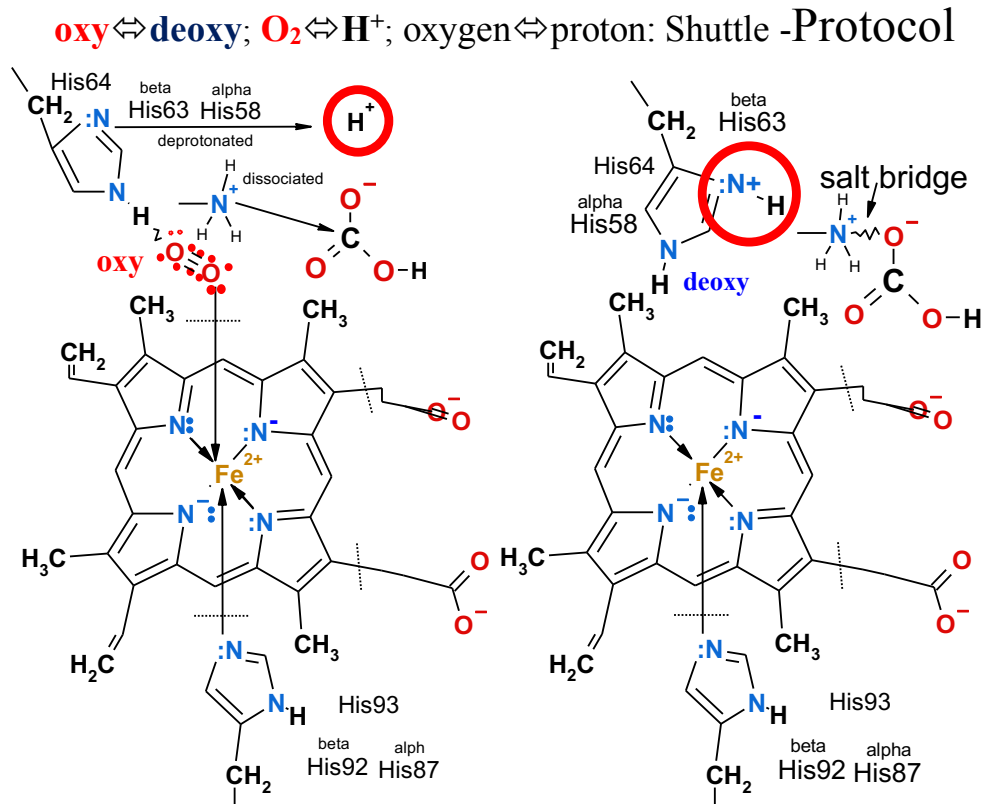


Hem pocket:

29 sphere Amino Acids

1-.....	16-.....
2-.....	17-.....
3-.....	18-.....
4-.....	19-.....
5-.....	20-.....
6-.....	21-.....
7-.....	22-.....
8-.....	23-.....
9-.....	24-.....
10-.....	25-.....
11-.....	26-.....
12-.....	27-.....
13-.....	28-.....
14-.....	29-.....
15-.....	Invisible 29 Amino acid G letter



Myoglobin is small monomeric 154 amino acids (human) molecule, which store oxygen molecules reserve in muscle cells.

Myoglobin is a small mono meric molecule that accumulates reserve of oxygen in the muscle tissues. Hemoglobin in red blood cells (erythrocytes) is concentration sensitive $O_2 \rightleftharpoons H^+$, HCO_3^- shuttle circulating from lungs with accumulated reserve of oxygen in exchange to equivalent Krebs cycle products H^+ , HCO_3^- amount. Circulating hemoglobin of blood plasma is in concentration sensitive equilibrium. Lungs saturates **arterial** blood up to concentration $[O_2] = 6 \cdot 10^{-5} M$ releasing equivalent amount Krebs cycle products H^+ , HCO_3^- breathing out CO_2 and in tissues **venous** blood concentration decreases up to $[O_2] = 0.426 \cdot 10^{-5} M$.

Cytosole concentration $[O_2] = 0.23 \cdot 10^{-5} M$ sensitive maintain myoglobin $O_2 \rightleftharpoons H^+$, HCO_3^- shuttle with oxygen binding fraction $\theta = 87\%$ which replace in Krebs cycle consumed oxygen by equivalent Krebs products H^+ , HCO_3^- amount.

M.A. Lopez and P.A. Kollman, Protein Sci., 2 (1993) 1975

Contents of file LOPEZ.KIN:

Kin.1 - Calpha trace of oxygen-bound myoglobin, with important features shown

Kinimage 1

is a Calpha trace of sperm whale myoglobin-**O₂**, the starting structure for this free energy perturbation study simulating the 'mutation' of oxygen-bound to carbonmonoxy-bound protein.

The **eight 8 alpha-helices** of the globin fold :

(A B C D E F G H) are highlighted progressively by the color spectrum.

29 residues

A 9~radius sphere around the iron(II) atom **heme**, which includes **29 residues** in the **heme** and **oxygen binding pocket**, is the portion used in the calculation; these residues can be specifically highlighted, except **Glycine 29 invisible** amino acid AA. Check **G** and find Glycine order number on the chain using **8 alpha-helices**.

The groups which exhibit the largest free energy changes upon mutation can be distinguished (distal His64(E7), proximal His93(F8), Phe43(CD1), Phe46(CD4), Thr67(E10), Val68(E11), heme).

View2 presents a similar perspective of the binding pocket to that in Figure 1a of the manuscript;

this is best looked at in stereo with 'Calphas' and 'helices' turned off.

Coordinates from Brookhaven Data Bank file: 1MBO.pdb

```
val1-leu2-ser3-glu4-gly5-glu6-trp7-gln8-leu9-val10-leu11-his12-val13-  
trp14-ala15-lys16-val17-glu18-ala19-asp20-val21-ala22-gly23-his24-gly25-  
gln26-asp27-ile28-leu29-ile30-arg31-leu32-phe33-lys34-ser35-his36-pro37-  
glu38-thr39-leu40-glu41-lys42-phe43-asp44-arg45-phe46-lys47-his48-leu49-  
lys50-thr51-glu52-ala53-glu54-met55-lys56-ala57-ser58-glu59-asp60-leu61-  
lys62-lys63-his64-gly65-val66-thr67-val68-leu69-thr70-ala71-leu72-gly73-  
ala74-ile75-leu76-lys77-lys78-lys79-gly80-his81-his82-glu83-ala84-glu85-  
leu86-lys87-pro88-leu89-ala90-gln91-ser92-his93-ala94-thr95-lys96-his97-  
lys98-ile99-pro100-ile101-lys102-tyr103-leu104-glu105-phe106-ile107-  
ser108-glu109-ala110-ile111-ile112-his113-val114-leu115-his116-ser117-  
arg118-his119-pro120-gly121-asp122-phe123-gly124-ala125-asp126-ala127-  
gln128-gly129-ala130-met131-asn132-lys133-ala134-leu135-glu136-leu137-  
phe138-arg139-lys140-asp141-ile142-ala143-ala144-lys145-tyr146-lys147-  
glu148-leu149-gly150-tyr151-gln152-gly153-
```

```
1 10 20 30 40 50 60  
1 VLSEGEWQLV LHVWAKVEAD VAGHGQDILI RLFKSHPETL EKFD RFKHLK TEAEMKASED  
61 70 80 90 100 110 120  
61 LKKHGVTVLT ALGAILK KKG HHEAELKPLA QSHATKHKIP IKYLEFISEA IIHVLHSRHP  
121 130 140 150 160 170 180  
121 GDFGADAQGA MNKALELFRK DIAAKYKELG YQG
```

http://www.ncbi.nlm.nih.gov/protein/1MBO_A

Ferrochelatase (protoheme ferrolyase, E.C. 4.99.1.1) is the terminal enzyme in heme biosynthesis and catalyzes the insertion of ferrous iron into protoporphyrin IX to form protoheme IX (heme). Due to the many critical roles of heme, synthesis of heme is required by the vast majority of organisms. Despite significant investigation of both the microbial and eucaryotic enzyme, details of metal chelation remain unidentified. In this work we present the first structure of the wild-type human enzyme, a lead-inhibited intermediate of the wild-type enzyme with bound metallated porphyrin macrocycle, the product bound form of the enzyme, and a higher resolution model for the substrate-bound form of the E343K variant. These data paint a picture of an enzyme that undergoes significant changes in secondary structure during the catalytic cycle. The role that these structural alterations play in overall catalysis and potential protein-protein interactions with other proteins, as well as the possible molecular basis for these changes, is discussed. The atomic details and structural rearrangements presented herein significantly advance our understanding of the substrate binding mode of ferrochelatase and reveal new conformational changes in a structurally conserved π helix that is predicted to have a central role in product release. Published online 2007 August 23. doi:[10.1016/j.jmb.2007.08.040](https://doi.org/10.1016/j.jmb.2007.08.040)

Heme is a cofactor found in essentially all aerobic organisms and a majority of anaerobes and facultative organisms. Most organisms that possess heme synthesize it themselves¹. With the exception of *Caenorhabditis elegans* and related helminthes², heme acquired via dietary sources is generally degraded to release free iron and is not utilized as a source of cellular heme³. While the traditional textbook roles for heme as a cofactor include hemo- and myoglobins, cytochromes and a handful of enzymes, considerable evidence has emerged that demonstrates a central role for heme in regulation of gene transcription^{4, 5}, as a gas sensor⁶, in the regulation of circadian rhythm⁷, during development⁸ and in RNAi processing⁹. Disordered heme metabolism can have profound developmental and health consequences^{10, 11}.

The terminal step in heme biosynthesis is the insertion of ferrous iron into protoporphyrin IX to make protoheme IX¹². This step, catalyzed by the enzyme ferrochelatase, thus represents the convergence of two cellular pathways: the synthesis of the organic macrocycle protoporphyrin and the supply of ferrous iron¹³. These two pathways are tightly regulated since their substrates are both chemically reactive and potentially damaging to the cell^{14, 15}. Ferrochelatase was the first enzyme activity to be identified with a recognized function as a biological metal chelator^{16, 17}. While there was considerable questioning early on about the physiological need for such an enzyme, it is now clear that chelataes and metal chaperones are key elements of biological systems^{18, 19, 20, 21, 22, 23}.

Ferrochelatases among all organisms are highly conserved at the level of tertiary structure although there is less than ten percent conservation at the level of amino acid sequence¹³. Crystal structures for the enzyme from *Bacillus subtilis*^{24, 25, 26, 27, 28}, *Saccharomyces cerevisiae*²⁹, and human^{30, 31, 32} have been published along with a number of structure/function studies^{33, 34, 35, 36, 37}. Based upon enzymatic studies that demonstrated strong competitive inhibition of ferrochelatase by *N*-alkyl porphyrins³⁸, Lavalee³⁹ proposed that *N*-alkyl porphyrins, because of their distorted macrocycle, may represent a transition state analog for the ferrochelatase reaction^{13, 40}. This proposal has been reviewed, discussed and refined, but has been generally accepted. Putative experimental validation for such a model came from studies with catalytic antibodies produced with *N*-methylmesoporphyrin (*N*-MeMP) as antigen. These catalytic antibodies bind porphyrin and in doing so distort the macrocycle so that divalent cations of the appropriate size are nonspecifically chelated into the porphyrin^{41, 42, 43}. A similar mode of action is envisioned for ferrochelation catalyzed by DNA- and RNAzymes^{44, 45}. It was, however, the series of structural studies on ferrochelatase from *B. subtilis* with bound *N*-MeMP that seemed to solidify this argument^{26, 28}. JMBFe-hem2QD207/JMBFe-hem2QD2.htm

Human ferrochelatase (E.C. 4.99.1.1) is a homodimeric (86 kDa) mitochondrial membrane-associated enzyme that catalyzes the insertion of ferrous iron into protoporphyrin to form heme. We have determined the 2.0 Å structure from the single wavelength iron anomalous scattering signal. The enzyme contains two NO-sensitive and uniquely coordinated [2Fe-2S] clusters. Its membrane association is mediated in part by a 12-residue hydrophobic lip that also forms the entrance to the active site pocket. The positioning of highly conserved residues in the active site in conjunction with previous biochemical studies support a catalytic model that may have significance in explaining the enzymatic defects that lead to the human inherited disease erythropoietic protoporphyria.

Ferrochelatase (protoheme ferro-lyase, E.C. 4.99.1.1) catalyzes the terminal step in the heme biosynthetic pathway, the insertion of ferrous iron into protoporphyrin IX to generate protoheme IX (heme). In most organisms, ferrochelatase is a membrane-associated protein of low abundance¹, but a few bacterial ferrochelatases, including the enzyme from *Bacillus subtilis*² (BFc), the structure of which has been reported³, are soluble proteins. Overall, the sequence homology among known ferrochelatases is <10%. The eukaryotic enzymes possess a 30–50-residue extension at the C-terminus that is not present in most bacterial

ferrochelatases¹. The function of this extension is unknown, but its removal from *Saccharomyces cerevisiae* or animal ferrochelatase results in the loss of enzyme activity⁴.

Although a catalytic model for ferrochelatase has been proposed based on a variety of experimental observations^{5,6,7,8}, details of the enzymatic mechanism as well as the nature of the specific amino acids involved in catalysis still remain largely unknown. Because the human ferrochelatase (HFc) differs considerably from the bacterial ferrochelatases reported to date⁹, it is essential to characterize the human protein to understand the structural basis of the inherited disease erythropoietic protoporphyria (EPP)¹⁰.

Overall structure

The structure of the mature, processed form of HFc has been determined at 2.0 Å resolution from the iron anomalous scattering signal using data collected on a rotating anode. The crystallized HFc contains a R115L mutation that has no measurable effect on kinetic parameters. The human enzyme exists as a homodimer with a molecular mass of ~86 kDa. Each subunit ([Fig. 1a](#)) contains residues 65–423 (GenBank D00726) and one [2Fe-2S] cluster, while residues 1–62 represent a mitochondrial targeting sequence that is removed during proteolytic processing. Three cholate detergent molecules were found in the active site pocket and there are 580 water molecules associated with the dimer. The monomer has an elongated shape with dimensions of ~65 ×45 ×40 Å³ and contains 47.7% helix and 13.5% β-sheet. It is folded into two similar domains, each with a four-stranded parallel β-sheet flanked by an α-helix in a β-α-β motif that is reminiscent of the fold found in the periplasmic binding proteins. The first domain (residues 65–247) is composed of helices α1–α7 and sheets β1–β4. Helices α2 and α3 together with a loop (residues 95–104) form a section of the active site pocket ([Fig. 1b](#)). The second domain (residues 248–423) consists of helices α8–α17 and sheets β5–β8. Residues 302–311 of this domain comprise a short loop that forms another section of the active site pocket. The C-terminal extension (residues 390–423) forms a helix-turn-helix structure that serves as part of the binding motif for the [2Fe-2S] cluster. The topological similarity ([Fig. 1c](#)) between the domains suggests that they have arisen from a gene duplication event. However, significant differences exist between the two domains, including an N-terminal section (residues 80–130) that forms part of the active site pocket, and a C-terminal extension (residues 390–423) that is involved in coordination of the [2Fe-2S] cluster and in stabilization of the homodimer.