CONFORMATIONS AND SINGLE-MOLECULE DYNAMICS OF NITRIC OXIDE SYNTHASE

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Nitric oxide synthase (NOS) functions by transfer of electrons sequentially from FAD to FMN in the reductase domain of the enzyme and then from FMN to the heme in the oxygenase domain of the opposing member of a homodimeric complex. Efficient electron transfer is activated by the calcium signaling protein calmodulin (CaM) and requires close proximity of electron-transfer donors and acceptors. The sequence of electron transfer therefore necessitates multiple conformational states of the enzyme, suggesting that the activity of the enzyme is conformationally gated. We have detected the presence of multiple conformational states of NOS by time-resolved detection of fluorescence from a fluorophore attached to CaM. 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. Single-molecule intensity trajectories reveal multiple fluorescence states with dynamics on the millisecond to second time scales. Analysis suggests sequential conformational interchange, with the longest-lived state being highly quenched, consistent with a conformation in which CaM is in close proximity with the heme groups. Analysis of conformational dynamics is underway.