that differences in structure in the N-terminal domain may account for differences in stability of the two proteins.

3397-Pos
Light Chain-Mediated Self-Association of Intrinsically Disordered Dynein Intermediate Chain
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Cytoplasmic dynein is a microtubule-associated protein with functions in cell division, positioning of organelles and the transport of cellular molecules. The dynein complex is composed of six subunits; but how these subunits assemble to form a functional complex is not entirely clear. In an on-going effort to understand complex assembly in cytoplasmic dynein, we have initiated structural studies of three of the subunits: IC74, the intermediate chain subunit and its light chain binding partners LCS and Tctex. These three subunits form a tight sub-complex at the base of the dynein particle where they are presumed to function as cargo adaptors or regulate the assembly of the complex.

We have previously reported that binding of LC8 to the intrinsically disordered N-terminal domain of IC74 leads to helix formation in a region downstream of the binding site. To better assess these structural changes several cysteine mutations that allowed monitoring of specific segments of the helix forming region were introduced into an IC74 construct (IC74-ICTLC). Fluorescence experiments on fluorophore-labeled ICTLC show that binding of the light chains induces self-quenching of the fluorophore. We attribute this self-quenching to close proximity of the ICTLC chains likely due to a modest IC-IC self-association. This modest IC-IC self-association is not observed in the absence of light chain binding. Thermodynamics of the IC74-light chain interactions indicate that while binding of LCS to IC74 is moderately weak (10 nM), having a pre-formed ICTLC cysteine cross-linked dimer or Tctex pre-bound to the ICTLC construct enhances the binding affinity (0.1-0.2 µM). Taken together, these results are consistent with a model where light chain binding coupled to IC-IC self-association could be important for stabilizing the dynein complex.

3398-Pos
Aquifer Aequolicus FlgM Protein Does Not Exhibit the Disordered Character of the Salmonella Typhimurium FlgM Protein
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Studies on the nature and function of Intrinsically Disordered Proteins (IDP) over the past ten years have demonstrated the importance of IDPs in normal cellular function. The flexibility of IDPs allows one IPD to assume multiple conformations or fold different protein-protein complexes, allowing a single protein to exhibit multiple functions. While many predicted IDPs have been characterized on an individual basis, an understanding of how different homologous proteins from different organisms has not been carefully studied. We now demonstrate that the FlgM protein from the thermophile Aquifer aequolicus exhibits significantly less disorder than the previously characterized FlgM protein from Salmonella typhimurium. FlgM is an inhibitor of the RNA transcription factor σ28, which is involved in regulation of flagella synthesis gene expression. Previous work has shown that the S. typhimurium FlgM protein is an intrinsically disordered protein, though the C-terminus becomes ordered when bound to σ28 or under crowded solution conditions. In this work, we demonstrate that, even under dilute solution conditions, that the A. aequolicus FlgM protein exhibits alpha-helical character. Furthermore, we use the fluorescence probe FIASH to show that the H2 helix is ordered, even in the unbound state, in contrast to the S. typhimurium FlgM protein, and the H1 and H2 helices appear to be associated in the absence of the σ28 protein. Taken together, our data demonstrates that the A. aequolicus FlgM protein, while flexible, does not exhibit the intrinsically disordered nature exhibited by the S. typhimurium FlgM protein.

3399-Pos
Analyzing the Self-Organizing Mechanism of Lysozyme Amyloid Fiber Formation
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The formation of amyloid fibers involves a number of different intermediates. By using separation techniques and analysis methods such as dielectric spectroscopy, AFM, and TEM, the aggregation steps of fiber formation were analyzed. An Agilent 4294A impedance analyzer and an Agilent 16452A liquid test fixture over a frequency range of 40Hz to 30MHz was used for dielectric spectroscopy. We approach amyloid fiber formation using the newly introduced colloidal model [1]. This model suggests that proteins aggregate into uniformly sized nano-spheres, driven by surface energy minimization. The uniform spheres then behave like a mono-dispersed colloidal suspension. Once the spheres have reached their critical diameter it is observed from microscopy that the colloidal growth stops. At this point the attractive forces that favor agglomeration are balanced by the barrier potential forces that retard agglomeration. The fully developed nucleation units then assemble in a linear fashion before finally evolving into mature amyloid fibers. The model postulates that the linear assembly arises from dipole-dipole interaction between nano-spheres. We analyze this assembly process in vitro using lysozyme from chicken egg whites in an acidic environment. In vivo, lysozyme has a propensity to form amyloid fibers in systemic amyloidosis diseases. Lysozyme amyloid fibers are synthesized in vitro and assembled into samples according to particle size. Our separation techniques yielded three samples: 1. a solution with a high concentration of monomeric lysozyme and small oligomers, 2. a solution composed of colloidal spheres and short fibers, and 3. a solution with a high concentration of mature amyloid fibers. The existence of these species in the three samples was confirmed with AFM, TEM, and Thioflavin-T binding assays. Results of dielectric analysis indicate intermediate sized aggregates have a higher dipole moment than small aggregates.


3400-Pos
Early Oligomer Formation of Alpha-Synuclein As Revealed by Fluorescence Correlation Spectroscopy
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Here we study the formation of early oligomers of α-synuclein by applying Fluorescence Correlation Spectroscopy (FCS). The idea is to use trace amounts (nM) of labeled protein in the presence of a large excess of unlabeled protein and follow the aggregation process by measuring the reduction in time of the diffusion coefficient of the fluorescent species. Synuclein with an engineered labeling (A140C) was labeled with Alexa488 and was used as a fluorescent probe in trace amounts (3-4 nM) in the presence of 100 µM unlabeled α-synuclein. The combination of short sampling times and repeated measurements produce a size distribution of the oligomers. Initially, a sharp peak is obtained (diffusion coefficient 114 ± 15 µm²/sec) corresponding to monomers. Subsequently a sharp transient population appears, followed by the gradual formation of larger sized distributions of higher oligomers. The process can be studied in time by following the reduction of the apparent monomer concentration. (Big aggregates are moving too slow to contribute to the fluctuations). The kinetics of this process can also be fitted with the Finke Watzky equation for a two state- two step mechanism (Morris et al., Biochemistry 2008, 47:2413-27), but the rate constants obtained from this process are different from the rate constants for turbidity formation, indicating the need for an intermediate state. The formation of the transient intermediate and the early oligomers is accompanied by a conformational change, as visualised using FRET between the donor labeled N-terminus and the acceptor labeled cysteine at position A140C.

3401-Pos
Structural and Functional Insights Into Lipid Binding by Oligomeric Alpha-Synuclein
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Oligomeric alpha-synuclein is considered to be the potential toxic species responsible for the onset and progression of Parkinson’s disease, possibly through the disruption of lipid membranes. Although there is evidence that oligomers contain considerable amounts of secondary structure, more detailed information on the structural characteristics and how these mediate oligomer-lipid binding are critically lacking. We have used tryptophan fluorescence spectroscopy to gain insight into the structural features of oligomeric alpha-synuclein and the structural basis for oligomer-lipid interactions. Several single tryptophan mutants of alpha-synuclein were used to gain site-specific information about the microenvironment of monomeric, oligomeric and lipid bound oligomeric alpha-synuclein. Acrylamide quenching and spectral analyses indicate that the tryptophan residues are considerably more solvent protected in the oligomeric form compared to the monomeric protein. In the oligomers, the negatively charged C-terminus was the most solvent exposed part of the protein. We have now used tryptophan fluorescence spectroscopy to gain insight into the structural features of oligomeric alpha-synuclein and the structural basis for oligomer-lipid interactions. Several single tryptophan mutants of alpha-synuclein were used to gain site-specific information about the microenvironment of monomeric, oligomeric and lipid bound oligomeric alpha-synuclein. Acrylamide quenching and spectral analyses indicate that the tryptophan residues are considerably more solvent protected in the oligomeric form compared to the monomeric protein. In the oligomers, the negatively charged C-terminus was the most solvent exposed part of the protein. Upon lipid binding a blue shift in fluorescence is observed for alpha-synuclein by applying Fluorescence Correlation Spectroscopy (FCS). The idea is to use trace amounts (nM) of labeled protein in the presence of a large excess of unlabeled protein and follow the aggregation process by measuring the reduction in time of the diffusion coefficient of the fluorescent species. Synuclein with an engineered labeling (A140C) was labeled with Alexa488 and was used as a fluorescent probe in trace amounts (3-4 nM) in the presence of 100 µM unlabeled α-synuclein. The combination of short sampling times and repeated measurements produce a size distribution of the oligomers. Initially, a sharp peak is obtained (diffusion coefficient 114 ± 15 µm²/sec) corresponding to monomers. Subsequently a sharp transient population appears, followed by the gradual formation of larger sized distributions of higher oligomers. The process can be studied in time by following the reduction of the apparent monomer concentration. (Big aggregates are moving too slow to contribute to the fluctuations). The kinetics of this process can also be fitted with the Finke Watzky equation for a two state- two step mechanism (Morris et al., Biochemistry 2008, 47:2413-27), but the rate constants obtained from this process are different from the rate constants for turbidity formation, indicating the need for an intermediate state. The formation of the transient intermediate and the early oligomers is accompanied by a conformational change, as visualised using FRET between the donor labeled N-terminus and the acceptor labeled cysteine at position A140C.