Detection of hemoglobin-radical derived nitrone adducts by Immuno-Spin Trapping

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Protein-derived free radicals, especially those derived from hemoproteins, play important roles in pathophysiological conditions, such as aging cancer, inflammation, atherosclerosis, neurodegenerative diseases and exposure to environmental hazards. Certainly, the earlier detection and characterization of hemoprotein-derived free radical could improve the human life quality and expectancy. In order to probe the involvement of a hemoglobin radical in the human oxyhemoglobin (oxyHb) or methemoglobin (metHb)/ H_2O_2 system, we have developed and utilized a new approach, "*Immuno-Spin Trapping*, (IST)" which combines the specificity and sensitivity of both spin trapping and antigen:antibody interactions. Previously, a novel rabbit polyclonal anti-DMPO nitrone adduct antiserum, which specifically recognizes protein radical-derived nitrone adducts, was developed and validated in our laboratory [Detweiler, 2002 #51]. In the present study, the formation of nitrone adducts on hemoglobin depended on the oxidation state of the iron heme, the concentrations of H_2O_2 and DMPO, and time as determined by an Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Western Blotting. The presence of reduced glutathione or L-ascorbate significantly decreased the level of nitrone adducts on metHb in a dose-dependent manner. Western blotting analysis showed that only the complete system (oxy- or metHb/DMPO/H_2O_2) generates epitopes recognized by the antiserum. The specific modification of tyrosine residues on metHb by iodination produced a significant decrease in antibody binding, while the thiylation of cysteine residues did not affect it at all. In comparison with either direct ESR or ESR spin-trapping detection of the tyrosine-centered protein-derived free radical in biological systems, IST showed the following advantages: i) exhibits higher sensitivity, ii) requires smaller samples, iii) gives the approximate molecular weight of the protein radical-derived nitrone adducts, iv) permits the assay of many samples at the same time, and v) does not require expensive ESR instruments to detect the radical adducts. In conclusion, IST showed the formation of tyrosyl radical(s) in human oxyHb and metHb exposed to *very low* concentrations of H_2O_2 and we reported here for the first time the detection of intracellular nitrone adducts in red blood cells, as a first application.