Quantitative spin trapping: superoxide generation by NAPDH cytochrome P-450 reductase

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The superoxide free radical plays an important role in cellular injury and signaling. Quantitative spin trapping using the spin trap 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) is a highly sensitive and specific method to measure superoxide generation by electron paramagnetic resonance. This method has been previously applied to quantitate superoxide generation from the enzyme xanthine oxidase and from activated leukocytes. The enzyme NADPH cytochrome P-450 reductase is also reported to generate superoxide in cells. It is present in high concentration in liver and is also found in a variety of other tissues. Depending on its magnitude this NADPH derived superoxide generation could have important biological effects. We present here the quantitation of superoxide generation by the NADPH cytochrome P-450 reductase We determined the concentration of the DEPMPO-O_2H adduct formed over time from the EPR spectra observed, by comparison to the spectrum of a standard solution of TEMPO. Then, we determined the decay rate of the adduct in this system following the addition of superoxide dismutase. After correcting for the adduct decay and compensating for the efficiency of DEPMPO for superoxide trapping, we could both estimate the magnitude and rate of superoxide generation from the enzyme. This method of quantitative spin trapping should be useful for measuring the magnitude of superoxide generation in a variety of enzymatic and cellular systems.