

Reaction of Human Hemoglobin and Peroxynitrite: Involvement of Protein Radicals on the Reaction Mechanism.

Natalia Romero¹, Rafael Radi¹, Edlaine Linares³, Ronald Mason⁴, Ohara Augusto³ and Ana Denicola².

¹Departamento de Bioquímica, Facultad de Medicina and ²Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay; ³Departamento de Bioquímica, Instituto de Química, Universidade de Sao Paulo, Sao Paulo, Brazil and Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina, USA.

We have previously demonstrated that even in the presence of physiological concentrations of CO₂, more than 60% of intravascular-generated peroxynitrite is able to diffuse and reach the inside of red blood cells (RBC). Once inside erythrocytes, the principal target of peroxynitrite will be oxyhemoglobin (oxyHb) due to its high concentration (20 mM heme) and rate constant ($k_2 = 2 - 3 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$). The final hemoprotein product was demonstrated to be methemoglobin (metHb) and the intermediacy of ferrylHb was established. During ferrylhemoglobin decay, a relatively stable and asymmetric EPR signal is detected, containing partially resolved hyperfine structure and broad low- and high-field shoulders. The relatively long half-life of the signal and its EPR parameters ($g = 2.005$; line width $> 20 \text{ G}$) identify the main radical species as a Hb bound tyrosyl radical. SDS-PAGE studies under non-reducing conditions and excess of hemoglobin shows dimerization products suggesting that not only tyrosine but also cysteine residues are involved in the reaction mechanism. Oxymetry studies show very little oxygen evolution when oxyHb reacted with equimolar concentration of peroxynitrite (less than 30% of metHb yields). However, oxygen yields were enhanced when reactive hemoglobin thiols (Cys β -93) were previously blocked with N-ethylmaleimide, suggesting that parallel globin reactions are likely to be consuming the oxygen liberated in the first steps of the reaction. Current studies are being performed with an anti-DMPO adduct antibody to further confirm the intermediacy of protein-derived radicals during the redox process.

Please attach this page when you submit your abstract

Presenting Author Name: Natalia Romero

Preferred presentation style:

Oral or Poster

Preferred session: choose one of the following sessions that best fit your abstract.

- | | |
|---|--|
| <input type="checkbox"/> Nonionizing Radiation--Derived Free Radicals | <input type="checkbox"/> Spin Trap Synthesis and Application |
| <input type="checkbox"/> Novel Methods of Free Radical Trapping | <input type="checkbox"/> Nitric Oxide Trap - Chemistry and Biology |
| <input type="checkbox"/> Nitroxide and Radical Trap Drugs | <input checked="" type="checkbox"/> Young Investigator Award Session |
| <input type="checkbox"/> Cellular Spin Trapping | <input type="checkbox"/> In Situ ESR |
| <input type="checkbox"/> In Vivo Spin Trapping | <input checked="" type="checkbox"/> Other |

Presentation method: Choose one of the following for your oral presentation if selected

- 35 mm slide projector
 Overhead projector
 Laptop with a LCD projector.

Please send your abstract and this page to:

Ms. Barbara Morse
LPC/NIEHS/NIH
P.O. Box 12233, MD F1-03
111 TW Alexander Drive
Research Triangle Park, NC 27709, USA
Phone: +1 919 541 3197
Fax: +1 919 541 5737
morse@niehs.nih.gov