Posters

Protein Conformation I

120-Pos

Fret Studies of the Conformational Changes in the 2b Sub-Domain of UvrD Helicase

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The *Escherichia coli* UvrD protein is a 3′ to 5′ superfamily 1 DNA helicase that functions in nucleotide excision repair and methyl-directed mismatch repair of DNA, as well as DNA replication of certain plasmids. UvrD uses the energy of ATP binding and hydrolysis to unwind double-stranded DNA (dsDNA) and translocate along single-stranded DNA (ssDNA) with biased 3′ to 5′ directionality. Single turnover pre-steady state DNA unwinding kinetics experiments have shown that the UvrD dimer is the active form of the helicase *in vitro*, although a UvrD monomer can translocate along ssDNA with the same directionality as used in unwinding. Crystal structures show that UvrD can exist in two dramatically different conformations, ''open'' in the apo state and ''closed'' when forming a complex with a 3′- ssDNA-dsDNA junction. The rotational orientations of the 2B domain differ in these two states by about 100 degrees. To study the conformational changes of the 2B domain, double cysteine mutants with one pair on 1B and 2B domains and another pair on 2A and 2B domains were constructed and labeled with a mixture of donor-acceptor fluorophores such that the movement of 2B domain results in either an increase or a decrease in FRET, depending on the positions of the labeled fluorophores. Our ensemble studies show that the 2B domain is in the closed conformation at low salt and swivels open at high salt in the absence of DNA. The open and closed conformations are in dynamic equilibrium. The binding of UvrD to ssDNA induces the open conformation of the 2B domain. The swiveling of the 2B domain is also coordinated with ATP binding and hydrolysis.

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Pyrene Fluorescence Analysis Offers New Insights Into the Conformation of the Lipoprotein-Binding Domain of Human Apolipoprotein E

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The C-terminal domain (CT) of apolipoprotein E (apoE) involved in the cholesterol homeostasis of plasma and the brain, functions in high affinity lipoprotein binding and protein self-association. The high-resolution structure of apoE N-terminal domain was reported in 1991; however that of the CT (residues 201-299) is unknown due to its self-association tendency. In our study, we employ site-specific fluorescence labeling to gain structural insights into lipid- free apoE CT at physiological concentrations (5-10 μg/ml). Pyrene, a spatially sensitive fluorophore, reports on proximity between desired sites by displaying unique spectral features. Pyrene maleimide was covalently attached to single cysteine-containing recombinant apoE CT at position 223 to probe the first predicted helical segment, and at 255 and 277 to probe the terminal helical segment. Regardless of the probe location, all three pyrene-labeled apoE CT variants displayed a dramatic excimer peak at 460 nm, indicating that two pyrene moieties are within 10 Å of each other. An intense peak at 387 nm (indicating that the probe is located in a highly hydrophobic environment) was additionally noted in all cases. The hydrophobicity of the pyrene moiety driving the helix-helix interaction was excluded when pyrene label at position 209, a predicted non-helical segment, did not display the above spectral features. Quenching by KI indicates that the accessibility to the probes was restricted. Our studies HenHkhindicate that parallel intermolecular helix-helix contacts exist throughout the entire CT in the lipid-free state. Upon binding to phospholipid/cholesterol vesicles, helix-helix interactions in pyrene labeled apoE CT are replaced by helix-lipid interactions yielding discoidal high density lipoprotein particles. This study presents the possibility of employing pyrene as a powerful new alternative to obtain complex structural and conformational information of proteins at physiologically relevant concentrations.

122-Pos

Identifing Unique Conformational Forms of Phosphofructokinase Using Fluorescence Phasor Analysis

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The two main parameters, phase angle and modulation, determined in frequency-domain fluorescence measurements, can be acquired with high precision. Unfortunately, when analyzing systems with complex decay mechanisms, error is often introduced by the imperfect modeling of this complexity. For questions that do not require a precise understanding of those mechanisms, the phasor approach allows a description of the system utilizing only the raw phase angle and modulation data with a corresponding improvement in reproducibility. In this investigation, we used phasor plots to describe the allosteric enzyme phosphofructokinase from E. coli (EcPFK). In our approach, we perform a direct transformation of the phase angle and modulation to the S and G function coordinates described in a Cartesian system as determined at an individual excitation modulation frequency. EcPFK contains a single tryptophan at position 311. Despite this simple composition, conventional fluorescence lifetime measurements of EcPFK exhibit complex decay behavior. The goal of this investigation is to describe the four species involved in the allosteric coupling between the substrate, fructose-6-phosphate (F6P) and the allosteric inhibitor, phosphoenolpyruvate (PEP), using the phasor approach. These four forms are: apo-EcPFK, EcPFK-F6P, PEP-EcPFK, and PEP-EcPFK-F6P). Special interest is on the ternary complex species (PEP-EcPFK-F6P) that is not considered in classic two-state models that attempt to explain the origin of allosteric behavior. The best results were obtained by exciting at 300 nm and collecting the fluorescence response at frequencies between 40 to 70 MHz. Our results show the presence of four unique conformations that correspond to the different ligated states of the enzyme. Notably, the ternary complex exhibits a unique phasor value, independent of whether it was formed by titrating the substrate followed by the inhibitor or vice versa. Supported by NIH Grant GM33216 and Welch Foundation Grant A1548.

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Protein Conformational Dynamics Detected Via Fluorescence Fluctuation Spectroscopy

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Fluorescence correlation spectroscopy (FCS) and cumulant analysis were applied to study the conformational dynamics of T4 Lysozyme (T4L) in solution. Previous EPR studies (Mchaourab et al., 1997)* have shown that T4L undergoes a hinge-bending motion in its native state - an oscillatory motion between an open and a closed conformation. To observe this motion on a single molecule level, we took advantage of the self quenching of the probe tetramethyl rhodamine (TAMRA) at short distances. Pairs of fluorescent probes were placed at specific residues predicted to undergo relative movement. FCS autocorrelation showed two components consistent with two conformational states of T4L in solution. Fits to a diffusion/kinetic model yielded a relaxation time in the range of 5 to 25 microseconds. Molecular brightness values obtained in the two conformations correlate with expected proximity in the structure and with distances between pairs of spin labels introduced at the same sites. We further found that the structural fluctuations as revealed by the autocorrelation curve are diminished when a substrate is bound to T4L. A novel finding is that the hinge motion modulates the dynamics of the long inter-domain helix. We are currently extending these studies to membrane transporters to detect and characterize functionally-relevant fluctuations in their structures.

*Mchaourab HS, Oh KJ, Fang CJ and Hubbell WL. 1997. Conformation of T4 Lysozyme in solution. Hinge-bending motion and the substrate-induced conformational transition studied by site-directed spin labeling. *Biochemistry* **36**:307-316.

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Conformational Transitions Associated with Electrochemically-Induced Redox Processes Through the Cytochrome C Oxidase Followed by Time-Resolved 2d-Surface-Enhanced Infrared Absorption Spectroscopy (tr-2d-Seiras)

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Electrochemically-induced redox processes of Cytochrome c oxidase (CcO) from *R. sphaeroides* were investigated using Surface-Enhanced

ATR-FTIR-Spectroscopy. The CcO with the his-tag attached to subunit II (SU II) was immobilized in a strict orientation on a two-layer gold film deposited on the ATR crystal of the IR spectrometer using the his-tag technology. A lipid bilayer was subsequently reconstituted by in-situ dialysis around the protein to yield a protein-tethered bilayer lipid membrane.
This system enabled us to observe the sequential electron transfer (eT) within the multi-redox-site membrane protein induced by electronic wiring to the gold surface using time-resolved (tr)-SEIRAS. Conformational transitions concerning a large number of single amino acids and also of secondary structures as a consequence of eT could be seen in a wide range of frequencies from 0.7 Hz to 2 kHz. A high resolution of the spectra was achieved by a combination of Two-Dimensional Infrared (2D IR) Spectroscopy and phase-sensitive detection. Kinetic constants were obtained by applying periodic potential pulses and recording spectral changes as a function of time. Methods were developed to separate these kinetic constants from the contribution due to charging currents.

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Thermodynamic Properties and Nmr Data Indicate An Inverse Calcium-Myristoyl Switch of Gcap2
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Guanylyl cyclase-activating protein-2 (GCAP-2) is a neuronal calcium sensor protein (NCS) present in vertebrate photoreceptor cells. Depending on the Ca^{2+} concentration, GCAP-2, and its homologue GCAP-1, inhibit or activate their target protein, the rod outer segment guanylate cyclase (ROS-GC). This plays an important role in shaping the photoreceptor light response. Like all members of the NCS, GCAP-2 is myristoylated at the N-terminus. This fatty acid modification is not essential for the basic function of GCAP-2, but required for full activation of the ROS-GC. Up to now, the biological role of this modification has not been fully understood. In order to gain insight into the Ca^{2+}-dependent conformational changes of GCAP-2, we measured the thermodynamic stability of the protein in dependence of Ca^{2+} binding and myristoylation by monitoring thermally and chemically induced folding / unfolding transitions of myristoylated and non-myristoylated GCAP-2. Stabilities observed for myristoylated and non-myristoylated GCAP-2 in absence of Ca^{2+} were indistinguishable. Addition of Ca^{2+} exerted a strong stabilising effect. This effect was more pronounced for the myristoylated GCAP-2 than for the non-myristoylated, indicating a structural role of the myristoyl moiety in Ca^{2+}-bound but not in the Ca^{2+}-free state. Furthermore, from deuterium solid state experiments we have evidence that the myristoyl moiety is highly flexible in the Ca^{2+}-free state when bound to liposomes. In contrast to the Ca^{2+}-myristoyl switch for the prototype NCS Recoverin, which exposes its myristoyl moiety in the Ca^{2+}-bound state, but buries it when Ca^{2+} is missing, the myristoyl moiety ofGCAP-2 appears to be fully solvent-exposed in the Ca^{2+}-free state. As we could show, myristoylation does not significantly enhance membrane binding of GCAP-2. These results are in agreement with a possible direct interaction of the myristoyl moiety with the target protein, the ROS-GC.

126-Pos
How Electron Transfer Is Linked to Conformational Transitions of Peptide Groups of the Cytochrome C Oxidase, a Study By 2d-Ir Spectro-Electrochemistry
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Synchronous 2D-IR spectrum Strong correlation between amide I of α-helices (1654 cm^{-1}) and β-sheets (1600, 1623, 1638, 1682 cm^{-1}) negative cross-correlation peak at 1620 vs. 1654 cm^{-1}
Fast scan cyclic voltammetry is used to follow the sequential electron transfer (eT) through the CcO immobilized in a biomimetic membrane architecture. CcO is immobilized via his-tag technology with the first electron acceptor, Cu_A directed toward the electrode, in a packing density optimized for fast eT to Cu_A. Kinetic constants of the sequential eT including protonations through the rest of the redox centers, heme a, a_3 and Cu_B are obtained by simulations of cyclic voltammograms measured at a wide range of scan rates using the software package MacSpice. Conformational transitions of peptide groups as a consequence of electrochemically-induced redox processes are investigated by static and time resolved 2D-surface-enhanced infrared absorption spectroscopy (tr-SEIRAS). Correlation of kinetic constants obtained from electrochemistry and tr-SEIRAS allows one to discriminate between conformational transitions regarding amino acids of the K and D proton input and exit channels and those regarding the protein backbone of α-helices and β-sheets.

127-Pos
Fitting To Lifetime Distributions in Photoacosutic Calorimetry
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Photoacoustic calorimetry (PAC) is emerging as a powerful technique for extracting volume and enthalpy changes associated with photo-triggered reactions in both chemistry and biology. Current PAC methods allow for the deconvolution of PAC waves using a deconvolution by reconvolution strategy. In this process, the calorimetric reference wave serves as an impulse function to which either a single exponential or multiple exponential functions are convoluted producing a simulated sample wave. The amplitude and rate constants of the associated exponentials are varied using selected parameter estimation algorithms and the reconvolution process repeated until an appropriate chi-square is achieved. Here we investigate a new PAC deconvolution process which allows for the sample acoustic waves to be fit using a variety of reaction models including stretched exponentials and lifetime distribution functions. Our new algorithm is tested against simulated data sets to provide fit validation.

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Comparison of Persistence Length Calculations of Model Collagen in Two and Three Dimensions To Afm Measurements
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Molecular-dynamics simulations are performed on a model collagen molecule in SPC water, with and without 100 mM NaCl. To calculate the persistence length, we find the center of mass of each amino acid. We then group the amino acids into triplets, representing each by the (unweighted) average of the three centers of mass. These center-of-mass positions are used as end points for directors. The time-averaged cosine between directors is found (by determining the scalar product of the directors) as a function of contour length between them. Additionally, two-dimensional projections of the three-dimensional images are constructed, in analogy to the experimental deposition of collagen onto a surface. Techniques for measuring and calculating persistence length from AFM images are used on the two-dimensional projection images, and results are compared to the model prediction and to actual experimental results.

129-Pos
Combining Single Molecule Optical Trapping and Small Angle X-Ray Scattering Measurements to Compute the Persistence Length of a Protein Alpha-Helix
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A relatively unknown protein structure motif forms stable isolated single alpha-helices, termed ER/K alpha-helices, in a wide variety of proteins and has been shown to be essential for the function of some molecular motors. The stability of the ER/K alpha -helix arises from the charge-charge interactions between its glutamic acid (E) and Arginine (R) or Lysine (K) side chains. The flexibility of the ER/K alpha-helix determines whether it behaves as a force-transducer, rigid spacer or flexible linker in proteins. The ER/K alpha-helix spans long distances with relatively few amino acid residues, has known salt and temperature sensitivity, and can be expressed in E. coli, making it an important tool in engineering proteins, provided its mechanical properties are clearly established. We have quantified the flexibility of the ER/K alpha-helix in terms of persistence length, namely the length scale over which it is rigid. We use single-molecule optical trapping and small angle x-ray scattering (SAXS), combined with Montecarlo simulations to demonstrate that the ER/K alpha-helix behaves as a worm-like-chain with persistence length of ~ 15 nm. This persistence length is dependent on the relative content of R and K residues in the ER/K alpha-helix. Knowledge of the persistence length enables us to define its function as a rigid spacer in a translation initiation factor, as a force-transducer in the mechanoenzyme myosin VI, and as a flexible spacer in the Kelch motif containing protein.