Roles of multiple-proton transfer pathways and proton-coupled electron transfer in the reactivity of the $\text{bis-Fe}^{\text{IV}}$ state of MauG

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The high-valent state of the diheme enzyme MauG exhibits charge–resonance (CR) stabilization in which the major species is a $\text{bis-Fe}^{\text{IV}}$ state with one heme present as $\text{Fe}^{\text{IV}}=\text{O}$ and the other as $\text{Fe}^{\text{IV}}$ with axial heme ligands provided by His and Tyr side chains. In the absence of its substrate, the high-valent state is relatively stable and returns to the diferric state over several minutes. It is shown that this process occurs in two phases. The first phase is redistribution of the resonance species that support the CR. The second phase is the loss of CR and reduction to the diferric state. Thermodynamic analysis revealed that the rates of the two phases exhibited different temperature dependencies and activation energies of 8.9 and 19.6 kcal/mol. The two phases exhibited kinetic solvent isotope effects of 2.5 and 2.3. Proton inventory plots of each reaction phase exhibited extreme curvature that could not be fit to models for one- or multiple-proton transfers in the transition state. Each did fit well to a model for two alternative pathways for proton transfer, each involving multiple protons. In each case the experimentally determined fractionation factors were consistent with one of the pathways involving tunneling. The percent of the reaction that involved the tunneling pathway differed for the two reaction phases. Using the crystal structure of MauG it was possible to propose proton–transfer pathways consistent with the experimental data using water molecules and amino acid side chains in the distal pocket of the high-spin heme.

MauG (1) is a diheme enzyme that catalyzes a six-electron oxidation required for posttranslational modification of a precursor of methylamine dehydrogenase (preMADH) (2) to complete the biosynthesis of its protein-derived cofactor (3) tryptophyl quinone (TTQ) (4). The hemes of MauG are unusual in several respects. One is a high-spin five-coordinate heme that is ligated by His35. The other is a low-spin six-coordinate heme with two ligands provided by His205 and Tyr294 (1, 5). The latter is, to our knowledge, the first example of natural His–Tyr ligation of a protein-bound heme cofactor, and the first example of Tyr ligation of a c-type heme. An intervening residue, Trp93, “connects” the two hemes (Fig. 1) via rapid electron transfer (ET) (6–9). A unique feature of MauG is that the oxidation of difer ferric MauG by $\text{H}_2\text{O}_2$, or of diferrous MauG by $\text{O}_2$, generates a high-valent $\text{bis-Fe}^{\text{IV}}$ state (8) in which the high-spin heme is present as $\text{Fe}^{\text{IV}}=\text{O}$ with the His35 ligand, and the other heme is present as $\text{Fe}^{\text{IV}}$ with the His–Tyr axial ligation retained (5, 10, 11). Formation of the $\text{bis-Fe}^{\text{IV}}$ state is accompanied by changes in the visible absorbance spectrum. One observes a decrease in intensity and shift of the Soret peak from 406 to 408 nm and appearance of minor peaks at 526 and 559 nm (Fig. 2) (9, 12).

Despite being a highly potent oxidant, the $\text{bis-Fe}^{\text{IV}}$ species displays extraordinary stability with a half-life of several minutes in the absence of its substrate (8). A basis for this stability was inferred from the observation of a near-infrared (NIR) electronic absorption feature centered at 950 nm that was observed in $\text{bis-Fe}^{\text{IV}}$ MauG (Fig. 2C). This spectral feature is characteristic of a charge–resonance (CR) transition phenomenon (6, 9). A model was presented in which the CR occurs in the absence of direct heme–heme contact by ultrafast and reversible ET between the two high-valent hemes, via hopping through the intervening Trp93 residue (9). In this model the high-valent form of MauG comprises an ensemble of resonance structures including compound ES-like and compound I-like forms of the hemes, with the $\text{bis-Fe}^{\text{IV}}$ as the dominant species.

The catalytic mechanism of MauG is unusual in that the preMADH substrate does not make direct contact with either heme but instead binds to the surface of MauG several angstroms away (5). Catalysis requires long-range ET to $\text{bis-Fe}^{\text{IV}}$ MauG from the residues on preMADH that are modified via a hole-hopping mechanism through Trp199 (13, 14), which resides at the MauG–preMADH interface (Fig. 1). Concomitant with this ET is the formation of free-radical intermediates on preMADH that go on to form the TTQ product (15). In the absence of preMADH, the autoreduction of the $\text{bis-Fe}^{\text{IV}}$ redox state to the difer ferric state leads to inactivation of MauG (16). Analysis of the damaged MauG revealed that this process involves the oxidation of three Met residues (108, 114, and 116) which are located 7.5–15.2 Å from the high-spin heme iron (Fig. 1) (17).

To further investigate the dynamic nature of the ensemble of resonance forms of MauG that comprise the high-valent state and the basis for its stability, temperature-dependence and kinetic solvent isotope effect (KSIE) studies were performed. These studies provide evidence for a redistribution within the ensemble of resonance structures before loss of CR stabilization of the high-valent redox state which is linked to the reduction to the difer ferric state. Thermodynamic analysis of the rates of reaction of these processes reveals that the rates of the initial redistribution of the ensemble of resonance structures and the subsequent loss of CR stabilization are determined by two separate KSIE processes. The first is a solvent isotope effect of 2.5 and the second is a solvent isotope effect of 2.3.
of CR stabilization exhibit different dependencies on temperature. This accounts for the fact that the early phase is only observable at lower temperatures. Proton inventories of the KSIE indicate that the rates of both the initial redistribution of the ensemble of high-valent species and the loss of CR stabilization are rate-limited by multiple proton-transfer (PT) steps involving two alternative pathways. The likely pathways are identified from the crystal structure of MauG.

Results

Temperature Dependence of the Kinetic Mechanism of the Conversion of the bis-Fe$^{IV}$ state of MauG to the Diferric State. The spectral changes that are associated with the formation of the bis-Fe$^{IV}$ redox state form occur quickly ($k > 300 \text{s}^{-1}$ at 30 °C) (12), and subsequently return over several minutes to the spectrum of the diferric MauG. The kinetics of the rates of change in absorbance associated with the return of the bis-Fe$^{IV}$ redox state to the diferric state was studied over a range of temperature from 15 °C to 35 °C. When this reaction was monitored at different temperatures it became evident that the kinetic mechanism of this process varied with temperature (Fig. 3 and Figs. S1–S3). At 35 °C a monophasic change in the spectral features in the Soret region from those of the bis-Fe$^{IV}$ state to the diferric state is observed which fits well to a single-exponential transition with a rate constant of $7.3 \times 10^{-3} \text{s}^{-1}$ (Fig. 3 A and B). At 15 °C one observes a biphasic change in which the maxima of the Soret peak initially decrease and shift from 408 to 406 nm before increasing in intensity. This process could not be fit by a single exponential, but fits well to a two-exponential transition with rate constants of $3.1 \times 10^{-3} \text{s}^{-1}$ and $1.5 \times 10^{-3} \text{s}^{-1}$ (Fig. 3 C and D). A spectral change also occurs in the far-visible region. The bis-Fe$^{IV}$ state initially exhibits $\alpha$ and $\beta$ Q bands at 524 and 560 nm, respectively. The intensity of these bands does not change during initial phase of the changes in the Soret region. However, during this time, an increase in absorbance centered around 642 nm appears (Fig. 3 E and F). Although this additional absorbance feature is weak, its appearance is consistent with the presence of compound I (18–21). A similar change in kinetic mechanisms was observed when monitoring the reaction in the NIR region. As shown in Fig. 4, at 30 °C one observes a monophasic decrease in the intensity of the peak at 950 nm in the NIR region, whereas at 15 °C one observes a lag period of no change in the intensity of the peak at 950 nm followed by a monophasic decrease in the intensity of that peak. The duration of this lag phase correlates with that of the first phase (decrease and shift in absorbance maximum) of the biphasic change in the Soret region. Thus, the sum of these data indicates that the initial reaction phase that is observed in the Soret region does not diminish the extent of CR stabilization. Instead, the initial changes in the absorbance features of the Soret peak at 15 °C reflect a change in the distribution of resonance structures that comprise the high-valent state, an event which precedes loss of CR stabilization. This redistribution includes a decrease in the presence of the true bis-Fe$^{IV}$ species with a concomitant increase in the presence of a compound I-like species.

The biphasic spectral change in the Soret region was most clearly observed at low temperatures. However, the inability to observe this initial phase at higher temperatures was not simply because the rate became too fast to observe. These reactions are occurring on a relatively slow time scale even at the higher temperatures. The inability to clearly observe the initial phase at higher temperatures is that the rates of the first and second phases of the overall reaction each vary differently with temperature (Fig. 5). The rate of the second reaction increases more rapidly with increasing temperature than does the initial reaction. Thus, at higher temperatures the rate of the first reaction is sufficiently slower than the second reaction such that there is no observable accumulation of the intermediate species. In contrast, at lower temperatures the rate of the first step is faster than the second step, so that one does observe an accumulation of the intermediate in the Soret region, as well as the lag preceding the decay of the NIR feature.

Thermodynamic Analysis of the Conversion of the bis-Fe$^{IV}$ State of MauG to the Diferric State. The dependence on temperature of the rate of each phase of this reaction was analyzed to determine the $E_a$ for the reaction (Fig. 5). The initial reaction described by the shift in the Soret peak exhibited $E_a = 8.9 \pm 0.3$ kcal/mol. The subsequent reaction described by the increase in intensity of the Soret peak.
exhibited an \( E_a = 19.6 \pm 1.1 \) kcal/mol. Thus, these two spectral changes are clearly describing two different reactions. Analysis of the rate of the decrease in the NIR region at 950 nm subsequent to the lag period yielded a value of \( E_a = 18.0 \pm 2.2 \) kcal/mol (Fig. S4), which is similar to that reported by Geng et al. (6). This value is within error of the \( E_a \) value for the second phase of the reaction observed in the Soret region, strongly suggesting that the increase in intensity of the Soret peak and decrease in intensity of the peak in the NIR region are describing the same reaction which correlates with the loss of CR stabilization and concomitant change in redox state.

**KSIE Studies of the Conversion of the bis-Fe\(^{IV}\) State of MauG to the Diferric State.** The rates of the reactions steps discussed above were determined in buffered D\(_2\)O to determine the KSIE for each phase of the reaction at 15 °C. For the initial reaction phase corresponding to the shift in the Soret peak, a KSIE of 2.5 ± 0.1 was observed. For the subsequent rate of increase in intensity of the Soret peak, a KSIE of 2.3 ± 0.2 was observed. To determine the basis for these KSIE values, proton inventory experiments (22–25) were performed. This method is based on the study of the dependence of the reaction rate on the atom fraction of deuterium in H\(_2\)O–D\(_2\)O mixtures as described by Eq. 1 (23–25), where \( k_x \) is the rate constant at atom fraction of deuterium equal to \( x \), \( k_0 \) is the rate constant at \( x = 0 \), and \( \phi_1 \) and \( \phi_2 \) are the isotopic fractionation factors for the transition and reactant states, respectively, for \( m \) or \( n \) PTs. In the case of a single proton transferred in the transition state there will be a linear relationship (Eq. 2). If two protons are transferred in the transition state this will result in a quadratic relationship (Eq. 3). If multiple PTs contribute to the rate the relationship reduces to Eq. 4.

\[
k_x = k_0 \left( \frac{1}{C_{TS}} \right)^m \left( 1 - x + x \phi_1^m \right) \left( 1 - x + x \phi_1^n \right) \]

\[
k_x = k_0 \left( 1 - x + x \phi_{TS}^m \right) \]

\[
k_x = k_0 \left( 1 - x + x \phi_{TS}^n \right) \]

\[
k_x = k_0 \left( \phi_{TS}^m \right)^x \]

As seen in Fig. 6, the data for each of the reaction phases significantly deviated from linearity and were not fit by Eqs. 2–4. The extreme curvature of the plot of the data suggests two alternative possibilities (25). One is that PT is occurring in the reactant state rather than the transition state. However, the magnitude of the KSIEs of 2.5 and 2.3 rules out this possibility as reported reactant state fractionation factors are between 0.55 and 1.28, and are typically unity (26). The other alternative is that multiple pathways are possible for the PTs. To test this possibility, the data for each reaction phase were fit by Eq. 5, which describes a model with two alternative pathways with each involving multiple PTs, where \( f \) is the fraction of contribution from the first pathway where \( m \) PTs occur, and \( (1 - f) \) is the fraction of contribution from the other pathway in which \( n \) PTs occur.

\[
k_x = k_0 \left( \frac{f}{C_{TS}} \right)^m \left( 1 - x + x \phi_{TS}^m \right) + (1 - f) \left( 1 - x + x \phi_{TS}^n \right) \]

The data for each reaction phase fit well to Eq. 5 and yielded the following values for the fitted parameters. The first reaction

![Fig. 3. Kinetic analysis of changes in the absorbance spectrum that are correlated with the conversion of the high-valent state of MauG to the difereric state. In A, C, and E the spectrum immediately after formation of the difereric state is red, and the spectrum after return to the difereric state is blue. The spectrum of an intermediate species, if present, is green. In B, D, and F the time course for the disappearance of the starting spectrum is red, the appearance of the final spectrum is blue, and the formation and decay of the intermediate spectrum is green. The kinetic plots depict global fits of the most statistically significant eigenvector of the SVD-reduced 3D data. The spectral changes were recorded and analyzed in the Soret region at 35 °C (A and B), in the Soret region at 15 °C (C and D), and in the far-visible region at 15 °C (E and F). Selected spectra recorded at different time points during the reactions and plots of the residuals associated with each of the global fits are shown in Figs. S1–S3.](image)

![Fig. 4. Time course of changes at 950 nm that are correlated with the spontaneous conversion of the high-valent state of MauG to the difereric state. Absorbance was monitored immediately following addition of stoichiometric H\(_2\)O at (A) 30 °C and (B) 15 °C.](image)
phase (Fig. 6A) was described by one pathway contributing $31 \pm 7\%$ with a $\Delta \phi^{TS} < 0.01$ and the other contributing $69 \pm 7\%$ with a $\Delta \phi^{TS} = 0.55 \pm 0.07$. The second reaction phase (Fig. 6B) was described by one pathway contributing $20 \pm 1\%$ with a $\Delta \phi^{TS} < 0.01$ and the other contributing $80 \pm 1\%$ with a $\Delta \phi^{TS} = 0.54 \pm 0.04$. Analysis of the data using models for three or more pathways did not improve the fit and therefore a more complex model than one involving two pathways was not considered.

Discussion

Two reaction steps in the conversion of the bis-Fe$^{IV}$ state to the diferric state were kinetically and spectroscopically described. Each step exhibits a similar KSIE which is described by a model for multiple PTs over multiple pathways. Despite these similarities, the initial reaction step involves no net transfer of electrons whereas the second reaction requires transfer of two electrons to the diheme system. A mechanism for the overall reaction that is consistent with the crystal structure of the diheme site of MauG is presented below.

High-valent hemes in proteins are typically present as compound I or compound ES, in which the ferryl iron is Fe$^{IV}$-O with a cation radical present on the porphyrin ring or a nearby Trp or Tyr residue, respectively (27). The bis-Fe$^{IV}$ state of MauG describes an alternative strategy by which to stabilize a high-valent heme iron. Several amino acid residues in the proximity of the two hemes have also been implicated in facilitating the formation and stabilizing the bis-Fe$^{IV}$ state (21, 28–32). The concept of CR stabilization of this high-valent state led to the description of it actually comprising an ensemble of resonance structures including the more traditional high-valent heme species, but with the true bis-Fe$^{IV}$ state as the dominant species (6, 9) (Fig. 7).

For the initial reaction phase that precedes the loss of CR stabilization, the most likely solvent-accessible site for PT involving the bis-Fe$^{IV}$ state is the ferryl heme oxygen. Precedence for such a protonation reaction is the autoreduction mechanism of ferryl globins, which is initiated by a protonated ferryl species and generates a variety of protein radicals (33–35). In contrast with that mechanism, this protonation event must be coupled to compensating electron transfers between the two hemes and Trp93 and possibly other nearby amino acid residues. This PT event results in an alteration of the distribution of the ensemble of high-valent species, while retaining the CR stabilization. Evidence supporting the redistribution of species in this reaction step is the increased absorbance around 642 nm, with the same rate as the blue shift of the Soret peak. This spectral change is consistent with conversion of at least some bis-Fe$^{IV}$ to a compound I-like species within the ensemble.

The protonation of the ferryl heme oxygen is consistent with the KSIE that indicates that solvent is involved in this reaction phase. However, the proton inventory for the first reaction phase indicates that several protons are involved in the step. As the ferryl heme oxygen can only accept two protons at the most, the inventory requires a more complex model to describe the PT. The data for this reaction were best fit to a two-pathway model, with each pathway involving multiple PTs. One pathway has a very small $\Delta \phi^{TS} (<0.01)$ and the other has a much larger $\Delta \phi^{TS}$ (0.55). The very small $\Delta \phi^{TS}$ value suggests a pathway that involves proton tunneling (26, 36–39), whereas the larger $\Delta \phi^{TS}$ value is in the range consistent with a primary effect that does not involve tunneling (36). Inspection of the crystal structure suggests two alternative pathways that would be consistent with these data.

In the crystal structure of the MauG-preMADH complex (5) a network of ordered waters is seen in the distal pocket of the high-spin heme that is oriented in two possible PT pathways (Fig. 8). These waters have also been observed in structures of crystals after soaking with reductant or H$_2$O$_2$ and after reaction with exogenous ligands. In Fig. 8, a ferryl heme oxygen was modeled based on the NO-bound MauG structure (40) and positioned for a 3.0-Å hydrogen bond to Gln103. This interaction was predicted by quantum-mechanical studies based upon the experimentally determined parameters from the Mössbauer spectrum of bis-Fe$^{IV}$-MauG (11). An ordered water (W0) is within 2.6 Å of the ferryl heme oxygen, as well as 2.8 and 3.0 Å from the carboxylic oxygens of Glu113. W0 is believed to be the direct proton donor to the ferryl heme oxygen. It is the common end point for two structured water networks which include seven waters and Glu113, Asn110, and Gln106, and delivers protons from bulk solvent water. One PT pathway includes W0, W1, and W2. W1 is 2.8 and 3.4 Å from carboxylate of Glu113 and the amide nitrogen of Asn110, respectively. W2 is 2.8 Å from amide oxygen of Gln106. This is the entry point for a proton from bulk water. Glu113, Asn110, and Gln106 can facilitate PT through the water network by optimally orienting the three waters via hydrogen bonding and serve as potential proton donors and acceptors. This highly structured pathway most likely is the one described by the very small $\Delta \phi^{TS}$. If the ordered waters are positioned in the perfect orientation to maximize vibrational movements of the hydrogen bonds, then one can imagine that proton tunneling can be associated with PT through this pathway. An alternative pathway branches off from W1 and traverses three waters (W4, W5, and W6). W4 is anchored by a 2.9-Å hydrogen bond with the amide nitrogen of Asn110. W6 is the entry point for a proton from bulk solvent. This pathway is less structured in that each water is not

Fig. 5. Thermodynamic analysis of the rates of change in the absorbance spectrum that are correlated with the spontaneous conversion of the high-valent state of MauG to the diferric state. Plots are shown for the first reaction phase (dashed line) and the second reaction phase (solid line). The lines are fits of the data by Eq. 7.

Fig. 6. Proton inventories of the KSIE on the first reaction phase (A) and the second reaction phase (B). The data are fit by Eq. 5 for the model of a transition state with two pathways, each involving multiple PTs. The other lines are simulations of the expected plots for a PT in the transition state of a single pathway involving the transfer of one (red, Eq. 2), two (green, Eq. 3), or multiple (blue, Eq. 4) protons.

participating in multiple hydrogen bonds with multiple amino acid residues. As such, it is less likely to support proton tunneling and is likely described by the larger $\phi^{TS}$.

The importance of these residues and the water network of the distal pocket of the high-spin heme is supported by previous site-directed mutagenesis studies. E113Q and Q103N mutations each affected the stability and the rate of the spontaneous decay of the high-valent state. An E113Q mutation disturbed the hydrogen bonding network from the ferryl oxygen to Asn110 (28). A Q103N mutation altered the water network in that an additional ordered water is present in the place of the amide nitrogen of Gln103 (30). Pro107 which also resides in the heme pocket was also shown to be important. Mutations of this residue disrupted the position and orientation of Glu113, which in turn altered the water network (29). Mutations of Pro107 as well as a Q103A mutation also increased the susceptibility of the three Met residues to autooxidation (15, 29).

The first reaction phase exhibits a relatively low $E_a$, yet the protonation step is still a relatively slow reaction. A likely explanation is that the reactive high-valent species in the CR-stabilized ensemble is a very minor species. As such, the reaction rate would be limited by the availability of the reactive species. Potential candidates for this reactive species are the compound I* and compound ES* shown in Fig. 7, in which the relatively basic Fe$^{III}$-O$^-$ on the high-spin heme would be susceptible to protonation. Furthermore, the water network in Fig. 8B would need to be perfectly aligned for the multiple PTs along the pathway that are coupled to the protonation event. If the species most susceptible for protonation is a very minor component of the CR-stabilized population (i.e., $K_{eq1} << 1$), and if the waters are only present in the proper orientation for PT for a very small a fraction of the time (i.e., $K_{eq2} << 1$), then these factors will significantly reduce the observed rate of the first reaction phase (i.e., $k_{obs} \sim k \times K_{eq1} \times K_{eq2}$).

The second reaction phase during which CR stabilization is lost and the hemes are reduced to the diferric state is a more complex reaction. It requires addition of two electrons and a second proton, with loss of water. The similar KSIE and proton inventory results for this reaction as for the first phase suggests that the same network of waters and residues in the distal heme pocket are involved in the PT in this reaction. Analysis of MauG which was inactivated by cycling between the bis-Fe$^{IV}$ and diferric state in the absence of substrate revealed that Met108 was the residue most susceptible to oxidation during this process (17). Thus, it is proposed that Met108 is the source of electrons that reduce the bis-Fe$^{IV}$. Met108 is located 7.3 Å from the heme oxygen. It follows that this reaction step involves proton-coupled long-range ET. A proton-coupled ET model is also consistent with the proton inventory and thermodynamic analysis results. The contribution of the tunneling pathway for this reaction is 20% compared with 31% for the first step. An explanation for this is that the highly structured orientation of the tunneling pathway may be slightly altered once the ferryl heme oxygen is protonated in the first reaction phase. A small alteration would be consistent with the decrease in the contribution of the tunneling pathway in the second reaction phase. The lower dependence on the tunneling pathway in
the second reaction phase can explain the different temperature dependencies of the two reactions. The first step which was more dependent on tunneling exhibited a shallower temperature dependence (Fig. 5), as expected, because tunneling rates are typically temperature independent (39).

The second reaction phase exhibited a larger $E_a$ than the first. This may reflect the fact that this PT reaction is coupled to long-range ET from Met108. Furthermore, at the Met108 site the ET would need to be coupled to a water (and subsequent protonation) or hydroxide attack to stabilize the resulting methionine cation radical (41–43). It is interesting to note that Geng et al. (6) previously used ET theory (44) to analyze the temperature dependence of the rate of disappearance of the bis-Fe$V^\text{III}$ state. That analysis yielded an ET distance that matched the distance between the two heme iron and Met108, consistent with the rate describing a long-distance ET reaction. However, it also exhibited an anomalously large reorganization energy that was not consistent with a true ET reaction. This may be explained by the fact that this is not a true ET reaction but a more complex proton-coupled ET reaction.

Methods

Recombinant MauG was expressed in Paracoccus denitrificans and purified as described previously (1). Studies were performed in 10 mM potassium phosphate, pH 7.5. Formation of bis-Fe$V^\text{III}$ MauG was achieved by addition of formate to MauG-H$_2$O$_2$. Reactions were monitored spectrophotometrically in the NIR range using a Beckman Coulter DU 800 spectrophotometer and in the visible range using an HP8452A diode array spectrophotometer run by the OLIS SpectralWorks/GlobalWorks software. Kinetic data collected in the rapid-scanning mode were reduced by factor analysis using the singular-value decomposition (SVD) algorithm and then globally fit using the fitting routines of Biochem Biophys Acta 1458(1):26–27.


Quine DM, Sutton LD (1993) Theoretical basis and mechanistic utility of solvent iso-


Shin S, Yukil ET, Sehanobish E, Wilmut CM, Davidson VL (2014) Site-directed muta-
genesis of Gln103 reveals the influence of this residue on the redox properties and


Shin S, Feng M, Davidson VL (2013) Mutation of Trp(93) of MauG to tyrosine causes

loss of bound Ca(2+) and alters the kinetic mechanism of trypophan trypoph-

Shin S, et al. (2015) A T67A mutation in the proximal pocket of the high-spin heme of

MauG stabilizes formation of a mixed-valent Fe(II)/Fe(III) state and enhances charge

resonance stabilization of the bis(Fe$V^\text{III}$) state. Biochim Biophys Acta 1847(7):709–716.


Lardinois OM, Ortiz de Montellano PR (2004) Autoreduction of ferryl myoglobin:

Discrimination among the three tyrosine and two tryptophan residues as electron


Bell RL, Truong TN (1997) Primary and solvent kinetic isotope effects in the water-


Gerritsen D, Limbach HH (1984) Kinetic isotope effects and tunneling in cyclic double


Schöneich C (2005) Methionine oxidation by reactive oxygen species: Reaction mecha-

Pogocki D, Serdiuk K, Schoneich C (2003) Computational characterization of sulfur-

containing three-electron-bonded radicals in methionine and methionine-containing


Schöneich C, Pogocki D, Hug GL, Bobrobski K (2003) Free radical reactions of me-