mainly due to a lack of relevant assays available at acidic pH. We have developed a single-molecule assay using force spectroscopy, which allows us to detect the reduction of single disulfides. The assay uses an Atomic Force Microscope to extend individual substrate proteins containing buried disulfides, thereby exposing the bonds to the solvent. When a disulfide bond is broken through reduction, this reaction is detected as a stepwise extension of the substrate polypeptide. This method enables measurement of reduction rates at a wide range of pH conditions. By altering the conformation of the disulfide through the applied strain on the substrate, we can dissect the mechanisms of enzymatic catalysis. Our results show that the enzymatic activity of GILT decreases as the strain on the substrate is increased. This feature is also seen in the enzyme thioredoxin but not in non-enzymatic reducing agents such as glutathione. These results shed light on the catalytic mechanism of GILT and establish single molecule force spectroscopy as a useful tool for characterizing enzymatic properties.

2327-Pos

Anisotropic Mechanical Response of the Enzyme Guanylate Kinase Perturbed by the DNA Molecular Spring

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Protein molecules are semi-rigid objects with organized but fluctuating conformation. For Guanylate Kinase, which catalyzes phosphoryl transfer between ATP and GMP, a large conformational change upon substrate binding occurs which is essential for enzymatic activity. With a DNA molecular spring stretching the molecule in distinct ways, we demonstrate that the enzymatic functions of substrate binding and phosphoryl transfer can be separately controlled mechanically.

Three different attachment points of the DNA spring on the surface of the protein were tested, corresponding to stretching the protein along three different directions. Using activity measurements with titration over substrate concentration, the kinetic parameters (i.e., binding affinity of substrates and catalytic rate constant) based on Michaelis-Menten kinetics were obtained in the presence and absence of the three different mechanical perturbations.

2328-Pos

Crystal Structure and Functional Analysis of Homocitrate Synthase, an Essential Enzyme in Lysine Biosynthesis

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Homocitrate synthase (HCS) catalyzes the first and committed step in the α-aminoadipate (AAA) pathway of lysine biosynthesis, which occurs in many fungi and certain archaea, and is a potential target for antifungal drugs. Here we report the crystal structure of the HCS apoenzyme from *Schizosaccharomyces pombe* and two distinct structures of the enzyme in complex with the substrate 2-oxoglutarate (2-OG). The structures reveal that HCS forms an intertwined homodimer stabilized by domain-swapping between the N- and C-terminal domains of each monomer. The N-terminal catalytic domain is comprised of a TIM barrel fold in which 2-OG binds via hydrogen bonds and coordination to the active site divalent metal ion, whereas the C-terminal domain is composed of mixed α/β-topology. In the structures of the HCS apoenzyme and one of the 2-OG binary complexes, a lid motif from the C-terminal domain covers the entrance to the active site of the neighboring monomer, whereas in the second 2-OG complex, the lid is disordered, suggesting that it regulates substrate access to the active site through its apparent flexibility. Steady state kinetic assays and *in vivo* yeast growth assays on wild-type enzyme and active site mutants allow us to elucidate its catalytic mechanism, including the residues implicated in catalysis. Together these results yield new insights into the mechanism and regulation of HCS, which provide a platform to identify small molecule inhibitors of HCS that may be optimized and used as anti-fungal agents.

2329-Pos

Kinetic Consequences of Mutations at an Allosteric Site in Arginase from the Thermophile *Bacillus caldovelox*

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Arginase is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. It is involved in ureagenesis and the control of arginine levels for production of proline, creatine, polyamines, and nitric oxide. The crystal structure of the enzyme from the extreme thermophile *Bacillus caldovelox* reveal a second arginine-binding site, located at the monomer-monomer interface. Binding of the guanidinum group of L-arginine by bidentate hydrogen bonds to Glu256 in one monomer and bifurcated hydrogen bond to Asp199 in the neighbor, was suggested to generate a catalytically competent conformation. Interestingly, in the rat and human liver arginases, an equivalent position is occupied by Arg-308, which is part of an S-shaped C-terminal motif, that is critical for oligomerization and cooperative response to the substrate. The bacterial arginase lacks this motif. To get some insight into the external site in *B. caldovelox* arginase, we examined the kinetic and structural consequences of mutations at Asp199 to asparagine and Glu256 to glutamine. Upon mutations, the hexameric subunit structure, affinity of the enzyme-manganese interaction, pH and temperature dependence of catalytic activity, thermal stability and tryptophan fluorescence properties of the enzyme were not altered. However, the hyperbolic kinetics exhibited by the wild-type enzyme (K_m= 3.5 mM) changed to cooperative for both variants (Hill coefficients of 1.5 ± 0.2). Results were not altered by agmatine (decarboxylated arginine) or low concentrations of guanidinium chloride. Our conclusion is that occupancy of the second site by L-arginine is not required for generation of a catalytically competent active site. Instead, by binding at the allosteric site, L-arginine acts as a typical allosteric activator. Thus, the intrinsic cooperative behavior is exhibited by the mutants because of their inabilities to bind the allosteric activator. Fondecyt 1070467.

2330-Pos

Catalysis Mechanism of Aminopeptidase from Streptomyces Griseus: A Quantum Mechanical/Molecular Mechanical Analysis

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Aminopeptidases are exopeptidases that catalyze the removal of N-terminal amino acids for peptides [1]. X-ray revealed that the streptomyces griesus aminopeptidase (SGAP) is a double zinc proteolytic enzyme [2,3] with strong preference for large hydrophobic amino acids. Two different schemes for the general catalytic pathway of SGAP are proposed [1,4]: OH- or H2O nucleophilic attack mechanism. We are investigating SGAP's catalytic mechanism by means of hybrid quantum mechanical/molecular mechanical calculations (AM1d/MM) and analogous small molecule module mechanism with both AM1/d and B3LYP/6-31++G(d,p) methods. A complex network of reaction pathways is generated so as to explore a variety of different putative reaction mechanisms. Our molecular dynamics simulations (SGAP binded with MET-ALA-ALA) for different protonation pattern in the active site indicate that the most probable scenarion is a nucleophilic attack by a Zn2+-bound hydroxide ion, with the GLU131 protonated. Small molecular model AM1 calculation with Gaussian 03 indicates that the Zn2+-bound hydroxide ion first attack the backbone C(O) of MET and then H of this hydroxide ion "migrate" to the adjacent N of ALA, then the C(O)-N peptide chain between MET and ALA is cleaved.

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2331-Pos

Substrate-Induced *Eisenia fetida* Protease Reactions Involve Both "Induced Fit" and "Lock and Key" Mechanisms

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The coupling between ligand binding and protein conformational change is the heart of biological network. "Lock and key" theory and "induced fit" theory were early contributions to our understanding for explaining how an enzyme binds to a substrate. It was accepted that the binding of a substrate to an enzyme is often accompanied by conformational changes of the enzyme. However, whether the substrate-induced complementary conformation is flexible or rigid after a catalytic reaction remains to be determined. By testing the enzyme activity and intrinsic fluorescence of a substrate non-specific *Eisenia fetida* protease-I with different substrates, we show that when this enzyme reacts with a first substrate, it utilises the "induced fit" mechanism. However, in its reaction with further substrates, either the "lock and key" or "induced fit" mechanisms will be used depending on the degree of conformational change required. In contrast to the high activity of the native protease, the chromozym-Th (or -Ch)-induced protease was unable to react with chromozym-U. Chromozym-U-induced enzyme, however, had high activity with chromozym-Th and chromozym-Ch. When low concentrations of GuHCl were used to disturb the conformation of the enzyme, only small changes in intrinsic fluorescence of the CTH-induced protease were