2230-Pos Board B200

Detection of Predominant Antiparallel Strand Registry in the Membrane-Associated HIV Fusion Peptide

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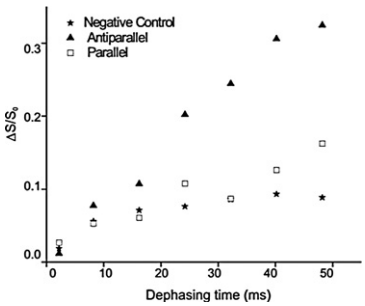
The HIV gp41 protein plays a significant role in the fusion of viral and host cell membranes. The ~16 N-terminal residues of gp41 form the apolar fusion peptide (HFP) that insert into the host cell membrane and play a critical role in fusion. A 30-residue peptide containing the HFP sequence was synthesized and was shown to induce fusion between vesicles. For HFP associated with membranes with physiological cholesterol content, solid-state nuclear magnetic resonance (SSNMR) measurements demonstrated a fully extended β strand conformation for the first 16-residues of HFP and that the HFPs were aggregated into a β sheet. In the present study, SSNMR was applied to probe for the relative population of parallel in-register strands and antiparallel strands in the β sheet region. Samples were prepared with a mixture of two HFPs, one of which contained a single ^{13}CO backbone label and one of which contained two sequential ^{15}N backbone labels. SSNMR measurements of ^{13}CO-^{15}N distances with different labeled HFPs showed that 50-60% of the strands in the β sheet were antiparallel and 5-15% were parallel. These data provide a clear structural model for the membrane-associated HFP.



2231-Pos Board B201

pH-Triggered Membrane Insertion Pathway of the Diphtheria Toxin T-Domain: 1. Insertion/Refolding Intermediate

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The T-domain of diphtheria toxin undergoes low pH-induced refolding that allows it to insert spontaneously into a lipid membrane. To gain insights into the general principles of non-constitutive membrane protein insertion we have examined pH-dependent membrane interactions of the T-domain using several spectroscopic techniques. Membrane topology of the NBD-labeled T-domain was studied using our fluorescence lifetime-based LysoUB quenching method. Our results reveal a well-defined translocation for NBD attached to a single cysteine in position 350 and no translocation for position 378C. We compared kinetics of membrane insertion and kinetics of binding of the T-domain. Energy transfer between donor-labeled T-domain and acceptor-labeled lipid vesicles demonstrates rapid membrane association for all pH values for which binding occurs. The insertion kinetics (measured with NBD probe attached at 369C) is significantly slower, and is also both pH- and lipid-dependent. This difference between FRET-based binding kinetics and NBD-based insertion kinetics indicates the presence of an interfacial intermediate on the insertion pathway of the T-domain. We examined the compactness of the fold of this intermediate using tryptophan quenching of bimane fluorescence. Bimane was attached at the 369C position, which places it in close proximity to the native W206, allowing one to monitor local unfolding of the protein. The pH-dependent reduction in quenching suggests that anionic lipids promote the unfolding of the intermediate on membrane interfaces. FCS measurements of the formation of the membrane-competent conformation of the T-domain indicate that the protonation of titratable residues occurs both in the bulk of the solution and near membrane interface. In an accompanying presentation (Rodnin et al., BPS Meeting 2009) we examine the role of several crucial histidine residues on the T-domain insertion pathway. Supported by NIH GM069783(-04S1).

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pH-Triggered Membrane Insertion Pathway of the Diphtheria Toxin T-Domain: 2. Role of Histidines

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The function of diphtheria toxin T-domain is to insert into the lipid bilayer and translocate the catalytic domain across the membrane in response to acidification of the endosome. In the accompanying presentation (Kyrychenko et al., BPS Meeting 2009) we have characterized the membrane insertion pathway of the T-domain WT. Here we examine the role of several crucial histidine residues in pH-dependent insertion by comparing WT to various mutants with one or more H-to-Q or H-to-R substitution(s). Thermal denaturation measured by CD indicates that all tested substitutions lead to lower stability of the protein. Kinetic measurements of guanidinum denaturation reveal that the folding branch of the chevron plot for the WT is independent of pH, while the unfolding branch becomes more prominent with acidification (a feature lost in some of the mutants). We have examined the insertion of the TH8-9 helical hairpin by following the topology of the NBD probe attached to C350, which is translocated across the bilayer in the WT. Interestingly, a single mutation of a remote histidine residue (H257R or H257Q) abolishes the insertion, while triple mutations of the H322, H323, H372, located on the top of the TH8-9 hairpin, do not affect the topology. This result is confirmed by emission measurements of the bimane probe placed in the middle of TH-9 helix: (1) WT-like blue shift for triple-R or triple-Q mutants and no shift for H257R or H257Q. The kinetics of membrane insertion, however, is affected in triple mutants: abolishing the charge (triple-Q mutant) results in slower insertion kinetics, while placing constant charges (triple-R mutant) in the same positions accelerates the insertion at intermediate pH. These results are consistent with the proposed role of His protonation in triggering membrane refolding. Supported by NIH GM069783(-04S1).

Molecular Chaperones

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Characterization of Substrate Binding to the Group II Archael Chaperonin from Methanococcus maripaludis (Mm-Cpn)

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Molecular chaperones are important for the proper folding and function of many proteins, and malfunction of chaperone-assisted folding has been implicated in several protein aggregation diseases. For this reason, chaperones have been identified as a potential area for drug development. An important step in developing specific therapeutic targets is the identification of the recognition site on the substrate as well as the binding site on the chaperone. In this study, we use a number of biophysical and biochemical methods to elucidate the binding site on a Human γ-Crystallin substrate and the recognition site on the Methanococcus maripaludis chaperonin (Mm-Cpn). A homologue of human chaperonins, Mm-Cpn has been shown to bind and inhibit the aggregation of several closely related members of the Human γ-Crystallin protein family including HγD- and HγC-crystallins. Although members of this protein family share a high degree of homology, Mm-Cpn suppression of HγD aggregation is twice as efficient as the suppression of HγC aggregation. It remains unclear whether this difference in suppression efficiency is linked to sequence-determined substrate binding kinetics or to differences in substrate aggregation kinetics. To better understand how sequence difference may affect aggregation and chaperone binding, we studied the chaperone-induced aggregation suppression for the isolated N-terminal and C-terminal domains of both HγD and HγC. Supported by an NIH Roadmap Award to the Center for Protein Folding Machinery (http://ncmi.bcm.tmc.edu/nanomedicine).

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Characterization of the Group II Chaperonin TriC derived from Human Cervical Adenocarcinoma (HeLa) Cells

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Molecular chaperones are key elements in protein folding pathways, and there are numerous proteins which are unable to fold in the absence of one or more molecular chaperones. Eukaryotic chaperones can be broadly separated into two families: chaperones, which sequester and protect unfolded proteins, but do not facilitate folding, and chaperonins, which require ATP, and actively fold nacent or misfolded proteins. The mammalian chaperonin TRiC, in addition to being required for actin and tubulin folding, binds and refold several disease causing proteins *in vitro*, including those associated with Alzheimer's disease, Von Hippel Landau tumor, and Huntington's disease. This refolding activity has revealed TRiC as a possible therapeutic agent for the treatment and prevention of aggregation diseases. We have purified TriC from cervical adenocarcinoma cells. To assess the properties of Human TriC, we are characterizing its interactions of with human γ Crystallins. Crystallins are a family of structural proteins found in the lens of the human eye, and aggregation of these proteins is thought to be the cause of cataract. *Methanococcus marapaludis* chaperonin Mm-Cpn, a homolog of human TriC, has been shown to both suppress aggregation of Human γ Crystallins, and refold the Crystallins to a native-like conformation. Suppression of γ Crystallin aggregation is being investigated using UV/Vis spectroscopy, and the ability of