Published in final edited form as:

Trends Biochem Sci. 2006 March ; 31(3): 135–142. doi:10.1016/j.tibs.2006.01.001.

Chelataes: distort to select?

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Abstract

Chelataes catalyze the insertion of a specific metal ion into porphyrins, a key step in the synthesis of metalated tetrapyrroles that are essential for many cellular processes. Despite apparent common structural features among chelataes, no general reaction mechanism accounting for metal ion specificity has been established. We propose that chelatae-induced distortion of the porphyrin substrate not only enhances the reaction rate by decreasing the activation energy of the reaction but also modulates which divalent metal ion is incorporated into the porphyrin ring. We evaluate the recently recognized interaction between ferrochelatase and frataxin as a way to regulate iron delivery to ferrochelatase, and thus iron and heme metabolism. We postulate that the ferrochelatase–frataxin interaction controls the type of metal ion that is delivered to ferrochelatase.

Introduction

Insertion of a specific divalent metal ion into a tetrapyrrole provides the basis for a multitude of cellular functions. The resulting metalated tetrapyrroles constitute the centerpiece for proteins involved in cellular processes ranging from oxygen and electron transport, signal transduction, photosynthesis, and sulfur and nitrogen metabolism to molecular rearrangements, methyl-group transfer reactions and regulation of transcription and translation [1–4]. Metalated tetrapyrroles such as the cofactors and prosthetic groups heme, chlorophyll, cobalamin (vitamin B₁₂) and coenzyme F₄₃₀ have a centrally chelated metal ion and arise from a common tetrapyrrole, uroporphyrinogen III. The chemical nature of the chelated metal ion varies; for example, Fe²⁺ is present in heme, Mg²⁺ in chlorophyll, Co²⁺ in cobalamin and Ni²⁺ in coenzyme F₄₃₀ [5–9].

Essential to the catalytic insertion of metal ions into various tetrapyrroles is a divergent group of enzymes called chelataes [5–9], which ensure specificity of the metal ion in

response to cellular requirements (Box 1). Although a model of the general reaction mechanism of porphyrin metalation has emerged [10–15], how chelataases bestow metal ion selectivity and specificity on porphyrin metalation remains unresolved.

Box 1

Chelataases: members of a 'functional' family?

Tetrapyrroles are organic molecules that contain four five-membered heterocyclic (pyrrole) rings, linked in a cyclic or linear array. Heme, chlorophyll, cobalamin (vitamin B₁₂), siroheme and coenzyme F₄₃₀ belong to a family of prosthetic groups that are characterized by their tetrapyrrole-derived nature and contain a central, complexed metal ion: Fe²⁺ in heme and siroheme, Mg²⁺ in chlorophyll and bacterio-chlorophyll, Co²⁺ in cobalamin, and Ni²⁺ in coenzyme F₄₃₀ [5,6,8,9]. All of these molecules are derived from a common tetrapyrrole, uroporphyrinogen III (Figure I), and require the function of specific enzymes or chelataases for the insertion of their metal ion (Figure I).

On the basis of structural, functional and genetic studies and results from genome sequencing projects [6,24], three main classes of chelataases are recognized: (i) ATP-dependent heterotrimeric chelataases, including protoporphyrin IX magnesium chelataase (ChlH-I-D or BchlH-I-D) involved in chlorophyll or bacteriochlorophyll biosynthesis [9], and aerobic cobaltochelataase (CobN-S-T) associated with cobalamin biosynthesis [6]; (ii) ATP-independent chelataases, including protoporphyrin IX ferrochelataase (HemH) involved in heme biosynthesis [5,16,33], sirohydrochlorin ferrochelataase (SirB) associated with siroheme biosynthesis [6,7], and anaerobic sirohydrochlorin cobaltochelataases (CbiK and CbiX) involved in cobalamin biosynthesis [6]; and (iii) multifunctional homodimeric chelataases associated with siroheme biosynthesis, including siroheme synthase (CysG) [8], and precorrin-2 dehydrogenase and sirohydrochloride ferrochelataase (Met8p) in yeast [24]. The enzymes in the third class house two enzymatic activities: dehydrogenase and chelataase [8,24].

Although there is no sequence similarity among chelataases and no significant sequence similarity (<12% overall sequence identity) between members of the ATP-independent chelataase class, the latter have similar overall topology and adopt a similar fold [5,6,16]. The ATP-independent chelataases possess two α/β domains with a similar Rossmann-type fold; the active site cleft is located between the N- and C-terminal domains and structural elements are contributed from both domains [5,6,33]. The different chelataases are thought to have similar enzymatic mechanisms, involving distortion of the tetrapyrrole, abstraction of two protons from the tetrapyrrole-derived substrate, and exposure of the pyrrole nitrogen atoms to the incoming metal ion substrate [6,16].

Figure I.

The relationship between tetrapyrrole biosynthetic pathways. Uroporphyrinogen III is the tetrapyrrole that is common to all tetrapyrrole biosynthetic pathways. 5-Aminolevulinate (ALA), a precursor of uroporphyrinogen III, derives from glycine and succinyl-CoA (in eukaryotes other than plants and the α subgroup of the photosynthetic purple bacteria) or glutamate (in plants and most bacteria). Class I, II and III chelataases are shown in blue, purple and yellow, respectively.

Among the chelataases, protoporphyrin IX ferrochelataase (protoheme ferrolyase, EC 4.99.1.1; hereafter called ferrochelataase) represents the most-investigated enzyme (Figure 1a). It

catalyzes the insertion of Fe^{2+} into protoporphyrin IX [16], the terminal step of the heme biosynthetic pathway. Distortion of porphyrin, which follows porphyrin binding to ferrochelatase, is a salient feature in the catalytic mechanism of this enzyme and embodies an ‘induced-fit’ [17] mechanism of catalysis.

Here we focus on the catalytic reaction of ferrochelatase as a model for all chelataes by exploring the interplay between distortion of the porphyrin substrate and metal ion insertion. We propose that chelataes, by differentially distorting the porphyrin substrate, modulate which metal ion is incorporated. In addition, we assess emerging roles associated with ferrochelatase – namely, its potential interaction with frataxin – and evaluate the significance of this protein–protein interaction with regard to the regulation of iron homeostasis and heme biosynthesis. We propose that protoporphyrin modulates the interaction between ferrochelatase and iron-loaded frataxin.

Porphyrin binding and distortion: towards catalysis of chelation

Distortion of porphyrin has long been recognized as a crucial step in the incorporation of metal ions into porphyrin molecules (Box 2). The crystal structure of ferrochelatase complexed with *N*-methylmesoporphyrin (*N*-MeMP), a potent inhibitor of ferrochelatase (dissociation constant, $K_d \approx 10$ nM) that mimics a strained substrate [18–20], shows that porphyrin binds in a deep cleft between two surfaces: one hydrophobic and one hydrophilic (Figure 1b). When used as a hapten, non-planar *N*-MeMP induces the formation of antibodies that catalyze the insertion of divalent metal ions into mesoporphyrin [19,21,22].

Box 2

Reaction mechanism of porphyrin metalation

More than 30 years ago, Hambright and Chock [11] proposed a mechanism for metal chelation involving formation of an outer-sphere complex between free-base porphyrin and a metal ion, followed by deformation of the porphyrin nucleus to generate an appropriate configuration for complexation of the metal ion (Figure I). Accordingly, the conformer would have two of the opposite pyrrole rings and lone-pair orbitals of the respective pyrrole nitrogen atoms pointing upwards, and the other two pyrrole rings would be pointing downwards with regard to the mean porphyrin plane (Figure Ia). Hambright and Chock [11] suggested that the coordinating ability of the lone pairs would be enhanced in this conformation, because the lone pairs would point away from the center of the macrocycle and the apparent acidity of the protons would be increased owing to elimination of the N–H tautomerism [11]. In summary, porphyrin distortion would heighten metal chelation, and deformation – rather than dissociation – would control porphyrin metalation [11].

Subsequent studies in solution and with enzymatic systems led to the formulation of a general reaction mechanism for the metalation of porphyrins [12–15]. The steps for the mechanism include, first, deformation of the porphyrin ring; second, outer-sphere association of the solvated metal ion and the porphyrin; third, exchange of a coordinated solvent molecule with the first pyrroline nitrogen atom; fourth, chelate-ring closure with liberation of additional solvent molecules to form the ‘sitting-atop complex’; fifth, first deprotonation of a pyrrole nitrogen atom; and last, deprotonation of the second pyrrole nitrogen with formation of the metalated porphyrin (Figure Ib).

Thus, induction of porphyrin distortion should be a reasonable role for a catalyst of metal ion insertion. Indeed, Lavalley [13] reasoned that ferrochelatase should catalyze porphyrin metalation mainly by facilitating porphyrin distortion and keeping the outer-sphere complex intact for the complete reaction to occur. Recent calculations of the

energetics associated with porphyrin distortion have shown that this step forms an integral part of the course of the ferrochelatase-catalyzed reaction [15]. Sigfridsson and Ryde [15] used combined quantum mechanical and molecular mechanical calculations to examine the energetics of porphyrin deformations in free porphyrins, ferrochelatase and the porphyrin–ferrochelatase complex. Their results clearly demonstrate that ferrochelatase-induced distortion of free-base protoporphyrin IX – the physiological substrate of ferrochelatase – is thermodynamically favorable. Their model supports a saddled structure for the porphyrin, in which two opposite pyrrole rings are slightly tilted upwards and the other two pyrrole rings are slightly tilted downwards.

Figure 1.

Reaction mechanism of porphyrin metalation. **(a)** Out-of-plane saddling deformation used to describe a nonplanar distortion of the porphyrin macrocycle. Note that the two opposite pyrrole rings with unprotonated nitrogen atoms (blue spheres) point upwards, and the other two pyrrole rings with protonated nitrogen atoms (blue spheres connected to white spheres) point downwards. **(b)** Steps in the reaction mechanism for incorporation of the metal ion (red) into porphyrin (pyrrole rings, green; unprotonated pyrrole nitrogen atoms, blue; protonated pyrrole nitrogen atoms with protons, yellow) include (i) deformation of the porphyrin ring; (ii) formation of the first metal–porphyrin bond, followed by other ligand-exchange steps leading to formation of a ‘sitting-atop’ complex (in which two pyrroline nitrogen atoms coordinate to the metal ion and two protons remain on the pyrrole nitrogen atoms); and (iii) sequential deprotonation of the two pyrrole nitrogen atoms coupled with formation of the metalated porphyrin.

Antibody 7G12, the result of five somatic mutations accumulated during affinity maturation of the germline precursor antibody, is an example of such a catalytic antibody [19,21,22]. Similar to ferrochelatase, antibody 7G12 has a porphyrin-binding cleft at the junction of two surfaces with different hydrophobicities [19,22]. Whereas binding of *N*-MeMP to *Bacillus subtilis* ferrochelatase leads to a conformational transition in ferrochelatase with widening of the binding cleft in a characteristic ‘induced-fit’ reaction mechanism [20], however, antibody 7G12 binds porphyrin in a ‘lock-and-key’ fashion without undergoing a conformational transition [19,21]. These findings indicate that, in contrast to ferrochelatase, the binding site of antibody 7G12 is rigid and pre-organized to bind a distorted porphyrin.

Although ferrochelatase and antibody 7G12 are both effective catalysts of porphyrin metallation, the specific interactions that lead to distortion of the macrocycle structure are almost certainly very different. Thus, in ferrochelatase, porphyrin rings B, C and D are held in a vice-like grip through interactions with conserved amino acid side chains (Figure 1b). For ring A, which is tilted, it is difficult to identify a single residue that might be responsible for this distortion. The binding pocket for this pyrrole is created by several residues, which, together with the strain generated by the tight binding of rings B, C and D, presumably produce the force necessary for tilting. Because tetrapyrroles are complex conjugated systems, however, tilting one of the rings will inevitably distort the other rings.

In antibody 7G12, *N*-MeMP and mesoporphyrin are bound in a cleft formed by the complementarity-determining regions (CDRs) L2 and L3 on one side and CDR H3 on the other [19,22]. The side chain of Asp100^L (Asp100 of the light-chain variable region of the

7G12 antibody) has an important role in interactions with the porphyrin. In the complex with mesoporphyrin (Protein Data Bank accession code 1NGW), the carboxylic oxygen of Asp100^L points towards the center of the ring and is located within hydrogen-bonding distance of the nitrogen atoms of rings A (2.8 Å), B (2.5 Å) and C (2.9 Å) [19,22]. The distance to the nitrogen atom of ring D is 3.7–3.8 Å, which is too long for a hydrogen bond.

A similar asymmetric interaction of a polar side chain with the pyrrole nitrogen atoms is adopted in macrocycle distortion by other proteins. For example, in the complexes of uroporphyrinogen decarboxylase, an enzyme of the heme biosynthetic pathway, with the coproporphyrinogen I and III isomers [23], the carboxylic oxygen of Asp86 shows an asymmetric distribution of distances to the nitrogen atoms of the four pyrrole rings: 2.8 Å, 2.9 Å, 3.1 Å and 3.4 Å. The tetrapyrrole lies against a surface built by conserved hydrophobic residues and adopts a domed conformation in which one pyrrole ring (with the longest distance to Asp86) is tilted out of the plane formed by the nitrogen atoms of the other three pyrroles. Phillips *et al.* [23] conclude that coordination of Asp86 to the pyrrole nitrogen atoms is one of the main factors that contributes to distortion of the tetrapyrrole ring.

We suggest that Asp100^L of the catalytic antibody has a similar function in distortion of the bound mesoporphyrin. A comparable mode of porphyrin distortion, involving a conserved aspartate residue, might be used by Met8p, a bifunctional dehydrogenase and ferrochelatase. The Met8p aspartate is located in the potential substrate-binding cleft and seems to perform dual functions in dehydrogenation and metalation [24]. Although anaerobic cobalt chelatase is structurally homologous to ferrochelatase (Box 1), the absence of structural information on substrate binding prevents a reliable analysis of the mechanism of substrate distortion in this enzyme. Nevertheless, the above analysis of porphyrin binding indicates that different binding modes probably result in different types of ring distortion, which in turn affect the reactivity of the macrocycle and its chelating ability.

Porphyrin binding and distortion in *Saccharomyces cerevisiae* and murine ferrochelatases and in antibody 7G12 have been directly assessed using resonance Raman spectroscopy [21,25–27]. In both enzymes and the antibody, porphyrin binding activates several assigned out-of-plane vibrational modes of the macrocycle. The frequency shifts in the structure-sensitive lines of the bound porphyrins relative to the unbound porphyrins reflect the type of distortion that is undergone by the porphyrin macrocycle. Significantly, γ_{15} , a saddling-symmetry (B_{2u}) out-of-plane mode, emerges with the binding of free-base protoporphyrin to murine ferrochelatase [27] and to the catalytic antibody [21]. A saddling deformation has also been identified as a chief component of the nonplanar distortion of either *N*-MeMP or Cu-*N*-MeMP bound to *B. subtilis* ferrochelatase in the crystal structures of these complexes [20].

In fact, the normal-coordinate structural decomposition of the porphyrins contained in the crystal structures accounts for how the different symmetric out-of-plane deformations (ruffled, saddled, domed, *x*- and *y*-waved, and propellered structures) contribute to the porphyrin distortion [27]. The main calculated contributions to the distortion of *N*-MeMP and Cu-*N*-MeMP bound to *B. subtilis* ferrochelatase are saddling, followed by *x*-waving, ruffling and doming [27]. We reiterate that saddling is an out-of-plane deformation that exposes both the protons and the lone pairs of the nitrogen atoms of the porphyrin macrocycle, in keeping with a porphyrin arrangement for metal insertion.

Metal ion binding and shuttling

Although the physiologically relevant reaction catalyzed by ferrochelatase is insertion of Fe²⁺ into the porphyrin ring, the enzyme can chelate other divalent metal ions (e.g. Zn²⁺,

Co^{2+} and Cu^{2+}) [16]. Curiously, in contrast to most ferrochelatases, the *B. subtilis* enzyme can chelate Cu^{2+} but not Co^{2+} [28]. Thus, understanding the metal-binding site was cited early on as a significant goal in defining the metal substrate specificity and the reaction catalytic mechanism [16].

Different spectroscopic techniques (e.g. extended X-ray absorption fine structure and Mössbauer), X-ray crystallography and site-directed mutagenesis have been used to characterize the metal-binding sites of ferrochelatase [5,29–35]. Mössbauer and X-ray absorption spectroscopies have indicated that ferrochelatase has an ionic coordination environment for Fe^{2+} with six nitrogen- and/or oxygen-containing ligands [29,30]. These results are consistent with a proposal, derived from kinetic studies of site-directed variants of yeast and human ferrochelatases, that a conserved histidine residue (His235 in yeast and His263 in human) is involved in binding the metal substrate [31,32]. The *B. subtilis* and yeast ferrochelatase crystal structures both support the coordination of a metal ion to the corresponding histidine (His183 in *B. subtilis* ferrochelatase) [5] (Figure 1c).

In particular, the crystal structures of yeast ferrochelatase complexed with either Co^{2+} or Cd^{2+} have revealed that three conserved amino acids (His235, Glu314 and Ser235) are involved in coordination of the metal ion [33]. His235 is the ligand at the shortest distance from the metal ion, thereby supporting its proposed role as the main metal-binding residue [33]. This view, however, is not undisputed. Analysis of the crystal structure of Co^{2+} -reacted human ferrochelatase revealed a metal-binding site that is located ~ 20 Å from His263 on the opposite side of the porphyrin-binding cleft [35]. Wu *et al.* [35] proposed a model in which the Fe^{2+} ion, after initially binding at this site (His230 and Asp383 in human ferrochelatase), translocates to the porphyrin-binding site through a conduit of conserved residues. In this model, the conserved active site histidine (His263 in human ferrochelatase) is responsible for abstraction of the porphyrin proton [16,35].

Determination of the structures of *B. subtilis* ferrochelatase complexed with either Cd^{2+} or Zn^{2+} has led to the clear identification of two metal-ion-binding and possibly interacting sites: one located at the surface of the molecule; and one located in the porphyrin-binding cleft, close to the distorted porphyrin ring A [34]. In fact, the nitrogen of ring A is directed towards His183 and Glu264 [34] (Figure 1c). The two metal-binding sites are located 7 Å apart, and the affinity of a metal ion towards each of them depends on the presence of a metal ion at the other site [34].

Lecerof *et al.* [34] proposed that a metal ion, by coordinating with acidic residues arranged along the helical edge of a π helix, would be shuttled from the site at the surface to the inner site. Similar to several other proteins in which π -helix residues function to coordinate metal ions required for enzymatic activity, the residues along the π helix in ferrochelatase might form a channel that connects the interior of the active site to the protein exterior [36]. Lecerof *et al.* [34] also advanced the hypothesis that channeling of the metal ion would require an exchange of ligands, initially between the solvent to which the metal is coordinated and the π -helix acidic residues, and ultimately between the protein ligands and the pyrrole nitrogen atoms, thereby culminating with insertion of the metal ion into the porphyrin macrocycle.

Interplay between porphyrin distortion and metal ion specificity

In non-enzymatic metalation, the relative order of divalent metal ion insertion into the porphyrin ring, $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Mn}^{2+} \sim \text{Co}^{2+} > \text{Fe}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} \gg \text{Mg}^{2+}$, matches the rates of solvent exchange for the ions [11]. Recently, Shen and Ryde [14] assessed the importance of ligand exchange in the non-enzymatic incorporation of either Fe^{2+} or Mg^{2+} into a porphyrin ring and found that the ligand-exchange step is rate limiting for the reaction. The estimated

activation energies associated with this step were ~51 and 72 kJ/mol for Fe^{2+} and Mg^{2+} , respectively. Metal desolvation and the role of ligand exchange in the catalytic mechanism of ferrochelatase or any other chelatase has been addressed only recently by Shipovskov *et al.* [37], who suggested that ferrochelatase accelerates the rate of ligand exchange for the metal.

For non-enzymatic porphyrin metalation, Shen and Ryde [14] also proposed that deprotonation might be facilitated by hydrogen-bond networks, which translocate the proton away from the 'sitting-atop complex' (Box 2). Although the nature of the catalytic base involved in proton abstraction in ferrochelatase or any other chelatase remains to be determined, it is expected that any amino acid involved in deprotonation will be located on the side of the porphyrin ring opposite to the metal ion. It is also possible that the proton is quickly released to the solvent after being removed by an amino acid, enabling the same amino acid to bind the metal ion before its insertion into the porphyrin. Alternatively, the porphyrin ring might be directly deprotonated by water molecules present in the porphyrin-binding cleft [14].

Independent experimental approaches have provided unequivocal evidence that porphyrin binding to ferrochelatase induces distortion of porphyrin [20,27]. Although axial ligation is not crucial for porphyrin distortion in the active site of ferrochelatase [20,27], however, the catalytic antibody [19] and probably Met8p [24] use axial ligation to induce distortion in the tetrapyrrole macrocycle. Given that the porphyrin-binding cleft is a common structural feature among chelatases [5,33,35], can differences in the mode of porphyrin binding and distortion explain the variation observed in metal specificity and catalytic activity? A strong argument in support of such a possibility is the high conservation of the porphyrin-binding pocket in ferrochelatases, which can be easily seen when the structures of *B. subtilis*, *Homo sapiens* and *S. cerevisiae* ferrochelatases are compared. The conserved residues on one side of the porphyrin ring (Ala182, His183, Ser184, Leu185, Pro186 and Met187 in *B. subtilis* ferrochelatase) can be superimposed on the corresponding residues of the yeast structure (Ala234, His235, Ser236, Leu237, Pro238 and Met239) with a root mean square deviation of 0.53 Å for the Ca atoms. This high degree of conservation in the cleft suggests that the mode and degree of porphyrin distortion are important parameters of the enzymatic reaction.

Of relevance, the degree of a specific nonplanar deformation of porphyrin contributes to the catalytic efficiency of ferrochelatase. Strikingly, the activation of an out-of-plane porphyrin vibration, γ_{15} , correlates with the catalytic efficiency of antibody 7G12 [21]. We propose that subtle structural differences among the active sites impose distinct degrees of porphyrin distortion and selective incorporation of the metal ion. Compelling evidence in support of this hypothesis indicates that metalation of *N*-MeMP by Cu^{2+} is more efficient than that by Zn^{2+} . This contrasts with the lower Michaelis constant for metalation of protoporphyrin IX by Zn^{2+} (17 μM) as compared with Cu^{2+} (170 μM) [37]. Thus, it seems that the different types of distortion of *N*-MeMP and protoporphyrin IX, when bound to ferrochelatase, affect the specificity of the enzyme towards the metal ion.

The chelatase-induced distortion of the porphyrin substrate substantiates particularly well the hypothesis formulated by Jencks [38] that 'the induction of strain in the substrate, the enzyme, or both plays a significant role in bringing about the observed specific rate acceleration'. The induction of strain in the enzyme-substrate complex decreases the activation energy of the reaction, but the cost of strain energy is a decrease in substrate-binding energy (i.e. the 'Circe effect') [38,39]. Thus, the relationship between ferrochelatase catalytic efficiency and the extent of the saddling undergone by porphyrin is consistent with the idea that catalysis involves straining of the substrate and evolution of the binding energy to lower the activation energy of the reaction. Furthermore, such a change in porphyrin and

protein conformations on complex formation [20] exemplifies an ‘induced-fit’ reaction mechanism.

The protein conformational transition on porphyrin binding to ferrochelatase might also have an additional function. Protoporphyrinogen IX oxidase, the enzyme preceding ferrochelatase in the heme biosynthetic pathway, has been proposed to form a complex with ferrochelatase across the inner mitochondrial membrane [40,41]. This possibly transient complex should facilitate the direct channeling of protoporphyrin IX to ferrochelatase. Thus, the change in protein conformation that follows porphyrin binding might control the affinity of ferrochelatase towards protoporphyrinogen IX oxidase, resulting in dissociation of the complex.

Ferrochelatase–frataxin interaction: a mechanism for iron delivery?

Under physiological conditions, efficient delivery of Fe^{2+} to ferrochelatase requires mechanisms to overcome both the limited bioavailability and the potential toxicity of iron. The mitochondrial protein frataxin can either make Fe^{2+} available to other proteins [42–44], thereby acting as a general iron chaperone, or convert surplus iron to a redox-inactive mineral [45], thereby providing a mechanism for iron detoxification. Growing evidence supports the hypothesis that frataxin is a facilitator of iron metabolism [46], promoting not only the biosynthesis and repair of iron–sulfur clusters [42,47] but also the biosynthesis of heme [48,49]. In *S. cerevisiae* knockout mutants lacking frataxin, ferrochelatase catalyzes the formation of Zn^{2+} -protoporphyrin but not heme. Thus, although ferrochelatase is catalytically competent, it cannot catalyze the insertion of Fe^{2+} into protoporphyrin, suggesting that frataxin has an important physiological role in controlling the availability of mitochondrial Fe^{2+} to ferrochelatase [50].

Frataxin is inferred to be an iron donor in heme biosynthesis with an estimated K_d of 1.7×10^{-8} to 4×10^{-8} M for ferrochelatase [50,51]. The ferrochelatase–frataxin interaction has been confirmed by *in vitro* ferrochelatase activity assays, which have established that Fe^{2+} -loaded frataxin supports heme formation by providing ferrochelatase with Fe^{2+} in the presence of atmospheric oxygen without reducing agents [43,45]. Determination of the crystal structure of human frataxin identified a conserved and predominantly hydrophobic region on the surface of the protein that was postulated to mediate protein–protein interactions [52]. More recently, He *et al.* [53], using NMR spectroscopy to determine the yeast frataxin solution structure, identified the frataxin helical plane as the possible ferrochelatase-binding surface.

Similar to the role of citrate in stabilizing the interaction between frataxin and aconitase [42], protoporphyrin might stabilize the frataxin–ferrochelatase complex and, in so doing, facilitate the ferrochelatase-catalyzed reaction and formation of heme. The relative concentrations of protoporphyrin and other substrates (citrate, sulfur) might regulate the extent to which frataxin delivers iron for heme and/or iron–sulfur cluster synthesis. Protoporphyrin probably becomes available to ferrochelatase through a complex formed, perhaps even transiently, with protoporphyrinogen oxidase (Figure 2). Protoporphyrin would then induce the interaction between ferrochelatase and iron-loaded frataxin, which in turn would deliver Fe^{2+} to ferrochelatase for insertion into the protoporphyrin ring to give heme.

In summary, the presence of frataxin would promote the use of Fe^{2+} by ferrochelatase. In a situation where frataxin is absent or defective, such as in the yeast knockout model or in individuals affected with the neurodegenerative disease Friedreich’s ataxia, ferrochelatase would not have Fe^{2+} available and would use other divalent metal ions such as Zn^{2+} as a metal ion substrate [48,49]. We therefore propose that protoporphyrin, along with

modulating the interaction between ferrochelatase and protoporphyrinogen oxidase, modulates the frataxin–ferrochelatase interaction.

Concluding remarks

We postulate that the distortion of porphyrin adopted during porphyrin binding to a chelatase, in addition to enhancing the reaction rate, controls the selection of the metal ion inserted into the porphyrin macrocycle. As a determinant of metal ion substrate specificity in chelatase-catalyzed reactions, the extent and type of porphyrin distortion adds a new dimension to the functional value of porphyrin deformation.

The interaction between ferrochelatase and frataxin assumes a crucial role in the ferrochelatase reaction in the cell, because frataxin, functioning as an iron chaperone, provides ferrochelatase with Fe^{2+} . We propose that frataxin interacts with ferrochelatase in a porphyrin-dependent manner, delivering Fe^{2+} according to cellular requirements. Finally, we postulate that protoporphyrin modulates the interaction between ferrochelatase and iron-loaded frataxin.

Future research on the type and degree of porphyrin distortion in relation to the chelatase-catalyzed reaction and on the biochemical and structural characterization of the ferrochelatase–frataxin partnership will be required to reveal how porphyrin distortion modulates the metal ion specificity of chelatases and how protein–protein interactions control the delivery of Fe^{2+} from frataxin to ferrochelatase.

Acknowledgments

We thank Tobias Karlberg for Figure 1. We apologize to investigators whose work could not be cited owing to space limitations. This work was supported by grants from the Swedish Research Council (to S. A.-K. and M. H.), the Muscular Dystrophy Association and National Institutes of Health (AG15709 to G. I.) and the American Cancer Society (RSG-96-05106-TBE to G.C.F). Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-AC04-94AL85000 (J.A.S.).

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Figure 1.

Porphyrin- and metal ion-binding sites in ferrochelatase. **(a)** Structure of *B. subtilis* ferrochelatase in complex with the transition-state inhibitor *N*-methylmesoporphyrin (*N*-MeMP). The structure is composed of two Rossmann-type domains (green and blue), in which a central four-stranded β sheet is flanked by α helices. A cleft defined by structural elements (red) from both domains accommodates the porphyrin- and metal-binding sites. The inhibitor *N*-MeMP is shown in the cleft (carbon atoms, yellow; oxygen, red; nitrogen, blue). On binding to the enzyme, porphyrin is distorted and the protein undergoes a conformational transition, during which the cleft is widened. This process is typical of an 'induced-fit' mechanism of catalysis. A fully hydrated Mg^{2+} ion is always located $\sim 8 \text{ \AA}$ from the nitrogen atom of the distorted ring A of the porphyrin (Mg^{2+} , green sphere; water, red spheres). **(b)** Interaction of *N*-MeMP with amino acids in the substrate-binding cleft of *B. subtilis* ferrochelatase. The side chains of the residues that contribute directly to the stabilization of porphyrin binding are shown in stick representation. Arg30 and Arg31 are on the surface of the molecule and contribute to neutralization of the negative charge of the propionic acid side chains of the porphyrin. Tyr13, Ile29, His183, Trp230 and Glu264 stabilize the position of the macrocycle in the cleft. The hydroxyl group of Tyr13 is directed towards the center of the ring and coordinates a metal ion in a complex of ferrochelatase with Cu^{2+} inserted into *N*-MeMP. Tyr26 and Leu43 constitute part of the switch region, which triggers the conformational transition in the enzyme on porphyrin binding. **(c)** Two metal-binding sites in *B. subtilis* ferrochelatase. The two sites are shown with a Zn^{2+} ion (gray sphere) and a fully hydrated Mg^{2+} ion (green sphere). The distance between the metal ions is $\sim 7 \text{ \AA}$. His183 and Glu264 are among the few residues that are invariant in all protoporphyrin IX ferrochelatases. The side chains of residues Glu272, Asp268 and Glu264 are aligned along a π helix (green) in a line connecting the two metal sites. Only a π helix can provide such an alignment of side chains. The π helix differs from the α helix in that it has 4.1 residues per turn and is stabilized by hydrogen bonds between residues i and $i+5$ (an α helix has 3.6 residues per turn and hydrogen bonds between residues i and $i+4$).

Figure 2.

Proposed model of modulation of ferrochelatase–frataxin interactions by protoporphyrin. **(a)** Protoporphyrinogen oxidase (PPO) makes protoporphyrin (PP) available to ferrochelatase (FCH) via a transient interaction (i). Frataxin (Fxn) then delivers Fe^{2+} to ferrochelatase (ii), which promotes its insertion into protoporphyrin to yield heme (iii). **(b)** In the absence of protoporphyrin, frataxin might donate Fe^{2+} to other proteins (e.g. aconitase) [42] or could incorporate surplus iron in a stable ferrihydrite mineral [43,45].