features that are very different from that of Hb A2 and Hb A, consistent with the altered functional properties.

3322-Pos

The Use of Glassy Films and Sol-Gel Matrices to Probe Nitrite Mediated Reactions of Met Hemoglobin

Camille J. Roche, Mahantesh Navati, M. Belen Cassera, David Dantsker, Joel M. Friedman.
Albert Einstein College of Medicine, Bronx, NY, USA.

There have been growing indications that under certain conditions hemoglobin (Hb) can undergo nitrite mediated reactions that result in the formation of bioactive forms of nitric oxide (NO) capable of reversing vasoconstriction due to NO scavenging. This process is especially relevant for the design of Hb based blood substitutes that typically cause vasoconstriction when administered. In this presented work the use of both trehalose-derived glassy films and silane derived sol-gel matrices are used to isolate both reactive intermediates and key steps in nitrite-mediated reactions of met Hb. The glassy films allow for controlled production NO within the glass and controlled access of the NO into the distal heme pocket of the met nitrite derivative of Hb. The use of the sol-gel allows for trapping either the T or R state forms of Hb and for facile separation of products (e.g. nitrosothiols such as GSNO) from the Hb containing sol-gel phase. The contributions of added NO and small thiol containing molecules (L-cysteine and glutathione) are exposed. The results are consistent with the formation of a relatively stable intermediate capable of forming S-nitrosothiols such as GSNO. The intermediate has properties consistent with one proposed by Gladwin, Kim-Shapiro[1] and coworkers which has the potent nitrosating agent N_2O_3 coordinated to a ferrous heme.

[1.] Basu, S., *et al*, *Nature Chemical Biology*, (2007) vol. 3, p.785.

3323-Pos

General Mechanisms for the Folding and Assembly of Myoglobins and Hemoglobins

David S. Culbertson, John S. Olson.
Rice University, Houston, TX, USA.

Mammalian myoglobin has served as the archetype globin for understanding the folding properties of single domain globins with the 3 on 3 helical fold. After removal of heme, the resultant apo-Mb shows a loss of structure in the proximal F helix and adjacent loops, and during acid or GdmCl-induced denaturation, apo-Mb populates at least one intermediate. In contrast, unfolding of holo-Mb appears to be a simple two-state process with little protein concentration dependence but the underlying mechanism is much more complex. The lack of protein concentration dependence implies that heme either interacts with the unfolded polypeptide, self-associates, or both. The observed steepness of the unfolding curves for holo met-Mb requires that the affinity of hemin for the intermediate and completely unfolded states must be at least be 1000 fold weaker than that for the native apo-state, and as a result, unfolding of holo met-Mb is governed primarily by the affinity of the folded native apo-state for hemin. The generality of this conclusion for holo-Mb has been tested in several other monomeric hemoglobins, including the miniglobin from *Cerebratulus lacteus* and the thermoglobin from *Aquifex aeolicus*.

Human hemoglobin unfolding is even more complex due to association of the α and β subunits into dimers and tetramers. Removal of hemin leads to formation of an apo-$\alpha_1\beta_1$ dimer and its unfolding appears to involve an intermediate whose stability is dependent on protein concentration. This dependence suggests the formation of a dimer intermediate with partially folded subunits still attached to each other through the $\alpha_1\beta_1$ interface. Folding and assembly of holo-Hb is even more complex because there are significant differences in hemin affinity between the α and β subunits, and between tetramers, dimers and monomers.

3324-Pos

Time Resolved Thermodynamic Studies of Ligand Binding/release to Sol-Gel Encapsulated Horse Heart Myoglobin

Carissa M. Vetromile, Randy W. Larsen.
University of South Florida, Tampa, FL, USA.

The successful confinement of proteins in solid state materials allows for a multitude of applications in the study of protein dynamics, as well as, advances in biotechnologies. The importance of bulk solvent and restricted space on the conformational dynamics of proteins can be identified by encapsulating the biomolecules (peptides, proteins, enzymezes, etc) in environments where both can be regulated. In addition, the affect of encapsulation on ligand binding and preservation of reactivity once encapsulated are of equal importance. With the use of Photoacoustic calorimetry (PAC) and Photothermal beam deflection (PBD) methods along with picosecond-millisecond transient absorption and fluorescence techniques we are investigating enthalpy, molar volume changes, and kinetics associated with with CO dissociation and rebinding to horse heart myoglobin encapsulated in sol-gels. Preliminary PAC data suggest that the dissociation of CO from HHMb results in four kinetic phases: <7ns, ~125ns, ~260ns, and ~600ns. We will discuss the differences in molar volume and enthalpy changes associated with the conformational dynamics of these events in sol-gels relative to native protein in aqueous solution.

3325-Pos

The Role of the Internal Disulfide Bond in the Conformational Dynamics of Neuroglobin

Luisana Astudillo[1], Pierre Sebban[2], Jaroslava Miksovska[1].
[1]Florida International University, Miami, FL, USA, [2]University of Paris XI, Orsay, France.

Neuroglobin (Ngb) is a member of the globin family expressed mainly in brain tissue of mammals and other vertebrates that plays a role in the neuronal response to hypoxia and ischemia. Human Ngb has two cysteine residues (Cys46 and Cys55) within the CD loop and the D helix of the protein that were shown to form an internal disulfide bond, however these two residues are replaced by Gly in rat Ngb and the internal disulphide bond is missing. Therefore, to investigate the impact of the internal disulfide bond on the dynamics and energetics in Ngb we used photoacoustic calorimetry and transient absorption spectroscopy and determined the time-resolved volume and enthalpy changes associated with CO rebinding to human Ngb, rat Ngb and a rat Ngb mutant with and engineered internal disulfide bond (Cys46rNgb). The relaxation of the protein structure associated with the ligand photo-release is fast (< 50 ns) and involves a decrease in the volume of the protein matrix. The enthalpy change associated to CO photo-dissociation for all samples studied was 19 kcal mol^{-1}, whereas the reaction volume changes for human Ngb and Cys46rNgb were roughly two times higher than for rat Ngb. The reaction volume changes obtained for human and Cys46rNgb were 13.4 ± 0.9 mL mol^{-1} and 10.3 ± 0.6 mL mol^{-1}, respectively, and 4.6 ± 0.3 mL mol^{-1} for rat Ngb. These results indicate that the presence of the engineered internal disulphide bond in Cys46rNgb leads to a structural volume change that is similar to that found for human Ngb indicating that the internal disulphide bond control, to some extend, conformational dynamics associated with the ligand binding to deoxy Ngb.

3326-Pos

Design and Characterization of an Enzymatically Active Amphiphilic Maquette Protein

Sarah E. Chobot, Gregory Wiedman, Christopher C. Moser, Bohdana M. Discher, P. Leslie Dutton.
University of Pennsylvania, Philadelphia, PA, USA.

Many questions still exist about how quinone molecules act as substrates for membrane oxidoreductase enzymes, as well as how quinones can act as a catalyst in energy conversion mechanisms. We apply our knowledge of electron tunneling and protein design towards defining the basic engineering requirements for quinone reactivity in natural membranes and heme proteins. We have synthesized and characterized a transmembrane, amphiphilic maquette protein, AP6, which extracts the basic structural components from Complex III necessary to perform transmembrane proton-coupled electron transfer. We have shown that our AP6 peptide assembles as a four-helix bundle protein and can potentially bind up to six bis-histidine ligated hemes tightly across a membrane interface. Given its sequence and heme binding capabilities, our AP6 design could accomplish a variety of potential functions, including: transmembrane electron transfer, electron transfer with aqueous proteins, proton-coupled electron transfer, or combining these, quinol-cytochrome *c* oxidoreductase activity. Through standard Complex III activity assays, we have demonstrated that AP6 has quinol-cytochrome *c* oxidoreductase activity in detergent micelles that is within two orders of magnitude of the activity of natural Complex III purified from *R. capsulatus*. This activity can be generated with a variety of reduced quinone substrates, and is dependent on the concentration of cytochrome *c* present. With no obvious quinone-binding site included in our protein design, AP6 provides clear evidence that a specific quinone-binding site within a membrane protein is not essential for generating significant quinol-cytochrome *c* oxidoreductase enzymatic activity from a heme protein.

3327-Pos

The Effect of Non-Coordinated Water in the Heme Pocket on the Ligand Binding Dynamics of Heme Proteins

Rosa L. Nguyen[1], Benjamin W. Lintner[2], Ignacio L. Pena[2], Pooncharas Tipgunlakant[2], Jayashree Soman[3], Ivan Birukou[3], John S. Olson[3], Daniel E. Asarnow[1], David S. Kliger[2], Robert A. Goldbeck[1], **Raymond M. Esquerra**[2].
[1]University of California, Santa Cruz, Santa Cruz, CA, USA, [2]San Francisco State, San Francisco, CA, USA, [3]Rice University, Houston, TX, USA.

Water molecules in internal protein cavities play fundamental roles in satisfying the H-bonding potentials of main chain atoms in turns, coils, and loops,