Isotopomer analysis of nitrous oxide produced by cytochrome P450nor from *Histoplasma capsulatum*

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Nitrous oxide (N_2O) is a potent greenhouse gas and is also involved in ozone layer destruction. Due to the rise of agricultural activities, the atmospheric concentration of N_2O is almost 20% higher than pre-industrial levels, increasing at a rate of ~0.25% per year. To account for the global N_2O concentration and establish effective policies related to N_2O production, we need to increase our understanding of microbial N_2O formation. Previous $\delta^{15}N$ and $\delta^{18}O$ characterization of the nitrification and denitrification processes provide insights for the fractionation of different pathways. However, $\delta^{15}N$-N_2O and $\delta^{18}O$-N_2O values alone are not enough to fully differentiate the two N_2O-generation pathways. Therefore, in addition to these values, site preference values are measured and have been successfully used to identify various bacterial N_2O sources. Because these isotope values are typically obtained from culture studies, however, they represent the net isotope fractionation of the entire process, including gas diffusion, enzymatic conversion, and product release. In most cases, the specific fractionation effects of the actual enzymatic steps remain unknown.

In our study we used trace gas-isotope ratio mass spectrometry with high precision to measure the four N_2O isotopomers ($^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, $^{14}N^{15}N^{18}O$, $^{15}N^{14}N^{18}O$) generated by purified P450 nitric oxide reductase (P450nor) from *Histoplasma capsulatum* over the course of an hour. The kinetic isotope effects, fractionation factors, and site preference values were calculated for the purified P450nor-catalyzed N_2O process. Our analysis indicated β-^{15}N of N_2O molecule has an inverse isotope effect but other atoms (α-^{15}N of N_2O and ^{18}O-N_2O) have normal isotope effects. The physiological and mechanistic implications of these results will be discussed.