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# Nitration and Inactivation of Tyrosine Hydroxylase by Peroxynitrite*

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**Tyrosine hydroxylase (TH) is modified by nitration after exposure of mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydrophenylpyridine. The temporal association of tyrosine nitration with inactivation of TH activity *in vitro* suggests that this covalent post-translational modification is responsible for the *in vivo* loss of TH function (Ara, J., Przedborski, S., Naini, A. B., Jackson-Lewis, V., Trifiletti, R. R., Horwitz, J., and Ischiropoulos, H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 7659–7663). Recent data showed that cysteine oxidation rather than tyrosine nitration is responsible for TH inactivation after peroxynitrite exposure *in vitro* (Kuhn, D. M., Aretha, C. W., and Geddes, T. J. (1999) *J. Neurosci.* 19, 10289–10294). However, re-examination of the reaction of peroxynitrite with purified TH failed to produce cysteine oxidation but resulted in a concentration-dependent increase in tyrosine nitration and inactivation. Cysteine oxidation is only observed after partial unfolding of the protein. Tyrosine residue 423 and to lesser extent tyrosine residues 428 and 432 are modified by nitration. Mutation of Tyr$^{423}$ to Phe resulted in decreased nitration as compared with wild type protein without loss of activity. Stopped-flow experiments reveal a second order rate constant of $(3.8 \pm 0.9) \times 10^3$ $M^{-1}$ $s^{-1}$ at pH 7.4 and 25 °C for the reaction of peroxynitrite with TH. Collectively, the data indicate that peroxynitrite reacts with the metal center of the protein and results primarily in the nitration of tyrosine residue 423, which is responsible for the inactivation of TH.**