem.* 2008; 283:31294–31302. [PubMed: 18790730]
- Cochran AG, Schultz PG. *Science.* 1990; 249:781–783. [PubMed: 2389144]
- Li Y, Sen D. *Nat. Struct. Biol.* 1996; 3:743–747. [PubMed: 8784345]
- Li Y, Sen D. *Biochemistry.* 1997; 36:5589–5599. [PubMed: 9154943]
- Conn MM, Prudent JR, Schultz PG. *J. Am. Chem. Soc.* 1996; 118:7012–7013.
- Jones MS, Jones OT. *Biochem J.* 1969; 113:507–514. [PubMed: 4308946]
- Gora M, Rytka J, Labbe-Bois R. *Arch. Biochem. Biophys.* 1999; 361:231–240. [PubMed: 9882451]
- Wu CK, Dailey HA, Rose JP, Burden A, Sellers VM, Wang BC. *Nat. Struct. Biol.* 2001; 8:156–160. [PubMed: 11175906]
- Davidson RE, Chesters CJ, Reid JD. *J. Biol. Chem.* 2009; 284:33795–33799. [PubMed: 19767646]
- Hunter GA, Sampson MP, Ferreira GC. *J. Biol. Chem.* 2008; 283
- Medlock AE, Carter M, Dailey TA, Dailey HA, Lanzilotta WN. *J. Mol. Biol.* 2009; 393:308–319. [PubMed: 19703464]
- Puy H, Gouya L, Deybach JC. *Lancet.* 2010; 375:924–937. [PubMed: 20226990]

29. Dailey TA, Dailey HA. J. Bacteriol. 2002; 184:2460–2464. [PubMed: 11948160]
30. Al-Karadaghi S, Hansson M, Nikonov S, Jönsson B, Hederstedt L. Structure (London, England : 1993). 1997; 5:1501–1510.
31. Karlberg T, Lecerof D, Gora M, Silvegren G, Labbe-Bois R, Hansson M, Al-Karadaghi S. Biochemistry. 2002; 41:13499–13506. [PubMed: 12427010]
32. Hansson MD, Karlberg T, Rahardja MA, Al-Karadaghi S, Hansson M. Biochemistry. 2007; 46:87–94. [PubMed: 17198378]
33. Medlock AE, Dailey TA, Ross TA, Dailey HA, Lanzilotta WN. J. Mol. Biol. 2007; 373:1006–1016. [PubMed: 17884090]
34. Karlberg T, Hansson MD, Yengo RK, Johansson R, Thorvaldsen HO, Ferreira GC, Hansson M, Al-Karadaghi S. J. Mol. Biol. 2008; 378:1074–1083. [PubMed: 18423489]
35. Lecerof D, Fodje M, Hansson A, Hansson M, Al-Karadaghi S. J. Mol. Biol. 2000; 297:221–232. [PubMed: 10704318]
36. Medlock A, Swartz L, Dailey TA, Dailey HA, Lanzilotta WN. Proc.Natl. Acad.Sci. (USA). 2007; 104:1789–1793. [PubMed: 17261801]
37. Crouse BR, Sellers VM, Finnegan MG, Dailey HA, Johnson MK. Biochemistry. 1996; 35:16222–16229. [PubMed: 8973195]
38. Ferreira GC, Franco R, Lloyd SG, Pereira AS, Moura I, Moura JJ, Huynh BH. J. Biol. Chem. 1994; 269:7062–7065. [PubMed: 8125912]
39. Franco, R.; Lloyd, SG.; Moura, J.J.G.; Moura, I.; Huynh, BH.; Ferreira, GC. Inorganic Biochemistry and Regulatory Mechanisms of Iron Metabolism. Ferreira, GC.; Moura, J.J.G.; Franco, R., editors. Weinheim: Wiley-VCH; 1999. p. 35–50.
40. Furukawa T, Kohno H, Tokunaga R, Taketani S. Biochem J. 1995; 310(Pt 2):533–538. [PubMed: 7544575]
41. Lloyd S, Franco R, Moura I, Moura JJG, Ferreira GC, Huynh BH. J. Amer. Chem. Soc. 1996; 118:9892–9900.
42. Schneider-Yin X, Gouya L, Dorsey M, Røufenacht U, Deybach JC, Ferreira GC. Blood. 2000; 96:1545–1549. [PubMed: 10942404]
43. Sellers VM, Johnson MK, Dailey HA. Biochemistry. 1996; 35:2699–2704. [PubMed: 8611576]
44. Sellers VM, Wang KF, Johnson MK, Dailey HA. J. Biol. Chem. 1998; 273:22311–22316. [PubMed: 9712849]
45. Shepherd M, Dailey TA, Dailey HA. Biochem J. 2006; 397:47–52. [PubMed: 16548850]
46. Wang Y, Shen Y, Ryde U. J. of Inorg. Biochem. 2009; 103:1680–1686. [PubMed: 19850353]
47. Ferreira GC, Andrew TL, Karr SW, Dailey HA. J. Biol. Chem. 1988; 263:3835–3839. [PubMed: 3346226]
48. Koch M, Breithaupt C, Kiefersauer R, Freigang J, Huber R, Messerschmidt A. EMBO J. 2004; 23:1720–1728. [PubMed: 15057273]
49. Masoumi A, Heinemann IU, Rohde M, Koch M, Jahn M, Jahn D. Microbiology. 2008; 154:3707–3714. [PubMed: 19047738]
50. Lecha M, Puy H, Deybach JC, Orphanet J. Rare Diseases. 2009; 4:19. [PubMed: 19744342]
51. Stumm W, Lee GF. Indust. and Engineering Chem. 1961; 53:143–146.
52. Sung W, Morgan JJ. Env. Sci. & Tech. 1980; 14:561–568.
53. Chen W, Dailey HA, Paw BH. Blood. 2010; 116:628–630. [PubMed: 20427704]
54. Hunter GA, Ferreira GC. J. Biol. Chem. 2010; 285:41836–41842. [PubMed: 20966079]
55. Hambright P, Chock PB. J. Am. Chem. Soc. 1974; 96:3123–3131. [PubMed: 4833462]
56. Inamo M, Kamiya N, Inada Y, Nomura M, Funahashi S. Inorg. Chem. 2001; 40:5636–5644. [PubMed: 11599964]
57. Lavalley DK. Mol. Struct. Energ. 1988; 9:279–313.
58. Yoon T, Cowan JA. J Biol Chem. 2004; 279:25943–25946. [PubMed: 15123683]
59. Al-Karadaghi S, Franco R, Hansson M, Shelnutt JA, Isaya G, Ferreira GC. Trends Biochem. Sci. 2006; 31:135–142. [PubMed: 16469498]

60. Bencze KZ, Yoon T, Millaan-Pacheco C, Bradley PB, Pastor N, Cowan JA, Stemmler TL. *Chem. Comm.* 2007;1798–1800. [PubMed: 17476391]
61. He Y, Alam SL, Proteasa SV, Zhang Y, Lesuisse E, Dancis A, Stemmler TL. *Biochemistry.* 2004; 43:16254–16262. [PubMed: 15610019]
62. Shi Z, Haddad R, Franco R, Shelnutt JA, Ferreira GC. *Biochemistry.* 2006; 45:2904–2912. [PubMed: 16503645]
63. Shipovskov S, Karlberg T, Fodje M, Hansson MD, Ferreira GC, Hansson M, Reimann CT, Al-Karadaghi S. *J. Mol. Biol.* 2005; 352:1081–1090. [PubMed: 16140324]
64. Sigfridsson E, Ryde U. *J. Biol. Inorg. Chem.* 2003; 8:273–282. [PubMed: 12589563]
65. McIntyre NR, Franco R, Shelnutt JA, Ferreira GC. *Biochemistry.* 2011; 50:1535–1544. [PubMed: 21222436]
66. De Matteis F, Gibbs AH, Smith AG. *Biochem. J.* 1980; 189:645–648. [PubMed: 7213349]
67. Ortiz de Montellano PR, Kunze KL, Cole SP, Marks GS. *Biochem. Biophys. Res. Commun.* 1980; 97:1436–1442. [PubMed: 6894236]
68. Ortiz de Montellano PR, Kunze KL, Cole SP, Marks GS. *Biochem. Biophys. Res. Commun.* 1981; 103:581–586. [PubMed: 7332558]
69. Dailey HA, Fleming JE. *J. Biol. Chem.* 1983; 258:11453–11459. [PubMed: 6688622]
70. Cole SP, Marks GS. *Mol. Cell. Biochem.* 1984; 64:127–137. [PubMed: 6390167]
71. Gamble JT, Dailey HA, Marks GS. *Drug Metab. Dispos.* 2000; 28:373–375. [PubMed: 10725301]
72. Franco R, Pereira AS, Tavares P, Mangravita A, Barber MJ, Moura I, Ferreira GC. *Biochem. J.* 2001; 356:217–222. [PubMed: 11336654]
73. Jencks, WP. *Catalysis in chemistry and enzymology.* New York: Dover; 1987. p. xvi-836.
74. Jentzen W, Ma JG, Shelnutt JA. *Biophys. J.* 1998; 74:753–763. [PubMed: 9533688]
75. Fleischer EB, Wang JH. *J. Am. Chem. Soc.* 1960; 82:3498–3502.
76. De Luca G, Romeo A, Scolaro LM, Ricciardi G, Rosa A. *Inorg. Chem.* 2009; 48:8493–8507. [PubMed: 19650629]
77. Huszank R, Horvath O. *Chem. Commun. (Camb.).* 2005:224–226. [PubMed: 15724193]
78. Huszank R, Lendvay G, Horvath O. *J. Biol. Inorg. Chem.* 2007; 12:681–690. [PubMed: 17333300]
79. Hambright, P. *The Porphyrin Handbook.* Kadish, KM.; Smith, KM.; Guillard, R., editors. Vol. 3. Academic Press; 2003. p. 129-210.
80. Hoggins M, Dailey HA, Hunter CN, Reid JD. *Biochemistry.* 2007; 46:8121–8127. [PubMed: 17566985]
81. Dailey, HA.; Dailey, TA. *The Porphyrin Handbook.* Kadish, KM.; Smith, KM.; Guillard, R., editors. Vol. 12. California, USA: Elsevier Science; 2003. p. 93-121.
82. Dailey HA, Wu CK, Horanyi P, Medlock AE, Najahi-Missaoui W, Burden AE, Dailey TA, Rose J. *Biochemistry.* 2007; 46:7973–7979. [PubMed: 17567154]
83. Sellers VM, Wu CK, Dailey TA, Dailey HA. *Biochemistry.* 2001; 40:9821–9827. [PubMed: 11502175]

The abbreviations used are

PPIX	protoporphyrin IX
SAT	sitting-atop complex

Figure 1.

Alignment of *B. subtilis* iron-bound (PDB# 2HR6; green) and human PPIX-bound (PDB# 2HRE; light blue) monomeric ferrochelatase crystal structures, showing the relative position of the substrates. The conserved glutamate and histidine that coordinate the iron atom are shown in yellow. In this analysis the iron atom is directly over and closest to the D-ring pyrrole nitrogen of PPIX.

Figure 2.

A schematic representation of three different possible mechanisms for ferrous iron acquisition by eucaryotic ferrochelatases. See text for details.

Figure 3. Surface map of the mitochondrial matrix side of a human ferrochelatase monomer in the open conformation with the π -helix unwound. The carbon atoms of the catalytic glutamate (E343) are colored yellow.

Scheme I.
The ferrochelatase catalyzed reaction