

A Trityl-Based Assay for the Detection of Superoxide in Biological Systems.

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Detection of superoxide radical is of critical importance for investigating the pathophysiological consequences resulting from altered cellular reactive oxygen homeostasis. In this study, we used the triarylmethyl (trityl) free radical, TAM Ox063, for the detection of superoxide by electron paramagnetic resonance (EPR) spectroscopy and spectrophotometry. TAM is paramagnetic (EPR active), highly soluble in water and exhibits a single sharp EPR peak in aqueous solutions. TAM reacts with superoxide with an apparent second order rate constant of $3.1 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. The specific reactivity of TAM with superoxide, which leads to loss of EPR signal, was used for the determination of superoxide concentration in enzymatic and cellular model systems. We also investigated the feasibility of a spectrophotometric assay of superoxide by taking advantage of the changes in the optical spectrum of TAM on reaction with superoxide. In conclusion, the use of a trityl radical for a quantitative assay of superoxide offers an alternative approach with certain advantages over other methods.