CONFORMATIONAL STATES OF NITRIC OXIDE SYNTHASE CHARACTERIZED BY TIME-RESOLVED FLUORESCENCE

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Nitric oxide synthase (NOS) catalyzes the formation of nitric oxide through a process that involves transfer of electrons sequentially from FAD to FMN in the reductase domain of the enzyme and then from FMN to a heme in the oxygenase domain of the partner enzyme in a homodimeric complex. Efficient NO synthase activity is enabled by binding of the calcium signaling protein calmodulin (CaM) to a CaM-binding domain situated between the reductase and oxygenase domains. Because the rate of electron transfer depends sharply on distance, electron transfer requires close proximity of electron-transfer donor and acceptor. The sequence of electron transfers in NOS therefore necessitates multiple conformational states of the enzyme, suggesting that the electron transfers are conformationally gated. We have used fluorescence-labeled CaM and time-resolved fluorescence to detect the presence of multiple conformational states of NOS. Fluorescence is quenched by FRET to the heme groups of the enzyme, and the extent of quenching depends on the conformational state of the enzyme. We used site-directed mutants of both NOS and CaM to assign fluorescence quenching states to conformational states of the enzyme. Mutations were chosen that have known effects on enzyme kinetics and/or subdomain interactions. The results suggest the presence of multiple conformation states in which CaM is in close proximity to the oxygenase domain. We suggest that CaM docking to the oxygenase domain facilitates interaction of the FMN domain with the partner oxygenase domain. Such a conformation would enable electron transfer from FMN to the heme.