Posters

Protein Conformation I

120-Pos

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

Haifeng Jia[1], Anita Niedziela-Majka[1], Sergey Korolev[2], Taekjip Ha[3], Timothy Lohman[1].
[1]Washington University School of Medicine, St. Louis, MO, USA, [2]St Louis University School of Medicine, St. Louis, MO, USA, [3]Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

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.

(This research was supported by the NIH (GM 045948).)

121-Pos

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

Arti B. Patel[1], Panupon Khumsupan[1], Vasanthy Narayanaswami[1,2].
[1]California State University Long Beach, Long Beach, CA, USA, [2]Center for the Prevention of Obesity, Cardiovascular Disease & Diabetes, Children's Hospital Oakland Research Institute, Oakland, CA, USA.

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

Mauricio D. Lasagna, Gregory D. Reinhart.
Texas A&M Univ, College Station, TX, USA.

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.

123-Pos

Protein Conformational Dynamics Detected Via Fluorescence Fluctuation Spectroscopy

Robel B. Yirdaw, Hassane S. Mchaourab, Shruti Sharma.
Vanderbilt University, Nashville, TN, USA.

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.

124-Pos

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)

Christoph Nowak[1,2], Wolfgang Knoll[2], Dieter Walz[3], Robert B. Gennis[4], Renate L.C. Naumann[1,2].
[1]Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany, [2]Austrian Institute of Technology, AIT, Donau-City Str. 1, 1220 Vienna, Austria, [3]Biozentrum, University of Basel, present address: Lerchenstrasse 21, 4059 Basel, [4]University of Illinois, Department of Biochemistry, 600 South Mathews Street, Urbana, IL 61801, USA.

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