ESR and HPLC-EC Analysis of Radical Intermediates

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Studies of oxidative stress have attracted considerable interest and have been the focus of much research in recent years. Cumulative oxidative damage to tissues has been implicated in a number of disease states, e.g. the aging process, cancer, and ischemia reperfusion. In the last two decades, the understanding of the molecular mechanisms of bio-oxidative damage was strongly enhanced with the advent of ESR spectrometry and the later development of the spin-trapping technique. The spin-trapping technique is based on the high rates of interaction of nitrones or nitroso compounds with radical species. The paramagnetic nitroxides formed as a result of the latter interaction exhibit ESR spectra that are "structural fingerprints" of the parent radical species. ESR analysis has been used for depicting free radical-dependent processes implicated in toxic pathways of metabolism of drugs and environmental chemicals. Extensive ESR spin-trapping studies in model systems led to the formation of spectral libraries, which in combination with computer programs for ESR spectra simulation, make virtually certain the structural identification of any physiologically relevant radical intermediate. The use of this technique in experiments *in vivo* and with intact cells, however, is restricted by the low stability of the nitroxides in cellular milieu. The disappearance of the ESR spectra of a nitroxide is generally due to its disproportion and/or metabolic transformation to ESR silent compounds. In this lecture, chromatographic methods for the separation and detction of ESR silent analytes derived from the spin adducts of glutathione tiyl, trichloromethyl, alkyl (C_{1-5}), and hydroxyl radicals will be presented.