The Ubiquinone-binding Site of the \textit{Saccharomyces cerevisiae} Succinate-Ubiquinone Oxidoreductase Is a Source of Superoxide*

Jing Guo and Bernard D. Lemire‡

From the Canadian Institutes of Health, Membrane Protein Research Group, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

Received for publication, June 16, 2003, and in revised form, September 3, 2003

Published, JBC Papers in Press, September 16, 2003, DOI 10.1074/jbc.M306312200

The mitochondrial succinate dehydrogenase (SDH) is a tetrameric iron-sulfur flavoprotein of the Krebs cycle and of the respiratory chain. A number of mutations in human SDH genes are responsible for the development of paragangliomas, cancers of the head and neck region. The \textit{mev-1} mutation in the \textit{Caenorhabditis elegans} gene encoding the homolog of the SDHC subunit results in premature aging and hypersensitivity to oxidative stress. It also increases the production of superoxide radicals by the enzyme. In this work, we used the yeast succinate dehydrogenase to investigate the molecular and catalytic effects of paraganglioma- and \textit{mev-1}-like mutations. We mutated Pro-190 of the yeast Sdh2p subunit to Gln (P190Q) and recreated the \textit{C. elegans mev-1} mutation by converting Ser-94 in the Sdh3p subunit into a Glu (S94E). The P190Q and S94E mutants have reduced succinate-ubiquinone oxidoreductase activities and are hypersensitive to oxygen and pararquat. Although the mutant enzymes have lower turnover numbers for ubiquinol reduction, larger fractions of the remaining activities are diverted toward superoxide production. The P190Q and S94E mutations are located near the proximal ubiquinone-binding site, suggesting that the superoxide radicals may originate from a ubisemiquinone intermediate formed at this site during the catalytic cycle. We suggest that certain mutations in SDH can make it a significant source of superoxide production in mitochondria, which may contribute to disease progression. Our data also challenge the dogma that superoxide production by SDH is a flavin-mediated event rather than a quinone-mediated one.

Succinate dehydrogenase (SDH)\(^1\) of the Krebs cycle and of the bacterial or the mitochondrial respiratory chain (MRC) is intriguing for its roles in energy generation and in mitochondrial-related diseases (1–3). SDH is an iron-sulfur flavoprotein of the inner membrane that functions as a succinate-ubiquinone oxidoreductase, oxidizing succinate to fumarate and reducing ubiquinone to ubiquinol (4–6). The yeast enzyme, like its mammalian counterpart, is encoded by four nuclear genes, SDH1–4 (4), and harbors a covalently attached flavin adenine dinucleotide (FAD), three iron-sulfur clusters, a \textit{b}-type heme (7), and 2 quinone-binding sites termed the proximal and distal sites (Q\(_p\) and Q\(_d\), respectively) (8, 9). Electrons flow from succinate to the FAD through the iron-sulfur clusters before entering the membrane subunits where quinone reduction occurs.

The membrane subunits have the most diversity in subunit structure and cofactor content largely determining the specific catalytic properties of each enzyme. The structures of the \textit{Caenorhabditis elegans} SDH and the closely related \textit{E. coli} and \textit{Wollinella succinogenes} fumarate reductases (FRD) have been determined (10–13). All flavoprotein and iron-sulfur subunits are highly conserved and adopt very similar structures. In contrast, the membrane subunits are not. The \textit{E. coli} SDH crystal structure revealed two membrane subunits with a single heme and a single bound ubiquinone (14); the \textit{E. coli} FRD also contains a pair of membrane subunits with two menaquinone molecules but no heme (10); the \textit{W. succinogenes} FRD has a single larger membrane subunit with two hemes, but quinone molecules were absent from the crystal (11).

Mutations in the human \textit{SDHA} gene can result in Leigh syndrome, an infantile-onset progressive neurodegenerative disease (1, 15). Mutations in the \textit{SDHB}, \textit{SDHC}, or \textit{SDHD} genes can result in paraganglioma (benign vascularized tumors in the head and neck) or pheochromocytoma (catechol-secreting tumors commonly occurring in the adrenal medulla) (16, 17). The carotid body, the most common tumor site for paraganglioma, senses oxygen levels in the blood (16). The striking phenotypic differences between \textit{SDHA} and \textit{SDHB–D} mutations suggest that SDH has additional functions, possibly in oxygen-sensing, besides its role as a succinate-ubiquinone oxidoreductase. The \textit{Caenorhabditis elegans mev-1(kn1)} mutation in the \textit{SDH3} homolog confers hypersensitivity to hyperoxia or to oxidative stress (18, 19). The mutation, which affects the Q\(_p\) site, leads to the overproduction of superoxide anions, the accumulation of aging markers, and a shortened life span. Iron-sulfur clusters are one-electron carriers, whereas quinones are fully reduced with two electrons. The order of the SDH electron carriers necessitates the formation of (at least transiently) a ubisemiquinone radical, which has been detected in SDH by electron paramagnetic resonance spectroscopy (20, 21). The \textit{mev-1} mutation may alter the stability or accessibility of the ubisemiquinone intermediate.

In this work, we used the yeast enzyme to model molecular and catalytic effects of introducing paraganglioma- and \textit{mev-1}-like mutations. The human \textit{SDHB} P198R mutation affects a conserved proline residue located near the [3Fe-4S] cluster and the Q\(_p\) site (22). We mutated Pro-190 in the yeast \textit{SDH2} gene to Arg, Leu, or Gln (P190R, P190L, and P190Q, respectively). We also mutated Ser-94 in the \textit{SDH3} gene to Glu (S94E) to mimic the \textit{C. elegans mev-1} mutation. The \textit{SDH2} P190Q and \textit{SDH3} S94E mutants displayed reduced SDH activities, hypoxia-sensitivity to oxygen and pararquat, and a marked increase in

\* This work was supported by the Canadian Institutes of Health Research Grant MT-15290. 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 should be addressed. Tel.: 780-492-4853; Fax: 780-492-0886; E-mail: bernard.lemire@ualberta.ca.

1 The abbreviations used are: SDH, succinate dehydrogenase; FRD, fumarate reductase; FAD, flavin adenine dinucleotide; MRC, mitochondrial respiratory chain; Q\(_p\) and Q\(_d\) proximal and distal quinone-binding sites, respectively.
superoxide production. Our results strongly suggest that the SDH Qp site, with its ubisemiquinone intermediate, can be a significant source of superoxide production.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, Media, and Culture Conditions—The parental strain JNY131 (ade2–1, leu2–3, his3–11,15, trpl–1, ura3–1, can1–100, mgm1–102, Mata, or Mata) was a kind gift of Dr. Jodi Nunnari (23). An SDH2 knockout of JNY131, JG1 (SDH2::HIS3), was constructed by replacing the entire SDH2 open reading frame with the HIS3 gene. Similarly, an SDH3 knockout, JG2 (SDH3::TRP1), was constructed by replacing the entire SDH3 open reading frame with the TRP1 gene. JG1 and JG2 can be complemented for growth on nonfermentable carbon sources with the plasmids pSDH2–45 and pSDH3–2, respectively. These plasmids contain the wild-type SDH2 and SDH3 genes cloned into the vector, YCplac33 (24). Site-directed mutagenesis was performed as described (25) and mutations confirmed by sequencing the entire gene (Department of Biochemistry Core DNA Facilities, University of Alberta, Edmonton, Alberta, Canada). The yeast media (YPD, YPG, SG, SD, and YPGal) have been described in Refs. 9 and 26. Bacterial strains were routinely grown on LB medium at 37 °C using ampicillin as the selectable marker. Oxygen sensitivity was measured on plates incubated at 30 °C in a jar continuously flushed at ~20 mℓ/min with pure oxygen.

Isolation of Mitochondria and Enzyme Assays—YPG-grown stationary phase cells were harvested by centrifugation and lysed in a Bead-Beater (BioSpec Products, Inc., Bartlesville, OK) with 0.5-mm acid-washed glass beads using five pulses of 1-min and five of 30-s duration interspersed with 5-min cooling periods. Complete protease inhibitor mixture tablets were added prior to cell lysis (Roche Diagnostics). Mitochondria were isolated by differential centrifugation and sucrose density centrifugation (27). Mitochondria were lysed by 10-fold dilution in sorbitol-free buffer and sonicated in ice water in a bath sonicator for 2 min. Succinate and glycerol phosphate dehydrogenase assays were performed as described (25) and mutations confirmed by sequencing the entire gene (Department of Biochemistry Core DNA Facilities, University of Alberta, Edmonton, Alberta, Canada). The yeast media (YPD, YPG, SG, SD, and YPGal) have been described in Refs. 9 and 26.

Mitochondria were isolated by differential centrifugation and sucrose density centrifugation (27). Mitochondria were lysed by 10-fold dilution in sorbitol-free buffer and sonicated in ice water in a bath sonicator for 2 min. Succinate and glycerol phosphate dehydrogenase assays were performed as described in Ref. 8. Superoxide generation was assayed as the superoxide dismutase-sensitive reduction of cytochrome c in the presence of 1 mM potassium cyanide (Imlay 1991). Thirty units of superoxide dismutase/ml (from bovine erythrocytes, catalog number S-2515, Sigma) were added in a parallel reaction. SDH is normally expressed at 30 °C immediately prior to analysis. When measuring the effect of stress on the site, Ubiquinone-2 and heme have been docked into a model of the yeast SDH generated using the E. coli SDH, the E. coli FRD, and the W. succinogenes FRD as templates and optimized with molecular dynamics. The [3Fe-4S] cluster is located in the Sdh2p subunit and is the electron donor for reduction of the bound quinone. Distances (shown in A) between the side chains and the quinone are indicated by the dashed lines.

FIG. 1. Location of the Sdh2p Pro-190 and Sdh3p Ser-94 residues at the Qp site. Ubiquinone-2 and heme have been docked into a model of the yeast SDH generated using the E. coli SDH, the E. coli FRD, and the W. succinogenes FRD as templates and optimized with molecular dynamics. The [3Fe-4S] cluster is located in the Sdh2p subunit and is the electron donor for reduction of the bound quinone. Distances (shown in A) between the side chains and the quinone are indicated by the dashed lines.

RESULTS

Choice of Mutations—The C. elegans mev-1 mutation has directly implicated nematode SDH as a significant source of superoxide (19). We wished to determine whether the yeast enzyme could also be a source of free radicals. In an alignment of the yeast and nematode Sdh3p sequences, Gly-71 of the C. elegans protein aligns with Ser-94, which we mutated to a glutamate (S94E) to mimic the mev-1 mutation. Residues 92–99 of Sdh3p contain a sequence (LSSLHRS) that closely resembles a motif associated with quinone-binding sites (LXXHXXXT) (bold letters indicate key amino acids) (31, 32). Thus, Ser-94 is likely in the vicinity of a quinone-binding site.

We also mutated SDH2 Pro-190 to Arg, Leu, and Gln. The P190R mutation in the human SDHB gene, which affects this conserved proline, results in pheochromocytoma (22). The analogous residue in the E. coli FrdB subunit (Pro-159) has also been mutated (33). The P159Q phenotype was very unusual; aerobic but not anaerobic activity of the mutant enzyme led to radical-mediated auto-inactivation. The P159Q mutation was proposed to perturb the Qp site allowing the ubisemiquinone intermediate to participate in hydroxyl radical generation (22).

The mev-1 and the FrdB P159Q mutations strongly suggest that free radicals are generated near the Qp site. We have modeled the three-dimensional structure of the yeast SDH based on the high-resolution structures of the E. coli SDH, the
paired MRC-dependent growth in the presence of 1.0 mM paraquat (Fig. 2B). The SDH3 S94E mutation conferred mild sensitivity to this stress (Fig. 2D).

We tested whether the two mutant SDH enzymes also conferred sensitivity to hyperoxia in yeast. Wild-type SDH2 or SDH3 genes supported growth in 100% oxygen, although somewhat more slowly than in air (Fig. 3). The SDH2 P190Q mutation completely abolished growth in the oxygen atmosphere, whereas the SDH3 S94E mutation conferred mild sensitivity (Fig. 3B). Therefore, both mutations render cells less able to withstand exogenous oxidative stresses. We believe the mutant enzymes are themselves a source of oxidative stress that taxes the defense mechanisms of cells, which are then less able to cope with the additional stress of hyperoxia or paraquat. We cannot, however, rule out the possibility that the mutations indirectly impair the resistance mechanisms of cells.

The Mutant Enzymes Have Reduced Quinone Reductase Activities—We purified mitochondria from the SDH2 P190Q and the SDH3 S94E mutant strains and their corresponding wild-type counterparts. We measured the succinate-dependent phenazine methosulfate-mediated reduction of dichlorophenolindophenol; this activity reflects enzyme assembly because it is dependent on the Sdh1p and Sdh2p subunits only and does not require catalytically functional membrane subunits (9, 30). We also determined the levels of covalent FAD in the mitochondria; SDH is the major covalent flavoprotein in Saccharomyces cerevisiae (36). The succinate-dependent reduction of decylubiquinone or of cytochrome c requires a fully functional SDH capable of transferring electrons to quinone acceptors. As shown in Table I, both the SDH2 P190Q and the SDH3 S94E enzymes are efficiently assembled; the FAD contents are greater than those of wild-type mitochondria, and the phenazine methosulfate-mediated reduction of dichlorophenolindophenol activities are diminished, although not severely. In contrast, the cytochrome c and the decyldubiquinone reductase activities are significantly reduced indicating that both mutations impair quinone reductase activities. A specific loss of quinone reductase activity is observed when the quinone-binding sites are perturbed (8, 9, 37).

![Fig. 2. MRC-dependent growth of wild-type and mutant strains in the presence and absence of paraquat. SDH2 (A and B) or SDH3 (C and D) knockout strains transformed with empty vectors (vec) or with wild-type (WT) or mutant SDH2 or SDH3 genes were grown overnight at 30 °C in YPG. Cultures were serially diluted with sterile water, and 10-μl aliquots were spotted onto SG plates without (A and C) or with (B and D) 1.0 mM paraquat. Plates were incubated at 30 °C in an air atmosphere and photographed after 6 days.](image1)

![Fig. 3. MRC-dependent growth of wild-type and mutant strains under normoxic or hyperoxic conditions. SDH2 or SDH3 knockout strains transformed with empty vectors (vec) or with wild-type (WT) or mutant SDH2 or SDH3 genes were grown overnight at 30 °C in YPG. Cultures were serially diluted with sterile water, and 10-μl aliquots were spotted onto SG plates. Plates were incubated at 30 °C in an air atmosphere and photographed after 6 days (A) or in a tank continuously flushed with 100% oxygen and photographed after 10 days (B). The SDH2 knockout strain transformed with the wild-type SDH2 gene is not shown, but its growth is not distinguishable from the SDH3 knockout transformed with the wild-type SDH3 gene.](image2)
Superoxide Production by the Yeast Succinate Dehydrogenase

**TABLE I**

| Enzyme | Strain |
|--------|--------|
|        | SDH2  | SDH2(P190Q) | SDH3 | SDH3(S94E) |
| Covalent FAD
| 82.1 ± 2.4 | 100 ± 6 | 118 ± 7 | 145 ± 10 |
| DCPIP reductase
| 1960 ± 106 | 1350 ± 119 (69%) | 1780 ± 157 | 1430 ± 136 (80%) |
| Cytochrome c reductase
| 2050 ± 226 | 350 ± 44 (17%) | 1720 ± 206 | 370 ± 42 (22%) |
| Decylubiquinone reductase
| 1320 ± 97 | 273 ± 19 (21%) | 1240 ± 180 | 360 ± 99 (29%) |

* Values represent the mean of at least 12 determinations ± S.E. | Activities are expressed as μmol of covalent FAD mg of protein⁻¹. Values represent the mean of at least 10 determinations ± S.E.

| Enzyme | Strain |
|--------|--------|
|        | SDH2  | SDH2(P190Q) | SDH3 | SDH3(S94E) |
| Cytochrome c reductase (−SOD) | 87 ± 16 | 10.8 ± 1.7 | 226 ± 13 | 47.4 ± 3.2 |
| Cytochrome c reductase (+SOD) | 83 ± 13 | 9.1 ± 1.0 | 221 ± 15 | 37.3 ± 3.9 |
| SOD-dependent change | -4.6% | -15.7% | -2.2% | -21.3% |
| p value | 0.41 | ≤0.001 | 0.42 | ≤0.001 |

* Values represent the mean of at least 12 determinations ± S.E.

* Activities are expressed as nmol of cytochrome c reduced min⁻¹ μmol of covalent FAD⁻¹. Values represent the mean of at least 20 determinations ± S.E.

**The Mutant Enzymes Are a Source of Superoxide Radicals**—Superoxide production was assayed (as the superoxide dismutase-sensitive cytochrome c reductase activity) in the presence of cyanide to inhibit cytochrome c oxidase (38). Two pathways of electron transfer can be used to reduce cytochrome c in the first, electrons flow through SDH to ubiquinone through the ubiquinol-cytochrome c oxidoreductase (complex III) and to cytochrome c; the second pathway involves a superoxide anion, which can directly reduce cytochrome c. Superoxide dismutase will inhibit the second pathway by converting superoxide into hydrogen peroxide. As seen in Table II, superoxide dismutase-insensitive component of that activity). Our data do not indicate any inhibition of superoxide formation at high succinate concentrations and are not consistent with the flavin auto-oxidation model. The dependence of superoxide generation on succinate concentration is strong evidence that SDH, rather than some other MRC component, is the site of superoxide production.

SDH and complex III have close structural and genetic interactions in yeast (40, 41), and complex III is a known source of oxygen-free radicals. We measured the glycerol phosphate-dependent reduction of cytochrome c in the presence and absence of superoxide dismutase to determine whether the SDH mutations affected complex III function. Glycerol phosphate is oxidized by the glycerol-phosphate dehydrogenase, an FAD-linked enzyme that, like SDH, reduces ubiquinone to ubiquinol (42). From ubiquinol, the pathway electrons follow to cytochrome c is identical to that for the succinate-dependent reaction. If complex III function had been altered by the SDH mutations, then we might expect a large superoxide dismutase-sensitive component associated with glycerol-phosphate cytochrome c reductase activity. As shown in Table III, this is not the case; superoxide dismutase has no significant effect on this activity in wild-type or in mutant mitochondria. This supports our conclusion that SDH is directly responsible for the production of superoxide.
Mitochondrial SDH is not normally considered a significant source of oxygen-free radicals (43–45). Yet, there are theoretical considerations and experimental observations that suggest otherwise. A ubisemiquinone intermediate is an obligatory part of the SDH catalytic mechanism; single electrons are transferred from the [3Fe-4S] center in the Sdh2p subunit to ubiquinone bound at the Qp site. Until another electron arrives, the enzyme must stabilize the ubisemiquinone radical and prevent its escape or inappropriate re-oxidation. Mutations in the vi-

**Figure 4. Dependence of activity on substrate concentration.** SDH was activated in phosphate-containing buffer before succinate-cytochrome c reductase activities were determined in the presence of increasing concentrations of succinate with or without superoxide dismutase. Activity values are expressed as turnover numbers (μmol of cytochrome c reduced min⁻¹ nmol of covalent FAD⁻¹); succinate concentrations are in mM. Data points were fit to a logarithmic function for illustration purposes only. A, the total succinate-cytochrome c reductase activities for the SDH2 (diamonds) and the SDH2 P190Q (squares) enzymes are fitted with solid and dotted curves, respectively. Values represent the mean of at least 7 determinations ± S.E. B, the total succinate-cytochrome c reductase activities for the SDH3 (diamonds) and the SDH3 S94E (squares) enzymes are fitted with solid and dotted curves, respectively. Values represent the mean of at least 7 determinations ± S.E. C, the superoxide dismutase-sensitive succinate-cytochrome c reductase activities for the wild-type SDH2 and SDH3 enzymes are plotted as diamonds and squares, respectively. Values represent the mean of at least 15 determinations ± S.E.
The mev-1 mutation in the C. elegans SDH, upon which we modeled our SDH3 S94E mutation, is a QP site mutation that results in the generation of superoxide (18, 19). Similarly, the SDH2 P190Q mutation is modeled on the E. coli FrdB P159Q mutation, which was proposed to produce hydroxyl radicals via a Fenton-type reaction (33). The Fenton reaction forms hydroxyl radicals from hydrogen peroxide (generated by the superoxide dismutase reaction) when cuprous or ferrous ions are present (44). Mitochondria contain both iron and copper, although both are normally complexed with proteins to prevent their participation in Fenton chemistry. The FrdB P159Q mutation is located close to the [3Fe-4S] cluster and may allow access of superoxide to iron atoms of the cluster leading to hydroxyl radical formation and subsequent enzyme inactivation under aerobic conditions (33). We have not observed a similar inactivation of the yeast Sdh3p S94E enzyme possibly reflecting a different structure near the yeast [3Fe-4S] cluster that prevents hydroxyl radical formation.

Complex I is a known source of oxygen-free radicals as it also generates semiquinones during its reaction cycle (44, 45), and reverse electron flow from SDH can provide the electrons for this to occur (46, 47). Three points argue against this possibility accounting for our results. First, S. cerevisiae does not have a multisubunit complex I-like enzyme with numerous iron-sulfur centers (42, 48). Rather, it has a single subunit NADH-ubiquinone oxidoreductase, Ndi1p (48). Purified Ndi1p apparently catalyzes a two-electron reaction, because semiquinone signals were not detected by electron paramagnetic resonance spectroscopy during a redox titration of the enzyme (49). Second, the mutant SDH enzymes are catalytically compromised for quinone reduction (Table I) and, thus, should also be inefficient in reverse electron flow. Finally, the production of superoxide radicals is proportional to the concentration of succinate (Fig. 4C) in the same way that the total succinate-cytochrome c reductase activity is proportional (Fig. 4, A and B). This would not be expected if the site of superoxide production was outside SDH itself.

The SDH mutations we introduced are localized near the QP site and may increase superoxide production in one of two ways. They may allow an increased flow of electrons out of the enzyme to oxygen, or they may allow the premature release of the ubisemiquinone intermediate and its subsequent reaction to form superoxide. We cannot distinguish between these mechanisms, but we favor the latter possibility, because conserved residues in the E. coli SDH located near Sdh3p Ser-94 are required for efficient quinone binding. When the E. coli SdhC Ser-27 and Arg-31 are mutated, succinate-ubiquinone reductase activity is lost, the enzymes can no longer be labeled by a benzoquinone photoaffinity probe, and the apparent affinities ($K_m$) for ubiquinone-2 are too low to be determined (50). The E. coli Ser-27 and Arg-31 correspond to the S. cerevisiae Sdh3p Ser-93 and Arg-97, respectively.

The E. coli FRD and SDH enzymes are believed to produce superoxide through the reaction of oxygen with the reduced FAD cofactor (39) rather than through the loss of electrons combined with superoxide dismutase-sensitive glycerol-phosphate cytochrome c reductase activities

| Enzyme                  | Strain          | SDH2 SDH2 (P190Q) | SDH3 SDH3 (S94E) | SDH3 SDH3 (S94E) |
|-------------------------|-----------------|-------------------|------------------|------------------|
| Cytochrome c reductase  | 21.0 ± 2.2      | 29.7 ± 2.0        | 137 ± 14         | 138 ± 5.3        |
| Cytochrome c reductase  | 20.1 ± 2.5      | 28.6 ± 1.9        | 136 ± 14         | 137 ± 6.7        |
| SOD-dependent change    | -4.3%           | -3.7%             | -0.7%            | -0.7%            |
| p value                 | 0.47            | 0.12              | 0.92             | 0.65             |

Activities are expressed as nmol of cytochrome c reduced min⁻¹ mg of protein⁻¹. Values represent the mean of at least 7 determinations ± S.E. SOD, superoxide dismutase.

Statistical significance was calculated using a two-tailed unpaired Student’s t test.
from quinones or from iron-sulfur centers. For these enzymes, high concentrations of succinate can fully suppress superoxide formation; the substrate binds near the FAD and is believed to obstruct the access of oxygen to the reduced flavin (39). Our data do not support this interpretation for the yeast SDH. At a concentration of succinate of 20 mM, the wild-type yeast SDH demonstrates a more than 2-fold increase in the rate of superoxide formation over that at 0.1 mM (Fig. 4C); in contrast, superoxide production by the E. coli SDH is reduced by ~10-fold at 20 mM succinate compared with its peak at 0.1 mM (39). Furthermore, when assayed under identical conditions, the fractions of superoxide dismutase-sensitive succinate-cytochrome c reductase activities of the mutant SDH enzymes are larger than their respective wild-types at all concentrations of succinate (not shown). The simplest explanation for our results is that the Q₈ site is a source of superoxide anions in the wild-type enzyme. The SDH2 P190Q and the SDH3 S94E mutations further increase superoxide production at the Q₈ site by perturbing its architecture.

In conclusion, our results demonstrate that mutated forms of a mitochondrial SDH can be significant sources of oxygen-free radicals. The mutations we investigated are located near the Q₈ site, strongly suggesting that this is the site of radical formation. Additional studies will be required to determine whether paraganglioma or other types of mutations can similarly increase the formation of superoxide radicals.

Acknowledgments—We thank Drs. Kayode Oyedotun and Zhangwei Zhao for helpful discussions.

REFERENCES
1. Ackrell, B. A. (2002) Mol. Aspects Med. 23, 369–384
2. Rustin, P., and Rigat, A. (2002) Biochim. Biophys. Acta 1553, 117–122
3. Rustin, P., Munnich, A., and Rotig, A. (2002) Eur. J. Hum. Genet. 10, 289–291
4. Lemire, B. D., and Oyedotun, K. S. (2002) Biochim. Biophys. Acta 1553, 102–116
5. Hederstedt, L., and Ohnishi, T. (1992) in Molecular Mechanisms in Bioenergetics (Ernster, L., ed) pp. 163–197, Elsevier Science Publishers, New York
6. Ackrell, B. A., Johnson, M. K., Gunsalus, R. P., and Cecchini, G. (1992) in Chemistry and Biochemistry of Flavoenzymes (Muller, F., ed) Vol. III, pp. 229–297, CRC Press, Inc., Boca Raton, FL
7. Oyedotun, K. S., and Lemire, B. D. (1999) FEBS Lett. 442, 203–207
8. Oyedotun, K. S., and Lemire, B. D. (2001) J. Biol. Chem. 276, 16938–16943
9. Oyedotun, K. S., and Lemire, B. D. (1999) J. Biol. Chem. 274, 23956–23962
10. Iversen, T. M., Luna-Chavez, C., Cecchini, G., and Rees, D. C. (1999) Science 284, 1961–1966
11. Lancaster, C. R., Kröger, A., Auer, M., and Michel, H. (1999) Nature 402, 377–385
12. Lancaster, C. R., Gross, R., and Simon, J. (2001) Eur. J. Biochem. 268, 1820–1827
13. Iversen, T. M., Luna-Chavez, C., Croal, L. R., Cecchini, G., and Rees, D. C. (2002) J. Biol. Chem. 277, 16124–16130
14. Yankovskaya, V., Horsefield, R., Turnroth, S., Luna-Chavez, C., Miyoshi, H., Leger, C., Byrne, B., Cecchini, G., and Iwata, S. (2003) Science 299, 700–704
15. Bourgeron, T., Rustin, P., Chretien, D., Birch-Machin, M., Bourgeois, M., Viegas-Pequignot, E., Munnich, A., and Rotig, A. (1995) Nat. Genet. 11, 344–349
16. Baysal, B. E. (2002) J. Med. Genet. 39, 617–622
17. Baysal, B. E., Willett-Broznic, J. E., Lawrence, E. C., Drozdlic, C. M., Savul, S. A., McLeod, D. R., Yee, H. A., Brackmann, D. E., Slattery, W. H., III, Myers, E. N., Ferrell, R. E., and Rubinstein, W. S. (2002) J. Med. Genet. 39, 178–183
18. Ishii, N., Fujii, M., Hartman, P. S., Tsuda, M., Yasuda, K., Seno-Matsuda, N., Yanase, S., Ayasawa, D., and Suruki, K. (1998) Nature 394, 684–697
19. Seno-Matsuda, N., Yasuda, K., Tsuda, M., Ohkubo, T., Yoshimura, S., Nakazawa, H., Hartman, P. S., and Ishi, N. (2001) J. Biol. Chem. 276, 41533–41538