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Regulation of electron transfer in nitric oxide synthase by conformational dynamics

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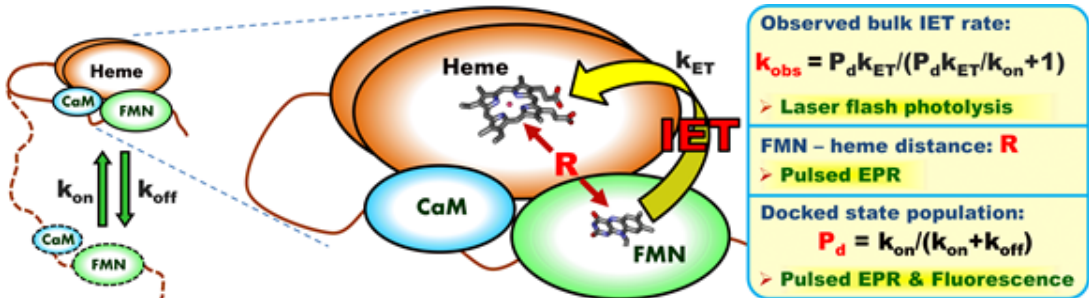
Mammalian nitric oxide synthases (NOSs) are a family of multi-domain redox enzymes responsible for producing nitric oxide (NO), an important signal and effector molecule. The NOS enzymes consist of multiple relatively rigid functional domains that are connected by flexible linkers. Each subunit has two modules joined by a calmodulin (CaM)-binding linker: (i) a catalytic heme domain, and (ii) a reductase domain with NADPH, FAD, and FMN binding sites in respective (sub)domains. CaM binding to NOS enables a conformational change, in which the FMN domain shuttles between the FAD and heme domains to deliver the NADPH-derived electrons to the active site heme center, thus allowing O_2 activation required for the NO synthesis.[1] A clear understanding of this large conformational change (Figure) is critical, since this step is rate-limiting in the NO production. Despite recent progress in revealing the architecture of full-length NOS proteins, the details of how CaM and the control elements function at the molecular level to regulate the NOS domain dynamics and control the interdomain electron transfer (IET) steps remain unclear. The large size and dynamic nature of NOS proteins necessitate an implementation of a synergistic approach (Scheme 1) combining the pulsed electron paramagnetic resonance (EPR) spectroscopy with functional and kinetics studies to achieve a molecular-level understanding of NOS regulation. We carried out pulsed EPR studies to determine some of the structures and conformational equilibria of NOS proteins.[2] The results are consistent with a model that the Ca^{2+}–CaM interaction causes CaM docking with the oxygenase domain. The low population of the docked state indicates that the CaM-controlled docking between the FMN and heme domains is highly dynamic. Additionally, to investigate the plausible structural re-arrangements and the domain interactions before and after the FMN-heme IET, molecular dynamic simulations were carried out on a model of a bi-domain oxygenase/FMN construct of human inducible NOS.[3] Our results indicate redox-dependent conformational changes that affect the distance between the heme and FMN centers. Moreover, specific residues important in the interdomain FMN/heme docking were identified on the FMN, heme and CaM domains. The predictions of the key interacting sites are supported by experimental data in literature. The computational work revealed the dynamic conformational changes of each domain, and provided plausible mechanism of the FMN domain motions. We have also provided an analytical solution of the kinetic equations describing the laser flash photolysis experiment.[4] The derived equations show an important role of the conformational dynamics in determining the bulk IET rate constant.



Scheme 1. Approaches to reveal the structural/conformational factors determining the FMN-heme IET.

Biography

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