

detected, in contrast to the native enzyme whose intrinsic fluorescence markedly increased. This indicates a relatively high rigidity of the substrate-induced enzyme compared with the native protease. It also indicates that the "induced fit" theory is not an adequate explanation of the mechanisms involved in such reactions. Utilising a "lock and key" mechanism for such secondary reactions may have adaptive value in that it facilitates high efficiency in enzymatic reactions.

2332-Pos

Peculiar Regulatory Role of Magnesium in Nucleotide Hydrolysis of dUTPases

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The dUTPase enzymatic activity is indispensable to efficiently reduce cellular dUTP/dTTP levels. Lack of the enzyme leads to erroneous uracil incorporation into DNA resulting in chromosome fragmentation and cell death. dUTPase is therefore reported to be a preventive DNA repair factor and a high-potential drug target in cancer. Although divalent metal ions are indispensable to the catalytic activity of numerous nucleotide hydrolyses, the increase in dUTPase steady-state activity is only twofold in the presence of magnesium. We had specific interest in investigating the influence of magnesium on the catalytic mechanism and the structure of human dUTPase, which is a completion of our previous study revealing the fundamental steps of the enzymatic cycle and providing a quantitative model for the mechanism. To address the above issue, a broad array of techniques were employed, such as transient kinetics, crystallographic and spectroscopic methods. We revealed that the homotrimeric human dUTPase has two structural metal-binding sites within the central channel of the enzyme with different binding affinities toward the magnesium ions. At the active sites, magnesium facilitates the formation of the catalytically competent gauche conformation of the alpha-phosphate group allowing the nucleophilic attack of catalytic water on the alpha-phosphorus atom. According to our current observations, the steady-state activity monitored in the absence of magnesium is a result of at least two parallel reaction series. One reaction pathway is consistent with our previous model of dUTPase catalysis and occurs very slowly without magnesium. The other possible pathway potentially involves hydrolysis initiated by nucleophilic attack on the beta-phosphorus atom.

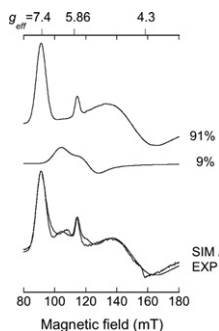
2333-Pos

Conservation of Active Site Geometry in Evolution of Iron Lipoygenases: EPR Studies

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Lipoxygenases employ a redox-active metal center (Fe or Mn) in electron-proton coupled reaction to initiate oxidation of unsaturated fatty acids. In this study, the iron center geometry is examined by EPR for two newly characterized bacterial lipoxygenases, and the data are compared with similar studies of the eukaryotic lipoxygenase-1 from soybean (Glycine max). Although the protein sequences of bacterial lipoxygenases (from *Pseudomonas aeruginosa* and *Shewanella woodyi*) are only 27 and 20% identical, respectively, to the soybean sequence, the sequences are highly similar in regions known to contribute side chains to active site cavities and to metal binding in the soybean protein. Remarkably, all three lipoxygenases reveal an identical set of iron EPR sub-spectra, but rates of inter-conversion of the sub-spectra differ. Multiple sub-spectra are also seen in ferrous-NO enzyme forms. Immediately after lipoxygenase iron is activated from ferrous to ferric, predominantly one of the EPR sub-spectra is observed, and this intermediate converts to multiple sub-spectra with time (minutes for soybean, longer for bacterial forms). The rate of formation of the first activated ferric state, the enzyme kinetics lag, and products of single substrate turnovers are compared.



2334-Pos

The N-Terminal Ig Domain of Endoglucanase Cel9A from the Thermoacidophilic *Alicyclobacillus Acidocaldarius* Enhances Protein Stability

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As part of our ongoing studies of thermophilic cellulases, we are examining the subfamily E1 of family 9 of glycoside hydrolases, members of which

have an N-terminal immunoglobulin (Ig)-like domain followed by the catalytic domain (CD). While the function of the Ig-like module has not been determined, deletion of the Ig-domain results in complete loss of enzymatic activity in the cellobiohydrolase, CbhA from *Clostridium thermocellum*. In this work we used simulation approaches to investigate the role of the (Ig)-like domain in the non-processive endonuclease Cel9A from the thermoacidophilic bacterium *Alicyclobacillus acidocaldarius* (Aa_Cel9A) for which the crystal structure has only recently been resolved. We are using molecular dynamics (MD) simulations to provide a dynamic view of Aa_Cel9A. Our goal is to try to piece together the available activity, kinetic, biophysical and structural information and to offer insights into domain interactions and domain motions that may be associated with Aa_Cel9A activity and stability. To examine the role of the Ig-domain, MD simulations combined with a simplified force field model were performed on the structure of Aa_Cel9A both with and without the Ig-like domain. Umbrella Sampling and free energy perturbation (UM/FEP) were also performed to obtain unfolding free energy landscapes for both cases. Both methods show that the Ig-like domain stabilizes the structure of the catalytic domain; thus, a major function of N-terminal Ig-like domain appears to be to confer thermostability. We also used the results of our MD simulations to study correlated motions among atoms in the Ig-like domain and atoms in the CD. Our preliminary results show that Ig-like domain motions are correlated with active site molecular motions, suggesting that the Ig-domain may be required for proper control and orientation of active site residues.

2335-Pos

First-Principles Study of Non-Heme Fe(II) Halogenase SyrB2 Reactivity

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We present here a computational study of reactions at a model complex of the SyrB2 enzyme active site. SyrB2, which chlorinates L-threonine in the syringomycin biosynthetic pathway, belongs to a recently discovered class of α -ketoglutarate, non-heme Fe(II)-dependent halogenases that shares structural and chemical similarities with hydroxylases. Halogenases and hydroxylases alike decarboxylate the α KG co-substrate, facilitating formation of a high-energy ferryl-oxo intermediate that abstracts a hydrogen from the reactant complex. The reaction mechanisms differ at this point, and mutation of active site residues fails to reproduce hydroxylating activity in SyrB2 or halogenating activity in similar hydroxylases. Using a density functional theory (DFT) approach with a recently implemented Hubbard U correction for accurate treatment of transition-metal chemistry, we explore probable reaction pathways and mechanisms via a model complex consisting only of the iron center and its direct ligands. We show that the first step, α KG decarboxylation, is barrierless and exothermic, while the subsequent hydrogen abstraction step has an energetic barrier consistent with that accessible under biological conditions. In the model complex we use, radical chlorination is barrierless and exothermic, while the analogous hydroxylation is found to have a small energetic barrier. The hydrogen abstraction and radical chlorination steps are strongly coupled: the barrier for the hydrogen abstraction step is reduced when carried out concomitantly with the exothermic chlorination step. Our work suggests that the lack of chlorination in mutant hydroxylases is most likely due to poor binding of chlorine in the active site, while mutant halogenases do not hydroxylate for energetic reasons. While secondary shell residues undoubtedly modulate the overall reactivity and binding of relevant substrates, we show that a small model compound consisting exclusively of the direct ligands to the metal can help explain reactivity heretofore not yet understood in the halogenase SyrB2.

2336-Pos

Pre-Steady-State Kinetic Analysis of the Elongation Mode of Dengue Virus RNA Polymerase Domain

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Dengue viral RNA polymerase replicates its positive single-stranded RNA genome in a primer-independent manner. The slow and inefficient initiation during replication masks the elongation mode. The aim of this work was to further characterize the mechanism of elongation towards an increased understanding of how the enzyme selectively recognizes different nucleotides. Transient kinetic methods were used to measure the microscopic rates of the reaction pathway comprised of enzyme and RNA binding followed by nucleotide binding and incorporation. After extended pre-incubation of the enzyme with double stranded RNA (12-mer primer with a 26-mer template), addition of a correct nucleotide resulted in a burst of single nucleotide incorporation,