iNOS-Independent SNO Generation at Low pH and SNO Prediction Using a Neural Network

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Nitrosylation is a form of post-translational modification, which provides modularity to protein function after translation, allowing cells to adapt to a variety of stimuli. However, unlike more “canonical” post-translational modifications, the motif and environmental conditions under which cysteine residues are nitrosylated are unclear. The chemistry of formation presupposes that there may be subsets of cysteines that are nitrosylated via different mechanisms. The most comprehensive database for SNO, dbSNO 2.0, indiscriminately collected literature reports for nitrosylation and thus cysteines do not follow a consistent set of environmental conditions and represent different populations of SNO. We hypothesize that different SNO populations may be generated by altering microenvironment of the cysteine residues. SNO protein formation was examined in RAW cells with and without LPS stimulation, while altering the intracellular environment using mitochondrial poisons (MP). Cells were treated with oligomycin, FCCP, or rotenone after 1 hour of stimulation with LPS. Treatment significantly increased nitrite release to the media. Both rotenone, the complex I inhibitor, and oligomycin, the ATP synthase inhibitor, significantly reduced nitrite production. FCCP did not alter nitrite production. Western blot of cell lysate confirms iNOS in LPS-stimulated cells for all treatments, with reduced banding in MP. Using Biotin Switch, we were able to show significant production of SNO proteins in response to LPS, which was unaltered by FCCP. However, rotenone completely abrogated SNO formation, while oligomycin forms SNO even in the absence of LPS. These studies indicate that nitrosylation is influenced by the intracellular environment, with cellular acidification being a major mechanism. Identification of the SNO-protein identities within cell lysate will allow one to create database for training a neural network for cysteines. To demonstrate viability of a neural network approach, an algorithm was trained on a subset of dbSNO entries, randomly selecting 20% of the trimmed sample as the validation set. A window size of 19 was discovered to return the optimal MCC for the following hyperparameters: two hidden layers, 50 neurons each layer, learning rate of 0.0005, L1 Regularization parameter of 5, and 2000 iterations. Sigmoid activation function was used for the hidden layers, TanH activation function for the output layer, and a quadratic cost function to backpropagate. A median MCC of 0.230, with a sensitivity of 0.661, a specificity of 0.569, a positive predictive value of 0.589, and a negative predictive value of 0.642 was obtained. *NIH ES005022 HL086621.*