Published in final edited form as:

Arch Biochem Biophys. 2014 February 15; 0: 112–118. doi:10.1016/j.abb.2013.10.004.

MauG, a diheme enzyme that catalyzes tryptophan tryptophylquinone biosynthesis by remote catalysis

Sooim Shin and Victor L. Davidson*

Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL 32827

Abstract

MauG contains two *c*-type hemes with atypical physical and catalytic properties. While most *c*-type cytochromes function simply as electron transfer mediators, MauG catalyzes the completion of tryptophan tryptophylquinone (TTQ) biosynthesis within a precursor protein of methylamine dehydrogenase. This posttranslational modification is a six-electron oxidation that requires crosslinking of two Trp residues, oxygenation of a Trp residue and oxidation of the resulting quinol to TTQ. These reactions proceed via a *bis*-Fe^{IV} state in which one heme is present as Fe^{IV}=O and the other is Fe^{IV} with axial heme ligands provided by His and Tyr side chains. Catalysis does not involve direct contact between the protein substrate and either heme of MauG. Instead it is accomplished by remote catalysis using a hole hopping mechanism of electron transfer in which Trp residues of MauG are reversibly oxidized. In this process, long range electron transfer is coupled to the radical mediated chemical reactions that are required for TTQ biosynthesis.

Keywords

Electron transfer; posttranslational modification; protein radical; peroxidase; oxygenase; high-valence iron

Introduction

Heme cofactors are used in biological systems for a wide range of biological functions including oxygen transport and storage via hemoglobin [1] and myoglobin [2], electron transfer [3], oxidation and oxygenation of substrates [4], peroxide reduction [5, 6] and ligand sensing [7]. The role of heme in oxygenases is incorporation of oxygen atoms from dioxygen into substrate. Examples of these enzymes, which typically contain *b*-type hemes include cytochrome P450s, secondary amine monooxygenase, prostaglandin H synthase, and tryptophan 2,3-dioxygenase. Cytochrome P450s are able to catalyze a wide variety of transformations [8]. This class of enzymes primarily catalyzes mixed-function oxidation reactions during which they break the dioxygen bond and catalyze the insertion of one oxygen atom into a substrate and reduce the second oxygen to a water molecule utilizing two electrons that are typically provided by NADH(P)H via a reductase protein [4]. Most

© 2013 Elsevier Inc. All rights reserved.

*Address correspondence to: Victor L. Davidson, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 6900 Lake Nona Blvd., Orlando, FL 32827 Tel: 407-266-7111. Fax: 407-266-7002. victor.davidson@ucf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

catalases and peroxidases also contain *b*-type hemes as cofactors. Catalases function to catalyze the decomposition of H_2O_2 to water and oxygen [9]. Heme-dependent peroxidases catalyze the two-electron oxidation of substrates by H_2O_2 and in doing so detoxify the peroxide [5]. The following are examples of these enzymes and their roles. Yeast cytochrome *c* peroxidase is a mitochondrial soluble protein, exerting a protective function against toxic peroxides. Ascorbate peroxidase removes H_2O_2 in chloroplasts and the cytosol of higher plants. Bacterial catalase-peroxidase features both peroxidase and catalase activities. Lignin peroxidases are involved in the oxidative degradation of lignin. Human myeloperoxidase plays a major role in the oxygen-dependent microbicidal system of neutrophils.

Bacterial diheme cytochrome *c* peroxidases (DCCPs) are a family of peroxidases which are distinct from the other heme-dependent peroxidases in that they contain two *c*-type hemes; one which functions as an electron transfer mediator and the other which plays a role in the peroxidatic reaction [10]. The *c*-type cytochromes are a distinct class of heme-containing proteins that are distinguished by the presence of a covalent thioether linkage between the porphyrin vinyl groups of the hemes and cysteine residues from the protein. These cysteines reside in a CXXCH *c*-type heme binding motif in the protein sequence [11].

MauG is an unusual *c*-type diheme protein [12]. Most *c*-type cytochromes function solely as mediators of electron transfer whereas MauG has catalytic activity. MauG exhibits several physical and functional properties which distinguish it from other *c*-type heme proteins. It does exhibit 30% similarity to DCCP, but MauG is not a simple peroxidase [13]. It catalyzes the posttranslational modification of another protein, methylamine dehydrogenase (MADH) [14], to generate the protein-derived cofactor, tryptophan tryptophyquinone (TTQ) [15]. The overall reaction that is catalyzed by MauG is a six-electron oxidation of the substrate protein resulting in crosslinking of two Trp residues, oxygenation of a Trp residue and oxidation of the resulting quinol to TTQ [16, 17]. In this respect MauG behaves more like a *b*-type heme enzyme than a *c*-type heme enzyme. The mechanism of catalysis by MauG is also unusual in that it requires formation of an unprecedented high-valence diheme *bis*- Fe^{IV} species [18] which does not make direct contact with the substrate [19] and requires long range electron transfer from substrate to heme to generate radical intermediates in the biosynthesis of TTQ [20, 21].

Physiological role of MauG

The gene which encodes MauG is located in the methylamine utilization (*mau*) gene cluster of several gram negative bacteria. The *mau* cluster contains the two structural genes for MADH as well as accessory proteins that are required for MADH biogenesis. The biosynthesis of MADH has been studied in *Paracoccus denitrificans* where the *mau* gene cluster contains 11 genes with the order of *mauRFBEDACJGMN* [22–24]. MADH is a heterotetramer consisting of two α subunits and two β subunits [25] which are encoded by *mauB* and *mauA*, respectively. MADH catalyzes the oxidative deamination of methylamine to formaldehyde and ammonia, a reaction which allows the host bacterium to use methylamine as a sole source of carbon, nitrogen and energy [26]. MADH donates the electrons which it extracts from methylamine to the *mauC* gene product, a type 1 copper protein named amicyanin [27], which in turn transfers electrons to cytochrome *c*-551i [28]. This natural ternary protein system has been used as a model with which to study interprotein electron transfer reactions [29–32]. The catalytic cofactor of MADH is TTQ [15]. It is not an exogenous cofactor but is instead derived from posttranslational modifications of the β subunits of MADH as evidenced from the crystal structure of MADH [25].

The posttranslational modifications which are catalyzed by MauG are a six-electron oxidation that requires formation of a covalent crosslink between residues β Trp57 and β Trp108, insertion of an oxygen atom into a mono-hydroxylated residue β Trp57, and oxidation of the resultant quinol into a quinone (Figure 1) [17]. In order to study MADH and TTQ biogenesis a recombinant expression system for MADH was established in which the genes *mauFBEDACJG* were introduced into *Rhodobacter sphaeroides* [33]. The role for MauG in TTQ biosynthesis was identified when it was shown that in the absence of *mauG* an MADH precursor protein (preMADH) was isolated from the recombinant expression system [16]. The preMADH lacked enzymatic activity and the visible absorption spectrum characteristic of the TTQ. Analysis of preMADH by mass spectrometry and isotope labeling studies revealed that preMADH contains a preTTQ intermediate in which β Trp57 is mono-hydroxylated at the C7 position and the covalent cross-link has not formed between two tryptophan residues [16, 34]. The subsequent crystal structure of the MauG-preMADH complex confirmed that this was indeed the structure of the preTTQ site [19]. Thus, while the mechanism by which the first hydroxylation of β Trp57 is not known, these studies demonstrated that in the absence of MauG, TTQ biosynthesis can proceed no further. As discussed below it has been demonstrated that MauG is able to catalyze the conversion of preTTQ on preMADH to TTQ when incubated with preMADH *in vitro*.

Properties of MauG

MauG has never been isolated from any native source including *P. denitrificans* which produces large amounts of MADH when grown with methylamine as a carbon source [26]. It was possible to express and isolate MauG from a recombinant homologous expression system using *P. denitrificans* [12]. The MauG protein possesses two covalently attached *c*-type hemes as predicted from the sequence which contains two CXXCH *c*-type heme binding motifs. The absorbance spectrum of MauG is typical of *c*-type cytochromes. The EPR spectrum of diferric MauG indicates that MauG contains one high-spin heme and one low-spin heme which exhibit *g* values that are atypical of *c*-type hemes. The high-spin heme signal is very similar to that of the heme oxygenase-heme complex and myoglobin. The low-spin heme signal is more similar to that of ligand complexes of cytochrome P450. Also, in contrast to typical *c*-type cytochromes, the fully reduced MauG reoxidizes in air and can bind CO [12].

MauG exhibits ~30% sequence similarity to DCCPs [10, 12] which also contain two *c*-type hemes, but the catalytic mechanisms of MauG differ greatly from those of DCCPs [13]. Comparison of the oxidation-reduction midpoint potential (E_m) values of different *c*-type hemes in cytochromes shows that the heme E_m values span a range over 1V, from +640 mV in cytochrome *c*₅₅₂ to -400 mV in cytochrome *c*₃ [35, 36]. In DCCPs, the E_m values of the two hemes are separated by more than 600 mV [10] which allows for a stable mixed-valence state which is an important intermediate in the catalytic cycle. In contrast, the two *c*-type hemes of MauG act in concert as a two-electron redox cofactor rather than as two independent hemes [37]. They exhibit redox cooperative behavior during conversion between the diferric and diferrous redox states. MauG exhibits two distinct E_m values of -159 mV and -244 mV, yet the two hemes are reduced and oxidized simultaneously [37]. Thus, these E_m values correspond to the sequential addition or removal of the first and second electrons to or from the diheme system. Furthermore, in contrast to DCCPs, MauG exhibits only weak peroxidase activity with a variety of cytochromes *c* and generic electron donors [12].

Although it has not been possible to crystallize MauG alone, the X-ray crystal structure of a complex of MauG and preMADH from *P. denitrificans* was determined at 2.1 Å resolution [19] (Figure 2). Complexes of MauG with the mature quinol and quinone TTQ forms of

MADH have also been structurally characterized [38]. Two molecules of the 42.3 kDa MauG interact with one molecule of preMADH in the MauG-preMADH complex. The latter is an $\alpha_2\beta_2$ heterotetramer in which each β subunit contains a pre-TTQ site. The structure of preMADH is exactly the same as that of mature MADH except for the TTQ site. One heme of MauG is five-coordinate and solvent accessible with His53 as an axial ligand (Figure 3). The other heme is six-coordinate with His205 and Tyr294 as axial ligand. This is the first example of a *c*-type heme with an axial Tyr ligand and the first example of any heme with His-Tyr axial ligation. The two heme irons of MauG are separated by 21 Å. Trp93 resides between two hemes, and a Ca^{2+} -binding site in MauG is located between the two hemes, and close to Trp93 (Figure 3). The structure of the diheme site of MauG is very similar to that of DCCPs. The positions of the two hemes, intervening Trp and Ca^{2+} are essentially identical [19]. The only structural feature that differs is the presence of the Tyr axial ligand in MauG whereas this ligand is a second His in DCCPs. Another striking feature of this structure is that there is no direct contact of the pre-TTQ substrate with either heme of MauG. The distance from the preTTQ site which is modified is approximately 40 Å from the five-coordinate heme iron and 19 Å from the six-coordinate heme iron (Figure 3).

Activation of MauG for oxidative catalysis

High-valence iron species in both non-heme iron and heme proteins are powerful oxidizing species in chemical and biological catalysis [39, 40]. In nature these are typically formed by reaction of O_2 with Fe^{II} or reaction of H_2O_2 with Fe^{III} . The process typically proceeds via a ferric hydroperoxy intermediate [4, 41], which may then lose water to yield a ferryl $\text{Fe}^{\text{IV}}=\text{O}$ species with a cation radical present on the porphyrin ring or axial amino acid ligand (known as Compound I). An alternative high-valence species is Compound ES, in which the $\text{Fe}^{\text{IV}}=\text{O}$ heme is coupled to an amino acid-based cation radical in close proximity to the heme. The catalytic high-valence form of MauG is neither Compound I nor Compound ES. Instead, MauG utilizes a *bis*- Fe^{IV} intermediate with one heme as $\text{Fe}^{\text{IV}}=\text{O}$ and the other as Fe^{IV} with axial His and Tyr ligands retained (Figure 4). This *bis*- Fe^{IV} species has been characterized by absorbance, EPR and Mössbauer spectroscopy [18, 42], computational studies [43, 44], and X-ray absorption studies [43]. The rate of formation of the *bis*- Fe^{IV} intermediate when MauG reacts with H_2O_2 occurs within the dead time of rapid mixing ($> 300 \text{ s}^{-1}$) [42]. In contrast to most high-valence iron species, the *bis*- Fe^{IV} species is remarkably stable as the spontaneous decay back to the diferric state requires several minutes [42]. This stability of this high-valence species is at least in part due to an unusual charge-resonance-transition phenomenon which occurs without direct contact between the two hemes. Instead, this phenomenon is a consequence of ultrafast and reversible electron transfer between hemes which occurs via hole hopping through the intervening Trp93 residue [45].

Kinetic mechanism of MauG dependent TTQ biosynthesis

The crystal structure of the MauG-preMADH complex requires that TTQ biosynthesis occurs by an unprecedented mechanism. Given the orientations of the proteins in the structure it is, not possible for the ferryl heme to interact directly with the substrate as is seen in the mechanism of cytochrome P450 enzymes. The relevance of the crystal structure was validated by the demonstration that addition of H_2O_2 to MauG-preMADH crystals resulted in synthesis of the mature TTQ cofactor *in crystallo* [19]. This means that the reaction occurs without any significant conformational change, indicating that electrons must be extracted from preMADH over a long distance to reduce high-valence oxidant of MauG. Furthermore, this result indicated that it is an oxygen atom from solvent that is inserted into βTrp57 and not the heme-bound oxygen. Subsequently, the exact order of three two-electron oxidation steps required for TTQ biosynthesis from preMADH (Figure 1) was

determined and reaction intermediates were characterized in solution and *in crystallo* [17]. The initial two-electron oxidation results in formation of the crosslink between the monohydroxylated β Trp57 and β Trp108. Freeze-quench techniques were used to trap an intermediate in this first step which was shown by high-frequency and high-field EPR analysis to be a tryptophan di-radical species. The interpretation of this result is that one radical is based on the monohydroxylated preMADH β Trp57 and the other on β Trp108, and that this is the intermediate that precedes crosslink formation [17].

A steady-state assay for the overall reaction of MauG-dependent TTQ biosynthesis from preMADH was developed in which TTQ formation was monitored by the increase in absorbance centered at 440 nm which is characteristic of TTQ in MADH [46]. This can be distinguished from the quinol form of MADH which absorbs at 330 nm, the semiquinone form which absorbs at 410 nm and preMADH has no visible absorbance [47]. When the steady-state reaction is initiated by addition of excess H_2O_2 it yields values of $k_{\text{cat}}=0.16 \text{ s}^{-1}$ and $K_{\text{m}}=1.7 \text{ }\mu\text{M}$ for preMADH [48] (Figure 5).

It has also been possible to study individual reaction steps in the process of MauG-dependent TTQ biosynthesis using transient kinetic techniques. The initial two-electron oxidation step was examined by mixing preMADH with *bis*- Fe^{IV} MauG, which had been formed by stoichiometric H_2O_2 [42]. The reaction (Figure 5) exhibits saturation behavior and a limiting first-order rate constant for the reaction of $k = 0.8 \text{ s}^{-1}$ and $K_{\text{d}} = 1.5 \text{ }\mu\text{M}$. Identical results were obtained if the reaction was instead initiated by mixing the preformed complex of MauG and preMADH with H_2O_2 . Thus, MauG obeys a random sequential kinetic mechanism in which the order of reactants does not matter. This distinguishes MauG from other heme-containing oxygenases which typically require substrate binding first to prime the enzyme for oxygen binding to the heme [49]. The reaction of *bis*- Fe^{IV} MauG with quinol MADH (Figure 5), the final two-electron oxidation step in TTQ biosynthesis, was also characterized [50]. Quinol MADH was generated by reduction by dithionite and mixed with *bis*- Fe^{IV} MauG. This reaction also exhibits saturation behavior and a limiting first-order rate constant for the reaction of $k = 20 \text{ s}^{-1}$ and $K_{\text{d}} = 11.2 \text{ }\mu\text{M}$ for quinol MADH. These results indicate that the rate of the initial two-electron oxidation (0.8 s^{-1}) is in the same range as that of the rate-limiting step in the overall six-electron oxidation process (0.2 s^{-1}). It is also apparent that the affinity of *bis*- Fe^{IV} MauG is much weaker for quinol MADH ($K_{\text{d}} = 11.2 \text{ }\mu\text{M}$) than for preMADH ($K_{\text{d}} = 1.5 \text{ }\mu\text{M}$). These data are consistent with the observation that MauG and preMADH co-elute during size-exclusion chromatography while MauG and MADH elute separately [51].

The role of the protein in stabilizing the *bis*- Fe^{IV} state of MauG

The His-Tyr ligation of the six-coordinate heme in the *bis*- Fe^{IV} state of MauG is the first example of a protein-bound heme that can stabilize an Fe^{IV} without an exogenous ligand. X-ray absorption spectroscopy and density function theory calculations have confirmed that this heme retains the tyrosinate ligand in the Fe^{II} , Fe^{III} and Fe^{IV} states [43]. Quantum chemical studies have also determined that the unusual Mossbauer parameters exhibited by the Fe^{IV} state of this heme are a consequence of the Tyr ligation [44]. To probe the significance of Tyr294, it was replaced with His by site-directed mutagenesis [19]. The crystal structure of the Y294H MauG-preMADH complex showed that this heme now has His-His axial ligation. Y294H MauG was able to interact with preMADH and function in a non-biosynthetic interprotein ET reaction between diferrous MauG and oxidized MADH [50], but it was unable to catalyze the TTQ biosynthesis reactions that require the *bis*- Fe^{IV} state. Spectroscopic data revealed that Y294H MauG could not stabilize the *bis*- Fe^{IV} state, but instead formed a compound I-like species which could not catalyze TTQ biosynthesis. In a parallel study, replacement of this Tyr with Lys generated a MauG variant in which there

was removal rather than replacement of the Y294 ligand, yielding a five-coordinate heme with only a single His ligand [52]. Y294K MauG also did not stabilize the *bis*-Fe^{IV} redox state, but instead formed a compound I-like species localized on the oxygen-binding heme. These data showed that Tyr is likely unique among amino acids in being able to stabilize a ferryl heme iron without an external ligand, and that the *bis*-Fe^{IV} state specifically, not just any high-valence Fe state, is required for TTQ biosynthesis. The explanation for these results was that the *bis*-Fe^{IV} state allows delocalization of the oxidizing power of the Fe^V equivalent to the heme iron which is nearer to preMADH (~19 Å away), whereas generation of the alternative compound I-like state in these mutants confines the oxidizing power to the more distant heme Fe (~40 Å away). This latter distance is too great to support the long range electron transfer required for MauG-dependent TTQ biosynthesis.

A novel form of enzyme catalysis that requires hole hopping-mediated long range ET

The distance between the modified residues of preMADH and the nearest heme iron of MauG is 19 Å, which is still a relatively long distance for biological electron transfer [53]. Electron transfer through proteins is typically thought to occur via direct electron tunneling which occurs in a single-step reaction from electron donor to acceptor [54, 55]. During electron tunneling, the amino acid residues that mediate electron transfer serve as a conductive matrix and do not undergo a change in redox state. An alternative mechanism for long range electron transfer is multi-step hopping [56]. During hopping, certain intervening amino acid residues are reversibly oxidized and reduced and serve as intermediate points for shorter electron tunneling segments, called hops. A multi-step hole hopping mechanism, in which the electron acceptor oxidizes an amino acid which in turn oxidizes the electron donor, will enhance the efficiency of electron transfer through proteins. Documentation of hopping through proteins has been difficult to achieve and this mechanism has been postulated for only a few systems [57]. Only a few amino acids (*e.g.*, Trp, Tyr, Cys) are able to undergo such reversible oxidation under biological conditions. Trp199 of MauG, which resides at the MauG-preMADH interface, is positioned midway between the residues on preMADH that are modified and the nearest heme. To test the possible role of Trp199 in mediating hopping, it was replaced with Phe [20], a residue that cannot be oxidized under biological conditions. The W199F mutation did not affect the spectroscopic and redox properties of MauG, its ability to stabilize the *bis*-Fe^{IV} state, or the MauG-preMADH structure. W199F MauG was shown to function in a non-biosynthetic ET reaction between MADH and MauG which does not require the *bis*-Fe^{IV} state. However, W199F MauG was unable to catalyze TTQ biosynthesis. It was postulated that Trp199 mediates multistep hopping from preMADH to *bis*-Fe^{IV} MauG during the long range electron transfer that is required for TTQ biosynthesis (Figure 6). Subsequent kinetic and thermodynamic analysis of the electron transfer reaction from preMADH to *bis*-Fe^{IV} MauG indicated that the experimentally-determined values of electronic coupling and electron transfer distance were consistent with describing a rate-determining hopping segment. In contrast, analysis of non-biosynthetic electron transfer reaction from diferrous MauG to oxidized MADH yielded data that would be expected for the longer single-step tunneling mechanism that does not involve hopping [21]. Hopping via Trp93, which resides between the two hemes of MauG, has also been proposed to account for the very rapid electron transfer between hemes in the *bis*-Fe^{IV} state which leads to charge-resonance stabilization of that species [45]. The sum of the data support a novel mechanism of remote catalysis in which a Fe^{IV}/Fe^{IV}=O species does not make direct contact with the substrate but instead uses long range electron transfer to oxidize Trp199, which in turn oxidizes preMADH, generating reactive radical intermediates on preMADH that form the covalent crosslink between substrate Trp side-chains and acquire the oxygen from solvent. The overall reaction has been tracked *in crystallo* [17] and

it was shown that the product of the initial two-electron oxidation reaction is the crosslinked monohydroxylated species shown in Figure 1. An early reaction intermediate was also trapped and shown by high-frequency, high-field EPR analysis to possess two distinct Trp-based radicals which reside on β Trp108 and β Trp57-OH [17]. This intermediate likely loses two protons and combines to form the crosslink.

The roles of residues in the distal pocket of the high-spin heme of MauG in controlling the reactivity of Fe^{IV}

Crystallographic studies demonstrated that Pro107 (Figure 7), which resides in the distal pocket of the high-spin heme of MauG, changes conformation upon binding of either CO or NO [58]. EPR studies also showed that NO exclusively binds to only that same heme [59]. Pro107 was converted to Cys, Val and Ser by site-directed mutagenesis [48]. The structures of each of these MauG variants in complex with preMADH were determined, as were their physical and catalytic properties and susceptibility to oxidative damage. The P107S mutation caused a structural change that resulted in the 5-coordinate high-spin heme being converted to an inactive 6-coordinate heme with a distal axial ligand provided by Glu113. P107C MauG was inactive and the crystal structure revealed that Cys107 had been oxidized to a sulfinic acid. It had been shown previously that native MauG undergoes inactivation when cycling between the *bis*- Fe^{IV} and diferric state in the absence of the preMADH substrate [60]. P107V MauG exhibited spectroscopic and catalytic properties that were similar to wild-type MauG, but was more susceptible to oxidative damage than native MauG, even in the presence of substrate. Thus, Pro107 is critical in maintaining the proper structure of the distal heme pocket of the high-spin heme of MauG to allow oxygen to bind, and direct the reactivity of the heme-activated oxygen during catalysis, thus minimizing the oxidation of other residues of MauG.

Two other residues of interest in the distal pocket of the high spin heme are Glu113 and Gln103. On the basis of the crystal structure of the MauG-preMADH complex with NO bound to the high-spin heme [58], it was suggested that Glu113 may be important in promoting cleavage of the O–O bond through protonation of oxygen intermediates bound to heme. Conversion of Glu113 to Gln by site-directed mutagenesis was shown to profoundly affect the redox properties of MauG [61]. MauG could be reduced by dithionite only to a mixed-valence state but not to the diferrous state, and addition of H_2O_2 generated a high-valence state which formed much more slowly than in *bis*- $\text{Fe}(\text{IV})$ MauG and which was less stable. It was concluded that the E113Q mutation disrupted the redox cooperativity between hemes that allows rapid formation of the diferrous state, and altered the distribution of high-valence species that participate in charge-resonance stabilization of the *bis*- Fe^{IV} redox state. E113Q. The structure of the distal distal pocket of the high-spin heme also suggests that Gln103 may be important in stabilizing the $\text{Fe}^{\text{IV}}=\text{O}$ by hydrogen bonding to the oxo. This hypothesis is supported by the results of a quantum chemical investigation that concluded that such a hydrogen bonding interaction accounted for Mössbauer spectroscopic parameters of the $\text{Fe}^{\text{IV}}=\text{O}$ heme in *bis*- $\text{Fe}(\text{IV})$ MauG [44].

A role for calcium in MauG

The structure of MauG reveals the presence of a Ca^{2+} which is positioned in the vicinity of the two hemes and which is connected to each heme via H-bonding networks that include bound waters. This structural feature is shared with the DCCPs. The tightly bound Ca^{2+} of MauG does not dissociate under physiological conditions but could be removed by chelators. The Ca^{2+} -depleted MauG showed no TTQ biosynthesis activity and exhibited altered absorbance, EPR and resonance Raman spectral properties. Re-addition of Ca^{2+} fully restored activity and the native spectral properties [62]. It was not possible to crystallize the

Ca²⁺-depleted MauG, but spectroscopic studies elucidated the nature of the Ca²⁺-dependent changes. X-band and high-field EPR spectroscopy, and Mössbauer spectroscopy were used to characterize the structure and unusual magnetic properties of the hemes in Ca²⁺-depleted MauG [63]. The results revealed reversible Ca²⁺-dependent changes in the nature and orientation of the axial ligands of the two hemes, which profoundly influences the axial ligation geometry and magnetic properties of both hemes. These findings provide insight into the correlation of the enzyme activity with the orientation of axial heme ligands, and describe a role for Ca²⁺ in maintaining this structural orientation that is required for activity.

Perspective

While hemes are a common cofactor in biology, the two hemes of MauG support catalysis by a mechanism that is thus far distinct among heme proteins. Despite their physical separation, the hemes function as a single two-electron cofactor rather than as independent hemes. More broadly, it is probably appropriate to also consider residue Trp93 and the bound Ca²⁺ as components of the diheme cofactor as well. Given that hole hopping via a distant Trp199 residue is also required for catalysis, the line between cofactor and the rest of the protein becomes blurred. Rarely does one see such a large portion of the host protein interacting in concert with the cofactor during catalysis.

Acknowledgments

Research from the author's laboratory was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award numbers R37GM41574 (VLD).

References

1. Connie CW, Hsia MD. *N Engl J Med*. 1998; 338:239–248. [PubMed: 9435331]
2. Ordway GA, Garry DJ. *J Exp Biol*. 2004; 207:3441–3446. [PubMed: 15339940]
3. Trumpower BL, Gennis RB. *Annu Rev Biochem*. 1994; 63:675–716. [PubMed: 7979252]
4. Sono M, Roach MP, Coulter ED, Dawson JH. *Chem Rev*. 1996; 96:2841–2888. [PubMed: 11848843]
5. Welinder KG. *Curr Opin Struct Biol*. 1992; 2:388–393.
6. Davies MJ, Hawkins CL, Pattison DI, Rees MD. *Antioxid Redox Signal*. 2008; 10:1199–1234. [PubMed: 18331199]
7. Gilles-Gonzalez MA, Gonzalez G. *J Inorg Biochem*. 2005; 99:1–22. [PubMed: 15598487]
8. Guengerich FP, Munro AW. *J Biol Chem*. 2013; 288:17065–17073. [PubMed: 23632016]
9. Nicholls P, Fita I, Loewen PC. *Adv Inorg Chem*. 2001; 51:51–106.
10. Pettigrew GW, Echalié A, Pauleta SR. *J Inorg Biochem*. 2006; 100:551–567. [PubMed: 16434100]
11. Barker PD, Ferguson SJ. *Structure*. 1999; 7:R281–290. [PubMed: 10647174]
12. Wang Y, Graichen ME, Liu A, Pearson AR, Wilmot CM, Davidson VL. *Biochemistry*. 2003; 42:7318–7325. [PubMed: 12809487]
13. Wilmot CM, Davidson VL. *Curr Opin Chem Biol*. 2009; 13:469–474. [PubMed: 19648051]
14. Davidson VL. *Adv Protein Chem*. 2001; 58:95–140. [PubMed: 11665494]
15. McIntire WS, Wemmer DE, Chistoserdov A, Lidstrom ME. *Science*. 1991; 252:817–824. [PubMed: 2028257]
16. Pearson AR, De La Mora-Rey T, Graichen ME, Wang Y, Jones LH, Marimanikkupam S, Agger SA, Grimsrud PA, Davidson VL, Wilmot CM. *Biochemistry*. 2004; 43:5494–5502. [PubMed: 15122915]
17. Yukl ET, Liu F, Krzystek J, Shin S, Jensen LM, Davidson VL, Wilmot CM, Liu A. *Proc Natl Acad Sci U S A*. 2013; 110:4569–4573. [PubMed: 23487750]

18. Li X, Fu R, Lee S, Krebs C, Davidson VL, Liu A. *Proc Natl Acad Sci USA*. 2008; 105:8597–8600. [PubMed: 18562294]
19. Abu Tarboush N, Jensen LM, Feng M, Tachikawa H, Wilmot CM, Davidson VL. *Biochemistry*. 2010; 49:9783–9791. [PubMed: 20929212]
20. Abu Tarboush N, Jensen LMR, Yukl ET, Geng J, Liu A, Wilmot CM, Davidson VL. *Proc Natl Acad Sci U S A*. 2011; 108:16956–16961. [PubMed: 21969534]
21. Choi M, Shin S, Davidson VL. *Biochemistry*. 2012; 51:6942–6949. [PubMed: 22897160]
22. van der Palen CJ, Slotboom DJ, Jongejan L, Reijnders WN, Harms N, Duine JA, van Spanning RJ. *Eur J Biochem*. 1995; 230:860–871. [PubMed: 7601147]
23. Chistoserdov AY, Boyd J, Mathews FS, Lidstrom ME. *Biochem Biophys Res Commun*. 1992; 184:1181–1189. [PubMed: 1590782]
24. van der Palen CJ, Reijnders WN, de Vries S, Duine JA, van Spanning RJ. *Antonie van Leeuwenhoek*. 1997; 72:219–228. [PubMed: 9403107]
25. Chen LY, Doi N, Durley RCE, Chistoserdov AY, Lidstrom ME, Davidson VL, Mathews FS. *J Mol Biol*. 1998; 276:131–149. [PubMed: 9514722]
26. Husain M, Davidson VL. *J Bacteriol*. 1987; 169:1712–1717. [PubMed: 3558322]
27. Husain M, Davidson VL. *J Biol Chem*. 1985; 260:14626–14629. [PubMed: 2997215]
28. Husain M, Davidson VL. *J Biol Chem*. 1986; 261:8577–8580. [PubMed: 3013855]
29. Davidson VL. *Acc Chem Res*. 2008; 41:730–738. [PubMed: 18442271]
30. Davidson VL, Jones LH. *Anal Chim Acta*. 1991; 249:235–240.
31. Chen L, Durley RC, Mathews FS, Davidson VL. *Science*. 1994; 264:86–90. [PubMed: 8140419]
32. Davidson VL, Jones LH. *Biochemistry*. 1996; 35:8120–8125. [PubMed: 8679563]
33. Graichen ME, Jones LH, Sharma BV, van Spanning RJ, Hosler JP, Davidson VL. *J Bacteriol*. 1999; 181:4216–4222. [PubMed: 10400578]
34. Pearson AR, Marimanikkuppam S, Li X, Davidson VL, Wilmot CM. *J Am Chem Soc*. 2006; 128:12416–12417. [PubMed: 16984182]
35. Paoli M, Marles-Wright J, Smith A. *DNA & Cell Biology*. 2002; 21:271–280. [PubMed: 12042067]
36. Reedy CJ, Elvekrog MM, Gibney BR. *Nucleic Acids Res*. 2008; 36:D307–313. [PubMed: 17933771]
37. Li X, Feng M, Wang Y, Tachikawa H, Davidson VL. *Biochemistry*. 2006; 45:821–828. [PubMed: 16411758]
38. Yukl ET, Jensen LM, Davidson VL, Wilmot CM. *Acta Crystallogr Sect F Struct Biol Cryst Commun*. 2013; 69:738–743.
39. Gumiero A, Metcalfe CL, Pearson AR, Raven EL, Moody PC. *J Biol Chem*. 2011; 286:1260–1268. [PubMed: 21062738]
40. Groves JT. *J Inorg Biochem*. 2006; 100:434–447. [PubMed: 16516297]
41. Kovaleva EG, Neibergall MB, Chakrabarty S, Lipscomb JD. *Acc Chem Res*. 2007; 40:475–483. [PubMed: 17567087]
42. Lee S, Shin S, Li X, Davidson VL. *Biochemistry*. 2009; 48:2442–2447. [PubMed: 19196017]
43. Jensen LM, Meharena Y, Davidson VL, Poulos TL, Hedman B, Wilmot CM, Sarangi R. *J Biol Inorg Chem*. 2012; 17:1241–1255. [PubMed: 23053529]
44. Ling Y, Davidson VL, Zhang Y. *J Phys Chem Lett*. 2010; 1:2936–2939. [PubMed: 20953337]
45. Geng J, Dornevil K, Davidson VL, Liu A. *Proc Natl Acad Sci U S A*. 2013; 110:9639–9644. [PubMed: 23720312]
46. Li X, Jones LH, Pearson AR, Wilmot CM, Davidson VL. *Biochemistry*. 2006; 45:13276–13283. [PubMed: 17073448]
47. Davidson VL, Brooks HB, Graichen ME, Jones LH, Hyun YL. *Methods Enzymol*. 1995; 258:176–190. [PubMed: 8524149]
48. Feng M, Jensen LM, Yukl ET, Wei X, Liu A, Wilmot CM, Davidson VL. *Biochemistry*. 2012; 51:1598–1606. [PubMed: 22299652]
49. Meunier B, de Visser SP, Shaik S. *Chem Rev*. 2004; 104:3947–3980. [PubMed: 15352783]

50. Shin S, Abu Tarboush N, Davidson VL. *Biochemistry*. 2010; 49:5810–5816. [PubMed: 20540536]
51. Li X, Fu R, Liu A, Davidson VL. *Biochemistry*. 2008; 47:2908–2912. [PubMed: 18220357]
52. Abu Tarboush N, Shin S, Geng J, Liu A, Davidson VL. *FEBS Lett*. 2012; 586:4339–4343. [PubMed: 23127557]
53. Moser CC, Keske JM, Warncke K, Farid RS, Dutton PL. *Nature*. 1992; 355:796–802. [PubMed: 1311417]
54. Gray HB, Winkler JR. *Q Rev Biophys*. 2003; 36:341–372. [PubMed: 15029828]
55. Marcus RA, Sutin N. *Biochim Biophys Acta*. 1985; 811:265–322.
56. Giese B, Graber M, Cordes M. *Curr Opin Chem Biol*. 2008; 12:755–759. [PubMed: 18804174]
57. Warren JJ, Ener ME, Vlcek A Jr, Winkler JR, Gray HB. *Coord Chem Rev*. 2012; 256:2478–2487. [PubMed: 23420049]
58. Yukl ET, Goblirsch BR, Davidson VL, Wilmot CM. *Biochemistry*. 2011; 50:2931–2938. [PubMed: 21355604]
59. Fu R, Liu F, Davidson VL, Liu A. *Biochemistry*. 2009; 48:11603–11605. [PubMed: 19911786]
60. Shin S, Lee S, Davidson VL. *Biochemistry*. 2009; 48:10106–10112. [PubMed: 19788236]
61. Abu Tarboush N, Yukl ET, Shin S, Feng M, Wilmot CM, Davidson VL. *Biochemistry*. 2013; 52:6358–6367. [PubMed: 23952537]
62. Shin S, Feng ML, Chen Y, Jensen LMR, Tachikawa H, Wilmot CM, Liu A, Davidson VL. *Biochemistry*. 2011; 50:144–150. [PubMed: 21128656]
63. Chen Y, Naik SG, Krzystek J, Shin S, Nelson WH, Xue S, Yang JJ, Davidson VL, Liu A. *Biochemistry*. 2012; 51:1586–1597. [PubMed: 22320333]

Highlights

MauG catalyzes the final three two-electron oxidation reactions to complete TTQ biosynthesis

The two hemes of MauG, Trp93 and a bound Ca^{2+} are critical for the function of the diheme cofactor

MauG forms a *bis*- Fe^{IV} redox state with one heme $\text{Fe}^{\text{IV}}=\text{O}$ and the other Fe^{IV} with His-Tyr ligation

Hole hopping via Trp199 of MauG is required to achieve remote catalysis of the protein substrate

Figure 1.

The role of MauG in TTQ biosynthesis. MauG catalyzes three consecutive two-electron oxidation reactions that result in the conversion of monohydroxylated β Trp57 and β Trp108 of preMADH to TTQ. This occurs via three two-electron oxidation steps in the order shown [17]. The posttranslational modifications are shown in red.

Figure 2. Overall structure of the complex between MauG (pink) and preMADH α -(yellow) and β -(green) subunits (pdb code: 3L4M). Heme porphyrins are indicated in hot pink stick and colored by atom. PreTTQ is indicated in green stick. Figure produced using PyMOL (<http://www.pymol.org/>).

Figure 3. Orientation of hemes, Trp93 and Ca²⁺ within the MauG-preMADH complex. A portion of the crystal structure is shown and distances are indicated. Figure produced using PyMOL (<http://www.pymol.org/>).

Figure 4. Conversion of the hemes of MauG from the diferric to *bis*-Fe^{IV} redox states. [O] represents oxidation equivalents that may be provided by addition of H₂O₂ or a reductant plus O₂.

Figure 5.
Kinetic parameters for steady-state and single-turnover reactions of MauG-dependent TTQ biosynthesis.

Figure 6. Proposed hopping pathway from preTTQ to the MauG hemes. Interaction distances are indicated by dash lines. Hemes, Try93, Trp199 and preTTQ are drawn in stick colored by atom. Heme irons are drawn as orange spheres. Figure produced using PyMOL (<http://www.pymol.org/>).

Figure 7. Amino acid residues of interest in the distal pocket of the high-spin heme of MauG. Figure produced using PyMOL (<http://www.pymol.org/>).