energies and huge preexponential factors. Neither the complex dependence of the binding rate on [Im] nor the thermodynamic and activation properties of the reaction are consistent with the popular model for *c*-type cytochromes in which ligand binding is preceded by a spontaneous opening of the heme cleft that becomes rate limiting at high concentrations. Instead, the dependence is better explained by initial binding of ligand that facilitates access to the heme by disruption of the H-bonded network of the heme domain.

3343-Pos

Electronic and Geometrical Structures of Hemes *a*/a_3 of the Bovine Cytochrome *c* Oxidase in the Fully-Reduced and Oxidized States Revealed by *ab Inito* Calculations

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In the respiration process, cytochrome *c* oxidase (C*c*O) plays a crucial role; i.e., it generates the gradient of the proton concentration between a matrix and an intermembrane space of the mitochondria, exploiting the free energy released by the catalysis where an oxygen atom is reduced to two water molecules. For this reaction, the required electrons/protons are transferred to the heme *a* site and the heme a_3-Cu_B site of C*c*O. Then, the catalytic reaction and proton pumping process are simultaneously occurred through these sites. In this way, the heme systems act as ''hub'', which is essential to understand the functional mechanisms of C*c*O.

In this study, to investigate the electronic structures of the heme systems, the Wannier function centers (WFCs) were employed. The WFCs correspond to the centers of maximally localized orthogonal functions termed the Wannier functions, and the concept of WFCs is similar to the Lewis view of the molecular bonds. Accordingly, this may provide convenient pictures of electronic structures based on chemical intuitions.

Using the bovine C*c*O structures, we constructed isolated models, each of which contains a heme and a histidine ligand, and performed geometry optimizations using *ab initio* DFT/HF hybrid all-electron calculations at the B3LYP functional level. Then, using the wave functions calculated for each of the heme systems, we obtained Wannier functions and WFCs. As a result, we have found that the imidazole ring of the histidine ligand are rotated in both the reduced and oxidized states, and are strongly associated with the polarization of the heme systems. In C*c*O, the hemes contact with the amino acid residues through van der Waals interactions, which could regulate the electronic structures of the heme systems.

3344-Pos

Examining Oxygen Accessibility in H-NOX Proteins with a Phosphorescent Ruthenium Porphyrin

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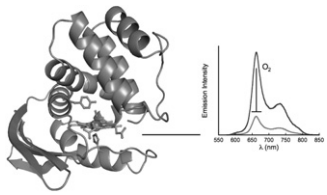
H-NOX (**H**eme **N**itric oxide and/or **OX**ygen) binding proteins are found in organisms from bacteria to humans and include soluble guanylate cyclase (sGC), the principle mammalian receptor for nitric oxide (NO). The ferrous heme of sGC selectively binds NO - completely excluding oxygen (O_2) binding - and resists oxidation in air. These features allow sGC to respond to NO even in aerobic cellular conditions. In the present study, we investigated the ability of O_2 to access the sGC heme pocket by incorporating a phosphorescent ruthenium porphyrin into two sGC domain truncations to serve as an internal O_2 sensor. A crystal structure obtained for a homologous H-NOX domain suggests that incorporation of the ruthenium porphyrin does not alter the H-NOX fold. In the sGC domains, differences were observed in both emission intensities and lifetimes +/- O_2, providing the first direct evidence that O_2 accesses the sGC heme pocket. In addition, the extent of O_2 quenching was found to parallel oxidation rates in the domains. Together these findings highlight the complexities of O_2 discrimination in sGC and point to the role of higher protein structure in partially protecting the heme from O_2.



3345-Pos

Structural Dynamics in Chloroperoxidase: A Photoacoustic Study

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Chloroperoxidase (CPO) is the most versatile heme containing enzyme that exhibits peroxidase, catalase, halogenase and monooxigenase activities. The heme iron coordination resembles to P450s enzymes whereas the composition of the distal pocket is more polar, containing Glu183 hydrogen bounded to His 105 and the heme pocket is connected with the surrounding solvent by two hydrophobic channels. To investigate the ligand-protein interactions in CPO we have employed time-resolved photoacoustic calorimetry and transient absorption study. Unlike other peroxidases, the CO photo-dissociation from CPO can be described as a two step process with distinct volume and enthalpy changes. The photodissociation of Fe-CO bond is associated with negligible volume change ($V = 1 \pm 8$ mL mol^{-1}) and a positive enthalpy change ($H = 48 \pm 34$ kcal mol^{-1}). Subsequent CO escape from the protein matrix is characterized by a positive volume change (25 ± 8 mL mol^{-1}) and insignificant enthalpy change (-2 ± 33 kcal mol^{-1}) and occurs with a lifetime of ~ 20 ns. Similar thermodynamic parameters were found in D_2O at pD 4.6 but the CO photo-release occurs two times slower (~ 50 ns) than in phosphate buffer. Presence of substrates (halides or cyclopentanedione) did not show a significant impact on the thermodynamic profiles associated with the ligand dissociation.

Membrane Protein Structure II

3346-Pos

Charge-Charge Interactions Promote Transmembrane Helix-Helix Association Depending on Sequence Context

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Folding and oligomerization of integral membrane proteins frequently depend on specific interactions of transmembrane helices. Interacting amino acids of helix-helix interfaces may form complex motifs and exert different types of molecular forces (Herrmann et al., 2009; Langosch and Arkin, 2009; Unterreitmeier et al., 2007). Here, a set of strongly self-interacting transmembrane domains, as isolated from a combinatorial library, was found to contain basic and acidic residues in combination with polar non-ionizable amino acids and C-terminal GxxxG motifs. Mutational analyses of selected sequences and reconstruction of high-affinity interfaces confirmed cooperation of these residues in homotypic interaction. Probing heterotypic interaction indicated the presence of interhelical charge-charge interactions. Further, simple motifs of an ionizable residue and GxxxG are significantly overrepresented in natural transmembrane domains and a specific combination of these motifs exhibits high-affinity heterotypic interaction. We conclude that intramembrane charge-charge interactions depend on sequence context. Moreover, they appear important for homo- and heterotypic interactions of numerous natural transmembrane domains.

Herrmann, J., J. Panitz, S. Unterreitmeier, A. Fuchs, D. Frishman, and D. Langosch. 2009. Complex patterns of histidine, hydroxylated amino acids and the GxxxG motif mediate high-affinity transmembrane domain interactions. J. Mol. Biol. 385:912-923.
Langosch, D., and I.T. Arkin. 2009. Interaction and Conformational Dynamics of Membrane-Spanning Protein Helices. Protein Sci. 18:1343-1358.
Unterreitmeier, S., A. Fuchs, T. Schäffler, R.G. Heym, D. Frishman, and D. Langosch. 2007. Phenylalanine Promotes Interaction of Transmembrane Domains via GxxxG Motifs. J. Mol. Biol. 374:705-718.

3347-Pos

Revisiting Hydrophobic Mismatch with Free Energy Calculations of Transmembrane Helix Tilting

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Membrane proteins are involved in various cellular processes via interactions between their helices and the surrounding lipid environments. It is energetically favorable for transmembrane (TM) domain to match the hydrophobic thickness of the lipid bilayer. We have utilized a helix tilt restraint potential and the free energy decomposition technique to characterize the microscopic forces governing tilting of various TM peptides in different conditions. To investigate the influence of hydrophobic length of TM helix on its tilting, we used a model peptide called WALP (acetyl-GW2(LA)nLW2A-NH2) by varying systematically the length of the peptide (WALP16, WALP19, WALP23, and WALP27). Also, to study the influence of membrane hydrophobic thickness on TM helix tilting, we used two kind of lipid bilayer (DMPC and POPC). Finally, we used arginine, lysine, and alanine as a flanking residue instead of tryptophan (acetyl-GX2(LA)8LX2A-NH2: X = R, K, and A) to examine the influence of various anchoring residues on TM helix tilting. The detail structural features and energetic contribution from hydrophobic (mis)match between lipid and peptides are presented and discussed.