

Protein NMR Spin Trapping with [Methyl-¹³C₃]MNP; Application to Equine Myoglobin

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Direct spin trapping studies of protein radical adducts are limited as a consequence of the long rotational correlation times and consequent broadening of the ESR resonances. It can be difficult to determine both the nature and number of adduct species present. NMR detection of reduced spin adducts represents an alternate approach that can potentially provide substantial structural insights into the nature of the adduct species. Application to protein adducts has the particular advantage that any chemical reactions of the trap which result in low molecular weight species are effectively eliminated from consideration through sample dialysis. We have recently utilized [methyl-¹³C₃]-MNP for the detection and analysis of tyrosyl spin adducts formed as a result of exposure of equine myoglobin to hydrogen peroxide. The methyl-¹³C label allows high detection sensitivity in two dimensions and narrow linewidths due to internal diffusion. For equine myoglobin, it is found that adduct formation involves a single residue - Tyr-103, and further that adduct formation occurs at the C-3 carbon of the amino acid. HMQC-NOESY experiments further reveal the proximity of the labeled methyl groups both to the three remaining tyrosyl ring protons as well as to the aromatic protons of the nearby Phe-106 residue. In order to fully exploit this methodology, a more complete understanding of the chemistry of reduced radical adducts will be required.