Regulation of Enzymatic Activity Occurs by Selection of Discrete Activity States

Nikos S. Hatzakis, Li Wei, Sune K. Jorgensen, Andreas H. Kunding, Per Hedegaard, Dimitrios Stamou.
Nanoscience Center University of Copenhagen, Copenhagen, Denmark.

Tight regulation of enzymatic activity is crucial for controlling a multitude of fundamental cellular processes. Yet the molecular level details underlying regulation often remain poorly understood (1). Here we employed single molecule activity studies to dissect the mechanistic origin of regulation of enzymatic activity (2). As a model system we employed a lipase and measured its activity as a function of accessibility to surface tethered liposomes (3,4), which are known regulators of its activity. Our results surprisingly revealed that the lipase does not follow a continuous distribution of activity rates (5,6) but rather oscillates between 2 states of different activity as the majority of signaling protein and ion channels do (7). We report for the first time both the interconversion rates between the 2 states and their inherent activity. Based on these we calculated the energetic landscape of the entire reaction pathway and identified that regulation of enzymatic activity does not occur via an increase in the enzymes inherent activity but rather predominantly originates from increased time spent on the highly active state. Because we found a number of structurally unrelated enzymes to exhibit an analogous behavior we anticipate that oscillation between a discrete number of activity states rather than a between a continuous distribution of them might be a generic mechanism potentially facilitating regulation of enzymatic activity.

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Decay of Compound ES in Dehaloperoxidase-Hemoglobin
Matthew K. Thompson1, Stefan Franzen2, Reza A. Ghilardi1, Brandon J. Reeder3, Dimitri A. Svistunenko2.
1North Carolina State University, Raleigh, NC, USA, 2University of Essex, Colchester, United Kingdom.

Dehaloperoxidase (DHP) is a respiratory hemoglobin (Hb) that catalyzes the conversion of trihalophenols to haloalcohols in the presence of hydrogen peroxide. Ferric heme states of the resting DHP and the free radical intermediates formed under H2O2 treatment were studied by low temperature EPR spectroscopy in the range of reaction time of 50 ms - 2 min at three different pH values. Two high spin ferric heme forms were identified in the resting enzyme associated with Compound ES has been assigned to Tyr34, the other radical - to the high-altitude hypoxic environment. Since it is widely accepted that myoglobin is not only an oxygen storage/deliver system, iso-II over expression has been related to other functions such as NO scavenger and/or nitrite reductase. In this work Molecular Dynamics simulations were applied to study the dynamics of 54K and 54E human myoglobins. Statistical analysis of internal cavities and their interconnections helped to highlight and compare the intrinsic dynamic behavior of these two proteins. Furthermore, the role of the solvent in the mutation proximity has been investigated and it was found that water molecules can quasi-allosterically modulate the dynamics of myoglobin distal region. Important differences have been found especially at the histidine gate, even if the two myoglobin isoforms differ at only one residue that, indeed, is not located at the gate itself. These differences suggest that the two more expressed human myoglobins might have a distinct and complementary role. This would well fit the literature scenario where NO scavenging is thought to be important at normoxic condition to retain mitochondrial respiration efficacy, while nitrite reduction is believed to be crucial under hypoxic stress. NO release by myoglobin would decrease mitochondrial oxygen consumption preserving the optimal O2 gradient across the cell but also ensuring sufficient ATP synthesis.

Protein Flexibility and Energy Flow During Enzyme Catalysis
Arvind Ramanathan1, Jose M. Borreguero1, Chakra S. Chennubhotla2, Pratul K. Agarwal1.
1Oak Ridge National Lab, Oak Ridge, TN, USA, 2University of Pittsburgh, Pittsburgh, PA, USA.

Enzymes are dynamic molecules. Although in the past enzymes were viewed as static entities, recent evidence from experimental, theoretical and computational work indicates that protein dynamics play a significant role in enzyme catalytic activity. Investigations of the free energy profile for several proteins such as cyslophilin A and dihydrololate reductase have revealed a network of protein motions that promote catalytic activity. Results indicate that these reaction-promoting motions are conserved as part of the enzyme fold across several species, even though they have low sequence similarity. Extending our study to a superfamily of enzymes, namely the dinucleotide binding Rossmann Fold proteins (DBRP), shows that in spite of having very low sequence homology and different structural features, the overall intrinsic dynamical flexibility of the superfamily is remarkably well preserved with respect to the catalytic step. The conformational coupling observed between exterior surface regions with the active site entails energetic coupling between them. To characterize this energetic coupling, we use an integrated information theoretic and biophysical approach to analyze residues that may constitute pathways through which energy may propagate from the flexible exterior surface regions to the active site of the protein. Our results reveal significant similarities in the energy flow pathways within the DBRP super-family. This study provides specific insights into how the DBRP super-family of proteins has evolved to catalyze hydride transfer reactions.

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