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

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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 NO₂ 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.
