2256-Pos Board B226
Allosteric Mechanism of Hemoglobin: Concerted Mechanisms or Graded Mechanisms
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Allosteric parameters (KT, KR, and Ln) of hemoglobin (Hb) are normally determined by thermodynamic analyses of oxygen-binding isotherma according to the Monod-Wyman-Changeux two-state concerted model [1]. KT and KR are independent of ligation states [1,2], whereas Ln is a function of the number of ligands bound. On the other hands, in structure-linked graded models such as the Koshland-Nemethy-Filmer sequential model [3], the Perutz steroechemical model [4], and the Eaton et al. tertiary two-state (TTS) model [5], the oxygen-affinity (Kn (n = 1, 2, 3, & 4)) of Hb is linked to certain ligation-induced structural changes (tertiary and/or quaternary) such as changes in salt bridges and T/R-quaternary structures. Then, the oxygen-binding processes become circular reversible kinetic processes, as schematically shown in the figure, rather than the truely reversible kinetic process as expected thermodynamically. Supported by NIH HL14508.
Reference:
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[2] Yonetani, Laberge BBA 1784 (2008) 1146-1158.
[3] Koshland, Nemethy, Filmer Biochemistry 5 (1966) 365-385.
[4] Perutz Nature 228 (1970) 726-739.
[5] Eaton et al. IUBMB Life 59 (2007) 586-599.

2257-Pos Board B227
Conformatinal Dynamics Of Cytochrome *c* Encapsulated In AOT Reverse Micelles
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Scalable interior volume of the reverse micelle provides a convenient way to investigate the impact of aqueous solvation and confinement on the structure, stability and dynamics of proteins and peptides. Here we report transient absorption study of CO rebinding to cytochrome c encapsulated in AOT reverse micelles. Encapsulation of cytochrome c is associated with the destabilization of the protein structure and concomitant dissociation of Met-80 from the heme iron as evident from the disappearance of the absorption band at 695 nm. Reduction of cytochrome c and subsequent exposure to CO result in the formation of CO bound protein with a Soret band located at 415 nm. Upon photodissociation, the ligand rebinding occurs as a two-step process with the first step having the lifetime of 7 μs and slower process with the lifetime of roughly 50 μs. No significant impact of the reverse micelle size (w = 10, 20 and 80) on the kinetics of CO rebinding to cytochrome c was observed. On the other hand, CO rebinding to the model compound, microperoxidase-11, is multi-phasic with 6 μs and 50 μs kinetics on the microsecond timescale and an additional kinetics on the millisecond timescale. These data show that the encapsulation of protein within the negatively charged reverse micelles results in the heterogenous population and/or distribution of protein within the reverse micelle.

2258-Pos Board B228
Substrate Stereoselectivity of Human Indoleamine 2,3-Dioxygenase
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Indoleamine 2,3-dioxygenase (IDO) and Tryptophan 2,3-dioxygenase (TDO) are two heme-containing enzymes that catalyze the oxidative cleavage of tryptophan (Trp) to N-formyl-kynurenine, the initial and rate-limiting step of the kynurenine pathway. Although IDO and TDO catalyze the same reaction, they exhibit distinct structural and functional features. TDO plays an important role in regulating homeostatic serum Trp concentrations, whereas IDO is involved in a wide spectrum of immune related pathophysiologies. It has been shown that immune cells express IDO to suppress pathogen growth by depleting the local Trp concentration and by producing cytotoxic metabolites. Ironically, IDO produced in the placenta and by cancer cells has also been implicated in inhibiting the proliferation of immune cells by similar mechanisms. To study the substrate-protein interaction in human IDO (hIDO), as compared to human TDO (hTDO), we have constructed and studied three mutants of hIDO, including S167H and F226Y (in which the two critical amino acids in the active site were mutated to mimic TDO), as well as trIDO (in which the N-terminal domain absent in TDO was truncated). The structural and enzymatic properties of each mutant were systematically examined with optical absorption and resonance Raman spectroscopies. The data were evaluated against the wild type hIDO and hTDO. It was concluded that: (1) the mutation of F226 to Tyr changes the substrate stereoselectivity to be ''TDO-like''; (2) the mutation of S167 to His causes the inactivation of IDO; and (3) the N-terminal domain of IDO is critical for Trp binding and activity. These studies will be discussed in the context of the dioxygen chemistry carried out by these two important heme-containing enzymes.

2259-Pos Board B229
The Unique Dioxygen Activation Mechanism of Human Indoleamine 2,3-Dioxygenase
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Human indoleamine 2,3-dioxygenase (hIDO) is an intracellular heme-containing enzyme, which catalyzes the initial and rate-determining step of L-tryptophan (L-Trp) metabolism via the kynurenine pathway in nonhepatic tissues. We have employed stopped-flow methods to study the L-Trp and oxygen binding kinetics of hIDO and the associated oxygen chemistry at pH 7.4 and 20 oC. We found that the binding rate constants of L-Trp to ferric and ferrous hIDO are 5.5*10(3) and 1.2*10(5) M-1s-1, respectively. In contrast to other dioxygenases or monooxygenases studied to date, under physiological conditions, most of hIDO binds dioxygen to form the oxy species first with a rate of 5.3*10(5) M-1s-1. It is followed by rapid binding of the substrate, L-Trp, with a rate of ca. 9.0*10(6) M-1s-1, to form the ternary complex, L-Trp-bound oxy species of hIDO.

Enzymes

2260-Pos Board B230
Substrate-Protein Interaction in Human Tryptophan dioxygenase
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The initial and rate-limiting step of the kynurenine pathway involves the oxidation of L-Trp to N-formyl kynurenine catalyzed by two hemeproteins, Trypophan 2,3 dioxygeanse (hTDO) and indoleamine 2,3-dioxygeanse (hIDO). Although hTDO and hIDO catalyze the same reaction, and show high structural homology, they are engaged in distinct physiological functions and show different biochemical properties. IDO has been implicated in diverse range of pathophysiological conditions, whereas TDO deals with the systemic regulation of the Trp flux in our body. Hence, understanding the differences between hTDO and hIDO offer invaluable information for the design of new inhibitors selective for hIDO. We have expressed, purified and characterized hTDO for the first time and demonstrated that the distal pocket of the two heme enzymes are distinctly different (JACS. 2007, 129, 15690-15701). In hTDO, the distal H76 residue is believed to act as an active site base to deprotonate the indole NH group of L-Trp, the initial step of the L-Trp oxidation reaction. In hIDO, this histidine residue is replaced by a serine. To investigate the role of the H76 residue in hTDO, we have constructed two mutants, in which the H76 is replaced by a serine or an alanine, and studied their structural and functional properties. Resonance Raman studies indicate that L-Trp is positioned in the active site by the ammonium, the carboxylate and the indole groups, via intricate H-bonding and hydrophobic interactions. This scenario is consistent with the observation that L-Trp binding significantly perturbs the electronic properties of the O2-complex of hTDO. The electronic properties of the active ternary complex of hTDO are found to be sensitive to the mutation of the H76 residue, highlighting the critical role of H76 in modulating the oxygen chemistry of hTDO.

2261-Pos Board B231
Insights Into The Mechanism Of The Cobalt Containing Nitrile Hydratase From *Geobacillus Pallidus*
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The crystal structures of the wild-type, Co(III) containing, nitrile hydratase and the mutant (βF36L / βL103S / βY127N / αD4G) enzyme from have been solved at resolutions of 1.4A and 1.15A respectively. Nitrile hydratases are noted for having an unusual cysteine claw structure at the active site. An important observation made in this paper is that cysteines α119 and α121 were both modified to cysteine sulfinic acid instead of the α121 cysteine sulfenic and α119 cysteine sulfinic acid reported previously. This was confirmed by MALDI-TOF mass spectroscopy.
The nitrile hydratases catalyse the conversion of nitriles to the corresponding amides. These enzymes underlie the industrial production ofacrylamide. In the cysteine claw structure an Fe(III) or Co(III) ion is octahedrally co-ordinated to three cysteines, two of which are oxidized, and two peptide backbone amide groups. The sixth ligand, *trans* to the unmodified cysteine is a water molecule or a hydroxide ion. The cysteine claw has the sequence CTLCSC in the iron case and CSLCSC in the cobalt case. The enzyme itself is a $\alpha_2\beta_2$ tetramer with the cysteine claw located in the α subunit and much of the active site contributed by the β subunit.
Our crystal structures are almost certainly an inactive form of the enzyme, being crystallized in 100 mM MES (2[N-Morpholino]ethanesulfonic acid) which

was found to be an inhibitor of the enzyme subsequent to the crystallization. However at present no evidence suggests that the inactivation is due to the oxidation of the active site cysteine sulfenic acid.
The structures give insight into the determinants of the specificity of the enzyme, suggesting that βphe55 and βphe55 obstruct access to the cysteine claw complex for larger substrates allowing the hydration of only smaller aliphatic nitriles.

2262-Pos Board B232
Crystal Structure Of Peroxide-bound Manganese Superoxide Dismutase
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The superoxide dismutase (SOD) enzymes are important antioxidant agents that protect the cells from reactive oxygen species (ROS). The SOD family is responsible for catalyzing the disproportionation of superoxide to oxygen and hydrogen peroxide. We report the first ever structure of a superoxide dismutase with bound hydrogen peroxide in the active site. Synchrotron X-ray diffraction data was collected from *Escherichia Coli* MnSOD crystals that were soaked in a cryosolution containing 0.008%(v/v) hydrogen peroxide and cryocooled to 100K. Structural refinement to 1.55 Å and close inspection of the active site revealed electron density for hydrogen peroxide in three of the four active sites. The hydrogen peroxide molecules are sideways bonded to the manganese in the position normally assumed by water or inhibitory hydroxide. The hydrogen peroxide molecules are present in active sites B, C and D. It was observed that MnSOD enzymes could accommodate two hydrogen peroxide molecules per active site in an antiprismo coordination geometry. Comparison of the peroxide-bound active site with the wild-type trigonal bipyramidal form shows a shifting of the gateway residues Tyr34 and His30, thereby preventing the escape of the bound ligands. The peroxide-bound form more closely resembles the active-site geometry of six-coordinate octahedral form (1D5N), where hydroxide ligands were trapped in the active site by cryocooling.

2263-Pos Board B233
Characterization of the Monomer-Dimer Equilibrium of Recombinant Histo-aspartic Protease from Plasmodium falciparum
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Malaria is a devastating disease that infects and kills 1-2 million people annually. Histo-aspartic protease (HAP) from *Plasmodium falciparum*, the most lethal of all Plasmodium parasites, is an intriguing aspartic protease due to its unique structure and its potential as an antimalarial target. Substantial effort has been devoted to investigate the structure function of this protease. The present study investigated the molecular state of HAP as related to enzymatic activity. Gel filtration chromatography indicated that recombinant Trx-tHAP fusion protein aggregated during purification and that aggregation could be prevented through the addition of 0.2% CHAPS. Using this latter technique as well as sedimentation velocity and sedimentation equilibrium ultracentrifugation, it was shown that the recombinant mature HAP (mtHAP), in which the His-tag, thioredoxin and prosegement were removed, exists in a dynamic monomer-dimer equilibrium in solution and the dissociation constant is 20-30 μM. Enzymatic activity data also indicated that HAP was most active as a monomer. The monomeric form of mtHAP showed a Km of 9.7 μM and a turnover number, kcat, of 0.044s-1 on the internally quenched fluorescent synthetic peptide substrate EDANS-CO-CH2-CH2-CO-Ala-Leu-Glu-Arg-Met-Phe-Leu-Ser-Phe-Pro-Dap-(DABCYL)-OH (2837b) at pH 6.5. Inhibition studies showed that the activity of mtHAP was completely inhibited by 1 mM PMSF and to a lesser degree by 10 μM ALLN, 10 mM EDTA and 10 mM 1,10-phenanthroline, and was inhibited strongly by ZnCl2 and to a lesser extent by NaCl and KBr. The effects of temperature and salts on the monomer-dimer equilibrium of mtHAP were also investigated by using sedimentation equilibrium ultracentrifugation.

2264-Pos Board B234
Analysis of Monomeric and Dimeric Phosphorylated Forms of PKR
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PKR (protein kinase R) is induced by interferon and is a key component of the innate immunity antiviral pathway. Upon binding dsRNA or dimerization in the absence of dsRNA, PKR undergoes autophosphorylation at multiple serines and threonines that activate the kinase. Although phosphorylation is known to enhance PKR dimerization, gel filtration analysis reveals a second, monomeric phosphorylated form. The monomeric and dimeric forms do not interconvert. The monomeric form dimerizes weakly with a Kd similar to unphosphorylated PKR. Isoelectric focusing and mass spectroscopy reveal that both the monomeric and dimeric forms are heterogeneous in their phosphorylation state. Equilibrium chemical denaturation analysis indicates that phosphorylation destabilizes the kinase domain by about 1.5 kcal/mol in the dimeric form but not in the monomeric form. Limited proteolysis also reveals that phosphorylation induces a conformational change in the dimeric form that is not detected in the monomeric fraction. The monomeric phosphorylated form binds dsRNA similarly to unphosphorylated PKR but the affinity is greatly reduced for the dimeric form. Despite these differences in biophysical properties, both phosphorylated forms are catalytically competent and are activated to phosphorylate the PKR substrate eIF2a in the absence of dsRNA. Thus, both monomeric and dimeric forms of phosphorylated PKR may participate in the interferon antiviral pathway.

2265-Pos Board B235
Structural Studies of Enzymatic Hydrolysis of Cellulose by Neutron Scattering and Reflectivity
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Improving the efficiency of enzymatic hydrolysis of cellulose is a key technological hurdle in reducing the cost of producing ethanol from lignocellulosic material. Typically, enzymatic hydrolysis proceeds to only a limited extent, high solution-to-solids ratios are required, and the rate of enzymatic hydrolysis typically decreases with time. A range of mechanisms have been proposed to explain these phenomena including product inhibition, denaturation of enzymes, nonproductive binding, and many others. We are studying the interaction of enzymes with cellulose to help unravel these mechanisms. Our studies include UV absorption and circular dichroism of enzymes in solution, small angle neutron and X-ray scattering (SANS, SAXS) of cellulose during hydrolysis, and neutron reflectivity (NR) of enzymes interacting with model cellulose surfaces. Insight from these studies should aid the development of more efficient enzyme systems and pretreatments.

2266-Pos Board B236
Expression And Purification Of A Stable, Monomeric Creatine Kinase
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Three isoform gene families of creatine kinase (CK) are present in animals. Two of these, mitochondrial and cytoplasmic CKs, are obligate oligomers . There is substantial evidence for functional interaction between subunits. Attempts at generating active, monomeric CKs have failed or in one case produced ephemerally active but unstable monomers. A third CK isoform, the so-called flagellar CK, is monomeric but is composed of three complete, contiguous CK domains. Each of these domains is catalytically competent but there is clear interaction between active sites (Hoffman et al., FEBS J 275: 646-654 [2008]). In the present effort, we have used a flagellar CK expression construct as a platform to engineer, express and purify a single domain, monomeric CK. Boundaries between the three domains (D1, D2 and D3) were identified by comparison of key catalytic residues and predicted secondary structural elements. A cDNA coding for D2 was amplified by PCR and inserted into an expression vector. Subsequent expression and purification yielded a recombinant CK which was stable as evidenced by the retention of activity over several weeks. Size exclusion chromatography showed that this CK was monomeric as expected, with a mass similar to the predicted Mr based on the amino acid composition. The engineering of a monomeric CK in the present effort clearly shows that oligomerization is not required for catalysis. Conventional wisdom supports the view that CKs evolved from a related phosphotransferase, arginine kinase (AK). AKs are typically monomeric. It seems likely that oligomerization occurred later in the evolution of CKs perhaps due to the selective pressure for targeting to and binding in intracellular compartments. (Supported by NSF grant IOB-0542236 to WRE).

2267-Pos Board B237
Role of Rim Tyr/Trp Residues in Interfacial Activation of Phospholipase C Enzymes
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The *Bacillus thuringiensis* phosphatidylinositol-specific phospholipase C (btPI-PLC) as a model system and mammalian PI-PLCδ1 without an intact PH domain have been examined for the contribution of rim aromatic groups to protein binding to vesicles and the correlation of this with catalytic activity. In the bacterial enzyme, two tryptophan residues (Trp47 in the two-turn helix B and Trp242 in a disordered loop) are critical for binding to interfaces; of the many several Tyr residues mutated, replacement with alanine at several sites (close to helix B as well as the active site) weakens membrane binding. For many of these residues the loss in binding affinity approximates what is