Understading the mechanism of Peroxynitrite induced nitration of *Escherichia coli* Manganese Superoxide Dismutase

N. Basak Surmeli[1], Nadia K. Litterman[1], Anush Karapetian[2], K. Padmakumar[2], Anne-Frances Miller[2] and John T. Groves[1]

[1]Department of Chemistry, Princeton University, [2]Department of Chemistry, University of Kentucky

The peroxynitrite anion is a strong oxidizing agent, formed by the diffusion-limited reaction of nitric oxide and superoxide. Peroxynitrite may represent an important mediator of inflammation-induced tissue injury and dysfunction by its ability to nitrate and oxidize biomolecules. Synthetic agents able to prevent this damage could be beneficial to human health[1][2]. Nitration of *Escherichia coli* manganese superoxide dismutase (Mn-SOD) by peroxynitrite was investigated, and demonstrated by spectral changes and MS analysis. Tyrosine nitration of Mn-SOD is followed by directly monitoring the nitrotyrosine chromophore. Addition of azide, an inhibitor of Mn-SOD, and fluorescein, a scavenger of NO_2 revealed that tyrosine nitration was Mn-mediated. Tyrosine nitration was also monitored in a Mn-SOD Y34F mutant because tyrosine-34 is in close proximity of the manganese ion at the active site. Tyrosine nitration is also examined in Mn-SODs in which the active site manganese is replaced by zinc, cobalt and iron. HPLC-MS studies of the tryptic digests of mono-nitrated Mn-SOD indicated that three out of seven tyrosine residues are susceptible to peroxynitrite mediated nitration: tyrosine-34, tyrosine-9 and tyrosine-11. In the presence of fluorescein, tyrosine 34 is the main target of nitration. The results clearly indicate that participation by the manganese ion causes the specific nitration of tyrosine 34. *Support of this research by the National Institutes of Health [GM036298] is gratefully acknowledged.*

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