The parameters that define flavin-based electron bifurcation

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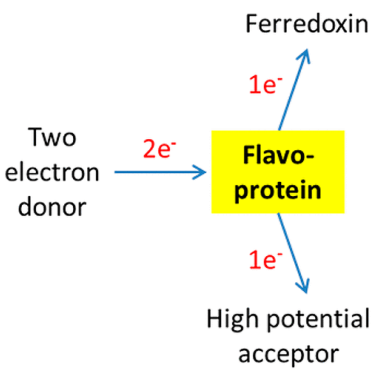
The term “Electron Bifurcation” (EB) has its origins as a component of the “Chemiosmotic Hypothesis” in the explanation of the functioning of what is coined the “Q cycle”. The basics of the “Chemiosmotic Hypothesis” are that the electron transport chain (ETC) couples the negative change in free energy from exergonic electron transfer reactions, from both mobile and fixed electron carriers, to drive the formation of a proton/ion gradient. The potential energy of the proton/ion gradient is then harnessed to drive the formation of chemical energy in the form of ATP, which is the unifying form of energy for life processes. The elegant interconversion between potential energy species provides the basis for defining biological energy conservation, fundamental to life. Phenomena satisfying this criterion were not recognized again for nearly forty years, when about a decade ago, EB re-emerged in the context of anaerobic metabolism. Several examples of flavin-based electron bifurcating enzymes have been described that involve the coupling of the oxidation of a two-electron donor such as the organic cofactor NAD(P)H coupled intimately to one electron reductions of a high potential acceptor and the low potential electron carrier ferredoxin (right). In anaerobic metabolism, where free energy cannot be squandered without dire consequences to cell viability, EB provides a high-fidelity mechanism to promote efficient energy conservation. Recent work from our research laboratories and others has provided the basis for defining the basic tenets that define flavin-based bifurcation. We suggest these tenants include 1) a bifurcating center capable of brokering both single and pairwise electron transfer reactions, 2) an energetic intermediate is required capable of driving a reduction reaction more negative than the average reduction potential of the two redox transitions of the bifurcating center, 3) a fixed and defined stoichiometry of 1:1 electrons transferred along different energetic paths, and 4) an energy landscape or specific mechanism that circumvents energy-wasting short-circuiting reactions. The talk will discuss the elegant enzyme architectures and the interesting physical properties and mechanistic features they have evolved to satisfy these criteria.



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The mechanistic determinants of catalytic bias in cofactor-based enzymatic oxidation-reduction reactions

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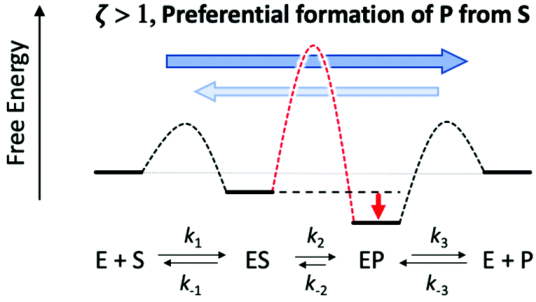

We have taken a unique angle to study enzyme mechanisms, emphasizing catalytic bias or the propensity of a biocatalyst (enzyme) to affect a disproportionate rate acceleration as a function of reaction directionality. The topic of catalytic bias is not addressed widely in the literature, where studies on enzyme kinetics often focus on a reaction in one direction or another. Catalytic bias is of significant interest in catalyst design, where there is a desire to affect the conversion of reactants to high-value products that can be manipulated away from equilibrium without a rapid conversion back to reactants. Catalytic bias could also be significant in human health, where metabolic defects result in enzyme variants that exhibit a bias that can result in the accumulation of undesirable intermediates or disrupt the normal functioning of feedback regulatory mechanisms. We will examine the mechanistic determinants of catalytic bias in two oxidation-reduction reactions: reversible hydrogen oxidation catalyzed by [FeFe]-hydrogenases and hydride transfer catalyzed by alcohol dehydrogenases. We have chosen these enzymes as model systems because 1) substantial catalytic bias has been observed in both [FeFe]-hydrogenases and alcohol dehydrogenase isozymes, 2) model systems have been developed in our laboratory and others allowing large scale purification of native and variant enzymes, 3) methods are established for the spectroscopic and kinetic characterization of enzymes and 4) oxidation-reduction reactions / reversible hydrogen oxidation / hydride transfer have been an ongoing interest of our laboratory. Our working hypothesis is that observed differences in catalytic bias are mainly a result of the relative stability of intermediates in enzyme variants. These differences are predominantly caused by variations in the amino acid environment of the active site, and as such, bias is controlled systematically with defined structural determinants. To test this hypothesis and delineate structural determinants, we have been placing site-specific amino acid substitutions in the active site of each enzyme system targeted at affecting the relative stabilization/destabilization of certain intermediate states. These substitutions, based in part on natural variation, broadly impact the electrostatics of specific regions that interact directly or affect indirectly either the active site metal cluster (H cluster) of [FeFe]-hydrogenase or the substrate binding Zn-site of alcohol dehydrogenase. Our results thus far support our hypothesis, and work continues to provide a more complete understanding of the mechanistic determinants of catalytic bias in cofactor-based oxidation-reduction reactions.

Reference:

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