Mitochondrial Aconitase Is a Source of Hydroxyl Radical

AN ELECTRON SPIN RESONANCE INVESTIGATION*

Received for publication, September 21, 1999, and in revised form, February 23, 2000

Jeanette Vásquez-Vivar†‡§§, B. Kalyanaraman§*, and Mary Claire Kennedy¶

From the ‡Department of Pathology, Cardiovascular Research Center, the §Biophysics Research Institute, and the ¶Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Mitochondrial aconitase (m-aconitase) contains a [4Fe-4S]2+ cluster in its active site that catalyzes the stereospecific dehydration-rehydration of citrate to isocitrate in the Krebs cycle. It has been proposed that the [4Fe-4S]2+ aconitase is oxidized by superoxide, generating the inactive [3Fe-4S]1+ aconitase. In this reaction, the likely products are iron(II) and hydrogen peroxide. Consequently, the inactivation of m-aconitase by superoxide may increase the formation of hydroxyl radical (·OH) through the Fenton reaction in mitochondria. In this work, evidence for the generation of ·OH from the reaction of m-aconitase with superoxide is provided using ESR spin trapping experiments with 5-dithiooxophosphoryl-5-methyl-1-pyrroline N-oxide and α-phenyl-N-tert-butylnitrone. Formation of free ·OH was verified with the ·OH scavenger Me₆SO, which forms methyl radical upon reacting with ·OH. The addition of Me₆SO to incubation mixtures containing m-aconitase and xanthine/xanthine oxidase yielded methyl radical, which was detected by ESR spin trapping. Methyl radical formation was further confirmed using [¹³C]Me₆SO. Parallel low temperature ESR experiments demonstrated that the generation of the [3Fe-4S]1+ cluster increased with increasing additions of superoxide to m-aconitase. This reaction was reversible, as >90% of the initial ac- onitase activity was recovered upon treatment with glu-tathione and iron(II). This mechanism presents a scenario in which ·OH may be continuously generated in the mitochondria.

There is much debate in the literature on the relative importance of hydroxyl radical (·OH) and peroxynitrite in free radical pathology (1). Clarification of the mechanism centered on this subject is of considerable importance, especially in mitochondria, cellular organelles that are constantly exposed to low levels of superoxide anion (2, 3). Several neurodegenerative diseases (e.g. Alzheimer’s disease, Parkinson’s disease, and Lou Gehrig’s disease or amyotrophic lateral sclerosis) and aging have been linked to mitochondrial oxidative damage that results in decreased mitochondrial function (3, 4). However, in neurodegenerative diseases (e.g., Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis) and aging, the [4Fe-4S]2+ cluster increased (5, 6). The toxicological significance of these species is clearly dependent on cell type, the biological targets, and their relationship to one another. One of the sensitive biological targets in oxidative damage to mitochondria is aconitase, an iron-sulfur protein that catalyzes the stereospecific dehydration-hydration of citrate to isocitrate in the Krebs cycle (7).

Aconitase activity in mitochondria has been reported to be a sensitive redox sensor of reactive oxygen and nitrogen species in cells (8–11). Aconitase contains a cubane-type [4Fe-4S]2+ cluster in its active site with three iron atoms bound to cysteinyl groups and inorganic sulfur atoms and a fourth labile iron atom (Fe-α). This Fe-α is unique in that it is not bound to a protein cysteine, but rather to a hydroxyl group of substrate and water (7). The labile Fe-α is released upon oxidation of the [4Fe-4S]2+ cluster with the concomitant formation of inactive [3Fe-4S]1+ enzyme. Aconitase is inactivated rapidly by superoxide (k ~ 10⁷ M⁻¹ s⁻¹) (12) in the presence and absence of substrate and relatively slowly by peroxynitrite (k ~ 10⁵ M⁻¹ s⁻¹) and NO (13, 14). However, the reaction between aconitase and peroxynitrite is strongly inhibited by the addition of substrate that binds to the enzyme with high affinity (14).

It was recently proposed that the reaction between mitochondrial aconitase (m-aconitase) and superoxide plays a major role in mitochondrial oxidative damage (15–17). During this reaction, it has been proposed that iron is released from m-aconitase as iron(II) with the concomitant generation of hydrogen peroxide. This facilitates the formation of “free” hydroxyl radical in mitochondria. In the presence of intracellular reducing agents (e.g., glutathione, ascorbate, and NADPH), iron(II) is reincorporated into the inactive form of m-aconitase to regenerate the active form. According to this proposal, hydroxyl radical should be continuously generated in mitochondria as a result of the reaction between superoxide and aconitase. However, the experimental verification of this intriguing mechanism has so far been lacking.

The objective of this study is to provide evidence, using ESR, for the formation of hydroxyl radical and inactive [3Fe-4S]1+ species from the reaction between superoxide and purified m-aconitase. Direct low temperature ESR was used to quantify [3Fe-4S]1+ species. Hydroxyl radical was detected by ESR spin trapping using a novel phosphorylated spin trap, 5-dithiooxophosphoryl-5-methylpyrroline N-oxide (DEPMPO), and a loop-gap resonator, which makes it possible to obtain ESR spectra using exceedingly small amounts of enzyme (19). Our results indicate that the reaction between m-aconitase and superoxide releases iron(II) from the [4Fe-4S]2+ cluster, which

* This work was supported by National Institutes of Health Grants RR01008, GM51831, CA77822, and HL47250 and by American Heart Association Grant-in-aid 9950629N. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence and reprints should be addressed: Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226. Tel.: 414-456-8095; Fax: 414-456-6515; E-mail: jvvivar@mcw.edu.

‡ The abbreviations used are: m-aconitase, mitochondrial aconitase; DEPMPO, 5-dithiooxophosphoryl-5-methyl-1-pyrroline N-oxide; DMPO, 5,5-dimethyl-1-pyrroline N-oxide; PBN, α-phenyl-N-t-tert-butylnitrone.

§ Note that [3Fe-4S]1+ is paramagnetic and hence ESR-active, whereas [4Fe-4S]2+ is diamagnetic and ESR-inactive.
can subsequently catalyze the formation of free hydroxyl radical. The biological implications of these reactions are discussed.

**EXPERIMENTAL PROCEDURES**

**Materials and General Methods**—Bovine heart mitochondrial aconitase was purified according to published procedures (20). Protein purity ($\geq 95\%$) was determined by dye staining of SDS-polyacrylamide gels. The cluster content was measured from iron, sulfide, and protein content analyses as described previously (21, 22). Active enzyme was prepared immediately before use by anaerobically incubating the protein with iron and dithionite. To remove excess reagent, the preparation was desalted anaerobically on Sephadex G-50 columns equilibrated with buffer (50 mM phosphate, pH 7.4). The same protocol was followed for reactivation studies, except that dithiothreitol or glutathione replaced dithionite as the reductant. Enzyme activity was measured following the formation of cis-aconitate from isocitrate at 240 nm, and the concentration was calculated using an extinction coefficient of 3.6 mM$^{-1}$ cm$^{-1}$. The assay was performed at 25 °C in 90 mM Tris-HCl buffer, pH 8.0, containing 20 mM L-isocitrate. Rates of superoxide generation by xanthine (1–0.5 mM)/xanthine oxidase (0.1 unit/mg of solid) system (Roche Molecular Biochemicals catalog no. 1048180) were determined by following the reduction of ferricytochrome c (50 $\mu$m) at 550 nm, and the concentration was calculated using an extinction coefficient of 21 mM$^{-1}$ cm$^{-1}$.

**Electron Spin Resonance Measurements**—Direct ESR spectra were obtained at 10–15 K using calibrated quartz ESR tubes. The spectra were analyzed on a Varian E109 Century Series spectrometer operating at 9.17-GHz and 100-kHz field modulation. A frequency counter (EIP Model 548) was used to determine frequency, and a gaussmeter (MH-110R Radiopan, NMR magnetometer) was used to determine field positions. Instrumental conditions for detecting the [3Fe-4S]$^{1+}$ cluster were as follows: microwave power, 0.1 milliwatts; modulation amplitude, 5 G; time constant, 0.128 s; and scan rate, 0.83 G/s. Quantification of spin concentration was carried out using a 1.0 mM copper perchlorate standard by comparing the double integral of the spectra and standard under similar conditions. ESR spin trapping experiments were recorded at room temperature on a Varian E109 spectrometer operating at 8.9-GHz and 100-kHz field modulation equipped with a loop-gap resonator (18). The use of a loop-gap resonator enabled the utilization of very small quantities of purified enzyme. ESR spin trapping experiments were analyzed at room temperature, and the instrumental settings were as follows: microwave power, 2 milliwatts; modulation am-

![Fig. 1. ESR spectra of incubations of DEPMPO with superoxide in the absence and presence of m-aconitase.](image)
incubation mixtures of m-aconitase with superoxide diminished the yield of DEPMPO-OH by \(-58\%\) (Fig. 2, trace C; cf. trace A). Control experiments demonstrated that isocitrate had no effect on the formation of DEPMPO-OH from incubation mixtures containing iron and xanthine/xanthine oxidase (data not shown). It is known that binding of substrate stabilizes the \([4\text{Fe}-4\text{S}]^{2+}\) cluster of aconitate and thereby protects the enzyme against inactivation (7, 23). It is therefore likely that the inhibitory effect of isocitrate on the formation of DEPMPO-OH is due to a decreased rate of iron release from the \([4\text{Fe}-4\text{S}]^{2+}\) cluster.

To investigate the contribution of hydrogen peroxide formed from superoxide on iron release from m-aconitase, spin trapping experiments were performed with a bolus addition of hydrogen peroxide. The reaction between hydrogen peroxide and m-aconitase generated DEPMPO-OH (Fig. 2, trace D), which represented \(\sim31\%\) of the yield obtained during the reaction of m-aconitase with superoxide. This result is consistent with the relatively slower second-order rate constant for the reaction of hydrogen peroxide with aconitate (\(\sim\text{to} \cdot \text{m}^{-1} \cdot \text{s}^{-1}\)) as compared with the rate constant for the reaction between aconitate and superoxide (\(\sim10^3 \cdot \text{m}^{-1} \cdot \text{s}^{-1}\)). Taken together, these results indicate that the reaction between m-aconitase and superoxide is primarily responsible for the release of iron from the \([4\text{Fe}-4\text{S}]^{2+}\) cluster, which subsequently catalyzes the generation of hydroxyl radical.

Effect of Dimethyl Sulfoxide on Radical Adduct Formation—Because 5,5-dimethyl-1-pyrroline N-oxide (DMPO)- and DEPMPO-hydroxyl adducts can be formed through several mechanisms, additional experiments were performed using a hydroxyl radical scavenger, dimethyl sulfoxide (Me$_2$SO). It is well known that hydroxyl radical reacts with Me$_2$SO to form methyl radical (\(\text{CH}_3\)); and therefore, the detection of the DMPO- or DEPMPO-methyl adduct is a diagnostic indicator of free hydroxyl radical formation (Reaction 1).

$$
\text{DEPMPO} + \text{OH} + (\text{CH}_3)_2\text{SO} \rightarrow \text{CH}_3\text{SO}_2\text{H} + \text{CH}_3 + \text{DEPMPO-CH}_3
$$

As shown in Fig. 3B, the addition of Me$_2$SO to incubation mixtures of m-aconitase and superoxide produced a DEPMPO-carbon-centered adduct whose ESR parameters are consistent with trapping of methyl radical. The identity of the trapped radical was confirmed using $^{13}$C-labeled Me$_2$SO. Substitution of $^{[13]}\text{Me}_2\text{SO}$ for $^{[13]}\text{Me}_3\text{SO}$ generated the $^{13}$C-labeled DEPMPO-methyl adduct, DEPMPO-$^{13}$CH$_3$ (Fig. 3C). In additional experiments, the spin trap a-phenyl-N-tert-butylnitrimine (PBN), which forms persistent spin adducts with carbon-centered radicals, was substituted for DEPMPO. As shown in Fig. 3D, no ESR signal was detected from the reaction between m-aconitase and superoxide in the presence of PBN. This is consistent with the instability of PBN-oxygen-centered adducts at physiological pH. However, the addition of Me$_2$SO to the incubation mixtures generated a spectrum corresponding to the PBN-methyl adduct (Fig. 3E), and a $^{13}$C-labeled PBN-methyl adduct was detected with $^{[13]}\text{Me}_2\text{SO}$ (Fig. 3F). These results demonstrate that the reaction between m-aconitase and superoxide generates free hydroxyl radical.

Direct ESR Analysis of \([3\text{Fe}-4\text{S}]^{2+}\) Cluster Formation during Reversible Inactivation of Mitochondrial Aconitate by Superoxide—The characterization and quantitative analysis of \([3\text{Fe}-4\text{S}]^{2+}\) species generated from the reaction of m-aconitase with superoxide were based on the comparative analysis with standards obtained from the stoichiometric oxidation of m-aconitase with ferricyanide (24) (Table I, part A). Aliquots from incubation mixtures of m-aconitase with superoxide were taken at
different time points and immediately frozen in liquid nitrogen to quench the reaction. As shown in Table I (part B), incubation of m-aconitase (100 μM) with superoxide generated at the rate of 95 μM min⁻¹ quantitatively oxidized the [4Fe-4S]²⁺ cluster to the [3Fe-4S]¹⁺ species and inactivated the enzyme. After 5 min of incubation, m-aconitase was fully converted to the [3Fe-4S]¹⁺ form, and only a residual enzyme activity was detected (Table I, part B). This result suggests that superoxide stoichiometrically oxidizes the [4Fe-4S]²⁺ cluster to form [3Fe-4S]¹⁺ and that a continued exposure of the enzyme to superoxide and hydrogen peroxide does not disassemble the cluster. As [3Fe-4S]¹⁺ upon reduction can incorporate iron(II) to regenerate the active [4Fe-4S]²⁺ form, the reversibility of the oxidation of m-aconitase by superoxide was investigated by examining the recovery of enzyme activity. The samples used in low temperature experiments were thawed and reactivated by anaerobic reduction with either glutathione (5 mM) or dithiothreitol (10 mM) in the presence of iron(II). As shown in Table I (part B), reactivation of samples from a 5-min incubation of m-aconitase with superoxide led to the recovery of 90% of the initial enzyme activity. This result demonstrates that inactivation of m-aconitase generates [3Fe-4S]¹⁺ and is therefore a reversible reaction. The addition of isocitrate (1 mM) to incubation mixtures of m-aconitase did not prevent superoxide-mediated oxidation of the cluster and loss of activity. This result indicates that although isocitrate causes a decrease in the formation of hydroxyl radical (Fig. 2), the extent of the inhibition is not high enough to prevent the inactivation of the enzyme. Under these conditions, therefore, the recovery of enzyme activity was maximal (Table I, part B).

As shown in Fig. 1, oxidation of m-aconitase by superoxide generated hydroxyl radical, a strong oxidant that can further oxidize amino acids critical for m-aconitase function. To investigate whether or not hydroxyl radical generated from m-aconitase contributes to the inactivation of m-aconitase by superoxide, the effect of hydroxyl radical scavengers on inactivation and reactivation of the enzyme was investigated. The addition of Me₂SO (10%, v/v) or DEPMPO (0.1 M) did not diminish superoxide-mediated formation of inactive [3Fe-4S]¹⁺ m-aconitase, and neither DEPMPO or Me₂SO prevented enzyme inactivation. A slight increase in m-aconitase activity was de-
Superoxide Reacts with Aconitase to Generate Hydroxyl Radical

**Table I**

| Experiment | Incubation time | [3Fe-4S]^{1+} | Residual activity | Recovered activity |
|------------|----------------|--------------|------------------|-------------------|
| A. Control | min            | %            | %                | %                 |
| B. m-aconitase (100 μM) | 0 | 3.8 | 100 | 90 ± 6 |
| + O₂ (95 μM min⁻¹) | 5 | 100 | <5.0 | 90 ± 6 |
| + Iodotride | 10 | — | ND | 77 ± 8 |
| + Me₆SO (10% v/v) | 5 | 95 | <2.0 | 100 |
| + DEPMPO (0.1 mM) | 5 | 99 | <2.0 | 99 |
| C. m-aconitase (10 μM) | 5 | 100 | — | — |
| + O₂ (95 μM min⁻¹) | 5 | 100 | ND | 70 |
| D. m-aconitase (100 μM) | 5 | 100 | <1.0 | 58 |

**Scheme 1.** Mechanism of superoxide-mediated generation of hydroxyl radical from m-aconitase (modified from Ref. 16).

The effect of superoxide on [3Fe-4S]^{1+} formation and the recovery of enzyme activity was further investigated in incubation mixtures containing m-aconitase at 10-fold lower concentrations than described above (Table I, part C). Under this condition, m-aconitase was oxidized to the [3Fe-4S]^{1+} species, but the recovery of enzyme activity was lower than that previously detected (Table I, part C; cf. part B). Hydrogen peroxide (1 mM) also oxidized [4Fe-4S]^{2+} aconitase to form the [3Fe-4S]^{1+} species. However, the recovery of enzyme activity was much lower than that measured for superoxide under similar incubation conditions (Table I, part D). These results indicate that inactivation of m-aconitase by hydrogen peroxide generated from superoxide dismutation or added as a bolus is due to oxidation of the [4Fe-4S]^{2+} cluster, leading to protein destabilization.

**DISCUSSION**

Superoxide-mediated Redox Cycling of Mitochondrial Aconitase—The intriguing hypothesis on the reaction between superoxide and [4Fe-4S]^{2+} clusters of dehydratases leading to hydroxyl radical formation has been previously proposed (12, 26). However, to date, the experimental verification of this hypothesis has not been provided. In this study, we present, for the first time, ESR spin trapping evidence for the generation of hydroxyl radical during superoxide-induced oxidation of m-aconitase (Figs. 1 and 3). This work was made possible because of recent breakthroughs in ESR technology and spin trap synthesis. First, the use of a loop-gap resonator in spin trapping has made it feasible to obtain ESR spectra from exceedingly small sample volumes (~10 μl) (27). As a result, we were able to perform a large number of experiments using highly purified m-aconitase. Clearly, this would not be possible with conventional ESR using cavity resonators since they use large sample volumes. Second, the newly synthesized DEPMPO spin trap reacts with superoxide to form a persistent DEPMPO-OOH adduct (28). Unlike DMPO-OOH, the DEPMPO-OOH adduct does not spontaneously decay to form the DEPMPO-OH adduct (18, 27). In this study, in which a mixture of superoxide and hydroxyl radical was generated, the use of DEPMPO has made spin trapping interpretations less confusing.

In addition to hydroxyl radical trapping, we demonstrated that the [4Fe-4S]^{2+} aconitase is quantitatively oxidized to the [3Fe-4S]^{1+} cluster by superoxide (Fig. 1 and Table I). From monitoring the ESR-active [3Fe-4S]^{1+} aconitase, we showed that superoxide releases only one iron atom from the [4Fe-4S]^{2+} cluster (Table I), suggesting that the reaction between superoxide and m-aconitase is stoichiometric. As shown in Table I, this was also confirmed by measuring the activity recovery. It is known that in the presence of cellular reductants such as thiols, m-aconitase recycles iron by incorporating iron(II) and enhances the generation of hydroxyl radical.

The effect of superoxide on [3Fe-4S]^{1+} formation and the recovery of enzyme activity was further investigated in incubation mixtures containing m-aconitase at 10-fold lower concentrations than described above (Table I, part C). Under this condition, m-aconitase was oxidized to the [3Fe-4S]^{1+} species, but the recovery of enzyme activity was lower than that previously detected (Table I, part C; cf. part B). Hydrogen peroxide (1 mM) also oxidized [4Fe-4S]^{2+} aconitase to form the [3Fe-4S]^{1+} species. However, the recovery of enzyme activity was much lower than that measured for superoxide under similar incubation conditions (Table I, part D). These results indicate that inactivation of m-aconitase by hydrogen peroxide generated from superoxide dismutation or added as a bolus is due to oxidation of the [4Fe-4S]^{2+} cluster, leading to protein destabilization.
rate (29) or ATP or ADP (30). These iron complexes and iron bound to DNA (31) represent a redox-active pool of iron capable of catalyzing free radical reactions including the site-specific generation of hydroxyl radical. In this study, we have shown that m-aconitase is a plausible physiological source of free iron during oxidative stress resulting from increased superoxide formation.

**Summary**—We have demonstrated that the reaction between m-aconitase and superoxide generates free hydroxyl radical. Superoxide releases iron(II) from the [4Fe-4S]2+ aconitate, forming the inactive [3Fe-4S]1+ aconitase and hydrogen peroxide, thus facilitating hydroxyl radical formation by the Fenton reaction. It is likely that the reaction between superoxide and m-aconitase may enhance mitochondrial oxidative damage associated with the pathophysiology of several chronic neurodegenerative and cardiovascular diseases.

**Acknowledgment**—We thank Dr. William E. Antholine for assistance in low temperature ESR experiments.

**REFERENCES**
1. Liochev, S. I., and Fridovich, I. (1999) *Free Radical Biol. Med.* 26, 777–778
2. Boveris, A., and Chance, B. (1973) *Biochem. J.* 156, 435–444
3. Wallace, D. C. (1999) *Science* 283, 1482–1488
4. Dykens, J. A. (1997) in *Mitochondria and Free Radicals in Neurodegenerative Diseases* (Flint, M. B., Howell, N., and Bodis-Wollner, I., eds) pp. 29–55 Wiley-Liss, New York
5. Huie, R. E., and Padjama, S. (1993) *Free Rad. Res.* 18, 195–199
6. Kissner, R., Nauser, T., Bugnon, P., Lye, P. G., and Koppenol, W. (1997) *Chem. Res. Toxicol.* 10, 1285–1292
7. Beinert, H., Kennedy, M. C., and Stout, C. D. (1996) *Chem. Rev.* 96, 2335–2373
8. Gardner, P. R., and Fridovich, I. (1991) *J. Biol. Chem.* 266, 19328–19333
9. Eisenstein, R. S., Kennedy, M. C., and Beinert, H. (1998) in *Metal Ions in Gene Regulation* (Silver, S., and Walden, W., eds) pp. 157–216, Chapman and Hill, Inc., New York
10. Gardner, P. R., and Fridovich, I. (1992) *J. Biol. Chem.* 267, 8757–8763
11. Gardner, P. R., Nguyen, D. D., and White, C. W. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 12248–12252
12. Flint, D. H., Tuminello, J. F., and Emptage, M. (1993) *J. Biol. Chem.* 268, 22369–22376
13. Castro, L., Rodriguez, M., and Emptage, M. (1994) *J. Biol. Chem.* 269, 29409–29415
14. Kennedy, M. C., Antholine, W. E., and Beinert, H. (1997) *J. Biol. Chem.* 272, 20340–20347
15. Fridovich, I. (1997) *J. Biol. Chem.* 272, 18515–18517
16. Gardner, P. R., Raineri, I., Epstein, L. B., and White, C. W. (1995) *J. Biol. Chem.* 270, 13399–13405
17. Liochev, S. I. (1996) *Free Radical Res.* 25, 369–384
18. Vásquez-Vivar, J., Hogg, N., Martásek, P., Karoui, H., Tordo, P., Pritchard, K. A., Jr., and Kalyanaraman, B. (1999) *Free Rad. Res.* 31, 607–617
19. Kennedy, M. C., Emptage, M. H., Dreyer, J.-L., and Beinert, H. (1983) *J. Biol. Chem.* 258, 11098–11105
20. Rozicka, F. J., and Beinert, H. (1978) *J. Biol. Chem.* 253, 2514–2517
21. Beinert, H., Emptage, M. H., Dreyer, J.-L., Scott, R. A., Han, J. E., Hodgson, K. O., and Thomson, A. J. (1983) *Proc. Natl. Acad. Sci. U. S. A.* 80, 393–396
22. Flint, D. H., and Allen, R. M. (1986) *Chem. Rev.* 86, 2315–2334
23. Kennedy, M. C., and Beinert, H. (1988) *J. Biol. Chem.* 263, 8194–8198
24. Brazzolotto, X., Gaillard, J., Pantopoulos, K., Hentze, M. W., and Moulis, J.-M. (1999) *J. Biol. Chem.* 274, 21625–21630
25. Liochev, S. I., and Fridovich, I. (1994) *Free Radical Biol. Med.* 16, 29–33
26. Frønck, W., and Hyde, J. S. (1982) *J. Magn. Reson.* 47, 515–521
27. Fréjaville, C., Karoui, H., Tuccio, B., LeMoigne, F., Culcasi, M., Pietri, S., Lauricella, R., and Tordo, P. (1995) *J. Med. Chem.* 38, 258–265
28. Konorev, E. A., Kennedy, M. C., and Kalyanaraman, B. (1999) *Arch. Biochem. Biophys.* 368, 421–428
29. Minotti, G., and Aust, S. D. (1987) *Free Radical Biol. Med.* 3, 379–387
30. Vile, G. F., and Winterbourn, C. C. (1988) *Biochem. Pharmacol.* 37, 2883–2897
31. Mello-Filho, A. C., and Meneghini, R. (1991) *Mutat. Res.* 251, 169–113
Mitochondrial Aconitase Is a Source of Hydroxyl Radical: AN ELECTRON SPIN RESONANCE INVESTIGATION
Jeannette Vásquez-Vivar, B. Kalyanaraman and Mary Claire Kennedy

J. Biol. Chem. 2000, 275:14064-14069.
doi: 10.1074/jbc.275.19.14064

Access the most updated version of this article at http://www.jbc.org/content/275/19/14064

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 29 references, 14 of which can be accessed free at http://www.jbc.org/content/275/19/14064.full.html#ref-list-1