Archives of Biochemistry and Biophysics 644 (2018) 37–46

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Ferrochelatase π-helix: Implications from examining the role of the conserved π-helix glutamates in porphyrin metalation and product release

Mallory E. Gillam, Gregory A. Hunter, Gloria C. Ferreira*

Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL, 33612, USA

ARTICLE INFO

Keywords:
Protoporphyrin ferrochelatase
Heme
Porphyrin
Enzyme mechanism
Erythropoiesis
Metalloenzyme

ABSTRACT

Protoporphyrin ferrochelatase catalyzes the insertion of Fe^{2+} into protoporphyrin IX to form heme. To determine whether a conserved, active site π-helix contributes to the translocation of the metal ion substrate to the ferrochelatase-bound porphyrin substrate, the invariant π-helix glutamates were replaced with amino acids with non-negatively charged side chains, and the kinetic mechanisms of the generated variants were examined. Analysis of yeast wild-type ferrochelatase-, E314Q- and E318Q-catalyzed reactions, under multi- and single-turnover conditions, demonstrated that the mutations of the π-helix glutamates hindered both protoporphyrin metalation and release of the metalated porphyrin, by slowing each step by approximately 30–50%. Protoporphyrin metalation occurred with an apparent pK_a of 7.3 ± 0.1, which was assigned to binding of Fe^{2+} by deprotonated Glu-314 and Glu-314-assisted Fe^{2+} insertion into the porphyrin ring. We propose that unwinding of the π-helix concomitant with the adoption of a protein open conformation positions the deprotonated Glu-314 to bind Fe^{2+} from the surface of the enzyme. Transition to the closed conformation, with π-helix winding, brings Glu-314-bound Fe^{2+} to the active site for incorporation into protoporphyrin.

1. Introduction

Heme is an essential cofactor in most organisms [1,2], and even parasitic organisms that lack heme-synthesizing enzymes compensate for this deficiency by acquiring heme or its precursor, protoporphyrin IX (PPIX) [3]. The terminal step of heme biosynthesis in metazoans, which is catalyzed by PPIX ferrochelatase (E.C. 4.99.1.1; hereon referred to as ferrochelatase), joins the porphyrin biosynthesis and iron transport pathways [4]. Cellular toxicity of the two physiological substrates, Fe^{2+} and PPIX, and high cellular demand for the heme product call for the ferrochelatase-catalyzed step to be highly regulated. Not surprisingly, ferrochelatase gene mutations can result in enzyme variants with decreased activity, as manifested in erythropoietic protoporphyria (EPP) [5–7]. Salient clinical features of EPP patients include PPIX accumulation in erythroid cells in the bone marrow and painful photosensitivity [6,8]. Elimination of the PPIX excess can also have taxing effects on the liver, causing liver damage and terminal failure in rare cases [9,10].

Ferrochelatases from various organisms, including human, *Mus musculus* (mouse) and *Saccharomyces cerevisiae* (yeast), and *Bacillus subtilis* coproporphyrin ferrochelatase have been kinetically and structurally characterized [11–16]. Despite the low amino acid sequence identity amongst the human and *S. cerevisiae* PPIX ferrochelatases and *B. subtilis* coproporphyrin ferrochelatase, the crystallographic structures of these proteins show that the overall tertiary structure is conserved [17–19]. The human, mouse, and yeast enzymes are homodimers [20–22], whereas *B. subtilis* coproporphyrin ferrochelatase is a monomer [23]. However, regardless of the oligomeric state and the porphyrin substrate (PPIX *vs*. coproporphyrin III), two similar domains in the monomeric unit are delimited by a cleft in which the porphyrin binds. Conserved residues in this cleft have important roles in catalysis [2,14,24–27]. Amongst these conserved residues are two glutamates located on the same side of a π-helix (Fig. 1), which is a secondary structural element also conserved within the chelatase family of proteins [28–30]. The crystal structures of human wild-type and mutated ferrochelatase and substrate- and product-bound ferrochelatase show that the π-helix adopts two conformations: unwound and wound [31]. Further, the different positioning of a large number of π-helix residue side chains in these structures helped to track the interconversion between the two conformations [27,32] and led to the proposal that unwinding of the π-helix is necessary for product release [32].

Both the crystal structures of *S. cerevisiae* ferrochelatase complexed with Co^{2+} or Cd^{2+} [17] and those of *B. subtilis* coproporphyrin ferrochelatase in complex with Zn^{2+} or Cd^{2+} [33] revealed two possibly

Abbreviations: MOPS, 3-[N-morpholino]propanesulfonic acid; PIPBS, piperazine-N,N′-bis(4-butanesulfonic acid); PPIX, protoporphyrin IX

* Corresponding author. Department of Molecular Medicine, Morsani College of Medicine, MDC 7, University of South Florida, Tampa, FL, 33612-4799, USA.
E-mail address: gferreir@health.usf.edu (G.C. Ferreira).

https://doi.org/10.1016/j.abb.2018.02.015
Received 16 November 2017; Received in revised form 17 February 2018; Accepted 20 February 2018
Available online 23 February 2018
0003-9861/

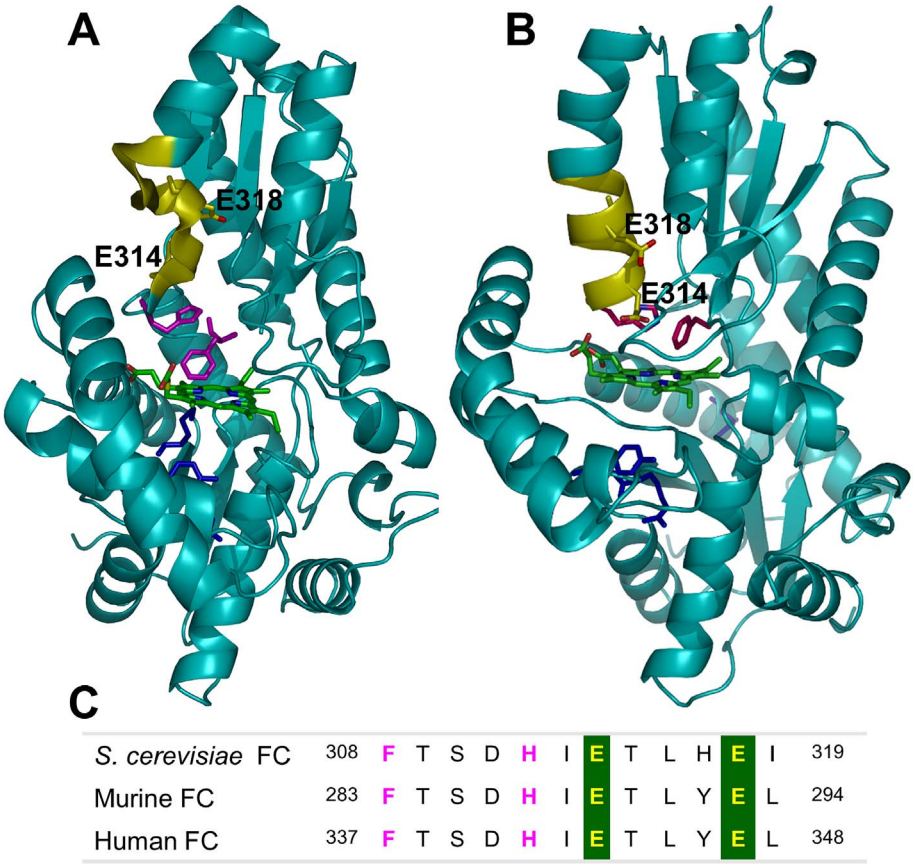

Fig. 1. Positioning of π-helix glutamates and other active site residues in the open and closed conformation of human ferrochelatase. Panel **A**. Ferrochelatase in "open" conformation with product heme bound at the active site (PBD #2QD2). Panel **B**. Ferrochelatase (monomer) in "closed" conformation with substrate PPIX bound at the active site (PBD #2HRE). The π-helix and glutamate residues (Glu-343 and Glu-347) examined in this study are highlighted in *yellow*. Phe-337/His-341 are in *magenta*. His-263 and Met-76/Arg-164/Tyr-165 are in *dark blue*. The amino acid numbering (Glu-314 and Glu-318) in panels A and B is for *S. cerevisiae* ferrochelatase, and it corresponds to human ferrochelatase Glu-343 and Glu-347. In our proposed model, when the π-helix unwinds, catalytic Glu-314 projects out of the matrix side of *S. cerevisiae* ferrochelatase, where it picks up an iron atom from and Fe^{2+} chaperone, and the winding of the π-helix brings Glu-314-bound Fe^{2+} to the active site for incorporation into PPIX. Panel **C**. Amino acid sequence alignment of ferrochelatase from three organisms covering π-helix amino acids pertinent to this study. The π-helix glutamates are indicated in *bold yellow* in dark green-shaded boxes and the secondary metal ion-binding site His and Phe are in *bold magenta*. FC, ferrochelatase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

interacting metal ion-binding sites 7 Å apart. The two sites, one at the surface of the protein and the other in the porphyrin-binding cleft, are connected through the π-helix. This structural arrangement is consistent with the initial hypothesis put forward by Al-Karadaghi and coworkers [33] to describe metal ion translocation from the surface to the active site of coproporphyrin ferrochelatase. Accordingly, the entering metal ion would exchange its solvent ligands with the coordinating, π-helix glutamates, and with the progressing ligand exchange along the π-helix acidic residues, the metal ion would reach the metal ion substrate-binding site and active site. There, the final ligand exchange, the substitution of the protein ligands for the pyrrole nitrogens, would occur and thus the metal ion would be inserted into the porphyrin macrocycle. The presence of an invariant His-Glu couple (*S. cerevisiae* ferrochelatase His-235 and Glu-314 and *B. subtilis* coproporphyrin ferrochelatase His-183 and Glu-264) in the inner or metal ion substrate-binding site corroborates with the findings from biochemical, spectroscopic and kinetic studies, which indicated that these residues are essential for metal ion binding and enzyme activity [15,24,25,34–36]. In fact, no Fe^{2+} was coordinated in the active site of *B. subtilis* coproporphyrin ferrochelatase when the invariant His-183 was mutated to Ala [25]. Nonetheless, the above interpretation for Fe^{2+} shuttling and coordination in the active site, prior to its incorporation in PPIX, does not have general acceptance [15,24,25,34–36]. Based on analyses of the crystal structures of human ferrochelatase and variants of the enzyme complexed with various divalent metal ions, Dailey, Lanzilotta and co-workers [16,19,37] postulated that human ferrochelatase His-263 (*S. cerevisiae* ferrochelatase His-235 and *B. subtilis* coproporphyrin ferrochelatase His-183), along with Glu-343 (*S. cerevisiae* ferrochelatase Glu-314 and *B. subtilis* coproporphyrin ferrochelatase Glu-264) function in proton abstraction from the porphyrin macrocyle and not in Fe^{2+} coordination. More recently, these investigators further proposed that solvent/water-filled channels serve as a "conduit" for the transport of Fe^{2+} from the outer surface of ferrochelatase to the active site [38]. According to their model, active site pocket Arg-164 and Tyr-165 (human ferrochelatase numbering), located opposite to His-263, coordinate the iron ion before porphyrin metalation. Arg-164 would also have a catalytic role by serving as a Lewis base [16]; thus, Fe^{2+} would enter the active site from the opposite face of the porphyrin at the non-conserved residues Met-76/Arg-164/Tyr-165 rather than via the conserved His-263 and Glu-343.

In agreement with the ferrochelatase crystal structures showing a second bound metal ion (Mg^{2+} or Cd^{2+}) [23,33], identification of a secondary conserved metal ion-binding site [14] also provided relevant information towards the understanding of the ferrochelatase chemical mechanism. A histidine (His-341 and His-287 in human and murine ferrochelatase, respectively), a perfectly conserved phenylalanine (Phe-337 and Phe-283 in human and murine ferrochelatase, respectively) and possibly other unidentified π-helix residues coordinate the metal ion in this secondary metal ion-binding site [14]. Kinetic characterization of murine ferrochelatase variants with either the coordinating His-287 or Phe-337 mutated offered an explanation for the ferrochelatase substrate inhibition being only observed at high, and non-physiological, metal ion concentrations [14] [Scheme 1]. The second metal ion-binding site residues appear to enhance ferrochelatase activity at low micromolar metal ion concentrations, but inhibit activity at higher, non-physiological concentrations [14]. In our mechanistic model, one of the conserved π-helix glutamates (*i.e.*, murine ferrochelatatase Glu-289 or *S. cerevisiae* ferrochelatase Glu-314) binds the iron atom in the open conformation of the PPIX-bound enzyme (E_oPP; unwound π-helix) [Scheme 1 [1] and Fig. 1A]. During the transition to the closed conformation, the conserved His-287 (in murine ferrochelatase or His-312 in yeast ferrochelatase) coordinates Fe^{2+} transiently with the conserved Phe-283 (in murine ferrochelatase or Phe-308 in yeast ferrochelatase) enhancing the binding affinity. Upon catalysis, the heme-bound enzyme (EcPr) reverts to the open conformation (E_oHeme), and then heme is