

Yang Y and Loscalzo J. S-nitrosoprotein formation and localization in endothelial cells. *Proc Natl Acad Sci* 2005; 102(1): 117-122.

Lay summary: Nitric oxide, a smooth muscle relaxer, can modify proteins through a process known as S-nitrosation that yields S-nitrosated proteins. Little is known about the source of NO or the target proteins that result, primarily due to technical difficulties. The authors have used a novel labeling and imaging method to localize S-nitrosated proteins to the mitochondria and peri-mitochondria and quantify their half-life in endothelial cells as approximately one hour. Furthermore, with their method, the authors were able to determine that intact mitochondrial function is essential for protein S-nitrosation to take place. When they caused endogenous NO production to change, the authors found that the abundance of S-nitrosated proteins changed in consequence. Using new tools of mass spectrometry they determined that a limited number of S-nitrosated proteins exist within endothelial cells and identified six of them, with the most abundant being GAPDH, suggesting a regulatory function for NO in carbohydrate metabolism that has been proposed but not previously characterized.

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Abstract

Protein S-nitrosation represents a recently described form of post-translational modification that is rapid and reversible. However, the analysis of protein S-nitrosation *in situ* has been difficult because of the absence of specific probes and the instability of cellular protein S-nitrosothiols. We developed a rapid and specific method for detecting endothelial S-nitrosoproteins patterned after the biotin switch method that involves thiol alkylation followed by reductive generation of thiols from S-nitrosothiols, which are then labeled with either a biotin- or Texas red-derivative of methanethiosulfonate. When we used this methodology, we found that S-nitrosated proteins can form within endothelial cells from an exogenous S-nitrosothiol donor or from endogenous production of NO by endothelial NO synthase. When we used confocal microscopy, we found that these S-nitrosoproteins exist mainly in the mitochondria and peri-mitochondrial compartment, and that their half-life is ≈ 1 h. Cellular S-nitrosated protein abundance changed as expected, with changes in activity of NO synthase, and with impairment of mitochondrial function and scavenging of peroxynitrite. We used a proteomic approach involving two-dimensional gel electrophoresis and mass spectrometry, and found that a limited number of S-nitrosoproteins exist in endothelial cells (S-nitrosoproteome) and identified GAPDH, vimentin, β -galactosidase, peroxiredoxin 1, β -actin, and ubiquitin-conjugating enzyme E2 among them. The most abundant S-nitrosated protein in the resting endothelial cell is GAPDH, suggesting a regulatory function for NO in glycolysis. These data offer methods and insights into identifying the protein targets of S-nitrosation reactions and their potential role in cell function and phenotype.