represent the first evidence for the long sought-after protein folding process triggered by photo-induced CO dissociation.

1174-Plat

Phosphorescence from Single Tryptophan in Amorphous Solid Human Serum Albumin Exhibits Solvent-Protein Dynamics Slaving

Andrew Draganski, **Richard D. Ludescher**.
Rutgers University, New Brunswick, NJ, USA.

The physical properties of amorphous biomolecules are important to the texture and stability of low-moisture foods, the stability of pharmaceuticals, the permeability of edible films, and the viability of organisms during anhydrobiosis. Protein stability is often improved via the inclusion of small-molecule excipients during freeze-drying and organisms overproduce sugars such as sucrose or trehalose during anhydrobiosis. The effect on internal protein dynamics caused by substitution of a protein's surface water molecules with small sugar molecules is unclear. To explore this question, we have analyzed tryptophan phosphorescence decays of human serum albumin (HSA) in the dry amorphous solid state. Phosphorescence is an ideal approach, as the long-lived triplet state of tryptophan is sensitive to the long time-scale molecular motions of proteins in the dry state. Human serum albumin (HSA) was chosen because it contains a single, buried tryptophan residue and thus can provide information on the local dynamics of a specific site in the interior of the protein. Amorphous protein films were prepared by spreading concentrated solutions of HSA with and without sugar onto quartz slides, followed by rapid drying and extensive desiccation. Phosphorescence intensity decays were collected and fit with multiple exponential functions. From the average lifetime of these fits the rates of non-radiative decay (kNR) of the triplet state were calculated; kNR is dependent on the microviscosity of the site and is thus a measure of molecular mobility of the HSA tryptophan site. At all temperatures this measure of molecular mobility was lower in the films containing sucrose. Break-point analysis of a kNR Arrhenius plot revealed two temperature regimes with a transition occurring at the glass transition temperature of sucrose. Research supported in part by the National Research Initiative of USDA-CSREES.

1175-Plat

Structural Dynamics of Light-Driven Proton Pumps

Magnus Andersson[1], Erik Malmerberg[2], Sebastian Westenhoff[2], Gergely Katona[2], Marco Cammarata[3], Annemarie B. Wohri[2], Linda C. Johansson[2], Friederike Ewald[4], Mattias Eklund[5], Michael Wulff[4], Jan Davidsson[5], Richard Neutze[2].
[1]University of California at Irvine, Irvine, CA, USA, [2]Gothenburg University, Gothenburg, Sweden, [3]European Synchrotron Radiation Facility, Grenoble, France, [4]European Synchrotron Radiation Facility, Grenoble, Sweden, [5]Uppsala University, Uppsala, Sweden.

In a recent publication (Andersson et al. (2009) *Structure*. 17(9):1265-75), we applied the emerging technique of time-resolved wide-angle X-ray scattering (TR-WAXS) to visualize the structural dynamics of two light-driven proton pumps, namely bacteriorhodopsin and proteorhodopsin, in real-time. Direct structural information was obtained over a time course of 360 ns to 100 ms. Our results establish that three conformational states are required to describe the respective photocycles of both proteins. Significant motions of the cytoplasmic half of helix F and the extracellular half of helix C are observed prior to the primary proton transfer event, which increase in amplitude following proton transfer. These results both simplify the structural description that have emerged from a range of biophysical techniques and reveal shared dynamical principals for proton pumping. Moreover, the measured magnitudes of the helical movements associated with the bacteriorhodopsin photocycle are larger than those anticipated by intermediate trapping studies. This demonstrates the effect of a crystal lattice on protein dynamics and shows the advantage of direct measurements in solution at room temperature.

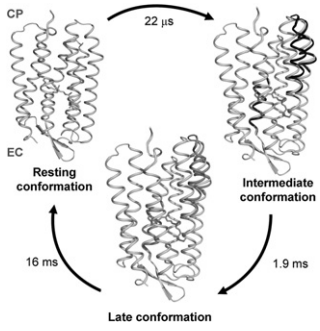

Figure 1. Schematic showing the observed helical rearrangements during the photocycle of bacteriorhodopsin.

1176-Plat

Mechanoenzymatics and Protective Mechanisms of Titins' Catalytic and IG Domains

Ulf Hensen[1], Lars Schaefer[1], Frauke Graeter[1], Joao M. Nunes[2], Daniel J. Mueller[2], **Helmut Grubmueller**[1].
[1]Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany, [2]Biotechnology Center, University of Technology, Dresden, Germany.

The giant titin filament controls many structural and functional properties of the sarcomere. Titin filaments connect M-line and Z-disc of the sarcomere and consist of four regions: the M-line, A-band, I-band, and Z-line. The different domains of titin (mainly immunoglobulin Ig and fibronectin-3 domains, and the catalytic domain titin kinase (TK)) exhibit dramatically different mechanical properties. We used atomistic molecular dynamics simulations to explore the coupling of mechanical stability with the enzymatic activity of titin kinase and the protective properties of Ig-domains. We showed that a unique autoinhibitory mechanism allows TK to act as a molecular force sensor, as relatively low forces already remove the autoinhibitory tail and prime the molecule for ATP binding. At much higher forces, the mechanical stability of Ig27 becomes important: In our studies, extensive dynamic force spectroscopy (DFS), Brownian dynamics, and molecular dynamics simulations worked together to examine mechanical stability of Ig27 under different loading rates. Our results suggest that Ig27 is perfectly suited to act as a molecular force buffer over a wide range of loading rates.

1177-Plat

Dynamics of apoB100-Containing Lipoproteins Determined by Incoherent Elastic Neutron Scattering

Christian Mikl[1], Judith Peters[2,3], Markus Trapp[2], Giuseppe Zaccai[4], **Ruth Prassl**[1].
[1]Institute of Biophysics and Nanosystems Research, Graz, Austria, [2]Institut de Biologie Structurale, Grenoble, France, [3]Université Joseph Fourier, Grenoble, France, [4]Institut Laue Langevin, Grenoble, France.

Apolipoprotein B100 (apoB100)-containing lipoproteins (very low density lipoprotein (VLDL) and low density lipoprotein (LDL)) are the principal fat and cholesterol carriers in blood. During metabolic conversion from VLDL to LDL, the particle size decreases (from ~ 80 nm to 20 nm) and lipid composition is changed, however, the amphiphilic apoB100 molecule remains bound to its lipoprotein particle and most likely compensates for structural changes due to its inherent conformational flexibility and dynamics .

Here, we report on motions in the time range of 100 ps to 1 ns in human-LDL, human VLDL and yolk-VLDL, which were recorded by elastic neutron-scattering temperature scans from 20K to 310 K using hydrated lipoprotein powders. The mean square displacement values $\langle u^2 \rangle$ were calculated from the scattering vector dependence of the elastic intensity *I(Q)*. The effective force constants $\langle k \rangle$, which are a measure for the resilience of the particle, were derived from the slopes in the $\langle u^2 \rangle$ vs. T scans. In the low-temperature harmonic regime we found no substantial differences between lipoprotein fractions ($\langle k \rangle$ ~1 N/m). Nevertheless, lipoproteins are softer compared to hydrated myoglobin powder (2 N/m) or purple membranes (1.7 N/m) [1]. Significant differences were observed with increasing temperatures. Both, human and yolk VLDL show two breaks in the scan with a steep increase in $\langle u^2 \rangle$ above 270K, whereas LDL shows a smooth behavior above a dynamic transition around 220K. Accordingly, at physiological temperatures VLDL-fractions are highly soft and mobile ($\langle k'' \rangle$ ~0.08 N/m) as compared to LDL ($\langle k' \rangle$ ~ 0.2 N/m). Sucrose, added as cryoprotectant, significantly modified the dynamics of VLDL, as it confers extreme stability to VLDL over the whole temperature range and substantially suppresses dynamic transitions.

[1] G. Zaccai, Science 288 (2000), 1604-1607

1178-Plat

Valine-Induced Packing Deficiencies of Transmembrane Domains Promote Helix Flexibility and Membrane Fusion

Christina Scharnagl, Stefan Quint, Simon Widmaier, Dieter Langosch.
Technical University Munich, Freising, Germany.

The helical transmembrane domains of fusion proteins are known to be functionally important and display an overabundance of helix-destabilizing Ile and Val residues. In an effort to systematically study the relationship of helix flexibility and fusogenicity, synthetic LV-peptides were designed whose hydrophobic core consists of Leu and Val residues at different ratios and at different positions (Hofmann et al., 2004; Poschner et al., 2009). The ability of the LV-peptides to fuse membranes increases with the content of helix-destabilizing residues. Molecular dynamics simulations were performed in order to characterize the backbone dynamics of these peptides in membrane-mimicking 80% TFE solvent and to relate the hydrogen-bond dynamics to experimental deuterium/hydrogen exchange kinetics. The analysis revealed that (i) the backbone dynamics of the helices increases systematically with Val content, (ii) that the impact of Val is due to stereochemical constraints within the helical structure and (iii) that side-chain packing mainly determines exchange kinetics. As a consequence, VxxV and VVxVV motifs promote helix destabilization whose relevance for membrane fusogenicity will be discussed.