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Dimerization of hydroxylated species of *m*-aminophenol by cytochrome *c* with hydrogen peroxide

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Abstract

The cytochrome *c* and hydrogen peroxide-dependent oxidation of *m*-aminophenol was investigated by electrochemistry and spectrophotometry. The results indicated that the hydroxylated species of *m*-aminophenol have at least two conjugated substituted groups on the ring system (most possibly, its oxidized form 2-hydroxy-4-iminoquinone), and that the degradation of cytochrome *c* by hydrogen peroxide can also be prevented in the presence of *m*-aminophenol. The hydroxyl radical scavengers, mannitol and sodium benzoate, almost completely eliminate the hydroxylation of *m*-aminophenol. But oxo-heme species scavenger, uric acid, does not inhibit the hydroxylation. Combining the results of mass spectrum, nuclear magnetic resonance and element analysis with that of spectrophotometry, electrochemistry and chemical scavengers, it is suggested that cytochrome *c* may act as a peroxidase, which facilitates the hydroxylation and subsequent dimerization of *m*-aminophenol.

Keywords: Cytochrome *c*; Hydrogen peroxide; Hydroxylation of aminophenol; Electrochemistry; Spectrophotometry; Dimerization

1. Introduction

It has been well known that cytochrome *P*450 mediates a wide variety of oxidation such as hydroxylation of aromatic compounds and hydrocarbons, epoxidation of olefins and oxidative *o*-dealkylation of ethers by activation of molecular oxygen [1,2]. These reactions, especially hydroxylation of aromatic compounds cannot easily be realized in purely chemical systems for generating active oxygen species in vitro [3–5]. The consensus mechanism for hydroxylation involves hydrogen atom abstraction from substrate by a high-valent iron-oxo intermediate followed by a bimolecular homolytic substitution step [6,7].

Cytochrome *c* or other hemoproteins can participate in the hydroxylation of *p*-nitrophenol [8], and oxidation of 2-keto-4-thiomethyl butyric acid [9], 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, 4-aminoantipyrine, and luminol [10], as well as the oxidation of *o*-methoxyphenol [11,12] in the presence of hydrogen peroxide. On the other hand, these proteins are degraded by hydrogen peroxide and hydroperoxides, with irreversible spectral changes [8,13]. However, the mechanisms of

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iron involvement and the active species participating have also not been well defined during the interaction of cytochrome *c* or other hemoproteins with hydrogen peroxide, hydroperoxides and reducing substrates. It is generally suggested that an excess of hydrogen peroxide degrades cytochrome *c* or other hemoproteins, releasing iron ions or mobilizing redox-active iron from the proteins that react with hydrogen peroxide to form a species as a hydroxyl radical or a bound hydroxyl radical ($Fe^{3+}-OH^{\cdot}$) or an oxo-heme complex ($Fe^{4+}=O$) [8–10,13–17]. Obviously, most details regarding the subject remain obscure.

In addition, hydrogen peroxide can be produced in tissues under a variety of conditions, in large part due to leakage from normal electron transport functions in mitochondria [18]. Yet, primary aromatic amines exhibit harmful toxicological properties [19]. Most aromatic amines are relatively inert and must be activated metabolically to form compounds which initiate chemical damage within organisms [20]. In many cases, this transformation is mediated by the cytochrome *P*450 located in the endoplasmic reticulum of liver and kidney cells. The more-polar compounds formed during the metabolic process may be eliminated from the body. These transformations may, in some cases, lead to the formation of species which are extremely reactive in the cellular environment [21,22].

Although aminophenol induces a type II spectral shift in the cytochrome *P*450 spectrum, a shift produced by a compound binding directly to the heme moiety of *P*450, cytochrome *P*450-catalyzed hydroxylation reaction of aminophenol cannot occur [23].

In the present investigation, we study the oxidation of *m*-aminophenol by cytochrome *c* with hydrogen peroxide using spectrophotometry and electrochemistry. The hydroxyl radicals were detected by their abilities to hydroxylate aromatic compounds with formation of specific hydroxylated product. Characterization of the isolated product indicated the hydroxylated product is dimerized.

2. Materials and methods

2.1. Materials

Cytochrome *c* (horse heart) from Sigma was used without further purification. Hydrogen peroxide (30%) from Beijing Chemicals was quantified by the titration method using $KMnO_4$ and by spectrophotometer with an absorption coefficient of 43.6 M^{-1} cm^{-1} at 240 nm [24,25]. 4*S*,4*S*′-dithiodipyridine (PySSPy) was obtained from Nakaral Chemicals. Mannitol, sodium benzoate, uric acid, and *m*-aminophenol were of analytical grade. All salts used were of analytical grade. All solutions were prepared with ultra-high purity water.

2.2. Instrument

Spectrometric studies were carried out with a Tracor Northern TN-6500 rapid scan spectrophotometer (Middleton, WI, USA) using 1 cm light path quartz cuvett. Electrochemical experiments were performed using a PARC model 173 potentiosat and a model 175 universal programmer (EG&G PARC, Princeton, USA). Cyclic voltammograms (CVs.) were recorded on a Gould 60,000 recorder. The electrochemical cell was a three-electrode system, the 4*S*,4*S*′-dithiobipyridine (PySSPy) modified gold electrode was used as a working electrode, a platinum foil as an auxiliary electrode, and an Ag/AgCl (saturated KCl) as a reference electrode. The potential of this reference electrode is +200 mV vs. the standard hydrogen electrode (SHE). All potentials are stated vs. an Ag/AgCl electrode. Analysis of the samples was performed on a Gilson gradient HPLC system (Gilson Medical Electronics) consisting of dual pumps and a Gilson 117 UV detector at 254 nm. The column used was a reverse-phase C18 column (4.0 mm × 200 mm i.d., 7 μm particle size) at a flow rate of 0.5 ml min^{-1}. The mobile phase was 50% methanol, 50% water. Element analysis was carried out at the Laboratory of National Spectrometry and Elec-

trochemistry in the Changchun Institute of Applied Chemistry. The mass spectrum was run on an LDI 1700-MALDI-TOF-MS. Nuclear magnetic resonance (NMR) spectra were run on a Varian Unity-400 MHz spectrometer. Spectra were obtained at room temperature from solutions of sample in methanol using tetramethylsilane as an internal standard.

2.3. Methods

The gold polycrystalline disc working electrode (99.99%, 1 mm diameter) sealed in a soft glass tube was polished with 1.0, 0.3 and 0.05 μm alumina paste to a mirror finish, and then was cleaned with hot sulfuric acid, washed by ultrasonication in high purity water. Prior to each experiment, the gold electrode was checked by comparing its electrochemical characteristics with the well-known CVs in the electrolyte solutions of either 0.5 M H_2SO_4 or 0.02 M $KClO_4$. Modification of PySSPy modified gold electrode for the direct electrochemistry of cytochrome *c* [26] was made by the dipping technique. The gold electrode was soaked in 1.0 mM PySSPy aqueous solution for 10 min after drying in air, and washed thoroughly with water to remove the weakly adsorbed PySSPy, then the modified electrode was placed in the normal electrochemical cell with 5 ml solution. All test solutions were prepared in 0.1 M phosphate buffer (pH 7.2) and purged with high purity argon. All experiments were carried out at room temperature (20 ± 1°C).

3. Results and discussion

3.1. Hydroxylation of m-aminophenol in the presence of hydrogen peroxide with cytochrome c

3.1.1. Electrochemical measurement for hydroxylation of m-aminophenol

It has been widely accepted that PySSPy modified gold electrode had the most effective promotion for cytochrome *c* in solution, and showing a reversible one-electron transfer process of the native protein [26–30]. The CV of 18.3 μM *m*-aminophenol on PySSPy modified gold electrode in 0.1 M phosphate buffer is shown in Fig. 1A. No observable redox peak current was obtained in the range of the potential applied, because the formal potential of *m*-aminophenol was 0.690 V [31]. After adding 64 μM cytochrome *c* into the solution, a typical reversible [32] waves corresponding to the one-electron redox process of heme Fe^{3+}/Fe^{2+} in cytochrome *c* was observed with the formal potential of 0.08 V, i.e., 280 mV vs. SHE [33], as shown in curve 1B. The peak current was

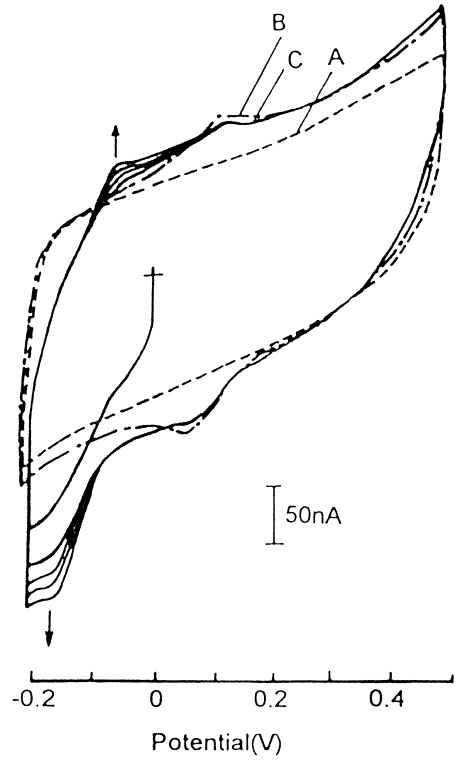


Fig. 1. The cyclic voltammograms for the hydroxylation of *m*-aminophenol during the reaction of cytochrome *c* and hydrogen peroxide. The working electrode was the 4*S*,4*S*′-dithiodipyridine modified electrode. The potential scan rate was 100 mV s^{-1}. All solutions contained 0.1 M phosphate buffer (pH 7.2). (A) 18.3 μM *m*-aminophenol; (B) 64 μM cytochrome *c* was added into the above solution; (C) 1.76 mM hydrogen peroxide was added into the above (B) solution again. The potential was successively swept.

Table 1
The scan rate relationship of the peak current for cytochrome *c*

ν (mV s^{-1})	$\nu^{1/2}$ [(mV $s^{-1})^{1/2}$]	i_p /nA
500	22.4	43.8
200	14.1	27.5
100	10.0	19.5
50	7.1	13.6

proportional to the square root of the potential scan rate (Table 1), which corresponded to a diffusion controlled process [32]. The CVs of cytochrome *c* in the presence of *m*-aminophenol was consistent with that in the absence of *m*-aminophenol.

As soon as 1.76 mM hydrogen peroxide was added into the bulk solution containing *m*-aminophenol and cytochrome *c*, and potential scan started simultaneously, an obvious change of CV was observed due to the addition of hydrogen peroxide (shown in curve C). An anodic peak around −0.06 V and a cathodic peak around −0.16 V increased gradually. The result exhibited a typical quasi-reversible [32] wave with the formal potential of −0.11 V, which displayed diffusional electron transfer. Meanwhile, the CV of cytochrome *c* was similar to that of curve B. In our previous report [16], we observed that the presence of hydrogen peroxide altered the CV of cytochrome *c*, the anodic peak of cytochrome *c* was obviously decreased, and a new anodic peak appeared at the more positive potential (0.34 V). Obviously, the quite different result was observed in presence of *m*-aminophenol. The above results suggested that the hydroxylation of *m*-aminophenol had taken place (identification of the hydroxylated species may be seen below) and it can be detected in situ by using cyclic voltammetry. On the other hand, electrochemical experiments showed no hydroxylation occurring in the absence of cytochrome *c* or hydrogen peroxide. Thus, the hydroxylating species were involved in the process, which may be produced through the interaction of cytochrome *c* and aminophenol with hydrogen peroxide.

3.1.2. UV–vis spectrometric measurement for hydroxylation of m-aminophenol

In order to further classify the redox couples with the formal potential of −0.110 V at curve C in Fig. 1, UV–visible spectra of the system were recorded, as shown in Fig. 2. Curve A showed the spectra of 9.6 μM ferricytochrome *c*. After addition of 0.417 mM *m*-aminophenol, no change was observed in the range of the wavelength recorded. After adding 4.0 mM hydrogen peroxide into the bulk solution containing *m*-aminophenol with cytochrome *c* and incubating for 2 min, a new absorption band appeared around 490 nm (shown in curve B). When the incubating time reached 10 min, the absorption band around 490 nm increased slightly (shown in curve B′). As the final concentration of hydrogen peroxide was increased to 0.018 M, the absorption band around 490 nm increased rapidly. But the absorption bands of ferricytochrome *c* at 408 and 531 nm were still observable (shown in curve C). It had been known that cytochrome *c* can be degraded by hydrogen peroxide and hydroperoxides, with ir-

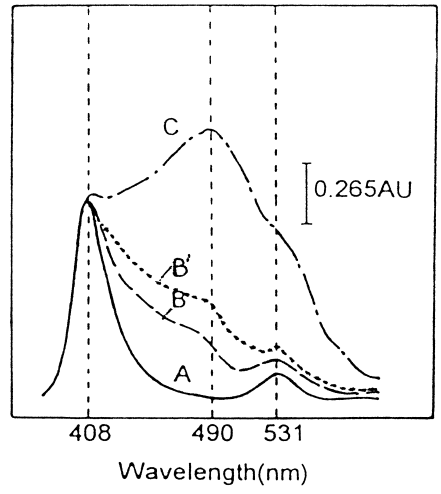


Fig. 2. UV–visible spectra for the hydroxylation of *m*-aminophenol by cytochrome *c* with hydrogen peroxide. All solutions contained 0.1 M phosphate buffer (pH 7.2). (A) Only 9.64 μM cytochrome *c*, or 0.417 mM *m*-aminophenol; (B) 4 mM hydrogen peroxide was added into the above solution and incubated for 2 min; (B′) the above solution (B) was incubated for 10 min; (C) the above solution (B) was added with hydrogen peroxide, whose concentration after mixing was 0.018 M, and incubating for 5 min.

reversible spectral changes and that a rapid bleaching reaction occurred immediately after mixing cytochrome *c* with hydrogen peroxide [8,13]. The above results exhibited that the degradation of cytochrome *c* can be prevented in the presence of *m*-aminophenol and its hydroxylated species, and the absorption maximum of the hydroxylated species was at 490 nm.

3.2. Inhibition for the hydroxylation of m-aminophenol

To estimate the kind of active species participating in the hydroxylation of aminophenol, a variety of scavengers were added into the aminophenol–cytochrome *c*–hydrogen peroxide system. The hydroxyl radical scavengers [8,14,16], mannitol and sodium benzoate at an excess concentration (0.1 M) almost completely eliminated the hydroxylation of *m*-aminophenol (Table 2) monitored at 490 nm, which suggested that a free hydroxyl radical was involved in the hydroxylation. Uric acid was an excellent scavenger of the oxo-heme species [10,34]. But no inhibition by uric acid ruled out the possibility of the oxo-heme species to be the hydroxylation agent, which showed that this system was quite different from the results in literatures [8,10,16] (in which a bound hydroxyl radical or a high oxidation state of an oxo-heme complex mediated the hydroxylation or oxidation reaction).

In general, cytochrome *c* can interact with hydrogen peroxide, giving rise to hydroxyl radical [13]. When cytochrome *c* reacted with hydrogen peroxide, it was proposed that a bound hydroxyl radical [8,13] formed by a site-specific reaction at the heme or activation of cytochrome *c* by hydrogen peroxide to a catalytically active species, a high oxidation state of an oxo-heme complex [10,16,17], mediated the oxidation or hydroxylation of substrates. In these cases, the hemoprotein was degraded and bleached by hydrogen peroxide with the apparent disappearance of the characteristic absorption bands for heme in the protein, which suggested that iron ions released or mobilized from the protein reacted with hydrogen peroxide to form the reactive species ($OH^{\cdot}$, Fe^{3+}–$OH^{\cdot}$ or $Fe^{4+}=O$) [8–10,13–17]. Identification of the hydroxyl radical was strengthened by its ability to hydroxylate *m*-aminophenol (giving dihydroxy aminobenzene) and its scavenging by the hydroxyl radical scavengers tested. But the absorption spectra in Fig. 2 of the reaction mixture indicated no apparent heme destruction. The electrochemical and spectrophotometric results also showed that hydrogen peroxide cannot oxidize *m*-aminophenol in the absence of cytochrome *c*. Thus, in the aminophenol–cytochrome *c*–hydrogen peroxide system, it was suggested that cytochrome *c* catalyzed hydrogen peroxide-dependent oxidation of *m*-aminophenol.

3.3. Preparation of the product of hydrogen peroxide-dependent oxidation of m-aminophenol

For the purpose of identifying the structure of the product of the hydroxylated species, preparative incubations of 50 ml contained 0.1 M potassium phosphate (pH 7.2), *m*-aminophenol

Table 2
The characteristics of the oxidized species of aminophenols

Oxidized species	λ_{max} (nm)	$E^{o'}$ (V)	Inhibition		
			Mannitol	Uric acid	Sodium benzoate
For *m*-aminophenol	490	−0.11	yes	yes	no

The system: 9.64 μM cytochrome *c*; 0.417 mM *m*-aminophenol; 0.018 M hydrogen peroxide.
Concentration of inhibitor added: 0.1 M mannitol; 0.1 M sodium benzoate; 0.05 M uric acid.
λ_{max} and $E^{o'}$ represent the maximum absorption wavelength and the formal potential.

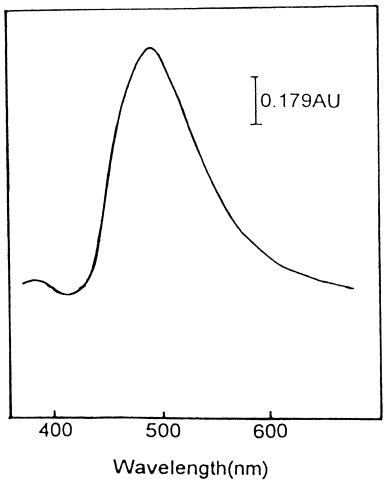


Fig. 3. UV–visible spectra for the isolated product of hydrogen peroxide-dependent oxidation of *m*-aminophenol. The solution contained 0.15 mM of the isolated product in 0.1 M phosphate buffer (pH 7.2).

(2.0 g), and hydrogen peroxide (2.0 ml, 30%) in a round-bottom flask equipped with a magnetic stirrer. The flask was chilled in an ice bath to approximately 10°C and cytochrome *c* (20 mg), dissolved in distilled water (1.0 ml), was added. After 15 min the ice bath was removed and the reaction was allowed to continue for 20 h. The $CH_2Cl_2/(CH_3)_2O$ (1:1, v/v) was added into the above solution to extract the hydroxylated species, and the organic phase was evaporated to dryness. The solid residue was recrystallized from dioxane/benzene giving the pure product (0.7 g, yield 35%). Fig. 3 shows the visible spectrum of the isolated product in 0.1 M phosphate buffer. An absorption band with maximum at 490 nm can be observed, which is in consistent with that of Fig. 2. The hydroxylated species should exist in its oxidized form in the presence of hydrogen peroxide. The results of the electrochemical study showed a quasi-reversible wave with the formal potential of -0.11 V also. Identification of the product may be seen below.

3.4. HPLC analysis

The HPLC separation of UV-absorbing *m*-aminophenol metabolites from hydrogen peroxide and cytochrome *c*-catalyzed reaction mixture, gives the retention times of *m*-aminophenol and the product as 5.65 and 9.86 min, respectively. These elution patterns of the compounds correspond exactly with the retention times of *m*-aminophenol and the above isolated product injected as methanol solutions. The HPLC analysis shows the isolated product as the only product.

3.5. Structure characterization of the isolated product

Mass spectrometry (MS) was used for molecular weight determination of the isolated product. As shown in Fig. 4, MS analysis of the isolated product indicated molecular ion consistent with the formation of one dimer (Mr. 242.4), which corresponds to a possible molecular formula, $C_{12}H_6N_2O_4$. Elemental analysis (EA) suggested a molecular formula, $C_{12}H_6N_2O_4$ (Calc.: C, 59.50; H, 2.50; N, 11.57; O, 26.43%. Found: C, 59.45; H, 2.58; N, 11.62%.). In previous studies, Tarcha et al.'s [35] result indicated that the product of the oxidation of *o*-phenylenediamine by hydrogen peroxide, catalyzed by horseradish peroxidase (HRP), was 2,3-diaminophenazine, which corresponds to a formula $C_{12}H_{10}N_4$ (Mr. 210). While Gallati and Brodbeck [36] assumed that 2,2′-diaminoazobenzene was the resulting product, which corresponds to a formula $C_{12}H_{12}N_4$ (Mr. 212). These previous reports indicated the HRP catalyzed

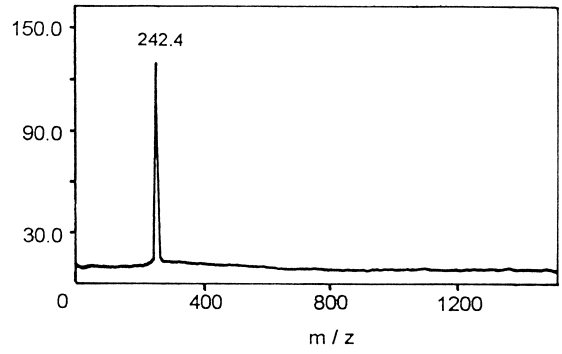


Fig. 4. Mass spectrum of the isolated product of *m*-aminophenol.

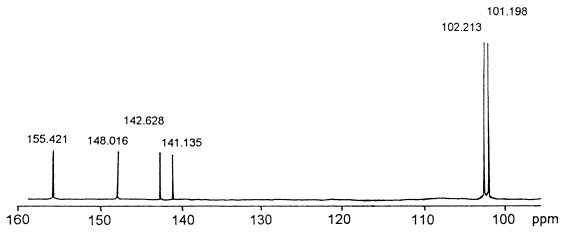
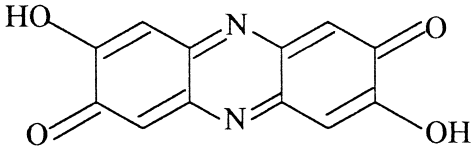


Fig. 5. Carbon-13 spectrum of the isolated product of *m*-aminophenol.

oxidation product consistent with the formation of a dimer. Our result exhibited that a dimer of the hydroxylated species of *m*-aminophenol perhaps was formed.

Carbon-13 NMR spectra of the isolated product are consistent with the more symmetrical structure. Fig. 5 shows the carbon-13 spectrum, which identifies the two methine carbons of the aromatic ring (102.213 and 101.198 ppm) and the four quaternary carbons of the aromatic ring (155.421, 148.016, 142.628 and 141.135 ppm). In a symmetrical structure, each signal corresponds to two carbon atoms; there are 12 carbons in the molecular structure of the product. The distortionless enhancement by polarization transfer (DEPT) pulse sequence [37] confirms the two methine carbons. Chemical shifts of the product suggest that phenazine ring involves in its structure, which includes 12 carbons.

Considering the results of MS, NMR, EA, UV–vis spectra, electrochemistry and chemical scavengers, a product structure such as Scheme 1 is conceivable. This product is named 2,6-diketo-3,7-di-hydroxy-phenazine. Its formation may contain two steps, *m*-aminophenol hydroxylates into 2-hydroxy-4-iminoquinone in the presence of hydrogen peroxide, and at the meantime, 2-hydroxy-4-iminoquinone polymerizes into 2,6-di-keto-3,7-di-hydroxy-phenazine.



Scheme 1.

4. Conclusion

Cytochrome *c* catalyzed the hydrogen peroxide-dependent oxidation and dimerization of *m*-aminophenol. A dimer of hydroxylated species of *m*-aminophenol was isolated from reaction mixtures. HPLC analysis indicated that *m*-aminophenol was converted to the dimer with ca. 100% selectivity. Chemical structure was determined by a combination of MS, NMR, EA, UV–vis spectra, electrochemistry and chemical scavengers. The results exhibit cytochrome *c* may act as a peroxidase during the hydroxylation and dimerization of *m*-aminophenol.

Acknowledgements

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References

[1] P.R. Ortiz de Montellano (Ed.), Cytochrome *P*450 Structure, Mechanism and Biochemistry, Plenum, New York, 1986.
[2] J.H. Dawson, Science 240 (1988) 433.
[3] J.R.L. Smith, P.R. Sleath, J. Chem. Soc., Perkin Trans. 2, (1983) 621.
[4] F. Chioccara, P. Di Gennaro, G. La Monica, R. Sebastiano, B. Rindone, Tetrahedron 47 (1991) 4429.
[5] W.-D. Woggon, H. Fretz, in: J.M. Coxon, (Ed.), Advances in Detailed Reaction Mechanisms, JAI Press, Greenwich, CT, Vol. 2, 1992, pp. 111–147.
[6] M. Newcomb, M.-H. Le Tadic-Biadatti, D.L. Chestney, E.S. Roberts, P.F. Hollenberg, J. Am. Chem. Soc. 117 (1995) 12085.
[7] M. Newcomb, M.-H. Le Tadic-Biadatti, D.A. Putt, P.F. Hollenberg, J. Am. Chem. Soc. 117 (1995) 3312.
[8] T.M. Florene, J. Inorg. Biochem. 23 (1985) 131.
[9] S. Harel, J. Kanner, Free Radic. Res. Commun. 5 (1988) 21.
[10] R. Radi, L. Thomson, H. Rubbo, E. Prodanov, Arch. Biochem. Biophys. 288 (1991) 112.
[11] A. Fujita, H. Senzu, T. Kunitake, I. Hamachi, Chem. Lett. (1994) 1219.
[12] I. Hamachi, A. Fujita, T. Kunitake, J. Am. Chem. Soc. 116 (1994) 8811.
[13] E. Cadenas, A. Boveris, B. Chance, Biochem. J. 187 (1980) 131.
[14] A. Puppo, B. Halliwell, Biochem. J. 249 (1988) 185.

[15] M.J. O'Connell, R.J. Ward, H. Baum, T.J. Peters, Biochem. J. 229 (1985) 135.
[16] Y. Zhu, J. Li, S. Dong, J. Chem. Soc., Chem. Commun. (1996) 51.
[17] P. Barr, R.P. Mason, J. Inorg. Biochem. 59 (1995) 441.
[18] I. Fridovich, in: D.L. Gilbert (Ed.), Oxygen and Living Processes, an Interdisciplinary Approach, Springer-Verlag, New York, 1981, pp. 250–272.
[19] J.L. Radomski, Annu. Rev. Pharmacol. Toxicol. 19 (1979) 129.
[20] C.L. Greenstock, in: Jennings, Cundall (Eds.), Progress in Reaction Kinetics, Pergamon, Oxford, 1982, pp. 73–135.
[21] J.R. Gillette, Drug Metab. Rev. 13 (1982) 941.
[22] M.R. Boyd, C.N. Statham, Drug Metab. Rev. 14 (1983) 35.
[23] J.B. Schenkman, H. Remmer, R.W. Estabrook, Mol. Pharmacol. 3 (1967) 113.
[24] M. Louis, Handbook of Analytical Chemistry, McGraw-Hill, New York, 1963.
[25] A.G. Hildebrandt, I. Rotts, M. Tjoe, G. Heinemeyer, Meth. Enzymol. 52 (1978) 345.
[26] I. Taniguchi, K. Toyosawa, H. Yamaguchi, K. Yasukouchi, J. Chem. Soc., Chem. Commun. (1982) 1032
[27] S. Srinivasan, Yu.A. Chizmadzhev, J.O'M. Bockris, B.E. Conway, E. Yeager, Comprehensive Treatise of Electrochemistry, Plenum, New York, Vol. 10, 1985.
[28] F.A. Schultz, I. Taniguchi (Eds.), Redox Mechanisms and Interfacial Properties of Molecules of Biological Importance, Proceedings of The Electrochemical Society, Pennington, NJ, Vol. 93–11, 1993.
[29] J. Bixler, G. Bakker, G. Mclendon, J. Am. Chem. Soc. 114 (1992) 6938.
[30] S.H. Willis, E.F. Bowden, G.J. Pielak, J. Inorg. Biochem. 51 (1993) 649.
[31] D.M. Radzik, P.T. Kissinger, Anal. Biochem. 140 (1984) 74.
[32] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Principles and Applications, Wiley, New York, 1980, pp. 213–236.
[33] R.H. Rice, Y.M. Lee, W.D. Brown, Arch. Biochem. Biophys. 211 (1983) 417.
[34] R.R. Howell, J.B. Wyngarden, J. Biol. Chem. 235 (1960) 3544.
[35] P.J. Tarcha, V.P. Chu, D. Whittern, Anal. Biochem. 165 (1987) 230.
[36] H. Gallati, H. Brodbeck, J. Clin. Chem. Clin. Biochem. 20 (1982) 221.
[37] D.M. Doddrell, D.T. Pegg, M.R. Bendall, J. Magn. Reson. 48 (1982) 323.