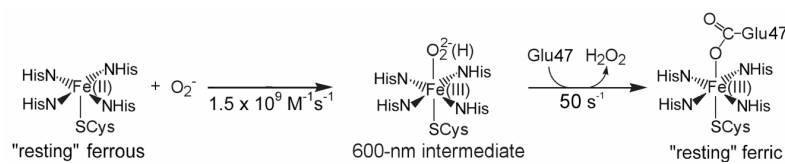


# Kinetic and Spectroscopic Investigations of the Mechanism of Superoxide Reductase from *Desulfovibrio vulgaris*

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The superoxide reductases (SORs) represent a newly emerging reductive rather than disproportionative paradigm for oxidative stress protection in air-sensitive bacteria. The SOR from *Desulfovibrio vulgaris* contains an [Fe(SCys)<sub>4</sub>], rubredoxin-like site in addition to the [Fe(NHis)<sub>4</sub>(SCys)] active site characteristic of all known SORs. The [Fe(SCys)<sub>4</sub>] site can be removed with no loss of functionality by a single mutation (C13S) (2). Our previous pulse radiolysis studies on reactions of the ferrous SOR site with superoxide detected an intermediate, which absorbs maximally near 600 nm, and



which decays to the resting ferric state as shown (1). The nature of the 600-nm intermediate, as well as the decay mechanism, remains unclear. We have developed stopped-flow and cryogenic methods for monitoring the reaction of the *D. vulgaris* SOR active site with superoxide. The stopped-flow UV-vis absorption time courses of both wild type and C13S SORs are fully consistent with the previous pulse radiolysis results, where only one intermediate was observed. The same intermediate is observed for the reaction of the ferrous SOR site with superoxide at -40 °C, where it is stable for up to 1 minute, in contrast to ~30 milliseconds at room temperature. EPR spectra of the reaction intermediate prepared under conditions similar to those used for the cryogenic UV-vis studies revealed two features ( $g = 4.15$  and  $g = 9.4$ ) that are distinct from those of the resting ferric state. These results provide new insights into the nature of the intermediate and the SOR mechanism.

1. Emerson, J. P., Coulter, E. D., Cabelli, D. E., Phillips, R. S., Kurtz, D. M., Jr. *Biochemistry* **2002**, *41*, 4348-4357.
2. Emerson, J. P., Cabelli, D. E., Kurtz, D. M. Jr. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100*, 3802-3807.