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1062-Plat
Regulation of Enzymatic Activity Occurs by Selection of Discrete Activity States
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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 (1). We accurately quantified 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.
1. Gardino A.K et al., Cell, 139, 1109 (2009).
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1063-Plat
Decay of Compound ES in Dehaloperoxidase-Hemoglobin
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Dehaloperoxidase (DHP) is a respiratory hemoglobin (Hb) that catalyzes the conversion of trihalophenols to dihaloquinones in the presence of hydrogen peroxide. Ferric heme states of the resting DHP and the free radical intermediates formed under H_2O_2 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 and assigned to the open and closed conformations of the distal histidine, His55. Two free radicals were found in DHP activated by H_2O_2: the radical associated with Compound ES has been assigned to Tyr34, the other radical - to Tyr38. The Tyr34 radical is formed with a very high relative yield (almost 100% of heme), atypical of other globins. The HPLC analysis of the reaction products showed a pH dependent formation of covalent heme-to-protein cross-links. The stable DHP Compound RH formed under H_2O_2 in the absence of substrates is proposed to be a state with the ferric heme covalently cross-linked to Tyr34. A kinetic model of the experimental data suggests that formation of Compound RH or the Tyr38 radical are two alternative routes of Compound ES decay. Which route is taken depends on the conformation of His55: in the less populated closed conformation, the Tyr38 radical is formed, but in the major open conformation, Compound ES decays yielding Compound RH, a product of safe termination of the two oxidizing equivalents of H_2O_2 when no substrate is available.

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Human Myoglobin: Two Isoforms that Differ at Single Residue. Their Different Dynamics Suggest Distinct and Complementary Role
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In Human up to five different myoglobin isoforms are expressed. Iso-I (~75-80%) and iso-II (~15-20%) are the more expressed ones and differ only at the 54th position, K54 and E54 respectively. It has been reported that myoglobin concentration in muscles is higher for high-altitude natives than sea-level populations, and that only iso-II is over-expressed among the myoglobin isoforms. This over-expression is thought to be one of the evolutive adaptation 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 though 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.

1065-Plat
Protein Flexibility and Energy Flow During Enzyme Catalysis
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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 enhancing catalytic activity. Investigations of the free energy profile for several proteins such as cyclophilin A and dihydrofolate 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.

PLATFORM AD: Molecular Dynamics

1066-Plat
Steered Molecular Dynamics Simulation of Kinesin Detachment from the Microtubule Surface
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The effects of timescale and force transducer stiffness on kinesin detachment from the microtubule surface, as represented by the alpha-beta tubulin dimer, have been investigated using Steered Molecular Dynamics (SMD) simulations. By decreasing the pulling speed and using a softer spring in the SMD simulation, a kinesin detachment force in the range of 100 to 150 pN is obtained. This result is in better agreement than previous SMD calculations with experimental measurements of the detachment force obtained using optical traps and atomic force microscopy.
Moreover, the differences between the simulated and experimentally measured kinesin detachment forces may be attributed to the experimental configuration a microbead attached to an 80 nanometer coiled-coil stalk. The stalk acts as a long moment arm on the neck liner of kinesin, thus causing underestimation of the actual detachment force at the kinesin-microtubule interface by a factor of 5 to 7. Taking into account the effect of the moment arm, the experimental results suggest an actual stall force for kinesin-microtubule binding in the range of 60 to 150 pN. This range of forces brackets the detachment force calculated in the SMD simulations.
It was also noted that different mechanisms of kinesin detachment from the tubulin dimer are observed for different selected values of the SMD pulling parameters. Specifically, at low pulling speed and for a soft spring stiffness, the detachment process reveals conformational changes that involve the translocation and rotation of the kinesin head as well as the switch II region.