INVOLVEMENT OF ALTERNATIVE RESPIRATORY PROTEINS IN SUPEROXIDE-DEPENDENT NITRIC OXIDE DEGRADATION BY PLANT MITOCHONDRIA

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Nitric oxide (NO) regulates mitochondrial respiration by inhibiting reversibly the cytochrome *c* oxidase. NO degradation is very important in controlling steady-state levels of NO, thereby affecting its regulatory functions. Here, we examined NO degradation in mitochondria isolated from potato tubers and *Arabidopsis thaliana* cells, and the contribution of plant alternative respiratory proteins, such as the external NAD(P)H dehydrogenases and the alternative oxidase, in this process. NO and oxygen concentrations were followed simultaneously with specific electrodes. NO degradation was faster in mitochondria energized with NAD(P)H than with succinate or malate. Accordingly, oxygen consumption was transiently inhibited by NO in NAD(P)H-energized mitochondria, in contrast to the persistent inhibition seen in the presence of succinate. NO degradation was abolished by superoxide dismutase, which suggested that NO was consumed by its reaction with superoxide anion (O_2^-). Antimycin-A stimulated and myxothiazol prevented NO consumption in succinate- and malate-energized mitochondria. Although favored by antimycin-A, NAD(P)H-mediated NO consumption was not abolished by myxothiazol, indicating that an additional site of O_2^- generation, besides complex III, stimulated NO degradation. Peroxide production assessed with Amplex Red showed that larger amounts of O_2^- were generated in NAD(P)H-energized mitochondria. The rates of NAD(P)H-mediated NO degradation and O_2^- production were stimulated by free Ca^{2+} concentration, indicating the involvement of Ca^{2+}-dependent external NAD(P)H dehydrogenases in O_2^- production, that favors NO degradation in potato tuber and *Arabidopsis* mitochondria. The alternative oxidase of *Arabidopsis* mitochondria, that is not inhibited by NO, diminished electron leakage from the respiratory chain, thereby decreasing NO consumption. These findings indicate the importance of alternative proteins of plant respiratory chain on mitochondrial NO homeostasis. Supported by FAPESP.
Keywords: nitric oxide; plant mitochondria; superoxide anion.