Interaction of Peroxynitrite with MetMb probed by Fluorescein

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Myoglobin is known to be susceptible to damage by peroxynitrite as one of the heme protein targets *in vivo*. Moreover, it has been reported that the bimolecular heme/peroxynitrite interaction results in the catalysis of peroxynitrite decomposition and myoglobin tyrosine nitration. In this study, we employed fluorescein as a hypersensitive nitration probe to investigate the interaction between peroxynitrite and metMb. To clarify the fundamental mechanism, the following interactions were explored in vitro in the absence of CO_2. The catalytic rate constant of peroxynitrite decomposition in the presence of metMb at pH 7.4 and 25 ^{o}C, is 1.0×10^4 M^{-1} s^{-1}. By examining the formation of nitrofluorescein mediated by peroxynitrite, we were able to probe the kinetics of NO_2 production after metMb/peroxynitrite interaction. Meanwhile, the yields of nitrofluorescein under these conditions allowed a determination of the extent of peroxynitrite transformation to NO_2 and nitrate. Following the above approaches, we found that metMb catalyzes the isomerization of peroxynitrite to nitrate, and to a less extent, NO_2. Kinetic simulation of these results indicates that freely diffusing NO_2 is produced during metMb-catalyzed peroxynitrite decay at a rate of 200 M^{-1} s^{-1}, which accounts for ~2% catalyzed peroxynitrite decay, compared to ~30% NO_2 evolution from peroxynitrite spontaneous decay. Hence, the total yields of the nitrated products decline. Furthermore, by detecting the formation of sulfmyoglobin, a ferryl myoglobin complex was identified as an intermediate during the course of metMb/peroxynitrite interaction. Collectively, using fluorescein to probe metMb/peroxynitrite reactions affords a novel approach to study hemeprotein/peroxynitrite interactions, which further shed light on NO-mediated hemeprotein reactions.