Factors Controlling the Substrate Specificity of Peroxidases: Kinetics and Thermodynamics of the Reaction of Horseradish Peroxidase Compound I with Phenols and Indole-3-acetic Acids

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ABSTRACT: The rates of oxidation of reducing substrates by heme peroxidases have previously been thought to be controlled only by their ease of oxidation. In the present study, we have compared the kinetics and thermodynamics of the oxidation of indole-3-acetic acid and derivatives and of phenols by horseradish peroxidase. Different dependencies of the reaction rates on the thermodynamic driving force reveal substrate specificity controlled by the enzyme−substrate complexes dissociation constants (Michaelis−Menten constants) and by the reorganization energies of electron-transfer within those complexes.

Heme peroxidases are widespread in eukaryotes and prokaryotes (Everse et al., 1991). They catalyze the oxidation of a broad range of substrates by organic peroxides or hydrogen peroxide, usually but not always via free-radical intermediates. Except in a few cases, they do not exhibit saturation kinetics, suggesting a loose enzyme−substrate interaction, i.e., a large Michaelis−Menten K_m constant. Within some classes of substrates, it has been shown that the rates of reaction correlate with substituent parameters (Job & Dunford, 1976, Dunford & Adeniran, 1986, Candeias et al., 1996). However, the reactivity of the enzyme toward structurally unrelated substrates can only be compared on the basis of the thermodynamic driving force of the reaction. This requires the determination of the reduction potentials of the substrate radicals and of the enzyme active intermediates. The latter have been estimated from stopped-flow measurements (Hayashi & Yamazaki, 1979; He et al., 1996). Reduction potentials of unstable, organic radicals are best determined by pulse radiolysis (Wardman, 1989).

In the present study, we have compared the kinetics and thermodynamics of the oxidation of indole-3-acetic acid and derivatives and of phenols by horseradish peroxidase (HRP).[1] The results lead to a new model of substrate specificity of peroxidases which combines Michaelis−Menten kinetics and the Marcus treatment of electron-transfer (Marcus & Sutin, 1985).

EXPERIMENTAL SECTION

Materials. Peroxidase from horseradish was purchased from Sigma (type VI-A) and used without further purification. Phenols, from Aldrich and Sigma, were purified by recrystallization. The indole-3-acetic acids were purchased from Aldrich or prepared as described previously (Candeias et al., 1996). The concentration of sodium chlorite solutions was calibrated spectrophotometrically using $\epsilon = 92\ M^{-1}\ cm^{-1}$ at 292 nm (Eriksen et al., 1981). All solutions were prepared with water purified by a Millipore Milli-Q system.

Methods. Stopped-flow measurements used a Hi-Tech SF-61 DX2 sequential-mix instrument equipped with a 100 W Xe lamp and two (detection and reference) photomultipliers (Brandenburg type 4409). The solutions were prepared in 10 mM phosphate buffer at pH 7.0 containing 0.1 M potassium nitrate. The drive syringes and the reaction cell were maintained at 25 °C by circulating water. Sequential mix mode was used. HRP was mixed with stoichiometric amounts of hydrogen peroxide in the age-loop; the compound I solution formed this way was mixed with the reducing substrate after 1 s. The final concentration of HRP in the cell was typically $\approx 0.5\ \mu M$, and the reducing substrate was in 10-fold excess, at least. Under these conditions, the conversion of compound I to compound II, monitored at 426 nm, followed exponential kinetics. The observed rates (k_{obs}), determined by nonlinear least-squares fit, varied linearly with the concentration of reducing substrate. The rate of reaction of compound I with the substrates ($k_{cpd\ I}$) was determined from the slope of this line. Up to the fastest rates observed ($k_{obs} \approx 500\ s^{-1}$), no evidence for saturation kinetics was found.

Measurement of reduction potentials of radicals by pulse radiolysis were performed using a 6 MeV linear accelerator that delivered 0.6 μs pulses. Doses per pulse of ca. 1.5 Gy were used, which corresponds to a concentration of radicals of $\approx 1\ \mu M$. The redox equilibriation with a reference couple was determined by kinetic spectrophotometry, before any radicals could otherwise decay (tens of microseconds) (Wardman, 1989).

RESULTS

The catalytic cycle of heme peroxidases, is initiated by the rapid oxygen transfer from a peroxide to the ferric enzyme (formally a two-electron oxidation), followed by two consecutive one-electron transfer steps (Dunford, 1991). In this cycle, the enzyme goes through two intermediates in addition to the (resting) ferric state (E): compound I (cpd I) and compound II (cpd II). In these, the iron is in the ferryl

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[1] Abbreviations: HRP, horseradish peroxidase; cpd I, compound I; cpd II, compound II.

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Table 1: Rates of Reaction of HRP Compound I with Phenols and Indoleacetic Acids and the Mid-Point Reduction Potentials of the Respective Radicals at pH 7.0

substrate and substituent		E/V[a]	$k_{cpd\ I}$/M^{-1} s^{-1}
phenols			
1	H	0.97[b]	$(2.8 \pm 0.3) \times 10^6$ [f]
2	*m*-CHO	1.05	$(8.3 \pm 0.6) \times 10^4$ [h]
3	*m*-OH	0.91[c]	$(1.2 \pm 0.1) \times 10^7$ [h]
4	*p*-CH_3	0.87[b]	$(4.2 \pm 0.2) \times 10^7$ [f]
5	*p*-Cl	0.94[b]	$(2.4 \pm 0.4) \times 10^7$ [h]
6	*p*-CN	1.17[b]	$(1.5 \pm 0.1) \times 10^4$
7	*p*-COO^-	1.04[b]	$(4.7 \pm 0.1) \times 10^5$
8	*p*-F	0.93[b]	$(1.2 \pm 0.2) \times 10^7$ [i]
9	*p*-I	0.96[b]	$(7.2 \pm 1.5) \times 10^7$ [i]
10	*p*-NH_2	0.42[b]	$(2.3 \pm 0.4) \times 10^8$ [f]
11	*p*-C_6H_5	0.96[d]	4.0×10^7 [g]
12	*p*-OCH_3	0.72[b]	$(1.3 \pm 0.2) \times 10^8$ [f]
indoleacetic acids			
13	H	0.96[e]	$(3.8 \pm 0.1) \times 10^3$
14	5-Br	0.98[e]	$(9.6 \pm 0.9) \times 10^2$
15	5-HO	0.82 [e]	$(7.1 \pm 0.3) \times 10^6$
16	5-OCH_3	0.96 [e]	$(2.2 \pm 0.1) \times 10^4$
17	2-CH_3	0.94 [e]	$(4.4 \pm 0.1) \times 10^5$
18	2-CH_3, 5-OCH_3	0.92 [e]	$(1.7 \pm 0.1) \times 10^6$
19	2-CH_3, 5-OCH_3, 6-OCH_3	0.85 [e]	$(3.9 \pm 0.1) \times 10^7$

[a] Estimated uncertainty ± 10 mV. [b] From Lind et al. (1990). [c] Calculated from the value in Armstrong et al. (1996). [d] Calculated from the value in Easton et al. (1996). [e] Calculated from the value in Candeias et al. 1996). [f] from Job & Dunford (1976). [g] From Hodgson and Jones (1989). [h] Our values are ≈2-fold higher than those given by Job and Dunford (1976). [i] Our value is 1 order of magnitude higher than that from Hodgson and Jones (1989).

(Fe^{IV}=O) state; the additional oxidizing equivalent in compound I is stored as an organic radical which can be at an oxidizable amino acid, as in cytochrome *c* peroxidase (Sivajara et al., 1989) or as a porphyrin radical cation, as in the peroxidase from horseradish used in this study (Dolphin et al., 1971):

$$E + H_2O_2 \rightarrow \text{cpd I} + H_2O \quad (1)$$

$$\text{cpd I} + SH \rightarrow \text{cpd II} + S^\bullet \quad (2)$$

$$\text{cpd II} + SH \rightarrow E + S^\bullet + H_2O \quad (3)$$

The three states of the enzyme have characteristic, intense absorption spectra (extinction coefficients on the order of ≈10^5 M^{-1} cm^{-1}), which allow the individual reaction steps 1−3 to be monitored by stopped-flow with spectrophotometric detection (cf. Experimental Section). In Table 1, we have compiled data on the reactivity of compound I with phenols and indoleacetic acids ($k_{cpd\ I}$) obtained by this method. Rate constants spanning over 5 orders of magnitude were observed.

At pH 7, the reduction of compound I to compound II is accompanied by protonation of the enzyme, whereas one-electron oxidation of phenols and indoles is generally associated with deprotonation of the incipient radical cations. Phenol radical cations have pK_a < 0 (Dixon & Murphy, 1976), and the pK_a values of the indoleacetic acid radical cations are in the range <4−7.7 (Candeias et al., 1996). Thus, the oxidation of these substrates by compound I (eq 2), involves complex prototropic equilibria. In order to investigate the effect of proton transfer on the rate of reaction, we compared the rate of the reaction of compound I with a phenol (3-hydroxybenzaldehyde) and with indole-3-acetic acid in water and deuterium oxide. Both enzyme and substrate were equilibrated with the deuterated solvent for ca. 1 h prior to the measurement to allow exchange of all the mobile protons, including the proton that is lost upon oxidation of the substrate. Only small kinetic isotope effects were found: $k_H/k_D = 1.23 \pm 0.04$ and 1.34 ± 0.07 for 3-hydroxybenzaldehyde and indole-3-acetic acid, respectively. Kinetic isotope effects $k_H/k_D > 10$ have been found when the proton/deuteron are exchanged during the rate-determining step (Bishop & Davidson, 1995); therefore, the small k_H/k_D values observed here suggest a different mechanism.

The thermodynamic driving force for reaction of compound I with the reducing substrates (eq 2) is the difference between the mid-point potentials of the compound I/compound II (cpd I/cpd II) and substrate radical/substrate ($S^\bullet$, H^+/SH) redox couples:

$$\Delta E = E(\text{cpd I/cpd II}) - E(S^\bullet, H^+/SH) \quad (4)$$

[Mid-point potentials are defined as the potential at which the concentrations of reduced, and oxidized forms of the couple are equal (Wardman, 1989)]. The mid-point potential of compound I at pH 7 has been estimated as E(cpd I/cpd II) = 0.879 V (Hayashi & Yamazaki, 1979, He et al., 1996),[2] on the basis of redox equilibration with hexachloroiridate ($IrCl_6^{2-}/IrCl_6^{3-}$).

Reduction potentials of a series of phenoxyl radicals have been determined by pulse radiolysis (Table 1). Here, the reduction potential of the *m*-formylphenoxyl radical was determined by equilibration with chlorine dioxide ($E°[ClO_2^\bullet/ClO_2^-]$ = 0.934 V; Wardman, 1989) at pH 13. *m*-Hydroxybenzaldehyde and $NaClO_2$ (sum of concentrations 1 mM) were dissolved in 10 mM aqueous NaOH containing 0.05 M sodium azide and saturated with nitrous oxide. Under these conditions, *m*-hydroxybenzaldehyde deprotonated to give the *m*-formylphenolate anion. Irradiation of these solutions formed the azidyl radical $N_3^\bullet$ (Alfassi & Schuler, 1985) which reacted both with chlorite and the *m*-formylphenolate anion (*m*−CHO−C_6H_4−O^-). The formation of *m*-formylphenoxyl radical (*m*−CHO−C_6H_4−$O^\bullet$) was evidenced by the increase of absorbance at 400 nm, which was complete ≈100 μs after the pulse. This plateau value of the absorbance (A_{400nm}) was found to depend on the concentrations of *m*-formylphenolate anion and chlorite, showing that a redox equilibrium was established:

$$ClO_2^- + m\text{−CHO−}C_6H_4\text{−}O^\bullet \rightleftarrows ClO_2^\bullet + m\text{−CHO−}C_6H_4\text{−}O^- \quad (5)$$

A nonlinear least-squares fit of the appropriate equations (Wardman, 1989) to a plot of A_{400nm} vs $[ClO_2^-]/[m\text{−CHO−}C_6H_4\text{−}O^-]$ (not shown) yielded the equilibrium constant $K_5 = 0.688 \pm 0.071$. The pK_a of *m*-hydroxybenzaldehde was determined by measuring the absorption at five wavelengths (238, 258, 276, 316, and 358 nm) of 70 μM deaerated aqueous solutions, buffered with 5 mM sodium borate at pH values between 6 and 12. The measurements at all the wavelenghts yielded ideal pK curves with inflection points at 9.08 ± 0.01. This value and the redox equilibrium with chlorite at pH 13 were used to determine the mid-point

[2] All reduction potentials are relative to the normal hydrogen electrode.

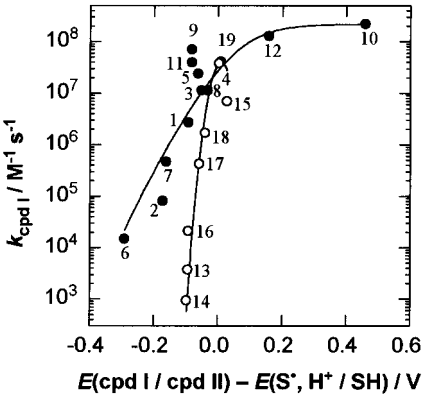

FIGURE 1: Dependence of the rate of reduction of horseradish peroxidase compound I ($k_{cpd\ I}$) by phenols (solid symbols) or indoleacetic acids (open symbols) on the thermodynamic driving force. The numbering of the points is given in Table 1 and the solid lines are the nonlinear least-squares fits described in the text with parameters given in Table 2.

potential of the *m*-formylphenoxyl radical at pH 7.0, E_7(*m*−CHO−C_6H_4−O$^•$, H$^+$/*m*−CHO−C_6H_4−OH) = 1.05 V.

The redox equilibria between radicals of indoleacetic acids and promethazine or hexachloroiridate were studied recently (Candeias et al., 1996). The reduction potential of the promethazine radical dication and the dependence on ionic strength have been subsequently re-evaluated (Candeias, 1997). Table 1 shows the revised values for the mid-point potentials of the indolyl radicals at pH 7.0 (Table 1) and ionic strength 0.1 mol dm^{-3}.

DISCUSSION

In Figure 1, the rates of reaction of HRP compound I with indoleacetic acids and phenols have been plotted against the thermodynamic driving force of the reaction at pH 7.0. Within each class of substrates (indoles or phenols) the reaction rates increase with increasing thermodynamic driving force. In addition, two other features are evident: (*i*) the dependence of the rates on the driving force is much steeper for the indoleacetic acids than for the phenols; and (*ii*) in the endothermic region the indoleacetic acids are much less reactive than phenols when the driving force of the reaction is similar. We conclude from these results that the rate of reaction is governed mainly by the oxidizability of the substrate within one substrate class, but substrates of different structure may be oxidized by compound I at very different rates, even if the thermodynamic driving force is similar. The enzyme does exhibit substrate specificity, at least with respect to the reduction of compound I.

Substrate specificity of an enzyme is a consequence of the formation of enzyme−substrate complexes with different dissociation constants corresponding to the Michaelis−Menten constants K_m. For the case of reduction of compound I:

$$\text{cpd I} + \text{SH} \rightleftarrows (\text{cpd I} - \text{SH}) \quad (6)$$

$$(\text{cpd I} - \text{SH}) \rightarrow \text{cpd II} + \text{S}^• \quad (7)$$

Saturation kinetics may be observed as a consequence of complex formation:

$$K_m = [(\text{cpd I} - \text{SH})]/\{[\text{cpd I}][\text{SH}]\} \quad (8)$$

$$k_{obs} = k_{ET}\,[\text{SH}]/\{K_m + [\text{SH}]\} \quad (9)$$

where k_{obs} is the observed rate constant at substrate concentration [SH] and k_{ET} is the rate of the electron (and proton) transfer step (eq 7). However, in this work, as well as in numerous previous studies (Dunford, 1991), no saturation kinetics were observed. Exceptions to this behavior have been reported for some substrates under particular conditions (Critchlow & Dunford, 1972), but in general k_{obs} depends linearly on the substrate concentration. This can be taken as evidence for large K_m values (loose enzyme−substrate complexes) such that:

$$k_{obs} \approx K_m^{-1} k_{ET}[\text{SH}] = k_{cpd\ I}[\text{SH}] \quad (10)$$

According to this hypothesis, the observed rate of reaction depends on (*i*) the dissociation constant of the enzyme−substrate complex (Michaelis−Menten constants K_m) and (*ii*) the rate of reaction of this complex. The first factor can explain the different rates observed with phenols and indoles of similar redox properties. However, the different slope of the semilogarithmic graph of the rate as a function of the driving force (Figure 1) is surprising.

In a pioneering study, Job and Dunford (1976) measured the rate of oxidation of a series of phenols and anilines by compound I and correlated them with Hammett (σ) or Brown−Okamoto (σ^+) substituent parameters. They found a linear correlation with σ but not with σ^+; since the Brown−Okamoto parameters introduce a correction for the stabilization of developing positive charges in the transition state, they concluded that the oxidation of the organic substrates by the enzyme intermediate proceeded either via electron transfer accompanied by proton transfer or via hydrogen atom transfer. This is consistent with the stoichiometry of the reaction (eq 2), which requires the deprotonation of the incipient substrate radical cation and the protonation of the enzyme. However, the small isotope effect observed in the present study implies that the proton transfer does not occur in the rate-determining step, but rather that the reaction is kinetically controlled by electron transfer. In the following, it will be demonstrated that the noncorrelation with σ^+ is a consequence of the electron-transfer mechanism.

According to the Marcus treatment of electron-transfer reactions, the steepness of the (parabolic) curve representing the dependence of the logarithm of the rate constant on the thermodynamic driving force is associated with the reorganization energy (λ), i.e., the energy necessary to take the reactants into the transition state conformation (Marcus & Sutin, 1985). This is expressed in eqs 11 and 12:

$$k_{ET} = k_0 \exp(-\Delta G^‡/RT) \quad (11)$$

$$\Delta G^‡ = \lambda(1 - F\Delta E/\lambda)^2/4 \quad (12)$$

where $\Delta G^‡$ is the activation free energy, k_0 is the rate of activationless electron transfer in the enzyme−substrate complex, *F* and *R* are the Faraday and gas constants, respectively, and *T* is the absolute temperature. The work terms have been neglected in first approximation, consistent with reaction within an enzyme−substrate complex. They would be expected to have a negligible effect on the steepness of the lines. Eqs 4 and 10−12 can be combined to yield an expression on the bimolecular rate of compound

Table 2: Reorganization Energies (λ) and Pre-Exponential Factors Estimated for the Oxidation of Substrates by Compound I

	λ/eV[a]	(k_0/K_m)/M^{-1} s^{-1}
phenols	0.5	5×10^9
indoleacetic acids	0.01	3×10^7

[a] 1 eV = 96.5 kJ mol^{-1}.

I with substrates (eq 13):

$$\log k_{\text{cpd I}} = \log(k_0/K_m) - (\lambda/4)(1 - \Delta E/\lambda)^2/0.059 \quad (13)$$

where ΔE is in V and λ is in eV. With electron-rich phenols, the rate of reaction has been suggested to approach a diffusion limit of $k_{\text{diff}} = 2.3 \times 10^8$ M^{-1} s^{-1}; this has been taken into account by calculating a corrected $k_{\text{cpd I}}'$ (eq 14):

$$1/k_{\text{cpd I}}' = 1/k_{\text{cpd I}} + 1/k_{\text{diff}} \quad (14)$$

The expression for $k_{\text{cpd I}}$ contains only two adjustable parameters, (k_0/K_m) and λ. A nonlinear least squares fit to the experimental points yielded the values listed in Table 2.

The reorganization energy for the oxidation of phenols estimated by this method ($\approx$0.5 eV) is on the order of the typical values for oxidation of organic compounds in aqueous solution. For example, recent measurements yielded λ = 0.6 eV (57.7 kJ mol^{-1}) for the electron transfer between phenoxyl radicals and phenylthiols (Armstrong et al., 1996). The value obtained with indoleacetic acids ($\approx$0.01 eV) is surprisingly low. In principle, different self-exchange rates between phenoxyl radicals/phenols and indolyl radicals/indoles could account for the observed differences in reorganization energy. To test this possibility we estimated the self-exchange rates (k_{11}) on the basis of the reported rates of reaction of forward (k_{12}) and reverse (k_{21}) electron transfer between $IrCl_6^{2-}$ and indole-3-acetic acids (Candeias et al., 1996). From the approximate cross-relation (eq 15) and the self-exchange rate of the couple $IrCl_6^{2-}/IrCl_6^{3-}$ ($k_{22} = 2 \times 10^5$ M^{-1} s^{-1}; Pelizzetti et al. 1979), we obtain $k \approx 2 \times 10^8$ M^{-1} s^{-1} for the self-exchange of indolyl radicals/indoles, a value typical for organic radicals (Armstrong et al., 1996).

$$k_{12} \approx (k_{11}k_{22}K_{12})^{1/2} \quad (15)$$

Studies by X-ray absorption (Penner-Hahn et al., 1986) and Mössbauer spectroscopy (Schulz et al., 1979) have shown no structural changes on the heme on reduction of compound I, compatible with a small reorganization energy. The electronic reorganization energy is also expected to be low since both compound I and compound II contain low-spin iron. Moreover, it is possible that the bound substrate minimizes even further the geometric rearrangements. From the low reorganization energy found in this study, it appears that the binding of the indoleacetic acids to the substrate pocket is in a geometry ideal for electron transfer. This brings the reorganization energy to the range of values associated with systems optimized for rapid electron transfer, such as the bacterial photosynthetic reaction center ($\leq$0.06 eV; Jia et al., 1993).

Despite the apparent optimization of the enzyme−indoleacetic acid complex in terms of minimization of reorganization energy, indoleacetic acids are oxidized slower by compound I than phenols forming radicals of similar reduction potential (Figure 1). This can be attributed to the (k_0/K_m) factor which, according to the fit procedure described, is 2 orders of magnitude lower for indoles than for phenols (Table 2). The reasons for this can be that either the indoleacetic acids bind at a larger distance from the heme or the Michaelis-Menten constants K_m for the enzyme−indoleacetic acids are larger. Rates of electron transfer decrease exponentially with increased distance between donor and acceptor, but NMR data suggest the heme−substrate distance is consistently $\approx$10 Å (Veitch, 1995). However, dissociation constants obtained from NMR and optical data vary from the nanomolar to the millimolar range, with indole-3-propionic acid at the top of this range (Veitch, 1995). This may explain the higher reactivity of phenols, despite the higher reorganization energy.

CONCLUSIONS

Evaluation of the kinetics and thermodynamics of the reduction of peroxidase compound I by phenols and indoleacetic acids reveals a much smaller reorganization energy in the latter case but also lower reactivity for equal thermodynamic driving force. These observations indicate the formation of enzyme−substrate complexes of different characteristics in each case. This hypothesis is supported by NMR data on some enzyme−substrate complexes (Veitch, 1995, Gilfoyle et al., 1996). We conclude that the substrate specificity of heme peroxidases is controlled by the reorganization energy of the electron-transfer step, which determines the redox dependence of the reaction rate within one substrate class, and by the dissociation constant of the enzyme−substrate complex (Michaelis−Menten constant), which influences the different reactivities of the enzyme toward structurally unrelated substrates.

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REFERENCES

Alfassi, Z. B., & Schuler, R. H. (1985) *J. Phys. Chem. 89*, 3359−3363.

Armstrong, D. A., Sun, Q., & Schuler, R. H. (1996) *J. Phys. Chem. 100*, 9892−9899.

Bishop, G. R., & Davidson, V. L. (1995) *Biochemistry 34*, 12082−12086.

Candeias, L. P. in *The Chemistry of N-Centered Radicals* (Alfassi, Z., Ed.) John Wiley & Sons, Chichester, U.K. (1997).

Candeias, L. P., Folkes, L. K., Porssa, M., Parrick, J., & Wardman, P. (1996) *Biochemistry 35*, 102−108.

Critchlow, J. E., & Dunford, H. B. (1972) *J. Biol. Chem. 247*, 3703−3713.

Dixon, W. T., & Murphy, D. (1976) *J. Chem. Soc., Faraday Trans. 2 72*, 1221−1230.

Dolphin, D., Forman, A., Borg, D. C., Fajer, J., & Felton, R. H. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 614−618.

Dunford, H. B. (1991) in *Peroxidases in Chemistry and Biology* (Everse, J., Everse, K. E., & Grisham, M. B., Eds.) pp 37−62, CRC Press, Boca Raton, FL.

Dunford, H. B., & Adeniran, A. J. (1986) *Arch. Biochem. Biophys. 251*, 536−542.

Easton, P. M., Simmonds, A. C., Rakishev, A., Egorov, A. M., & Candeias, L. P. (1996) *J. Am. Chem. Soc. 118*, 6619−6624.

Eriksen, T. E., Lind, J., & Merényi, G. (1981) *J. Chem. Soc., Faraday Trans. 1 77*, 2115−2123.

Everse, J., Everse, K. E., & Grisham, M. B. (1991) *Peroxidases in Chemistry and Biology*, CRC Press, Boca Raton, FL.
Gilfoyle, D. J., Rodriguez-Lopez, J. N., & Smith, A. T. (1996) *Eur. J. Biochem. 236*, 714−722.
Hayashi, Y., & Yamazaki, I. (1979) *J. Biol. Chem. 254*, 9101−9106.
He, B., Sinclair, R., Copeland, B. R., Makino, R., Powers, L. S., & Yamazaki, I. (1996) *Biochemistry 35*, 2413−2420.
Hodgson, M., & Jones, P. (1989) *J. Biol. Chem. 3*, 21−25.
Jia, Y., DiMagno, T. J., Chan, C. K., Wang, Z., Du, M., Hanson, D. K., Schiffer, M., Norris, J. R., Fleming, G. R., & Popov, M. S. (1993) *J. Phys. Chem. 97*, 13180−13191.
Job, D., & Dunford, H. B. (1976) *Eur. J. Biochem. 66*, 607−614.
Lind, J., Shen, X., Eriksen, T. E., & Merényi, G. (1990) *J. Am. Chem. Soc. 112*, 479−482.
Marcus, R. A., & Sutin, N. (1985) *Biochim. Biophys. Acta 811*, 265−322.
Pelizzetti, E., & Metasti, E. (1979) *Inorg. Chem. 18*, 583−588.
Penner-Hahn, J. E., Eble, K. S., McMurry, T. J., Renner, M., Balch, A. L., Groves, J. T., Dawson, J. H., & Hodgson, K. O. (1986) *J. Am. Chem. Soc. 108*, 7819−7825.
Schulz, C. E., Devaney, P. W., Winkler, H., Debrunner, P. G., Doan, N., Chiang, R., Rutter, R., & Hager, L. P. (1979) *FEBS Lett. 103*, 102−105.
Sivajara, M., Goodin, D. B., Smith, M., & Hoffman, B. M. (1989) *Science 245*, 738−740.
Veitch, N. C. (1995) *Biochem. Soc. Trans. 23*, 232−239.
Wardman, P. (1989) *J. Phys. Chem. Ref. Data 18*, 1637−1755.

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