Metabolism-based Transformation of Myoglobin to an Oxidase by BrCCl₃ and Molecular Modeling of the Oxidase Form*

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The stoichiometric reductive debromination of BrCCl₃ to a trichloromethyl radical by myoglobin caused the prosthetic heme to become covalently cross-linked to the protein moiety and transformed myoglobin from an oxygen storage protein to an oxidase. This was shown in experiments in which oxygen consumption was measured during reoxidation of the altered myoglobin in the presence of ascorbate or an enzymatic reducing system containing diaphorase and NADH. Redox cycling eventually led to loss of the protein-bound heme adduct and oxidase activity of myoglobin. We have used molecular modeling and the known structure of the protein-bound heme adduct to identify probable mechanisms for transformation of myoglobin to an oxidase. Based on these modeling studies, the most likely structure of the experimentally observed adduct involves ligation to the heme iron of the nitrosonium atom of histidine 97 and/or that of histidine 64. The model structures revealed access of solvent to the heme active site, which could facilitate oxygen reduction. The transformation of myoglobin and perhaps other hemoproteins to oxidases may have toxicological importance in causing the tissue damage resulting from exposure to various xenobiotics and endogenous chemicals as well as explaining how hemoproteins are inactivated during catalysis.

The formation of altered heme products by active site-generated reactive intermediates is thought to account for the inactivation of hemoproteins, such as cytochrome P-450 and myoglobin, by a variety of chemicals (1). Previous studies of the major altered heme adducts formed from the reaction of BrCCl₃ with myoglobin (Scheme I, compounds 1–4) (2–4) unexpectedly revealed that a protein-bound heme adduct (compound 4), which accounted for 60% of the altered heme (5), was still active (6). Specifically, the protein-bound heme adduct could be reduced under anaerobic conditions to the ferrous form and rapidly reoxidized in the presence of air to the ferric form (6). This suggested that the protein-bound heme adduct had oxidase activity (4). These findings are consistent with a recent report on the H₂O₂-mediated transformation of myoglobin to an oxidase concurrent with the formation of a protein-bound heme adduct (7).

In this study, we report oxygen consumption catalyzed by this altered protein under multiple turnover conditions with a stoichiometry of an oxidase. Although the nature of the transformed protein has been previously discussed (4), this study is the first to describe the oxidase activity and to model the three-dimensional structure of the hydrated protein. The predicted structures can account for the oxidase activity. This study should lead to a better understanding of the molecular determinants controlling hemoprotein reactivity and their involvement in toxicological processes.

EXPERIMENTAL PROCEDURES

Materials—Diaphorase, catalase, superoxide dismutase and NADH, methylene blue, and sperm whale myoglobin were purchased from Sigma, Fluka, Calbiochem, Fisher, and U. S. Biochemical Corp., respectively.

General Methods—Spectra were taken on an 8450A diode array spectrophotometer (Hewlett-Packard) and an SLM Aminco DW-2000 spectrophotometer. NADH oxidation was measured by the loss of absorbance at 340 nm (ε = 6.22 mm⁻¹). Oxygen consumption was monitored with a Clarke-type electrode on a Yellow Springs Instruments 5300 monitor. In experiments in which the stoichiometry was determined, NADH oxidation and oxygen consumption were measured from the same sample with the use of a quartz cuvette fitted with an oxygen electrode. HPLC¹ was performed with the use of a Waters instrument (Millipore Corp., Milford, MA) consisting of a 600E gradient system controller and a 490E variable wavelength detector. The data were collected with the use of a Nelson 760 series system. All samples were dried under vacuum in a Speed Vac apparatus (Savant Instruments, Inc., Farmingdale, NY).

Preparation of BrCCl₃-altered Protein-bound Heme Adduct of Myoglobin—Photoreduced myoglobin (147 µM) was treated with BrCCl₃ (150 µM or 3 mM) under anaerobic conditions in 50 mM potassium phosphate, pH 7.4, as previously described (2, 3). After 60 min, the sample was opened to air and placed on ice. Unreacted BrCCl₃ was removed from samples that were treated with excess BrCCl₃ (3 mM) by chromatography on a Sephadex G-25M column (PD-10, Pharmacia LKB Biotechnology Inc.) equilibrated with water. A partially purified preparation of protein-bound heme adduct devoid of acid-dissociable heme products was prepared by 2-butanone extraction of the BrCCl₃-treated myoglobin reaction mixture (3, 8). Apomyoglobin was also prepared from untreated myoglobin by the same method and reconstituted with heme and acid-dissociable heme adducts as previously described (9), except that an apoprotein concentration of 0.42 mg/ml in a total volume of 2.5 ml was used. Unbound heme was removed by chromatography on a PD-10 column equilibrated with water. Samples were stored at 4 °C.

Measurement of Oxidase Activity of BrCCl₃-altered Myoglobin—Aliquots of the reaction mixture, apomyoglobin, partially purified protein-bound heme adduct, or apoprotein reconstituted with altered hemes were added to a cuvette containing a catalytic amount of diaphorase (100 µg/ml) and catalase (1.0 µg/ml) in a total volume of 1.0 ml of 40 mM potassium phosphate, pH 7.4. After preincubation for 5 min, ascorbate (10 mM) was added to start the reaction. An enzymatic reducing system containing diaphorase (100 µg/ml, 1.70 units), methylene blue (1.0 µM), superoxide dismutase (4.0 µg/ml, 14 units), and catalase (1.0 µg/ml, 67 units) was substituted for ascorbate in experiments where the stoichiometry was measured. In these experiments, the mixture was preincubated for 5 min before diaphorase was added. Oxygen consumption was monitored using a Clark-type oxygen electrode with a Clark-type oxygen electrode and a Yellow Springs Instruments 5300 monitor. The data were collected with the use of a Nelson 760 series system.

¹The abbreviations used are: HPLC, high performance liquid chromatography; SD, steepest descent; CG, conjugate gradient.
5.0 min, and then NADH was added (2.0 µl) at a final concentration of 120 µM to start the reaction. NADH oxidation, oxygen consumption, and visible spectra were measured on the same sample. As indicated in the text, experiments in the absence of superoxide dismutase and catalase were also performed.

Computer Simulations—Several heme-myoglobin adducts were studied using the molecular dynamics and modeling tools supported in the CHARMM program (10). The carboxymyoglobin model contained 2536 atoms with explicit hydrogen atoms. The simulations included 350 water molecules to effectively hydrate the protein (11). A surface aspartic acid at position 122 was replaced by an asparagine based upon incorrect sequence analysis of myoglobin (12). However, the modeling results should be insensitive to this mutation (13). The calculations used the Polygen all-atom parameter set (Polygen parameter file for CHARMM version 20), slightly modified so as to provide a planar heme in the native structure (14). Electrostatic interactions were simulated with a constant dielectric of 1.0. Electrostatic forces (not energies) were switched to zero from 8.0 to 12.0 Å, and Lennard-Jones interactions were "shifted" to zero at 12.0 Å.

The heme-myoglobin adducts were created from an initial structure of carboxymyoglobin derived from a 150-ps simulation performed previously at 300 K (11). The atomic coordinates were averaged over the final 100 ps of this simulation and energy-minimized. The CO ligand was deleted from this initial structure. A harmonic restraint of 0.2 kcal/mol/Å² was then imposed on the 459 backbone atoms (N, C, and Ca). The covalent bond between the heme iron and Nt of the proximal histidine (His-93) was broken using slow-growth homology modeling while performing 200 steps of steepest-descent (SD) energy minimization. That is, the energy of the system was described as a linear combination of the initial and final system energies. The weight given the energy of the final system (with iron-histidine bond broken) was continuously increased from zero to one. The energy was minimized while the bond was slowly replaced by nonbonded interactions.

This structure with the proximal bond broken was further energy-minimized with 100 steps of SD followed by 100 steps of conjugate-gradient (CG) energy minimization.

Nt of His-93 was then bound to the α-carbon of the heme vinyl, and the CCl₃ group was appended. These structural changes were "slowly" introduced by performing three consecutive sets of 100 steps of restrained SD with a harmonic restraint on all non-CCl₃ atoms of 20.0, 2.0, and 0.2 kcal/mol/Å², respectively. All atoms were then freed of harmonic restraint, and the conformational energy of the heme-myoglobin adduct was further minimized using 300 steps of CG.

The distance between the heme iron of the altered adduct and the N atom of the nearest histidines was 4.5, 5.1, and 6.3 Å for histidines 64, 93, and 97, respectively. We consequently studied six different iron-histidine linkages: His-64N₇, His-64N₆, His-93N₇, His-97N₆, His-97N₆, and the double linkage involving Nt of both His-64 and His-97. The adduct with no histidine bound to the iron was also analyzed. The initial myoglobin structure (with CO removed) was studied as a control. Although an improper dihedral term was added to the potential energy to enforce planarity of the proximal histidine bond to the vinyl carbon atom, no bias for planarity was introduced when binding histidines to the iron. Next, the hydrated structures were energy-minimized with 100 SD and 200 CG and allowed to relax during 5 ps of molecular dynamics at 300 K using a 1-fs time step. The structure with 2 histidines bound to the iron was formed by binding His-97 to the His-64-bound structure obtained after 5 ps of dynamics. An additional 5 ps of dynamics were then simulated with the 2 histidines bound to the iron. Finally, the eight structures were energy-minimized with 100 SD and 1000 CG. The resultant root mean square energy gradients were <0.073 kcal/mol/Å. Conformational strain of the heme was characterized by deleting all non-heme atoms and comparing the initial heme energy to that after 100 SD and 1000 CG in vacuum. The final root mean square energy gradients of the isolated hemes were <0.0006 kcal/mol/Å. Upon minimization, five of the eight heme complexes reached the same energy of 1.54 kcal/mol, presumably corresponding to the global energy minimum of heme. The other three heme energies were <2.8 kcal/mol. For each of the eight structures, the strain energy of the heme (Estrain) was taken to be the initial heme energy (after deleting all non-heme atoms) minus 1.54 kcal/mol.

RESULTS

Transformation of Myoglobin to Oxidase—NADH oxidation (Fig. 1A) and oxygen consumption (Fig. 1B) catalyzed by BrCCl₃-treated myoglobin in the presence of an NADH diaphorase metmyoglobin reducing system, which couples NADH oxidation to metmyoglobin reduction through methylene blue as an electron carrier, were measured. The BrCCl₃-treated myoglobin catalyzed NADH oxidation and oxygen consumption at a much higher rate than that of untreated...
myoglobin (control). However, because of the appreciable rates observed for the control sample, due to the autoxidation of methylene blue, the exact rates were not calculated. The stoichiometry of the reaction was determined by measuring the amount of oxygen consumed after complete utilization of NADH. The BrCCL$_3$-altered myoglobin oxidized 1 mol of NADH/mol of oxygen consumed, whereas in the presence of superoxide and catalase, 2 mol of NADH were consumed per mol of oxygen. These results are consistent with the formation of H$_2$O$_2$. The stoichiometry was doubled in the presence of catalase due to the dismutation of H$_2$O$_2$ to water and oxygen.

Characterization of Oxidase—The BrCCL$_3$-altered myoglobin reaction mixture was found to catalyze oxygen consumption in the presence of ascorbate (Fig. 1C). In contrast to the diaphorase system described above, only a slow rate of oxygen consumption and NADH oxidation was seen in control samples. Thus, ascorbate was used to further characterize the oxidase activity of the BrCCL$_3$-altered myoglobin. Under these conditions, the initial rate of oxygen consumed by BrCCL$_3$-treated myoglobin was 1.57 nmol/min/nmol of myoglobin, which is at least 12-fold over that of the rate of controls (0.13 nmol/min/nmol of myoglobin). This difference would be even greater if corrected for the rate of oxygen consumed by apoprotein solution (0.15 nmol/min/nmol). The rate of oxygen consumed for a partially purified preparation of protein-bound heme, devoid of heme or other dissociable heme products, could more than account for the rate observed for the reaction mixture (Fig. 1D). In addition, apomyoglobin reconstituted with heme and BrCCL$_3$-altered dissociable heme metabolites did not catalyze oxygen consumption (Fig. 1D).

To determine the effect of redox cycling on heme and the
altered hemes, aliquots from the reaction mixtures were taken before (Fig. 2A) and after (Fig. 2B) redox cycling with ascorbate. The protein-bound heme product (peak 4) was completely degraded after redox cycling of the reaction mixture (Fig. 2B). A partially purified preparation of protein-bound heme gave the same results when it was incubated with ascorbate (data not shown). Heme (peak 2) and the dissociable heme products (peaks 1 and 5, corresponding to compounds 1 and 5, respectively, of Scheme I) were resistant to degradation.

**Modeling of Oxidase**—Table I summarizes the results of the molecular modeling of the BrCCl₃-altered myoglobin. Although the covalent structure of the heme adduct and its ability to form a bishistidine complex have been described (4), the histidine residues involved in the complex have not been identified. After covalently bonding the ε-nitrogen of His-93 to the heme I vinyl, six different iron-histidine complexes were modeled. The histidine atom(s) bound to the iron were either the ε- or δ-nitrogen of residue 64, either the ε- or δ-nitrogen of residue 97, the δ-nitrogen of residue 93, or the ε-nitrogens of both residues 64 and 97. When ligated to the iron, the ε-nitrogens of histidines 64 and 97 produced a lower energy heme than did the δ-nitrogens. Consequently, not all complexes involving δ-nitrogens were modeled. The six iron-histidine structures, the adduct with no histidines bound, and the initial myoglobin structure were each characterized in terms of the distance (rₑ-Mb) between the heme iron and the center of mass of the globin matrix, the mass-weighted root mean square deviation (ΔₑMₑ) of the 2461 globin atoms from those of the x-ray structure (15), the mass-weighted root mean square out-of-plane distance (ΔₑAₑ) of the 25 central heme atoms, and the strain energy of the heme (Eₑₑₑₑₑₑ). The final conformational energy of the protein-water systems ranged from -12,336 to -12,284 kcal/mol. Direct comparison of these total energies is difficult because the differences in atomic connectivity among the structures correspond to different energy surfaces, and there was a large variation in the solvent energy.

As expected, the 93δ- adduct in which the proximal histidine was bound both to the heme vinyl group and to the heme iron exhibited high values of ΔₑAₑ and Eₑₑₑₑₑₑ, indicating a highly strained heme. The 64δ- adduct also had a nonplanar high-energy heme. Both the heme and the protein matrix were less perturbed in the 64ε-structure than in the 64δ-structure. Similarly, the 97ε-structure appeared more stable than the 97δ-structure. Of the two structures with a single Nε bound to the iron, that with His-97 bound showed the least heme deformation and therefore seems the most likely structure of the experimentally observed adduct. The bishistidine adduct with residues 64 and 97 bound to the iron showed the least heme strain of all the modified structures investigated.

**DISCUSSION**

Our laboratory has recently reported that oxidative modification of myoglobin by stoichiometric amounts of H₂O₂ leads to cross-linking of the heme to the polypeptide and transformation of this hemoprotein to an oxidase (7). In this study, we have shown that alteration of myoglobin by a trichloromethyl radical metabolite of BrCCl₃ similarly transformed the hemoprotein to an oxidase. A single turnover of BrCCl₃ (or, in effect, a stoichiometric amount of the trichloromethyl radical) was sufficient to give a protein-bound heme adduct, which accounted for 60% of the heme metabolized (Scheme I; for structure, see Scheme I, compound 4) (5). Unlike the other heme products that were not covalently bound to the protein (for structures, see Scheme I, compounds 1, 3, and 5), only covalently bound product catalyzed the oxidase reaction. We have not investigated whether covalent modification of the apoprotein, aside from the cross-linking of heme, plays a role in the oxidase state. However, it must have at most a minor role since the protein-bound heme adduct could more than account for the total oxidase activity observed from the BrCCl₃-treated myoglobin.

A more detailed investigation on the mechanism and identification of the molecular determinants involved in the transformation of myoglobin to an oxidase was undertaken with the use of the known structure of the adduct and with molecular modeling and simulations. The covalent structure of the
cross-linked heme has been elucidated by peptide mapping, mass spectrometry, and NMR as an adduct of an imidazole nitrogen of His-93 bound to the α-carbon of the heme ring I vinyl group, which has been altered by one carbon and two chlorines derived from BrCCl₃ (3, 4). This altered myoglobin has been shown to be more susceptible to hydrolysis by trypsin, an indication that tertiary structural changes to a less globular or more open state have occurred (6). Furthermore, spectral changes in the visible region indicated an alteration in the heme active site to a bishistidine complex, similar to that of cytochrome b₅ (4). However, unlike cytochrome b₅, the altered myoglobin complex could bind carbon monoxide, implying a weaker bis complex. With these considerations, we modeled the structures of the altered myoglobin (Fig. 3, B–D) using the tools supported in the CHARMM program and compared them to the crystal structure of native carboxymyoglobin. It was concluded that the most likely structure for the bishistidine adduct involves the ε-nitrogens of residues 64 and 97 (Fig. 3B). Of the modeled adducts, this bis complex showed the least heme strain in terms of the energy (Estrain) and the root mean square out-of-plane distance (Δ plane). The heme strain was reduced, in part, by the translation of the heme group relative to the protein such that the heme is only partially embedded within the protein (Fig. 3B). Prior to binding of carbon monoxide and presumably of oxygen as well, this six-coordinate complex must be transformed to a pentacoordinate complex probably by the loss of residue 64 (Fig. 3C). Histidine 97 appeared to bind more favorably to the iron as judged by the strain energy of the resulting heme complexes. The other possibility, with histidine 64 bound to the heme, is also shown (Fig. 3D). In any case, the resulting ferrous oxygen complex quickly autoxidized to form a superoxide anion radical with subsequent dismutation to H₂O₂, thereby giving rise to the oxidase activity.

Although autoxidation can occur with normal myoglobin, this rate is very slow compared to the rate catalyzed by the protein-bound heme adduct of myoglobin (at least 12-fold greater). The rate enhancement can be rationalized from the predicted structures of the altered myoglobins determined in this study (Fig. 3). The greater steric accessibility of the heme active site to water or anions is known to promote autooxidation of myoglobin and hemoglobin (16, 17). Anions are thought to facilitate autooxidation by nucleophilic attack at the ferrous deoxygenated heme to give an Fe²⁺ anion complex, which can then reduce oxygen by an as yet undefined mechanism (16). Alternatively, a direct reduction of solvent protons at the heme iron that could, in turn, reduce unbound oxygen has also been suggested (18). In either case, the greater accessibility of the solvent to the heme could readily be seen in our models since the calculations were performed on altered myoglobin hydrated by 350 water molecules to approximate the true nature of the fully hydrated protein. In fact, it appeared that a solvent channel leading from the aqueous environment to the heme active site was formed in both structures of the transformed protein (Fig. 3, C and D). It has been suggested from crystallographic and mutagenesis studies of cytochrome P-450CAM that formation of a solvent channel may serve to promote H₂O₂ formation in lieu of normal substrate metabolism (19). In addition, although specific calculations were not performed, the predicted structure of the transformed protein revealed that His-64 (Fig. 3C) or His-97 (Fig. 3D) may act as an endogenous nucleophile and promote autoxidation, as has been proposed for the Tyr and Asp site-directed mutagens of the distal histidine of myoglobin (17). Furthermore, the stabilization of the bound oxygen by hydrogen bonding to the distal histidine may be disrupted.

This study has shown that solvent is actively involved in the relationship between protein structure and function. The experimental and modeling results have demonstrated that a modified protein-bound heme structure can lead to a more solvent-exposed active site, resulting in dramatically different function. Moreover, since a variety of xenobiotics as well as endogenous compounds are known to cause the cross-linking of heme to protein (3, 6, 20–24), these modeling results take on added significance in light of the potential toxicological importance of this adduct in eliciting oxidative stress by formation of H₂O₂ (Scheme II).

Although we have focused on the formation of the oxidase state, the subsequent degradation of the protein-bound heme may also play a toxicological role in releasing free iron, a known prooxidant (Scheme II) (25–32). The destruction of the protein-bound heme adduct after multiple catalytic cycles is consistent with previous findings for the H₂O₂-mediated reaction (7). This degradation pathway involves the loss of the Soret absorbance of the altered heme, probably due to the oxidative destruction of the porphyrin ring. The oxidative degradation of cytochrome P-450 heme is known to lead to
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Fig. 3. Active site of myoglobin (control, A) and of predicted structures for histidine-ligated altered myoglobin (B), histidine-97 ligated altered myoglobin (C), and histidine-64 ligated altered myoglobin (D). The heme is depicted in green. Histidines 92, 97, and 64 are in blue, red, and yellow, respectively. Water molecules are shown with oxygen and hydrogen atoms in red and white, respectively.
formation of protein-bound products (21) as well as ring-cleaved products, such as hematinic acid, methylvinylmaleimide, and dipyrrolic propentdehydopents (33). Since cleavage of the heme ring requires multiple oxidative events, protein-bound heme adducts (by nature of their oxidase function) may be intermediates in the formation of ring-cleaved products for cytochrome P-450 as well as other hemoproteins (Scheme II).

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REFERENCES
1. Ortiz de Montellano, P. R., and Correia, M. A. (1983) Annu. Rev. Pharmacol. Toxicol. 23, 481-503
2. Osawa, Y., Higet, R. J., Murphy, C. M., Cotter, R. J., and Pohl, L. R. (1989) J. Am. Chem. Soc. 111, 4462-4467
3. Osawa, Y., Martin, B. M., Griffin, P. R., Yates, J. R., Shabanowitz, J., Hunt, D. F., Murphy, A. C., Chen, L., Cotter, R. J., and Pohl, L. R. (1990) J. Biol. Chem. 265, 10340-10346
4. Osawa, Y., Higet, R. J., Bax, A., and Pohl, L. R. (1991) J. Biol. Chem. 266, 3208-3214
5. Osawa, Y., Fellows, C., and Higet, R. J. (1992) in Synthesis and Application of Isotopically Labelled Compounds 1991, Proceedings of the Fourth International Symposium (Buncel, E., and Rabalda, G. W., eds) pp. 415-420, Elsevier Science Publishers B.V., Amsterdam
6. Osawa, Y., and Pohl, L. R. (1989) Chem. Res. Toxicol. 2, 131-141
7. Osawa, Y., and Korzekwa, K. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 7081-7085
8. Tenle, F. W. J. (1959) Biochim. Biophys. Acta 35, 543
9. Antonini, E., Brunori, M., Caputo, A., Chiancone, E., Panelli, A. R., and Wyman, J. (1964) Biochim. Biophys. Acta 79, 284-292
10. Brooks, B. R., Brucoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., and Karplus, M. (1988) J. Comp. Chem. 4, 187-217
11. Steinbach, P. J., Loncharch, R. J., and Brooks, B. R. (1991) Chem. Phys. 158, 383-394
12. Edmundson, A. B. (1965) Nature 206, 883-887
13. Phillips, G. N., Jr., Archini, R. M., Springer, B. A., and Sígar, S. G. (1990) Proteins: Structure, Function, and Genetics 7, 358-365
14. Loncharch, R. J., and Brooks, B. R. (1990) J. Mol. Biol. 215, 439-455
15. Korzun, J., Wilt, S., Kaplanski, M., and Petzko, G. A. (1986) J. Mol. Biol. 192, 133-154
16. Wallace, W. J., Houtchens, R. A., Maxwell, J. C., and Coughy, W. S. (1982) J. Biol. Chem. 257, 4966-4977
17. Springer, B. A., Egeberg, K. D., Sígar, S. G., Rohlf, R. J., Mathews, A. J., and Olson, J. S. (1990) J. Biol. Chem. 264, 3057-3060
18. Perutz, M. F. (1988) Trends Biochem. Sci. 14, 42-44
19. Raag, R., Martinis, S. A., Sígar, S. G., and Poulos, T. L. (1991) Biochemistry 30, 11429-11439
20. Davies, H. W., Brit, S. G., and Pohl, L. R. (1986) Chem.-Biol. Interact. 58, 345-362
21. Guengerich, F. P. (1986) Biochem. Biophys. Res. Commun. 138, 193-198
22. Correia, M. A., Decker, C., Sugiyma, K., Caldera, P., Bornheim, L., Wrighton, S. A., Rettie, A. E., and Trager, W. F. (1987) Arch. Biochem. Biophys. 258, 436-451
23. Catalano, C. E., Choe, Y. S., and Ortiz de Montellano, P. R. (1988) J. Biol. Chem. 264, 10534-10541
24. Ortiz de Montellano, P. R. (1990) Pharmacol. Ther. 48, 95-120
25. Trotta, R. J., Sullivan, S. G., and Stern, A. (1983) Biochem. J. 212, 759-772
26. Gutteridge, J. M. C. (1986) FEBS Lett. 201, 291-295
27. Puppo, A., and Hallibew, B. (1985) Free Radical Res. Commun. 4, 415-422
28. Frasset, M. R., Engelmann, R. M., Jones, R. M., and Das, D. K. (1989) Biochem. J. 263, 751-756
29. Ferral, M., Ciccotti, L., and Comporni, M. (1989) Biochem. Pharmacol. 38, 1819-1825
30. Sullivan, J. L. (1989) Am. Heart J. 117, 1177-1188
31. Reddy, B. R., Kiton, R. A., and Fryklenk, K. (1989) Free Radical Biol. & Med. 7, 45-52
32. Sakaida, I, Kyle, M. E., and Farber, J. L. (1990) Mol. Pharmacol. 37, 435-442
33. Schreger, W. H., Harris, T. M., and Guengerich, F. P. (1985) Biochemistry 24, 3254-3263