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