Title

Crowd Control: Effects of Macromolecular Crowding on Early Events in Protein Folding

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controls their function is not understood. Here, we study the contribution of individual CH domains to the actin-binding function of utrophin's tandem CH domain. Co-sedimentation assays indicate that the C-terminal CH2 domain binds weakly to F-actin when compared with the full-length tandem CH domain, consistent with the published results on tandem CH domains. However, the surprise came from the CH1 domain. Isolated CH1 binds strongly to F-actin when compared with the full-length tandem CH domain. These results indicate that CH2 has a negative influence on actin-binding when it is linked with CH1. Thus, the obvious question that arises is why tandem CH domains require CH2, when CH2 is reducing their actin-binding efficiency. To answer, we probed the thermodynamic stabilities of individual CH domains. Isolated CH1 domain is unstable and is prone to serious aggregation. Isolated CH2 is very stable, even more stable than that of the full-length tandem CH domain. This makes utrophin's tandem CH domain as the first example where an isolated domain is more stable than the full-length protein. These results indicate that the main function of CH2 is to stabilize CH1 at the expense of decreasing the actin-binding efficiency. Consistently, the proposed structure of utrophin's tandem CH domain based on earlier X-ray studies indicates a close proximity between the C-terminal helix of CH2 and the N-terminal helix of CH1, and this helix in CH2 becomes more dynamic in the full-length protein when compared with that in the absence of CH1, suggesting a mechanism by which CH2 stabilizes CH1 despite the decrease in actin-binding function.

2365-Pos Board B57

Characterization of Human, Mouse, and Frog Rhodopsin Microdomains Within Native Disc Membranes

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Rhodopsin is the light-activated receptor located in the disc membranes of rod photoreceptor cells in the retina and initiates vision via the phototransduction signaling cascade. There are divergent views on rhodopsin's quaternary organization within native disc membranes. The classical view posits that rhodopsin molecules function as freely diffusing monomers. However, recent evidence suggests that rhodopsin oligomerizes and forms higher order structures within the membrane. An accurate description of signaling events in phototransduction and of associated disease mechanisms is reliant on a comprehensive understanding of how rhodopsin is organized within native disc membranes. The aim of the current study was to determine and quantify the physiological arrangement of several vertebrate rhodopsins within their native disc membranes using atomic force microscopy (AFM). AFM is a microscopic method that allows for the imaging of membrane proteins in their native environment under physiological conditions. Disc membranes, with 90% of the total protein content comprised of rhodopsin, were isolated from human, mouse, and frog ocular tissue. AFM images of single-bilayer disc membranes revealed that these vertebrate disc membranes have similar topographies. Topographic features in these images indicate that rhodopsin is organized into microdomains and that the formation of these microdomains is not an effect of a low temperature environment. The microdomains formed by rhodopsin from each species were quantified and comparatively analyzed. By characterizing these microdomains, a baseline has been established for the organization of rhodopsin in human, mouse, and frog disc membranes. These characterizations of microdomain rhodopsin organization may serve as a guide in future investigations which will need to address the importance and role of such an organization on phototransduction and diseased states.

2366-Pos Board B58

Reconstitution of the 26S Proteasome Reveals Functional Asymmetries in its Heterohexameric AAA+ Unfoldase

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The 26S proteasome is the major protease in eukaryotic cells responsible for selective protein degradation to mediate protein quality control and regulation. However, the detailed mechanisms by which the proteasomal heterohexameric AAA+ unfoldase drives ATP-dependent protein degradation remain poorly understood. Delineating the roles of the six distinct ATPase subunits in substrate processing has been hindered by limitations in working with endogenous proteasomes due to misassembly or lethal degradation defects. We therefore developed a heterologous expression system to produce the unfoldase subcomplex from Saccharomyces cerevisiae in Escherichia coli and reconstituted the proteasome in vitro to perform systematic mutational analyses of the individual ATPase subunits. Our studies demonstrate that the six ATPases have distinct functions in degradation, corresponding to their positions in the spiral staircases adopted by their large AAA+ domains in the absence or presence of substrate. ATP hydrolysis in subunits at the top of the staircases is critical for substrate engagement and translocation. Whereas the unfoldase relies on this vertical asymmetry for substrate processing, interaction with the peptidase exhibits a pronounced three-fold symmetry. Only three ATPase subunits, arranged in alternate positions within the unfoldase ring, contain a conserved C-terminal hydrophobic/aromatic/unspecified (HbYX) motif that is critical for both peptidase binding and gate-opening, whereas the C-terminal tails of the interjacent ATPase subunits are dispensable. Our study provides an initial glimpse into the potential importance of the spiral staircase configurations of proteasomal ATPase subunits in substrate processing and highlights how the 26S proteasome may deviate from simpler, homomeric AAA+ proteases.

Protein Folding and Chaperones I

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Crowd Control: Effects of Macromolecular Crowding on Early Events in Protein Folding

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Earlier time-resolved far-UV TRCD/TRORD studies have shown that electron-transfer triggered folding of reduced cytochrome *c* (redcyt *c*) from the GuHCl-induced unfolded ensemble in dilute phosphate buffer involves kinetic partitioning: one fraction of molecules folds rapidly, on a sub-microsecond time scale, while the remaining population folds more slowly [Chen et al. (2003) *J. Phys. Chem. A*, 107,8149-8155]. With the addition of 220 mg/mL dextran 70 (spherical-like structure), the population of the fast folding step is greatly reduced [Chen et al. (2012) *Biochemistry* 51, 9836-9845]. However, this fast phase is not significantly affected when sucrose is used to introduce the same microviscosity as that of the dextran solution. These results, as well those from corresponding coarse-grained simulations, suggest that excluded volume effects, but not viscosity, due to macromolecular crowding compacts the unfolded state ensemble such that cyt *c*'s access to fast-folding conformations is decreased in crowded conditions. Presented here will be studies that examine the effects of other crowding agents of different size and shape (rod-shaped Ficoll 70) and concentration (100 mg/mL dextran 70) on the kinetics of redcyt *c* folding. How the His33-Fe-His18 heme configuration, which was previously correlated with the fast folding population under buffer conditions, is affected by the presence of macromolecular crowders that facilitate compaction of the unfolded state is also examined.

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Validation of the Conservation and Robustness of Folding Initiation Sites in Evolutionarily Related Proteins

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Despite the recent progress in the protein folding field, how a given amino acid sequence folds into its unique structure is still unclear.
According to KA Scott et. al., for Immunoglobulin and Fibronectin-like fold, which are evolutionarily unrelated but have the same topology, share a folding mechanism consisting of 4 key residues and their peripheral residues.
However, there are also some cases that two evolutionarily related proteins have different folding mechanisms (for example, PDZ2 and PDZ3 whose sequence identity is nearly 30%).
In this study, we aim to investigate whether such folding segments (i.e., folding initiation sites playing important roles for the folding from the denatured state to the transition state) really exist in evolutionarily related proteins by our sequence analysis methods and validate the results with the experimental phi values that provide a degree of the contribution for the structural formation in the transition state for each residue.
Our sequence analysis methods are based on the inter-residue average distance statistics and already applied to several proteins, like leghemoglobin, FABP, azurin, and TIM-barrel proteins; they returned reasonable results with HD exchange experiments or phi-value analyses.
According to the current study, for the Ferredoxin-like proteins we treat here, the predicted folding initiation sites have good agreements with experimental phi values, and the application to their evolutionarily related proteins returns a suggestion that the location of folding initiation sites are more conservative than sequence.
In addition to these analyses, we also investigate the robustness of folding initiation sites by comparing the results of our analyses between naturally evolved proteins, which are under the pressure of nature, and artificially evolved proteins, which are not under the pressure of nature.
The details will be reported in the conference.