Protein-protein radical transfer reactions in peroxide-treated myoglobin

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Protein radicals can lead to cross-linking of the protein, cleavage of the protein backbone and the formation of protein peroxy radicals and protein peroxides. These radical reactions can be physiologically relevant. In most situations, however, protein radicals facilitate pathological or toxicological processes. Despite their importance, the factors and mechanisms that control the formation, localization, delocalization, and propagation of protein free radicals remain obscure.

The reactions of sperm whale and horse heart metmyoglobin (MetMb) with H_2O_2 have served as a useful model for the investigation of protein radicals. The early observation by EPR of protein radicals in the reaction of MetMb with H_2O_2 has been more precisely defined by recent work [1,2]. Site-specific mutagenesis, in conjunction with protein digestion, peptide sequencing, mass spectrometry, and EPR spectroscopy, has established that protein radicals delocalize readily, even to distant sites within the globin molecule, once they are generated at a well-defined site, i.e. in the vicinity of the heme iron atom. The radicals have been localized to tyrosine and/or tryptophan residues that effectively stabilize the odd electron through extended delocalization over unsaturated bonds.

In the present work, we have investigated whether the protein radicals thus generated can transfer from one globin molecule to another and whether this mechanism of radical propagation can produce protein radicals in the second molecule of globin distinct from that formed by radical generation within the protein itself. Characterization of these reactions provides valuable information regarding the poorly understood mechanisms of radical transfer between proteins.

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[2]. Degray J. A., Gunther, M. R., Tschirret-Guth, R., Ortiz de Montellano, P.R. and Mason, R. P., *J. Biol. Chem.,* **272**, 2359-2362, 1997