PEROXIREDOXIN 1 COORDINATION TO DINITROSYL IRON COMPLEX

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Protein S-nitrosation is a reversible post-translational modification that has been associated with a variety of physiological and pathological processes. However, the mechanisms responsible for S-nitrosation in vivo remain debatable, and there are limited kinetic studies of S-nitrosation reactions. Since peroxiredoxins (Prx) have been proposed to transnitrosate other thiol proteins, we decided to compare the kinetics of Prx1 S-nitrosation by nitrosoglutathione (GSNO) and dinitrosyl-diglutathionyl-iron complex \([\text{Fe(NO)}_2(\text{GS})_2]^+\). The reactions were followed by the decay of the intrinsic fluorescence of the enzyme. Prx1 was shown to be transnitrosated by GSNO with a second-order rate constant of \(k_{1+\text{NO}} = (15.4 \pm 0.4) \text{ M}^{-1} \text{s}^{-1}\) and \(k_{2+\text{NO}} = (1.7 \pm 0.4) \text{ M}^{-1} \text{s}^{-1}\) at pH 7.4 and 25°C. The kinetics of the reaction between Prx1 and \([\text{Fe(NO)}_2(\text{GS})_2]^+\) provided second-order rate constants of \(k_1 = (2.97 \pm 0.06) \times 10^2 \text{ M}^{-1} \text{s}^{-1}\) and \(k_2 = (1.42 \pm 0.02) \text{ M}^{-1} \text{s}^{-1}\) at pH 7.4 and 25°C. Attempts to separate the excess of the iron complex from the protein were not successful. Thus, EPR analysis at room temperature were performed to evaluate a possible interaction of \([\text{Fe(NO)}_2(\text{GS})_2]^+\) with Prx1. In contrast with the EPR spectra of free \([\text{Fe(NO)}_2(\text{GS})_2]^+\) that shows a single symmetrical line at \(g = 2.03\), the EPR spectra of the solution containing the protein exhibited an anisotropic signal, likely rhombic \((g_z = 2.04; g_y = 2.03; g_z = 2.01)\). Apparently, a Prx1 dinitrosyl complex was formed instead of the expected Prx1-SNO. This same behavior was observed with Prx2, Prx1 mutant C83SC173S and C52S, establishing that the peroxidatic thiol participates in the interaction. These data suggest that peroxiredoxin thiol can replace the GS ligand coordinating to the iron center forming a stable DNIC of high molecular weight. These complexes may be involved nitric oxide storage and/or protein S-nitrosation.

Key words: S-nitrosation; peroxiredoxin; dinitrosyl iron complex.

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