Compartment-specific Protection of Iron-Sulfur Proteins by Superoxide Dismutase*

Received for publication, July 16, 2003, and in revised form, September 10, 2003
Published, JBC Papers in Press, September 12, 2003, DOI 10.1074/jbc.M307700200

Fanis Missirlis‡, Jianguo Hu‡, Kim Kirby§, Arthur J. Hilliker‡, Tracey A. Rouault†, and John P. Phillips§

From the §Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20892, the ‡Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario N1G 2W1, Canada and the ¶Department of Biology, York University, Toronto, Ontario M3J 1P3, Canada

Iron and oxygen are essential but potentially toxic constituents of most organisms, and their transport is meticulously regulated both at the cellular and systemic levels. Compartmentalization may be a homeostatic mechanism for isolating these biological reactants in cells. To investigate this hypothesis, we have undertaken a genetic analysis of the interaction between iron and oxygen metabolism in *Drosophila*. We show that *Drosophila* iron regulatory protein-1 (IRP1) registers cytosolic iron and oxidative stress through its labile iron sulfur cluster by switching between cytosolic aconitase and RNA-binding functions. IRP1 is strongly activated by silencing and genetic mutation of the cytosolic superoxide dismutase (*Sod1*), but is unaffected by silencing of mitochondrial *Sod2*. Conversely, mitochondrial aconitase activity is relatively insensitive to loss of *Sod1* function, but drops dramatically if *Sod2* activity is impaired. This strongly suggests that the mitochondrial boundary limits the range of superoxide reactivity in vivo. We also find that exposure of adults to paraquat converts cytosolic aconitase to IRP1 but has no effect on mitochondrial aconitase, indicating that paraquat generates superoxide in the cytosol but not in mitochondria. Accordingly, we find that transgene-mediated overexpression of *Sod2* neither enhances paraquat resistance in *Sod1*-null mutants. We conclude that in vivo, superoxide is confined to the subcellular compartment in which it is formed, and that the mitochondrial and cytosolic SODs provide independent protection to compartment-specific protein iron-sulfur clusters against attack by superoxide generated under oxidative stress within those compartments.

Iron and oxygen are indispensable but potentially harmful elements of aerobic life. Individually, their reactivity has been harnessed through association with a variety of proteins and the regulation of iron and oxygen metabolism constitutes one of the major triumphs of molecular evolution (1). Iron sulfur cluster proteins function in electron transport during oxidative phosphorylation and metabolism, but can also serve as iron and oxygen sensors (2). For instance, iron regulatory protein-1 (IRP1) exerts its dual activities through the reciprocal use or disassembly of its cubane iron sulfur [4Fe-4S] cluster; the holoprotein functions as a cytosolic aconitase, whereas the apoprotein is an RNA-binding translational regulator (1, 3). The stability and functionality of IRP1 as a translation regulator is affected not only by iron levels, but also by oxidative stress, which induces IRP1 to bind iron responsive elements (IREs) located on the 5’ and 3’ untranslated regions of target genes (4, 5). Although it is established that [4Fe-4S] cluster proteins can be specifically inactivated by superoxide (O$_2$^•−) (6–8), the questions of whether the IRP1 [4Fe-4S] cluster reacts with O$_2$^•− and the cellular origin of this O$_2$^•− have not yet been elucidated (9–11).

Studies in *Saccharomyces cerevisiae* have suggested an important role for cytosolic and mitochondrial superoxide dismutases (SODs) in iron metabolism (12, 13). In addition to its function in the cytosol, *Sod1* localizes in the mitochondrial intermembrane space and appears to also contribute to mitochondrial superoxide scavenging (14). Conversely, overexpression of the mitochondrial SOD2 was shown to compensate for lack of the cytosolic enzyme in a set of experiments assessing resistance to freeze-thaw stress (15). Although these results may point to some extent of functional redundancy between the two enzymes, other aspects of the *Sod1Δ* phenotype, such as vacuolar fragmentation, cannot be rescued by *Sod2* overexpression (16). Moreover, only recombinant bacterial FeSOD that is targeted to yeast mitochondria can rescue *Sod2Δ*, but it cannot rescue *Sod2Δ* if the mitochondrial targeting sequence is omitted and FeSOD is expressed in cytosol (17, 18). These results suggest a functional compartmentalization of superoxide metabolism and have broad implications for both physiologic redox signaling and cellular oxidative stress (19). However, the question of whether mitochondrially derived superoxide normally transfers into the cytosol (20, 21), or if this is an abnormality associated only with apoptosis (22–24) remains controversial.

To address these questions, we used the cytosolic (IRP1) and mitochondrial aconitases as compartment-specific markers of O$_2$^•− reactivity in conjunction with genetic modulation of superoxide dismutase levels in the cytosolic and mitochondrial compartments. We present evidence that, in *Drosophila*, these compartments define and limit the range of O$_2$^•− reactivity.

**EXPERIMENTAL PROCEDURES**

*Drosophila Stocks—*Drosophila* was cultured at 25 °C on standard cornmeal agar medium. *Sod1(100)* and *Sod1(2ts)* represent null-activity alleles of the *Sod1* gene (25, 26). For RNA interference studies, UAS-

*This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) (to J. P. P. and A. J. H.) and by the Intramural program of the National Institute of Child Health and Human Development (NICHD). 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.*

1 To whom correspondence should be addressed. Tel.: 519-824-4120 (ext. 52796); Fax: 519-837-2075; E-mail: jphillip@uoguelph.ca.
Sod2 cDNA (30) co-injected with pUAST transgene-mediated overexpression of Sod2 cytosol, leads to IRP1 activation, in contrast to silencing of mitochondrial Sod1. Note that a robust activation of IRE-binding activity of IRP1 follows depletion of iron by deferoxamine treatment, whereas addition of iron to the medium diminishes this activity. Thus, Drosophila IRP1 registers change in iron levels in an analogous manner to IRP1 of mammals.

RNA mobility shift assays were performed on Schneider 2 cell lysates, following a 16 h treatment with either 50 and 20 μM of the iron chelator deferoxamine (lanes 1 and 2), no treatment (lane 3), or 20 and 100 μg/ml of ferric ammonium citrate (lanes 4 and 5). Radioactive human ferritin H chain IRE was used as a probe; each lane was loaded with 8 μg of total protein. Addition of 2-mercaptoethanol (2-ME) after lysis reveals the total amount of activable IRP1 (lanes 6–10, respectively). Note that a robust activation of IRE-binding activity of IRP1 follows depletion of iron by deferoxamine treatment, whereas addition of iron to the medium diminishes this activity. Thus, Drosophila IRP1 registers change in iron levels in an analogous manner to IRP1 of mammals.

Sod1IR2 and UAS-Sod2IR strains (27) were crossed to daG32Gal4 (Flybase: P(GAL4-da.G32)), which provided widespread expression of the UAS-transgenes and corresponding silencing of the respective endogenous Sod genes. All RNAi experiments presented here were reproduced with different independent transgene insertions and by crossing to another ubiquitous driver line, tub-Gal4 (Flybase: P(GAL4-tubP)). For transgene-mediated overexpression of Sod2, UAS-Sod2 transformants were generated by standard embryo injection methods (28) using a pUAST transformation vector (29) carrying a full-length Drosophila Sod2 cDNA (30) co-injected with pΔ2–3) helper plasmid into w1 recipient embryos. Other Gal4-driver lines used in this study include D42-Gal4, which drives expression in the motorneurons (31), and MHC-Gal4, which drives expression in muscle (gift from G. Boulianne, University of Toronto).

**Drosophila Cell Culture**—Schneider II cells were maintained at 25 °C in Schneider’s Drosophila medium (Invitrogen). Sixteen hours prior harvesting, either 20 and 50 μM deferoxamine or 20 and 100 μg/ml ferric ammonium citrate (Sigma) were added to the medium.

**RNA Mobility Shift Assay**—Whole flies or cell pellets were homogenized directly in band shift buffer (40 mM KCl, 25 mM Tris HCl, pH 7.5) containing 1% Triton X-100, 5 mM dithiothreitol, and protease inhibitors. Extracts were centrifuged twice at >16000 × g on a bench-top centrifuge (4 °C), and the supernatant was immediately used for further analysis. Protein concentrations were determined using the Bradford reagent (Bio-Rad, Hercules, CA). Ten μg of total protein were added to a final volume of 12.5 μl of band shift buffer with or without 2% 2-mercaptoethanol, which activates IRP1 in vitro. The samples were incubated for 5 min at room temperature with 12.5 μl of a reaction mixture containing 20% glycerol, 0.2 units/μl Super RNAsine, 2 μg/μl yeast tRNA, 200 μg dithiothreitol, and 32P-labeled IRE from the human ferritin H chain gene (2000 counts/μl). Twenty μl of the reaction mixture were loaded onto a 10% acrylamide/Tris borate EDTA (89 mM Tris, 2-mercaptoethanol, 10 mM EDTA, pH 8.0) gel, run at 200 V for 2 h, then the gel was dried and exposed for autoradiography. A single band was observed, which could be competed out by addition of 10-fold excess of cold IRE probe (data not shown).

**Aconitase Activity Assay**—Mitochondrial and cytosolic aconitase activities were assayed jointly in whole-fly extracts after electrophoretic separation. Thirty adult males were homogenized in 120 μl of extraction buffer (0.6 mM MnCl2, 2 mM citric acid, 50 mM Tris-HCl, pH 8.0) and centrifuged at 16,000 × g. Aliquots were electrophoresed on Sepraphore III membranes (Pall). Aconitase activity was detected chromogenically by incubating the membrane in 100 mM potassium phosphate, pH 6.5, 1 mM NADPH, 2 mM isocitrate acid, 1.2 mM 2,6-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 0.3 mM phenazine methosulfate, 25 mM MgCl2, 5 units/ml isocitrate dehydrogenase. All chemicals were purchased from Sigma.

**SOD2 Activity Assay**—Adult males, 24–72 h old, were homogenized in buffer-1 (50 mM sodium phosphate, pH 7.4, 0.1 mM EDTA). The extract was sonicated for 10 s to rupture mitochondria and centrifuged at 13,000 × g. The supernatant was incubated in 60 mM diethylthiocarbamic acid for 1 h at room temperature to inactivate SOD1. SOD2 activity was then determined spectrophotometrically by monitoring the autoxidation of 6-hydroxydopamine (6-HD) at 490 nm and 37 °C in 500 μl buffer-1 containing 0.1 mM 6-HD (32). Protein concentration was determined using the Bradford reagent (Bio-Rad).

**Paraquat Exposure**—w1 or w1;UAS-Sod2IR+/daG32Gal4+ adult
FIG. 3. Genetic diminution of SOD2 and SOD1 leads to selective inactivation of mitochondrial and cytosolic aconitase, respectively. Mitochondrial and cytoplasmic aconitase activities in whole-fly extracts were assayed simultaneously following electrophoretic separation. Genotypes are the same as in Fig. 2. A, cytosolic aconitase activity is scarcely detectable in the Sod1 mutant in contrast to its respective control. Note that hampering the removal of cytosolic O$_2^-$ (A and C) inactivates specifically the cytosolic aconitase without affecting its mitochondrial counterpart. Reciprocally, interference with the mitochondrial O$_2^-$-scavenging enzyme (B) inhibits mitochondrial aconitase activity without affecting its cytosolic counterpart.

FIG. 4. Paraquat exposure selectively converts cytosolic aconitase to IRP1 but has no effect on mitochondrial aconitase and its toxicity is not mitigated by mitochondrial Sod2. A, augmentation of SOD2 by expression of a UAS-Sod2 transgene. SOD2 activity was assayed in extracts of 2–4 days old adults treated with diethylthiocarbamate to inactivate SOD1. Genotypes assayed were: w$^+$; da$^{G32}$Gal4/+ (open circle), w$^+$; UAS-UM41/+; da$^{G32}$Gal4/+ (open square), w$^+$; da$^{G32}$Gal4,Sod1x39 (closed square), and w$^+$; UAS-UM41/+; da$^{G32}$Gal4,Sod1x39 (open square). B, overexpression of Sod2 does not enhance adult resistance to paraquat. The 3 genotypes are as described in A. Survivors were enumerated after 48 h of continuous exposure. C, paraquat exposure reduces cytosolic but not mitochondrial aconitase activity. Sod1- adults (w$^+$; da$^{G32}$Gal4/+ and da$^{G32}$Gal4,Sod1x39) were exposed to 0, 5, or 10 mM paraquat and collected after a 16-hour non-lethal exposure. Extracts were prepared and assayed for aconitase as described in the legend to Fig. 3. Cytosolic aconitase activity is reduced by about half with both 5 and 10 mM exposure. We also note a small but consistent increase in mitochondrial aconitase at these exposure levels. Overexpression of Sod1 or Sod2 has no detectable effect on the activity of either aconitase (data not shown). D, RNA mobility gel shift assays on the same extracts show robust activation of IRE-binding activity of IRP1 following a 16-hour exposure to paraquat.

FIG. 5. Overexpression of Sod2 does not compensate for genetic loss of Sod1. Strains overexpressing Sod2 in a Sod1 mutant genetic background were constructed using the Sod1$^{--}$ mutant allele. The UAS-Sod2 transgene, UM41 (see Fig. 4A), was expressed using the da$^{G32}$ (ubiquitous), D42 (adult motorneuron), and MHC (muscle) Gal4 drivers. The genotypes assayed were w$^+$; da$^{G32}$Gal4,Sod1x39, da$^{G32}$Gal4,Sod1x39/Sod1x39 (closed circle), Sod1 mutant, w$^+$; UAS-UM41/+; da$^{G32}$Gal4,Sod1x39/Sod1x39 (closed square), w$^+$; UAS-UM41/+; D42Gal4,Sod1x39/Sod1x39 (open triangle), w$^+$; UAS-UM41/+; MHCGal4,Sod1x39/Sod1x39 (cross). Expression of Sod2 has no discernable effect on Sod1 mutant lifespan. In contrast, widespread expression of the human Sod1 by the da$^{G32}$Gal4 driver in the Sod1 mutant background (triangles; w$^+$; UAS-hSod1/+; da$^{G32}$Gal4,Sod1x39) gives a robust restoration of lifespan.

Drosophila Cells Interconvert IRP1 and Cytosolic Aconitase in Response to Iron—Drosophila melanogaster expresses two IRP1 homologues with 86% identity that are co-expressed during development (33). These IRP1 homologues can bind to iron-responsive elements of succinate dehydrogenase B (34) and ferritin 1 heavy chain homologue (35) and, in this respect, are more similar to mammalian IRPs than to the recently described Caenorhabditis elegans homologue (36). We asked if conservation of this molecular interaction functionally extends to iron-dependent regulation of Drosophila IRP1 by performing RNA mobility shift assays on extracts of Drosophila Schnei-
der-II cells cultured with varying concentrations of iron. Fig. 1 shows that chelation of iron by deferoxamine greatly enhances binding of IRP1 to radiolabeled IRE. In contrast, addition of ferric ammonium citrate, which reconstitutes the [4Fe-4S] cluster, converts IRP1 to its aconitase form. This assay does not discriminate between the two Drosophila IRP1 proteins. Taken together, these results suggest that the [4Fe-4S] cluster switch that mediates the functional interconversion between a translation regulator and a cytosolic aconitase in response to iron levels is evolutionarily conserved between Drosophila and mammals.

IRP1 Binding Activity Is Enhanced in the Absence of SOD1—To determine whether cytosolic O$_2^-$ specifically reacts with the [4Fe-4S] cluster of IRP1, we used a Drosophila-null mutant for the cytosolic Sod1 (25) to generate a cytoplasmic environment of high constitutive oxidative stress. We predicted that this cytosolic environment would promote the enhanced binding of IRP1 to IRE through the O$_2^-$-mediated loss of the [4Fe-4S] cluster of IRP1 holoprotein. Indeed, extracts of flies lacking cytosolic Sod1 activity show markedly increased IRP1 binding activity in comparison to Sod1$^+/+$ controls with an otherwise identical genetic background (Fig. 2A).

IRP1 Binding Is Activated by Genetic Diminution of SOD1, but Not SOD2—We then asked whether O$_2^-$ that originates in mitochondria will react with IRP1 localized in the cytoplasm. As for other eukaryotes, Drosophila possess a form of SOD (SOD2) that is confined to mitochondria (30). However, because no Sod2 mutant has been reported that could be used to create a mitochondrial environment of high oxidative stress, we utilized transgenic strains developed earlier in this laboratory that silence Sod2 by means of RNAi (27). For controls, we generated strains with transgenically silenced Sod1$^+/+$ (see “Discussion”), IRP1 is predicted to be unaffected when Sod2 expression is silenced. Fig. 2B shows that IRP1 binding to IREs is indeed unaffected when Sod2 expression is silenced. Fig. 2B shows that IRP1 binding to IREs is indeed unaffected when Sod2 is silenced. In contrast, silencing of Sod1 by the same mechanism leads to robust activation of IRP1 (Fig. 2C), in keeping with the activation of IRP1 seen in the Sod1$^-/-$ mutant (Fig. 2A).

Mitochondrial and Cytosolic Aconitases Are Selectively Inactivated by Loss of SOD2 and SOD1, Respectively—Having established that IRP1 activity is unaffected by mitochondrial O$_2^-$, we asked if silencing Sod2 would exhibit any corresponding effect on cytosolic aconitase activity. As shown in Fig. 3B, silencing of mitochondrial Sod2 has no discernible effect on cytosolic aconitase activity. In contrast, loss of SOD1 activity by mutation or RNAi-mediated silencing strongly inactivates cytosolic, but not mitochondrial, aconitase (Fig. 3, A and C). Collectively, the results presented in Figs. 2 and 3 argue strongly for a compartmentalized redox environment in which the reactivity of O$_2^-$ generated within either the mitochondrial or cytosolic compartment is limited to protein iron-sulfur substrates residing within the same respective compartment.

Resistance to Paraquat Toxicity Is Unaffected by Augmentation of SOD2—In view of the compartmentalized effects conferred by selective diminution of SOD1 and SOD2 in the cytosolic and mitochondrial compartments, respectively, we asked if the two enzymes function differentially in the context of whole organism biology as was previously shown for the thioredoxin antioxidant defense system of Drosophila (37, 38). To address this question, we produced UAS-Sod2 transformants that ectopically express Sod2 through use of the UAS/Gal4 system (29). Using the da-Gal4 driver to broadly overexpress Sod2 produces a 3-fold elevation of mitochondrial SOD2 activity (Fig. 4A). We then assessed the protective effect of Sod2 overexpression against the toxicity of paraquat, a widely used O$_2^-$-generating agent (25). In sharp contrast to the robust paraquat-resistance conferred by augmentation of SOD1 (31, 39, 40), augmentation of SOD2 activity provided no increased protection against the toxicity of paraquat (Fig. 4B). This result was also seen with augmentation of SOD2 using motorneuron or muscle Gal4 drivers (data not shown) and is consistent with previously published results on the failure of Sod2 overexpression to provide resistance to hyperoxia, heat stress, and starvation (41).

Paraquat Exposure Converts Cytosolic Aconitase to IRP1 but Has No Effect on Mitochondrial Aconitase—We then examined the compartment-specific impact of paraquat exposure on the cytosolic and mitochondrial aconitases. Wild-type flies (Sod1$^+/+$, Sod2$^+/+$) were exposed to the same concentrations of paraquat as in Fig. 4B, but for a shorter, non-lethal time (16 as opposed to 48 h), and IRP1 binding and aconitase activities were assayed. A decrease in cytosolic aconitase activity and a reciprocal increase in IRP1 binding activity confirmed that, as expected, paraquat exposure causes an increased flux of O$_2^-$ in the cytosol but not in mitochondria (Fig. 4, C and D). The failure of Sod2 overexpression to afford additional protection against paraquat toxicity (Fig. 4B) may thus result from the confinement of paraquat reactivity to the cytosol and the inaccessibility of SOD2 to this compartment.

Augmentation of SOD2 Does Not Rescue the Sod1-null Mutant—Finally, we asked whether enhancing the capacity of mitochondria to detoxify O$_2^-$ through elevated SOD2 activity could rectify the debilitating and lifespan-shortening effects of the Sod1$^-/-$ mutation. A negative answer to this question would reinforce the findings above that the cytosol and mitochondria represent functionally isolated O$_2^-$ compartments in Drosophila. To answer this question, we overexpressed Sod2 in the Sod1$^+/+$ genetic background using ubiquitous, motorneuron or muscle-specific Gal4 drivers and determined the lifespan of the resulting adults (Fig. 5). The results clearly demonstrate that, in striking contrast to the ecotypic expression of Sod2 confers no discernible effect on the severely truncated lifespan of Sod1$^-/-$ adults (Fig. 5). Neither does it rectify the unusually high pupal mortality of Sod1$^+-/-$ (data not shown). We thus conclude that the role of SOD2 in mitigating cytosolic oxidative stress in general and scavenging cytosolic O$_2^-$ in particular is negligible.

**DISCUSSION**

These studies represent the first in vivo analysis in a higher eukaryote of the consequences of genetic modulation of cytosolic and mitochondrial SODs on the function of iron-sulfur proteins that reside within those respective compartments. These iron-sulfur proteins are both important cellular targets and sensitive indicators of superoxide-mediated oxidative stress. The distinct subcellular partitioning of the cytosolic and mitochondrial aconitases and the availability of methods to assay these two activities concurrently in the same extract provided the opportunity to investigate whether superoxide generated in the mitochondrial compartment can affect an iron-sulfur protein in the cytosol and vice versa. Using a combination of genetic and pharmacological methods to generate compartment-specific oxidative stress, we examined the responses of the cytosolic and mitochondrial aconitases. The results are consistent with the interpretation that in *Drosophila*, cytosolic aconitase is unaffected by superoxide generated in mitochondria and conversely, that mitochondrial aconitase is unaffected.

3 J. Hu, K. Kirby, A. J. Hilliker, and J. P. Phillips, manuscript in preparation.
by superoxide generated in the cytosol. We interpret these results to mean that containment of O$_2^-$ within the compartment of its origin along with the SOD specific to that compartment is a central feature of reactive oxygen homeostasis in Dro sophila.

How this homeostatic mechanism might relate to the severe debilitating phenotypes that characterize Sod1$^{-/-}$ mutants of Dro sophila (25, 26) as compared with the relatively benign phenotype of the corresponding Sod1$^{-/-}$ mutant in mice (42) is unclear at present. In principle, the liberation of intracellular iron resulting from the reaction of unscavenged O$_2^-$ with [4Fe-4S] clusters (12, 13, 43), could also contribute to some of the complex phenotypes of Sod1$^{-/-}$ mutants (25). In this regard, it is interesting to note that mutations in iron-sulfur cluster assembly genes have been shown to suppress the Sod1 deficiency phenotype of S. cerevisiae (44), suggesting that reduced iron sulfur protein expression can abrogate O$_2^-$ toxicity. Whether mutations in homologous genes would have similar effects on Sod1$^{-/-}$ phenotypes in Dro sophila remains an open question.

The results described here contrast with a recent in vitro study of isolated mammalian mitochondria. Han et al. (21) present evidence that superoxide can be released from rat heart mitochondria cultured in vitro through voltage-dependent anion channels. Biological differences between Dro sophila and rat heart mitochondria, the use of different methods used to detect and modulate superoxide flux, and in vitro versus in vivo conditions are all potential contributors to these differences. Further experiments will be required to clarify this matter.

These studies set the stage for investigating of the role of Sod1, Sod2, and oxidative stress in iron metabolism in Dro sophila. The results presented here underscore the importance of compartment boundaries in maintaining reactive oxygen homeostasis in Dro sophila and could have therapeutic implications in relation to the oxidative stress component of inflammatory disease.

Acknowledgments—We thank Dr. Mary Lilly for kindly providing food supplies and facilities for fly maintenance and fly pushing at Cell Biology and Metabolism Branch/NICHD, Dr. Esther Meyron-Holtz for technical advice with RNA mobility shift assays, and Dr. Wing-Hang Tong for critical reading of this manuscript.

REFERENCES

1. Rouault, T. A., and Klausner, R. D. (1996) Trends Biochem. Sci. 21, 174–177
2. Fillebeen, C., and Pantopoulos, K. (2002) Redox Rep. 7, 15–22
3. Gardner, P. R., Fridovich, I. (1991) J. Biol. Chem. 266, 19328–19333
4. Flint, D. H., Ramezani, M. H., and Emtage, P. (1999) J. Biol. Chem. 274, 22269–22276
5. Gardner, P. R., Raineri, I., Epstein, L. B., and White, C. W. (1995) J. Biol. Chem. 270, 13399–13405
6. Liu, Y., Pedersen, C., Day, B. J., and Patel, J. M. (2001) J. Neurochem. 78, 746–755
7. Tabuchi, A., Funaji, K., Nakatubo, J., Fukushi, M., Tsuchiya, T., and Tsuda, M. (2003) J. Neurosci. Res. 71, 504–515
8. Kawasaki, A., and Fridovich, I. (1991) J. Biol. Chem. 266, 17414–17419
9. Rouault, T. A., and Klauser, R. D. (1996) Trends Biochem. Sci. 21, 174–177
10. Fillebeen, C., and Pantopoulos, K. (2002) Redox Rep. 7, 15–22
11. Gardner, P. R., and Fridovich, I. (1991) J. Biol. Chem. 266, 19328–19333
12. Flint, D. H., Ramezani, M. H., and Emtage, P. (1999) J. Biol. Chem. 274, 22269–22276
13. Gardner, P. R., Raineri, I., Epstein, L. B., and White, C. W. (1995) J. Biol. Chem. 270, 13399–13405
14. Rouault, T. A., and Klauser, R. D. (1996) Trends Biochem. Sci. 21, 174–177
15. Fillebeen, C., and Pantopoulos, K. (2002) Redox Rep. 7, 15–22
16. Gardner, P. R., and Fridovich, I. (1991) J. Biol. Chem. 266, 19328–19333
17. Flint, D. H., Ramezani, M. H., and Emtage, P. (1999) J. Biol. Chem. 274, 22269–22276
18. Liu, Y., Pedersen, C., Day, B. J., and Patel, J. M. (2001) J. Neurochem. 78, 746–755
19. Kawasaki, A., and Fridovich, I. (1991) J. Biol. Chem. 266, 17414–17419
20. Rouault, T. A., and Klauser, R. D. (1996) Trends Biochem. Sci. 21, 174–177
21. Fillebeen, C., and Pantopoulos, K. (2002) Redox Rep. 7, 15–22
22. Gardner, P. R., and Fridovich, I. (1991) J. Biol. Chem. 266, 19328–19333
23. Flint, D. H., Ramezani, M. H., and Emtage, P. (1999) J. Biol. Chem. 274, 22269–22276