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

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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

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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.

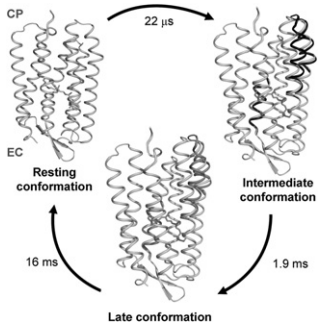

Figure 1. Schematic showing the observed helical rearrangements during the photocycle of bacteriorhodopsin.

1176-Plat

Mechanoenzymatics and Protective Mechanisms of Titins' Catalytic and IG Domains

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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

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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

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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.

Hofmann, M.W., K. Weise, J. Ollesch, A. Agrawal, H. Stalz, W. Stelzer, F. Hulsbergen, H. deGroot, K. Gerwert, J. Reed, and D. Langosch. 2004. De novo design of conformationally flexible transmembrane peptides driving membrane fusion. Proc. Natl. Acad. Sci. U S A 101:14776-14781.
Poschner, B.C., S. Quint, M. Hofmann, and D. Langosch. 2009. Sequence-specific conformational dynamics of model transmembrane domains determines their membrane fusogenic function. J. Mol. Biol. 386:733-741.

Platform Z: Mechanosensitive & TRP Channels

1179-Plat

Outer Pore Domain of TRPV1 Ion Channel is Required for Temperature-Independent Step During Temperature-Activation

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TRPV1 is the founding and best-studied member of the family of temperature-activated transient receptor potential ion channels (thermoTRPs). Voltage, chemicals, and heat allosterically gate TRPV1. Molecular determinants for TRPV1 activation by capsaicin, allicin, acid, ammonia, and voltage have been identified. However, many years after the discovery of TRPV1, the structures and mechanisms mediating temperature-sensitivity remain unclear. Recent studies of the related channel TRPV3 identified residues within the pore region required for heat activation. Here we describe both random and targeted mutagenesis screens of TRPV1 to identify single point-mutations that specifically affect temperature-activation. The mutations found are all located in the outer pore region, in close proximity to but distinct from residues previously implicated in acid-activation. Electrophysiological analysis shows that mutations affect a temperature-independent step that is part of the temperature-gating pathway. These results suggest that the outer pore plays a general role in heat-sensitivity of thermoTRPs.

1180-Plat

Temperature-Driven Activation of Thermotrps: A Distinct Pathway Involved

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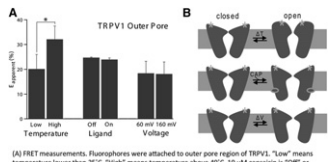
A group of thermosensitive transient receptor potential (ThermoTRP) channels, with high temperature sensitivity of channel gating, are the cellular temperature sensors. ThermoTRPs are polymodal receptors. Besides temperature they are gated by voltage, ligand, extracellular pH and other stimuli. How temperature changes drive activation conformational rearrangement remains unknown. Here we combine functional, mutational, and site-directed fluorescence studies to demonstrate that temperature-dependent activation uses a pathway distinct from those for ligand- and voltage-dependent activation.

We observed that neither strong depolarization nor application of capsaicin could significantly alter thermodynamics of temperature-driven TRPV1 activation. In addition, voltage and ligand exhibited additive gating effects over temperature gating. Indeed, a TRPV1 mutant in which part of the outer pore region was replaced by an artificial sequence showed virtually no temperature sensitivity but maintained near normal capsaicin sensitivity. Furthermore, site-directed FRET measurements showed that conformational changes in outer pore can only be induced by heating, but not by voltage or ligand. Together these observations suggest that a distinct pathway for temperature to gate TRPV1 involves the outer pore region.



(A) FRET measurements. Fluorophores were attached to outer pore region of TRPV1. "Low" means temperature lower than 25°C, "High" means temperature above 40°C. 10 μM capsaicin is "Off" or "On". * indicates $P < 0.05$ (B) A schematic representation of the proposed gating conformational changes in TRPV1 induced by heat and capsaicin. The green stars represent fluorophores attached to the outer pore.

1181-Plat

Role Of Pip2 On Ca^{2+}-Dependent Desensitization of Trpv2

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TRPV2 is a member of the transient receptor potential superfamily of ion channels involved in chemical and thermal pain transduction. Unlike the related TRPV1 channel, TRPV2 does not appear to bind either calmodulin or ATP in its N-terminal ankyrin repeat domain. In addition, it does not contain a calmodulin-binding site in the distal C-terminal region, as has been proposed for TRPV1. Importantly, though, we have found that TRPV2 undergoes Ca^{2+}-dependent desensitization similar to TRPV1, suggesting that the mechanism of desensitization may be conserved in the two channels. To elucidate the molecular mechanism underlying Ca^{2+}-dependent desensitization in TRPV2 we used whole-cell recordings of F-11 cells transiently transfected with TRPV2. We found that prolonged applications of the TRPV2 agonist 2-APB led to nearly complete desensitization of the channel in the presence of extracellular Ca^{2+}. In contrast, no desensitization was observed in the absence of Ca^{2+}. TRPV2 desensitization was not altered in whole-cell recordings in the presence of calmodulin inhibitors or upon co-expression of mutant calmodulin, suggesting that CaM does not play a major role in Ca^{2+}-dependent desensitization of TRPV2. Interestingly, simultaneous confocal imaging and electrophysiological recording of whole cells expressing TRPV2 and a fluorescent PI(4,5)P2 binding probe showed a high degree of temporal correlation between the Ca^{2+} induced desensitization and depletion of PI(4,5)P2. Thus, Ca^{2+} influx through TRPV2 is sufficient to trigger a dramatic decrease in PI(4,5)P2 levels, presumably by activating PLC. We propose that the decrease in PI(4,5)P2 levels upon channel activation underlies at least a major component of Ca^{2+}-dependent desensitization of TRPV2.

1182-Plat

TRPM8 Cation Channel. Effects of Voltage, Cold and Menthol on Single-Channel Gating

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Single-channel patch-clamp recording allows mechanistic insights into fundamental ion channel properties. Thus, a single kinetic model can formally describe the complex interactions during poly-modal activation of the channels. For TRPM8, activation depends on temperature, voltage and chemical signaling [1, 2], and such modeling provides the most comprehensive approach to the study of the channel. In this study, we examined the influence of voltage, cold and menthol on TRPM8 gating using patch-clamp recording techniques. In HEK293 cells stably expressing TRPM8, single-channel currents were measured (filtered at 2 kHz and sampled at 10 kHz) in cell-attached patches at different voltages (−100 to 140 mV), temperatures (20 or 30°C) and menthol concentrations (10 or 100 μM) (n = 7-11). As has been reported for whole-cell TRPM8 currents [3], shifts in the voltage-dependent single-channel open probability curve toward less positive potentials were induced by cold or menthol. Thus, the potential for half-maximal activation was reduced from 162.4 to 116.1 mV during cooling from 30 to 20°C, with a further shift to 52.8 mV with 100 μM menthol. To investigate the relationship between these modulators, we used different techniques - HJCFIT [4], QuB [5] and 2D fitting [6] - to develop a single-channel kinetic model aiming to identify the most likely potential-, menthol- and cold-regulated transitions. A model with 5 closed and 2 open states showing correlation between brief openings and long closings and between brief closings and long openings, was able to describe our macroscopic and single-channel data. Interestingly, temperature and menthol mimicked voltage-dependent activation of the channel at the model level by increasing the probability of transitions from long closed states to brief ones. This is the first complete kinetic model based on single-channel data for any of the TRP channels.

1183-Plat

Phosphoinositide Regulation of TRPM8 Channels in Planar Lipid Bilayers

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The cold and menthol receptor TRPM8 is regulated by membrane phosphoinositides. To study the effects of lipids directly on the channel, we have reconstituted the purified TRPM8 in planar lipid bilayers. This system allows full control of the lipid composition in our experiments. The reconstituted channel was activated by menthol or cold, and its activity depended on the presence of specific phosphoinositides. In the presence of menthol, TRPM8 exhibited the highest probability of opening in the presence of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] with Po ~ 0.89 at +100 mV and Po ~ 0.4 at −100 mV. Less channel activity was induced by phosphatidylinositol 3,4-bisphosphate [$PI(3,4)P_2$] with Po ~ 0.53 at +100 mV and Po ~ 0.2 at −100 mV. Phosphatidylinositol 3,4,5-bisphosphate [$PI(3,4,5)P_2$] resulted in irregular TRPM8 channel currents and lower open probability with Po ~ 0.21 at +100 mV and Po ~ 0.087 at −100 mV. Among the tested lipids the lowest TRPM8 channel activity was induced by phosphatidylinositol 4-phosphate [PI(4)P] with Po ~ 0.12 at +100 mV and Po ~ 0.019 at −100 mV. The lipid specificity profile in lipid bilayers is very similar to that observed in excised patches. We have also studied the activation of TRPM8 channels in lipid bilayer with cold. Cooling the system with reconstituted TRPM8 channels also required the presence of $PI(4,5)P_2$. The main shift in the channel behavior was observed in the temperature range from 21°C to 18°C where the channel showed drastic changes in the open probability from 0.05 to 0.85 at +100 mV.