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Review

Tryptophan tryptophylquinone biosynthesis: A radical approach to posttranslational modification ☆

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ABSTRACT

Protein-derived cofactors are formed by irreversible covalent posttranslational modification of amino acid residues. An example is tryptophan tryptophylquinone (TTQ) found in the enzyme methylamine dehydrogenase (MADH). TTQ biosynthesis requires the cross-linking of the indole rings of two Trp residues and the insertion of two oxygen atoms onto adjacent carbons of one of the indole rings. The diheme enzyme MauG catalyzes the completion of TTQ within a precursor protein of MADH. The preMADH substrate contains a single hydroxyl group on one of the tryptophans and no crosslink. MauG catalyzes a six-electron oxidation that completes TTQ assembly and generates fully active MADH. These oxidation reactions proceed via a high valent *bis*-Fe(IV) state in which one heme is present as Fe(IV)=O and the other is Fe(IV) with both axial heme ligands provided by amino acid side chains. The crystal structure of MauG in complex with preMADH revealed that catalysis does not involve direct contact between the hemes of MauG and the protein substrate. Rather it is accomplished through long-range electron transfer, which presumably generates radical intermediates. Kinetic, spectrophotometric, and site-directed mutagenesis studies are beginning to elucidate how the MauG protein controls the reactivity of the hemes and mediates the long range electron/radical transfer required for catalysis. This article is part of a Special Issue entitled: Radical SAM enzymes and Radical Enzymology.

1. Introduction

Tryptophan tryptophylquinone (TTQ) is the protein-derived cofactor of certain amine dehydrogenases [1]. Protein-derived cofactors are catalytic or redox-active centers of proteins that are formed by post-translational modification of one or more amino acid residues [2,3]. TTQ is formed by a post-translational modification of two tryptophan residues of the polypeptide chain. This review will focus on the biosynthesis of TTQ in methylamine dehydrogenase (MADH) from *Paracoccus denitrificans*. In that enzyme, two atoms of oxygen are incorporated into the indole ring of residue βTrp57 and a covalent bond is formed between the indole rings of βTrp57 and βTrp108 (Fig. 1).

MADH catalyzes the oxidative deamination of methylamine to formaldehyde plus ammonia [4] and transfers the electrons derived from the amine substrate to a type 1 copper protein, amicyanin [5,6]. In doing so MADH allows the host organism to use methylamine as a sole source of carbon, nitrogen and energy. Crystal structures have been determined of MADH alone [7], MADH in complex with amicyanin [8], and MADH in complex with amicyanin and cytochrome *c*-551i [9], the electron acceptor of amicyanin in this soluble electron transfer chain. MADH is a heterodimer of two 45 kDa α subunits and two 14 kDa β subunits, the latter each possessing TTQ [7] (Fig. 1). TTQ is critical for both the catalytic and redox properties of MADH as it physically bridges active site chemistry, the oxidative deamination of methylamine, and surface mediated electron transfer to amicyanin.

The biosynthesis of MADH requires not only the post-translational modifications to generate TTQ, but also formation of six disulfide bonds in the β subunit, export of the protein subunits to the periplasm, and assembly of protein subunits. The genes encoding the α and β subunits of MADH are located in the methylamine utilization (*mau*) gene cluster [10]. The *mau* cluster of *P. denitrificans* has 11 genes with a gene order of *mauRFBEDACJGMN* [11]. The α and β subunits of MADH are encoded by *mauB* and *mauA*, respectively, and *mauC* [12] encodes the electron acceptor for MADH, amicyanin. Deletions of either *mauF* [11], *mauD* [13], *mauE* [13] or *mauG* [11] resulted

Abbreviations: MADH, methylamine dehydrogenase; TTQ, tryptophan tryptophylquinone; preMADH, the biosynthetic precursor protein of MADH with incompletely synthesized TTQ; *bis*-Fe(IV) MauG, redox state of MauG with one heme as Fe(IV)=O and the other as Fe(IV); E_m, oxidation-reduction midpoint potential; Compound I, cpd I; Compound ES, cpd ES.

☆ This article is part of a Special Issue entitled: Radical SAM enzymes and Radical Enzymology.

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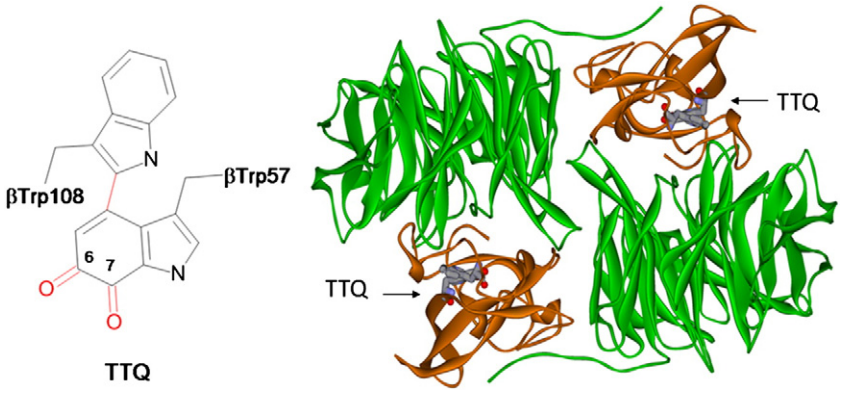

Fig. 1. The protein derived tryptophan tryptophylquinone (TTQ) cofactor of methylamine dehydrogenase (MADH). The structure of TTQ is shown on the left with the posttranslational modifications of residues βTrp57 and βTrp108 colored red. The crystal structure of MADH is shown on the right the α subunits in green and the β subunits in orange. The TTQ of each β subunit is displayed as sticks. The coordinates used for this figure are from PDB ID: 2BBK.

in loss of both MADH activity and the ability of the bacterium to grow on methylamine. In the first three deletions, no MADH protein subunits could be detected in cell extracts. Cells with the *mauG* deletion were lacking in MADH activity and were unable to grow on methylamine, but in this case it was shown by Western Blot analysis that near wild-type levels of the MADH β subunit were expressed [11]. This suggested that MauG might play a role in TTQ formation.

2. The role of MauG in TTQ biosynthesis

A key step in beginning to elucidate the role of MauG in TTQ biosynthesis was the development of a recombinant expression system for MADH. A plasmid which contained the structural genes for MADH as well as *mauFEDG,* the genes required for MADH biosynthesis, was placed in *Rhodobacter sphaeroides* and active recombinant MADH with the correctly synthesized TTQ cofactor was isolated from these cells [14]. To specifically test the role of MauG in TTQ biosynthesis, the *mauG* gene was inactivated in this expression system by site directed mutagenesis. The MADH which was isolated from this altered expression system was inactive and lacked the visible absorption spectrum characteristic of the TTQ cofactor. Analysis of this inactive form of MADH by mass spectrometry revealed that the majority species that was isolated was a biosynthetic intermediate of MADH with incompletely synthesized TTQ containing βTrp57 which was mono-hydroxylated and with no covalent cross-link to residue βTrp108 [15] (Fig. 2). This species was designated preMADH. Incubation of preMADH in vitro with purified MauG and oxidation equivalents provided by molecular oxygen plus an electron donor, or by H_2O_2, resulted in completion of TTQ biosynthesis and formation of active MADH [16]. Thus, while the mechanism by which the first oxygen is inserted into residue βTrp57 is not known, it is known that MauG is required to complete TTQ biosynthesis from that intermediate point. The position of insertion of the second oxygen into βTrp57 was determined by $^{18}O_2$ labeling studies of the MauG-dependent biosynthetic reaction to be the C6 position [17] and it follows that preMADH is hydroxylated exclusively at the C7 position.

3. Physical properties of MauG

When *P. denitrificans* is grown with methylamine as the sole carbon source, the cells are induced to produce large amounts of MADH. However, the MauG protein had never been detected in extracts of these cells. To enable study of MauG, a homologous expression system for expression of this protein was developed in *P. denitrificans* [18]. The MauG isolated from this expression system was shown to be a 42.3 kDa protein which possesses two *c*-type hemes, as was predicted from the gene sequence that contains two CXXCH motifs in which the two Cys residues form covalent thioether linkages to the heme and the His provides an axial ligand [18]. The visible absorption spectra of diferric and diferrous MauG are typical of those of *c*-type cytochromes. The EPR spectrum of fully oxidized MauG reveals that the two ferric hemes are present in a distinct spin state; a high-spin ferric heme that is ligated with a His ligand and a low-spin ferric heme that is six-coordinate with two protein ligands [18]. The crystal structure of the MauG-preMADH complex (Fig. 3) confirmed the presence of a five-coordinate and six-coordinate heme and revealed that the low-spin heme possessed a His–Tyr ligand set [19]. Natural Tyr–His ligation to a *c*-type heme

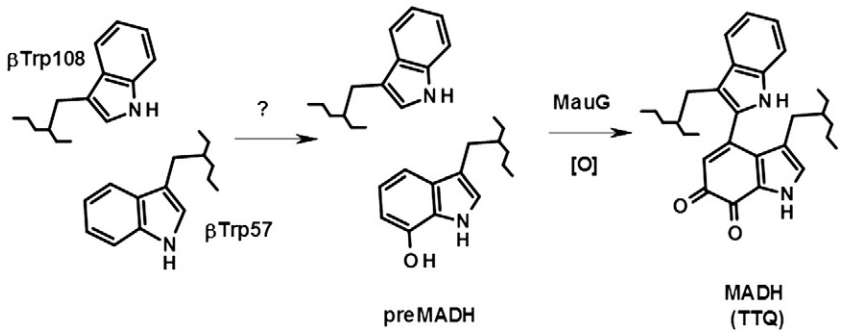

Fig. 2. The role of MauG in TTQ biosynthesis. MauG catalyzes the conversion of monohydroxylated βTrp57 and βTrp108 of preMADH to TTQ. Oxidation equivalents ([O]) may be provided by O_2 plus an electron donor or by H_2O_2.

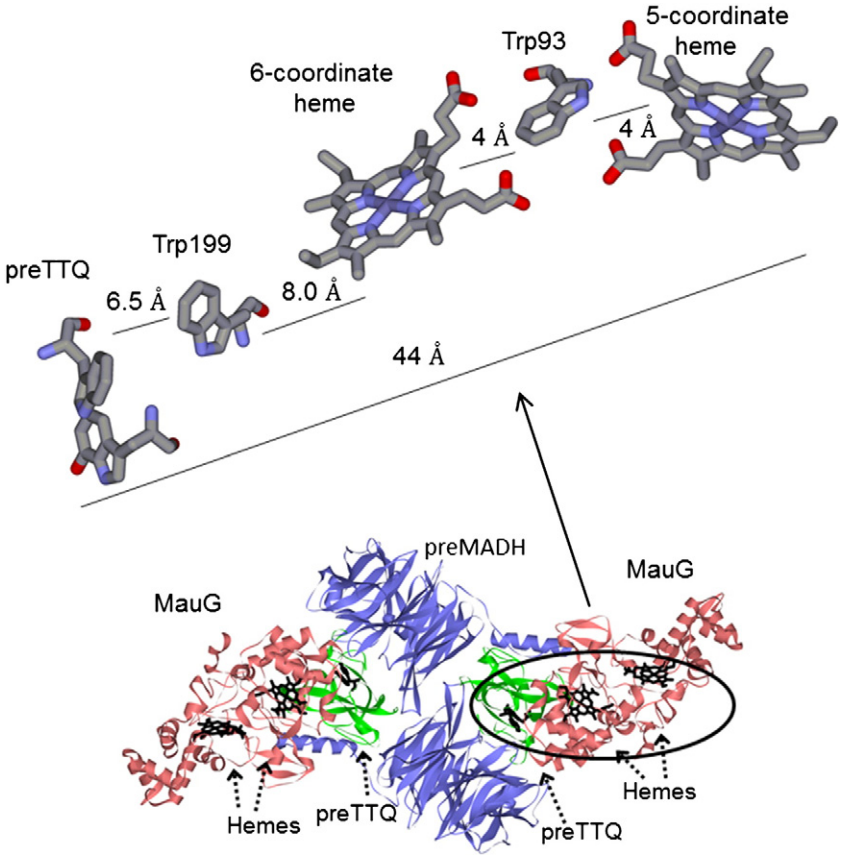

Fig. 3. Structure of the MauG-preMADH complex. The overall crystal structure of the MauG-preMADH complex (PDB ID: 3L4M) is shown with MauG colored red; preMADH α subunit colored blue, and preMADH β subunit colored green. The hemes of MauG and βTrp108 and mono-hydroxylated βTrp57 of preMADH are drawn in a stick representation and colored black. A portion of the crystal structure is enlarged and shown which includes residues of the β subunit of MADH which are posttranslationally modified to form TTQ (preTTQ), the two hemes of MauG and intervening residues Trp93 and Trp199 of MauG. The distances which separate these residues and hemes are indicated.

had not previously been described, and MauG is the first known example of a *c*-type heme with axial ligation by Tyr.

During redox titrations of MauG that were monitored by absorption and EPR spectroscopy, the two hemes were oxidized and reduced simultaneously rather than sequentially, which indicates that the intrinsic oxidation-reduction midpoint potential (E_m) values for the Fe(III)/Fe(II) couple of each of the two hemes are equivalent. As such, a clear cut mixed valence state is never observed. However, the hemes display negative redox cooperativity, such that the E_m values for the sequential addition or removal one electron to or from the diheme system are different, i.e., −159 and −254 mV [20]. This redox cooperativity indicates that facile equilibration of electrons occurs between the two hemes allowing it to function as a diheme unit that is a two-electron redox cofactor. Despite this communication between hemes, EPR studies indicated that the two hemes are not spin-coupled and therefore, the two hemes must be distantly located even though they efficiently share electrons. The physical separation of the two hemes was evident in the crystal structure of the MauG-preMADH complex [19] with the two Fe ions separated by ~21 Å, with the heme edges within 10 Å of each other. Residue Trp93 is located midway between the hemes and thus well-positioned to mediate electron transfer between the hemes (Fig. 3).

It was also shown by EPR spectroscopy that when diferrous heme reacts with NO, binding of NO is exclusively to the high-spin heme [21]. The six-coordinate heme does not directly bind exogenous molecules and the distal Tyr ligand remains bound to the heme during the chemical reaction of the five-coordinate heme. This study also showed that the His–Tyr ligation remains unchanged at the fully reduced diferrous state. Subsequent determination of the structures of CO and NO adducts of the preMADH-MauG complex described the binding of these exogenous ligands exclusively to the five-coordinate heme [22].

4. Characterization of the high valance state of Fe in MauG

The most interesting feature of the redox properties of MauG is the high valance state which it stabilizes and utilizes to oxidize preMADH. When MauG reacts with one equivalent of H_2O_2, a new stable species is formed [23] which spontaneously returns to the diferric state over minutes. Appearance of this species in the absorption spectrum of MauG is characterized by a Soret peak shift from 405 to 407 nm (Fig. 4A). The X-band EPR spectrum of diferric MauG displays two heme signals, a high-spin ($g = 5.57, 1.99$) and low-spin ($g = 2.54, 2.19, 1.87$). After mixing with H_2O_2, both high- and low-spin signals disappear and a new radical signal can be observed at $g = 2.003$ with a peak-to-peak width of 1.3 millitesla (Fig. 4B). The EPR characterization of the $g = 2.003$ radical suggests that it is an organic free radical. However, quantitation from spin double integration is that the radical signal represents ca. 1% of the protein and does not nearly compensate for the loss of the two ferric heme EPR signals.

When ^{57}Fe-labeled MauG is treated with H_2O_2 the resultant Mössbauer spectra shows the appearance of two sharp lines in addition to a broad, magnetically split feature associated with ferric heme. Upon subtraction of the ferric species, the resulting spectrum is fitted by two quadrupole doublets with the following parameters: Species 1 with isomer shift (δ_1) of 0.06 mm/s and Species 2 with quadrupole splitting parameter (ΔE_{Q1}) of 1.70 mm/s and $\delta_2 = 0.17$ mm/s and

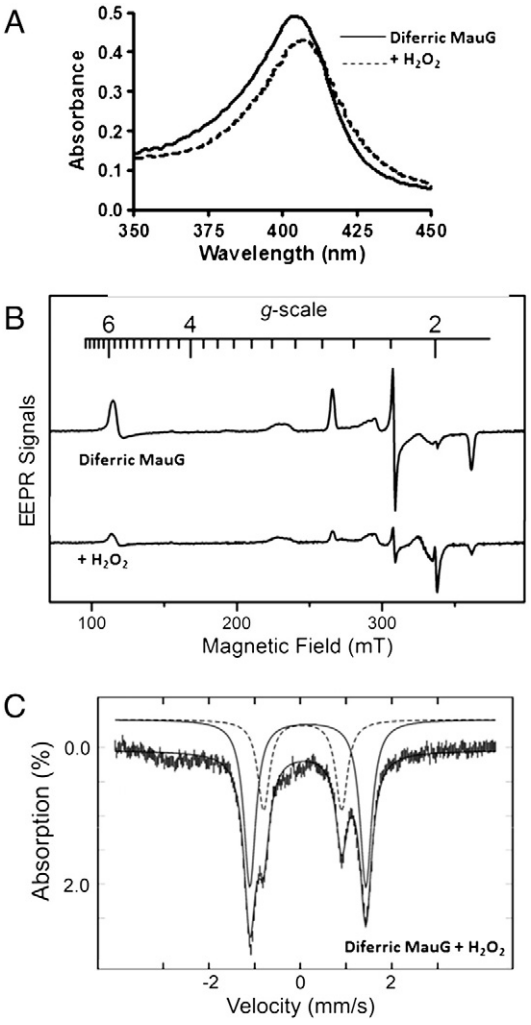

Fig. 4. Spectroscopic properties of the *bis*-Fe(IV) state of MauG. A. Absorbance spectra of diferric MauG before and after addition of H_2O_2 to generate the *bis*-Fe(IV) state. B. EPR spectra of diferric MauG before and after addition of H_2O_2 to generate the *bis*-Fe (IV) state . C. The Mössbauer spectrum of *bis*-Fe(IV) MauG formed after addition of H_2O_2 and subtraction the contributions to the spectrum of residual diferric MauG is shown as hashed marks. The simulated quadrupole doublets are shown in solid and dashed lines on top of the experimental data.

$\Delta E_{Q2} = 2.54$ mm/s (Fig. 4C). The isomer shift values are typical of Fe (IV) species and the quadrupole splitting parameter of Species 1 is in the range typically observed for ferryl and protonated ferryl species. The quadrupole splitting parameter of Species 2 is unusually large ($\Delta E_{Q2} = 2.54$ mm/s) and was assigned to a six-coordinate heme with two axial amino acid ligands [23].

A subsequent theoretical quantum chemical study [24] found that the unusual Mössbauer properties of both Fe(IV) species originated from novel structural features of the enzyme. The structures of the diferric heme sites in the crystal structure of the MauG-preMADH complex were used as a starting point for modeling the *bis*-Fe(IV) MauG species. The calculated ΔE_Q and δ values from this study for both hemes were in good agreement with experimental values determined by Mössbauer spectroscopy. The modeling supported the conclusion that the His/Tyr ligand set determined the unusually large ΔE_Q value exhibited by the six-coordinate Fe(IV) heme. The modeling also suggested that the Fe(IV) = O heme moiety is stabilized by a H-bond to an active site residue, which could account for the unusual stability of this high valent state in MauG.

It is noteworthy that the oxidizing power of MauG can be shared by its two hemes when the Fe atoms are separated by 21 Å. A

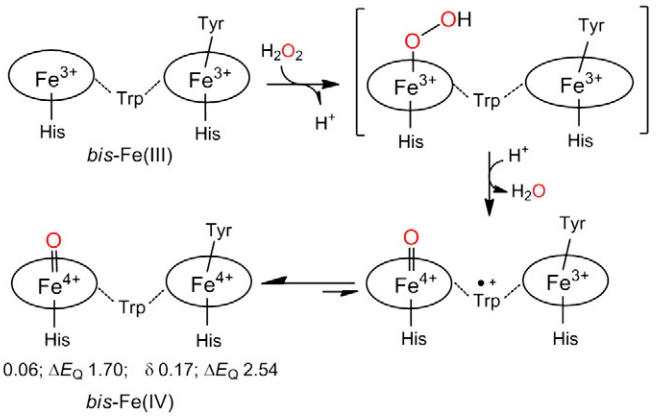

Fig. 5. Proposed scheme for the formation of the *bis*-Fe(IV) redox state after addition of H_2O_2 to MauG. The Mössbauer parameters for *bis*-Fe(IV) MauG are listed below their respective Fe(IV) hemes.

proposed mechanism for the formation of the *bis*-Fe(IV) state after addition of H_2O_2 is shown in Fig. 5. The initially formed peroxo intermediate loses water to yield the Fe(IV) = O heme and oxidation of the Trp residue which lies between the hemes. This Trp cation radical is present only transiently as it oxidizes the six-coordinate heme to yield the *bis*-Fe(IV) species which was characterized by Mössbauer spectroscopy. As discussed earlier, the EPR spectrum of the *bis*-Fe (IV) MauG included a radical signal that accounted for only ~1% of the protein. To accommodate this result, the final step in *bis*-Fe(IV) formation is drawn as an equilibrium, heavily favoring *bis*-Fe(IV) but with a small percentage of the MauG in the intermediate state with the Trp93 cation radical which would explain the EPR results.

Intuitively, one would anticipate that oxidation of the His-Tyr ligated ferric heme would yield a ligand radical (Tyr in this case) or a porphyrin radical. Surprisingly, the six-coordinate heme of MauG stabilizes a ferryl species without an exogenous oxo group. This is very unusual; because the negatively charged oxo group was seen in all previously characterized ferryl heme species in proteins and was considered to be crucial for stabilization of the high-valence charge of the Fe ion.

While a six-coordinate Fe(IV) heme species with two axial amino acid ligands had never been observed in a protein, analogous *bis*-ligated high-valent inorganic porphyrin model compounds have been generated [25,26]. These species exhibited a small isomer shift value and large quadruple splitting value. Consistent with the Mössbauer data for *bis*-Fe(IV) MauG, evidence for a porphyrin or Tyr ligand radical was not seen in EPR and absorption spectra of this MauG intermediate. Apparently, a Tyr ligand radical coupled with an Fe(III) ion is not a favored intermediate in MauG, and importantly, the Tyr ligand appears to be sufficient to stabilize the high-valence state of the Fe(IV) heme. It is not clear when the term ferryl became synonymous for the Fe(IV) = O species rather than the term oxoferryl. With the discovery and characterization of the *bis*-Fe(IV) state of MauG, it is now debatable whether the term ferryl should be reserved for Fe (IV) like ferrous and ferric for Fe(II) and Fe(III), respectively, and oxoferryl for Fe(IV) = O.

5. Why use *bis*-Fe(IV) for catalysis rather than Compound I or Compound ES?

A common function of enzymes is to promote specific chemical transformations of small organic compounds. The specificity of each enzyme is typically derived from the substrate binding pocket which does not allow other chemical substances to properly position at the active site to undergo catalysis. Heme containing enzymes that

utilize oxygen for oxidation and oxygenation reactions typically employ a high-valent Fe intermediate, known as Compound I (cpd I), which is an oxoferry1 ion, *i.e.*, Fe(IV)=O coupled with a π-cation radical located on the porphyrin ring (Fig. 6). Thus, cpd I transiently stabilizes two oxidizing equivalents above the resting Fe(III), and it is chemically equivalent to an Fe(V) species. Until now, true Fe(V) intermediates have only been proposed in a few cases for inorganic model systems and have never been observed in any biological reactions. Similar to an Fe(V) species, cpd I also carries two oxidizing equivalents, which are stored not just in the metal ion but in the entire heme moiety. During the reaction cycle, the enzyme active site often needs reorganization and conformational changes in order to activate substrates or stabilize transient intermediates. The charge distribution to the porphyrin ring endows cpd I with greater chemical stability than Fe(V). This is a great advantage for promoting biological reactions because cpd I is reactive enough to transform the substrate but not so reactive to cause non-specific oxidation prior to substrate orientation and active site reorganization. Cpd I seems to be the most common naturally evolved strategy for storing oxidizing power on a heme moiety. Another form of the high-valent Fe species in heme proteins is Compound ES (cpd ES), which is composed of an Fe(IV)=O heme and amino acid-based cation radical in close proximity to the high-valent heme. The compound ES description is based on the initial characterization from cytochrome *c* peroxidase [27]. In cytochrome *c* peroxidase, the cation radical is found at a Trp residue rather than at the porphyrin. Likewise, cpd ES is an intermediate state with two oxidizing equivalents above the original ferric state. Cpd ES is reportedly more stable than cpd I, because of the spatial separation of the two oxidizing equivalents [28]. The formation of cpd ES rather than cpd I may be a requirement for substrates of large size. In cytochrome *c* peroxidase the reducing equivalents come from cytochrome *c* and thus protein-protein interactions are also a part of its catalytic process.

The spin-uncoupled *bis*-Fe(IV) species found in MauG is an unprecedented Fe intermediate. Its role in MauG-dependent catalysis appears to be not only stabilization of an Fe(V)-equivalent state, but also delocalization of the oxidizing equivalents over a large area of the protein. The requirement for Tyr to provide the distal axial ligand is supported by site-directed mutagenesis of Tyr294 of MauG [29]. Conversion of this Tyr to a His resulted in a protein with a His-His ligated heme rather than a His-Tyr ligated heme. Y294H MauG was not able to stabilize Fe(IV) on the His-His coordinated heme. Rather than forming a *bis*-Fe(IV) species, addition of H_2O_2 to this variant resulted in formation of a cpd I-like species which was stable but unable to oxidize preMADH. While the *bis*-Fe(IV) species and cpd I may be similarly potent oxidants, in the former the spatial separation of the two oxidizing equivalents delocalizes the oxidizing power over a large area. As the catalytic reaction of MauG requires protein-protein interactions and long range remote catalysis, the extension of the oxidizing equivalent to the second heme shortens the distance required for the long range electron transfer that is a part of this unusual reaction [29]. This explains why the *bis*-Fe(IV) species in MauG can oxidize preMADH whereas cpd I in Y294H MauG cannot. Use of the *bis*-Fe(IV) species provides a novel natural strategy to oxidize a large substrate that cannot be accommodated in a traditional enzyme active site.

6. Kinetic mechanism of MauG-dependent TTQ biosynthesis from preMADH

To demonstrate the kinetic competence of the *bis*-Fe(IV) MauG intermediate it was mixed with preMADH and the reaction was monitored by absorption and EPR spectroscopy. These experiments showed rapid return to the diferric MauG state after reaction with preMADH. The EPR spectrum of the reaction product mixture also showed the appearance of a new stable radical signal that was based on what was the preMADH substrate. The identity of that preMADH-based radical intermediate is still under investigation. While the chemical reaction mechanism of MauG-dependent TTQ biosynthesis remains to be elucidated, considerable progress has been made in characterization of the kinetic mechanism of MauG-dependent TTQ biosynthesis using both steady-state kinetic and single turnover kinetic approaches.

The conversion of preMADH to MADH with the mature TTQ cofactor requires three two-electron oxidations to achieve the crosslink formation, insertion of the second oxygen and oxidation of the quinol to the quinone. While the exact sequence of these events has not been established there is good evidence that the final step in this process is the oxidation of quinol MADH to the quinone (TTQ) [16]. In the spectroscopic assay of steady-state MauG-dependent TTQ biosynthesis from preMADH, a transient intermediate with a λ_{max} at 330 nm was observed early in the reaction before the steady-state accumulation of the product which exhibits a λ_{max} at 440 nm. As the fully reduced quinol MADH exhibits an absorption maximum at 330 nm [30] it was postulated that the quinol form of TTQ was an intermediate in TTQ biosynthesis.

Two MauG catalyzed steady state reactions were characterized, one using preMADH as a substrate [16,31] and the other using quinol MADH as a substrate [32]. In each case product formation was monitored by the increase in 440 nm that is associated with the appearance of TTQ. The former reactions exhibited a k_{cat} of 0.2 μM and a K_m of 6.6 s^{-1}, while the latter exhibited a k_{cat} of 4.1 s^{-1} and a K_m of 11.1 μM. Single turnover kinetic studies of the initial two-electron oxidation of preMADH, and the final two-electron oxidation of quinol MADH, by *bis*-Fe(IV) MauG were monitored by the absorbance changes associated with the reduction of *bis*-Fe(IV) MauG to the diferric state. The oxidation of preMADH exhibited a limiting first order rate constant of 0.8 s^{-1} and a K_d of <1.5 μM [33] while the oxidation of quinol MADH exhibited a limiting first order rate constant of 20 s^{-1} and a K_d of 11.2 μM [32]. By changing the order of mixing of each substrate with MauG and H_2O_2 it was demonstrated that each reaction exhibited a random kinetic mechanism, in which the order of addition to MauG of H_2O_2 and either preMADH or quinol did not matter. Such a random mechanism is in contrast to that typically seen for heme-dependent oxygenases [34].

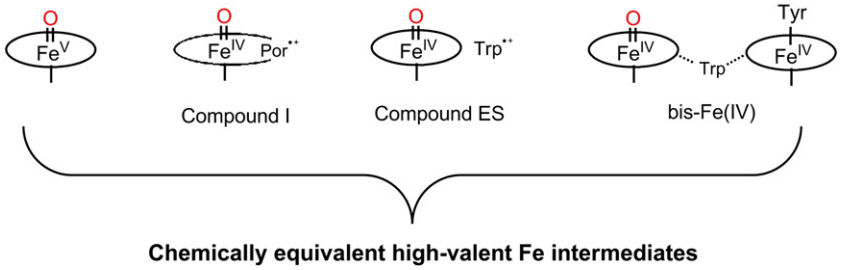

Fig. 6. Comparison of the Fe(V) equivalents, compound I, compound ES and *bis*-Fe(IV). Por indicates that the radical in cpd I resides on the porphyrin ring.

7. Suicide inactivation of MauG

The random-binding kinetic mechanism exhibited by MauG [33] may be necessitated by the fact that the co-substrate is specific amino acid side chains within a 119 kDa precursor protein. While the presence or absence of preMADH has no influence on the reactivity of MauG towards oxidation equivalents, MauG is inactivated when supplied with oxidation equivalents in the absence of preMADH. Repeated oxidation of diferric MauG (i.e., formation of *bis*-Fe(IV) followed by spontaneous decay to the diferric state) leads to loss of catalytic activity and inactivation of heme as judged by absorption spectroscopy and pyridine hemochrome assay [25]. The loss of enzymatic activity did not occur concomitant with heme inactivation, but instead loss of activity preceded heme inactivation. This suggests that the initial loss of enzymatic activity involves oxidative damage to critical amino acid residue (s) rather than to heme, and that the hemes retain their redox reactivity after the initial loss of enzymatic activity. The radical scavenger hydroxyurea protects against inactivation, consistent with the inactivation proceeding via a radical mechanism. Suicide inactivation or oxidative damage is sometimes observed with other iron and heme-dependent oxygenases. In contrast to MauG, heme-dependent monooxygenases tend not to be reactive toward oxygen in the absence of co-substrate. For example, with cytochrome P450 enzymes, binding of substrate triggers a conformational change that allows the high-spin heme to bind and activate oxygen [30]. Thus, the kinetic mechanisms and mechanisms of avoidance and susceptibility to suicide inactivation exhibited by MauG are distinct from those of other heme-dependent and non-heme Fe-dependent enzymes which activate oxygen.

8. Evidence for a hopping mechanism of long range electron transfer during catalysis

The rate constants of 0.2 s^{-1} and 20 s^{-1} for the reactions of *bis*-Fe(IV) MauG with preMADH and quinol MADH, respectively, most likely describe the long range electron transfer that is required for catalysis. These values are surprisingly large given that the distance from the electron donors that are oxidized to the hemes. The crystal structure of the MauG-preMADH complex [19] revealed that βTrp57 and βTrp108 do not make direct contact with either heme of MauG (Fig. 6). The distance between the side-chain of βTrp108 of preMADH and the iron of the oxygen-binding five-coordinate heme is 40.1 Å, and the closest distance to the iron of the six-coordinate heme is 19.4 Å. This is the catalytically competent structure, as it was shown that addition of H_2O_2 to MauG-preMADH crystals causes synthesis of the mature TTQ cofactor *in crystallo*. As discussed earlier, extending the oxidative power to the closer six-coordinate heme shortens the electron transfer distance. Recent site-directed mutagenesis studies have also suggested that this long range electron transfer occurs by a mechanism of hole hopping via Trp199 of MauG, which lies midway between the six-coordinate heme of MauG and the residues on preMADH which are oxidized [35]. Thus it appears that both hemes and at least two Trp residues of MauG are working in concert to couple long range electron transfer to a radical mediated mechanism of catalysis.

9. Chemical reaction mechanism

The conversion of preMADH to oxidized MADH with the mature TTQ cofactor is a six-electron oxidation. The process requires oxygen insertion (two-electrons), cross-linking of βTrp57 and βTrp108 side-chains (two-electrons), and oxidation to the quinone (two-electrons). The precise order of events and mechanistic details remain to be elucidated. It is known that the initial reaction of *bis*-Fe(IV) MauG with preMADH results in the formation of a radical species that is based on preMADH [23]. The results obtained thus far suggest that the structure of the preMADH protein is facilitating the catalytic steps. The role of MauG appears to be the generation of radical intermediates on the preMADH substrate. The substrate itself then likely directs the crosslink formation and incorporation of oxygen from solvent during the multi-step biosynthetic process. Further studies will hopefully elucidate the order and mechanistic details of the sequential oxidation reactions that are required for MauG-dependent TTQ biosynthesis.

Acknowledgements

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