Attempt to Model the Kinetics of Formation and Decay of Nitrone Superoxide Spin Adducts

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Superoxide, one of the most important radicals formed in aerobic organisms, and other reactive oxygen species have been proposed to be involved in a wide range of pathological processes, such as DNA damage, lipid peroxidation, inflammation, or neuron death. Spin trapping using nitrones, in conjunction with EPR spectroscopy, is one of the best techniques for detecting free radicals in biological systems. Therefore, many efforts have been devoted to the elaboration of new and always more efficacious nitrones to trap superoxide in aqueous media. Until now, the principal aim of many groups of researchers was to improve the superoxide adduct stability. In this field, many results have demonstrated that the presence of an electron withdrawing group, such as a phosphonate or a carboxy-ester, in β-position towards the nitrone function significantly improved the persistence of the superoxide spin-adduct. However, what is really important is not to obtain more persistent spin adducts, but to reach higher spin adduct concentrations, and this can also be done by increasing the spin trapping rate. Thus, when various nitrones are compared as regards their efficacy in detecting superoxide, not only the adduct decay rate but also the trapping kinetics must be considered.

Whereas it is quite easy to measure a superoxide adduct life time, it is much more difficult to study the superoxide trapping kinetics. The method of kinetic competition with another nitrone or with SOD is not easily implemented and often gives rough results. So there is a real need to elaborate an efficacious method to determine the superoxide trapping rate.

For this purpose, superoxide was produced in buffered solution using a standard xanthine / xanthine oxidase system in the presence of a nitrone. The nitrone concentration used must be low enough to observe a competition between the spin trapping and the dismutation of the superoxide radical. An EPR spectrum of the superoxide adduct formed was thus recorded at steady intervals in the presence of an internal standard, in order to calculate the spin adduct concentration. Modeling the kinetic curve thus obtained must permit to evaluate simultaneously the rate constants for the spin trapping of superoxide and for the adduct decay. Finally, we tried to apply this new method to various phosphorylated and non-phosphorylated nitrones.