of the distal histidine is due to the stability of the final bound state rather than an intrinsic bond strength difference. The implications of increased dynamics on the distal side of the pocket after ligand binding will be discussed in terms of ligand migration inside GHB and related globins.

Support by NSF grant MCB 0843439.

**1206-Pos** Board B116

**Molecular Activation by Peroxidases**

Koroush Sasan, Stefan Franzen.

The peroxidase class of enzymes has been known to activate substrates for electron transfer from nearly a century. The precise consequences of that electron transfer have been in less detail than the initial even. Herein, we examine the kinetic and structural studies indicate that peroxidase enzymes are capable of oxidation of 2,4,6-trichlorophenol (2,4,6-TCP) to 2,6-dichloro-1,4-benzoquinone (2,6-DCQ). There are two dehaloperoxidase-hemoglobins of *Amphirite ornata*, DHP A and B, that both have peroxidase activity superior to hemoglobin- or myoglobins previously studied. One consequence of the further oxidation of 2,6-DCQ is the formation 2,6-dichloro-3-hydroxy-1,4-benzoquinone (2,6-DCQOH). The secondary product, 2,6-DCQOH, has been observed by UV-vis spectrophotometry and 1H-13C NMR. The results are consistent with the spontaneous oxidation of 2,6-DCQ in the of hydrogen peroxide ion (H2O2+). Based Arhenius analysis of the kinetics, the activation energy of spontaneous hydroxylation (Ea = 32.4 kJ/mol) is significantly lower than the activation energy of oxidation 2,4,6-TCP (Ea = 56.3 kJ/mol). 2,6-DCQOH Density functional theory (DFT) calculations were employed to differentiate between two possible hydroxylation mechanisms, leading to different products, 1) 2,6-DCQOH and 2) 2,3-dihydroxy-6-cloro-1,4-benzoquinone. The formation rate of the 2,6-DCQ product appears to affect the secondary hydroxylation rate. We have probed the effect of alternative activation using peroxy acids, such as meta-chloroperoxybenzoic acid, to test the role played by activation and electron transfer in the formation of products by the dual function hemoglobin-dehaloperoxidase. These effects are modeled structurally using a variety of methods including docking, and structural studies of interaction of the substrate with the protein.

**1207-Pos** Board B117

**Reaction Mechanism of Heme-Based Dioxygenases**

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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 (NFK), the initial and rate-limiting step of the kynurenine pathway. Until recently it was generally believed that the heme dioxygenase reaction follows a base-catalyzed mechanism. Based on this mechanism, the reaction is initiated by deprotonation of the indoleamine group of Trp by an active site base. It is followed by electrophilic addition of the heme-bound dioxygen to the C2=C3 bond of the indole moiety of Trp, leading to a heme-bound 3-indolylperoxo intermediate, which subsequently converts to the product NFK, via a dioxetane intermediate or a Criegee type of rearrangement. In this work, we develop to use continuous-flow resonance Raman spectroscopy, combined with stopped-flow UV-Vis spectroscopy, to investigate the dioxygenase reaction carried out by IDO and TDO. Surprisingly, a ferryl intermediate was detected during the IDO reaction at 0.2 s. The presence of this intermediate supports a new mechanism, in which the two atoms of dioxygen are sequentially incorporated into the substrate via a two-step reaction. The ferryl intermediate is not observable during the TDO reaction, highlighting the structural differences between the two types of dioxygenases, as well as the importance of the stereoelectronic factors in modulating the reactions.

**1208-Pos** Board B118

**Mutagenesis Study on the Conformation of Distal Histidine in Dehaloperoxidase-Hemoglobin**

Shu Jiang, Stefan Franzen.

Dehaloperoxidase-hemoglobin (DHP A), found in terebellid polychaete Amphiulitae ornata, acts as a bi-functional protein. It has the globin fold and acts as an oxygen carrier, but it can also act as the peroxidase, which can oxida-tively dehalogenate halophenols found in benthic ecosystems to the corre-sponding quinones. The distal histidine His55 is the only residue that determines the substrate and heme ligand binding function. His55 also plays a key role in peroxidase catalysis. His55 has two conformations, open conforma-tion and closed conformation, which is related to inhibitor and substrate binding respectively. In myoglobin, Phe36 and Arg45 have been suggested the critical residues effect the C alpha - C beta ratio of distal His64. Moreover, in myoglobin the residue adjacent to His 64 (the distal histidine) is a glycine (Gly65). Although this is a conserved feature in many globins, DHP A has a threonine in this position. To understand the role of the amino acid in this position the mutants T56G, T56A, T56S and T56V were pre pared. Kinetic assay results show that all of these mutants have higher activity than wild-type DHP A. Molecular dynamics simulations, Resonance Raman spectroscopy, X-ray crystallography and other methods will be presented to understand how a change in the Thr56 position affects the both the conformation of His55 and peroxidase activity. The inhibitor in DHP A binds in an unusual internal binding site. We will determine the inhibitor binding constants and measure the enzyme inhibition in kinetic assays to understand the role played by the distal histidine conformation inhibition as well.

**1209-Pos** Board B119

**Effect of H55D Mutation on Kinetics and Structure of Dehaloperoxidase-Hemoglobin A**

Junjie Zhao, Vesna de Serrano, Rania Dumari, Matt Thompson, Stefan Franzen.

The H55D mutant of dehaloperoxidase-hemoglobin A (DHP A) has been prepared to mimic the H64D mutant of Sperm Whale myoglobin (SWMb), which has the highest peroxidase activity of any myoglobin mutant studied to date. The high peroxidase activity has been attributed to the rapid formation as well as the prolonged lifetime of compound I following addition of H2O2. However, unlike H64D SWMb, the H55D mutant of DHP A has ~13-fold lower peroxidase activity towards oxidation of 2,4,6-trichlo-rophenol (TCP) into 2,6-dichloroquinone (DCQ) in the presence of H2O2. The origin of the lower rate constant may be the solvent-exposed conformation of D55, which has the effect of removing the acid-base catalyst necessary for heterolytic cleavage O-O bond in Fe-bound H2O2. These observations further support the hypothesis that the flexibility of distal histidine in wild-type DHP A, plays a crucial role in enzyme function. Remarkably, the H55D mutant also shows inhibition by 4-bromophenol (4BP) in the oxidation of TCP, which is substantiated by the X-ray crystal structure of 4BP-bound to the internal inhibitor binding site observed in wild type DHP A and B. The inhibitor complex is an unusual internally bound molecule, completely surrounded by the protein. Compound ES, a tyrosine radical species associated with the oxoferryl intermediate detected in DHP A, is not observed in H55D. Thus, the H55D mutant is a key test case for the role of electron transfer in the DHP A mechanism.

**1210-Pos** Board B120

**Identification of a New Charge-Transfer Transition through the Partial Unfolding of Cytochrome C under Mild Acidic Conditions**

Emanuela Frabdi, Jonathan Soffer, Reinhard Schweitzer-Stenner.

Cytochrome c, a model protein, has been isolated from a wide variety of prokaryotic and eukaryotic sources. It is commonly believed that proteins behave identically if it is extracted from the same source. This investigation illustrates the unexpected conformational change on the exposure of oxidized equine heart cytochrome c to TCA, as opposed to the preparation from acetic acid, both obtained from Sigma-Aldrich and dissolved at a concentration of 0.5 mM in 1.0 mM monobasic phosphate buffer. Without further purification an earlier unfolding event was obtained with the TCA exposed protein (pH 7.0, Temp. 338K). To unravel this enigma a variety of purification methods were employed utilizing partial unfolding-refolding protocols. The complete oxidation of cytochrome c was achieved by adjusting the pH of the sample to 11.5, neutralizing the positive patches on the protein surface. After complete oxidation, the protein was passed over a Sephadex G10 column. Subsequently, the pH of the solution was adjusted back to 8.0, which was the starting point of a titration which covered the region between 8.0 and 4.0. Surprisingly we observed a weak absorption band at 625nm, which increased with decreasing pH, reflecting the protonation of a titratable group (pK~5.2). This value strongly indicates a H33 as the involved residue. Normally, this band is indicative of the population of a ferric high spin state of the heme iron. However, in our case the 695nm band, which is an indicator of the intactness of the Fe(III)-M80 bond, remained present without any loss of intensity. Our observation suggest the stabilization of an excited charge transfer state, possibly resulting from an A2u->dπ transition by the positive charge on H33 in a heme cavity modified by the initially present TCA.