Kinetics and thermodynamics in the protein-ligand interactions during activity in the bifunctional FAD synthetase from Corynebacterium ammoniagenes

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M. Sebastián[I], A. Serrano[I], A. Velázquez-Campoy[I], **M. MEDINA**[I]

[I]Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, and Institute of Biocomputation and Physics of Complex Systems, Universidad de Zaragoza, Zaragoza, Spain

Prokaryotic bifunctional FAD synthetases (FADSs) are bimodular enzymes exhibiting ATP:riboflavin kinase (RFK) activity in its C-terminal module and FMN:ATP adenylyltransferase (FMNAT) activity in its C-terminal. These activities provide the organism with the flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) cofactors. The RFK activity of FADS from *Corynebacterium ammoniagenes* (*Ca*FADS) has a strong inhibition at mild concentrations of the RF substrate. Selective inhibition of key enzymes is a common tool to regulate metabolic pathways. Since FMN and FAD act as cofactors in a plethora of flavoproteins and flavoenzymes in all living organisms, inhibition of the RFK activity in some family members might contribute to the flavin cellular homeostasis, and, therefore, it is a topic worthy of study. Here we use a truncated *Ca*FADS variant that only contains the C-terminal RFK module, being it similarly functional in the RFK activity as the full length enzyme. The steady-state characterization of this variant indicates that besides inhibition by the RF substrate, both of the reaction products, ADP and FMN, also inhibit the RFK activity. The use of pre-steady-state kinetics collectively with isothermal titration calorimetry allows us to present a kinetic and thermodynamic explanation of such inhibitory behavior related to ligand binding that is coherent with the structural conformational changes occurring during the RFK catalysis in *Ca*FADS. Furthermore, these methods can be also used to evaluate the behavior of the full-length enzyme.