

EPR spin-trapping investigation into the kinetics of glutathione oxidation by the superoxide radical

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The ability of glutathione (GSH) to scavenge the superoxide radical is a matter of serious contention in the literature: reported values for the second-order rate constant range from 10^2 to greater than $10^5 \text{ M}^{-1} \text{ s}^{-1}$. The physiological implications of this discrepancy will determine, for example, whether or not glutathione can compete with Mn-superoxide dismutase for reaction with the radical in the mitochondrial matrix, leading to formation of the potentially harmful glutathionyl radical (Reaction 1).



Several authors have investigated the kinetics of glutathione oxidation by superoxide using spectrophotometric assays, based on competition between either ferricytochrome c or epinephrine for reaction with the radical. However, these approaches have received criticism because the contributions of various secondary reactions to the overall kinetics have been largely overlooked. In the present investigation, we have used EPR spectroscopy to monitor competition between GSH and the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) for reaction with superoxide. Interpretation of the EPR spectra (and the kinetics) was complicated by the finding that the DMPO superoxide radical-adduct undergoes a rapid, two-electron reduction to the hydroxyl radical-adduct by GSH. Taking this and other factors into consideration, we estimate the second-order rate constant for the oxidation of glutathione by superoxide to be $\sim 200 \text{ M}^{-1} \text{ s}^{-1}$.

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