A Second FMN-binding Site in Yeast CPR suggests a Novel Mechanism of Electron Transfer by Diflavin Reductases

Larissa M. Podust, Michael R. Waterman, *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, USA.* E-mail: larissa.m.podust@vanderbilt.edu

NADPH-cytochrome P450 reductase (CPR) transfers two reducing equivalents from NADPH via FAD and FMN to the large super family of microsomal cytochrome P450 monooxygenases (CYPs) in one-electron transfer steps. Mechanism of electron transfer by diflavin reductases remains elusive and controversial. We determined the crystal structure of CPR from *Saccharomyces cerevisiae*, which is functionally active toward its physiological substrate cytochrome P450 and discovered a second FMN-binding site at the interface of the connecting and FMN-binding domains. We propose that during catalytic turnover a single FMN molecule shuttles twice between two protein sites that accommodate two different semiquinone forms, neutral (blue) and anionic (red). Oscillating between two sites FMN presumably swings along the interface between the reductase domains circumscribing about half a circle of the 10 Å radius around invariant D187 as the center of rotation, so that the FMN N5-reference atom relocates approximately 20 Å, while the ribityl moiety remains within interaction distances from the carboxyl of D187 and T71. Yeast CPR loses the ability to support the catalytic function of CYP51 upon substitution of D187 or T71 with alanine. We believe that the proposed mechanism will move forward our understanding of electron transfer by diflavin reductases (including nitric oxide synthase (NOS)) since these electron transporters are highly homologous genetically, structurally, and functionally to CPR.

Keywords: diflavin reductase, electron transfer, FMN-binding