

Application of a Trityl-based Radical for Measuring Superoxide Generation in Biological Systems

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A new trityl-based narrow line EPR probe, TAM OX063, has been investigated for possible superoxide detection in aqueous solutions. The probe is non-reactive toward most of the common oxidants/reductants that occur in biological systems. However, it is observed to react specifically with radical species such as superoxide, nitric oxide, and alkylperoxyl radical. Its reaction with hydroxyl radicals, however, was non-specific possibly due to the high reactivity of the hydroxyl radical. A competitive kinetic method was used to calculate the second order rate constant of superoxide with TAM. The specific reactivity of TAM toward superoxide has been utilized to measure superoxide generation in chemical, enzymatic and cellular systems. The 'spin-loss' method of superoxide detection and quantification using EPR appears to have several advantages over the conventional nitron-based spin trapping or hydroxylamine oxidation methods.