38-Plat Ammonium Transport Mechanisms in the Amt/Rh Protein Family

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Transport of ammonium across cellular membranes is a fundamental physiological process. Although ammonium is highly toxic to animals, it is the preferred source of nitrogen for most microorganisms. Ammonium transport is mediated by a family of ubiquitous membrane proteins (Amt), found in all domains of life and homologous to animals Rhesus (Rh) proteins. Based on the X-ray structure of the *E. coli* AmtB transporter, it was concluded that the conduction mechanism involves the single file diffusion of electro-neutral ammonia (NH_3) molecules, excluding the presence of water molecules in the narrowest portion of the pore (Khademi et al., Science 2004). This lead the Amt/Rh proteins to be generally considered as ammonia conducting channels. However, this model neglects the fact that X-ray diffraction experiments have shown electronic density in the pore lumen for crystals grown in both presence and absence of ammonium salt (Zheng et al., PNAS 2004). Furthermore, free energy calculations suggest that the hydrophobic pore of AmtB is able to stabilize a file of water molecules at positions in excellent agreement with the experimental electronic density (Lamoureux et al., Biophys. J. 2007). The possible presence of water molecules in the pore lumen of AmtB calls for the reassessment of the so far accepted permeation model. Functional experiments on plant ammonium transporters and rhesus proteins suggest a variety of permeation mechanisms including the passive diffusion of NH_3, the antiport of NH_4^+/H^+, the transport of NH_4^+, or the cotransport of NH_3/H^+ (Javelle et al., J. Struct. Biol. 2007). In the light of some recent functional and simulation studies on the AmtB transporter, we discuss these mechanisms and illustrate how they can be reconciled with the available high resolution X-ray data.

Platform D: Membrane Proteins I

39-Plat Determining the Conductance of the SecY Protein Translocation Channel for Small Molecules

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The channel formed by the SecY complex must maintain the membrane barrier for ions and other small molecules during the translocation of membrane or secretory proteins. We have tested the permeability of the channel using planar bilayers containing reconstituted purified E. coli SecY complex. Wild type SecY complex did not show any conductance for ions or water. Deletion of the "plug", a short helix normally located in the center of the SecY complex, or modification of a cysteine introduced into the plug, resulted in transient channel openings; a similar effect was seen with a mutation in the pore ring, a constriction in the center of the channel. Permanent channel opening occurred when the plug was moved out of the way by disulfide bridge formation. These data show that the resting channel on its own forms a barrier for small molecules, with both the pore ring and the plug required for the seal; channel opening requires movement of the plug (Saparov et al., 2007).

References

Saparov, S.M., Erlandson, K., Cannon, K., Schaletzky, J., Schulman, S., Rapoport, T.A., and Pohl, P. (2007). Mol. Cell *26*, 501–509.

40-Plat Role of Histidine Protonation in the pH-Triggered Membrane Insertion of Diphtheria Toxin T-Domain

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Diphtheria toxin T-domain inserts into the lipid bilayer and translocates the catalytic domain across the endosomal membrane in response to acidification. Similar to other self-inserting proteins (colicins, annexin B12), T-domain requires negatively charged lipids for proper insertion, suggesting a possible role for cationic residues. We hypothesize that protonation of histidines plays an important role in

(A) destabilizing the aqueous state of the T-domain and
(B) assisting in interfacial refolding via charge-charge interaction with anionic lipids.

We test this hypothesis by replacing one or several His's either with neutral Gln's or charged Arg's. We examine the folding and thermal stability of the mutants in solution using circular dichroism and test their membrane activity in an ANTS/DPX vesicle leakage assay. We also used several fluorescence quenching techniques to characterize the compactness of the fold in solution and topology of the membrane-inserted state. Our data indicate that the T-domain can tolerate multiple substitutions without losing its native-like CD-appearance and pore-forming activity. Substitutions of all 6 His's, or either of the N-terminal (223, 251, 257) or C-terminal (322, 323, 372) His's with Gln's slowed down leakage kinetics. Multiple replacements with Arg's were more likely to produce a misfolded inactive protein, but a C-terminal triple mutant had unaltered pore-forming activity. Interestingly, some single Arg mutants (*e.g.*, H257R) showed an increased activity over the WT at moderately acidic pH values, suggesting that positive charge at this position will promote membrane interactions of the T-domain. Neutralizing the charge at the same position (H257Q) resulted in slow leakage. These differences are consistent with our expectations of the role of histidine protonation in the formation of the interfacial intermediate state, a postulated general intermediate of the insertion pathway of non-constitutive membrane proteins.

Supported by NIH GM069783(-04S1).

41-Plat Towards Atomic Resolution of TM Domain Structure of Human Glycine Receptor

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The entire four transmembrane (TM) domains of human glycine receptor (GlyR) α1 subunit were spectroscopically resolved by NMR in lyso-LPPG micelles. A parameter-correlation approach was devised to characterize the structure and dynamics of the four TM domains. The resolved NMR peaks are correlated by their contribution to the histograms of the ^{15}N R_2/R_1, ^{15}N-{^1H} heteronuclear NOE (hetNOE), and paramagnetic probe-induced chemical shift changes. Three clusters in the hetNOE histogram, corresponding to hetNOE values of <0.35, 0.35–0.5, and >0.5, are classified. The middle cluster is further divided into two based on the corresponding R_2/R_1 values. 46% of the NMR peaks are associated with hetNOE >0.5 and R_2/R_1 ratio of 17–35, with a R_2/R_1 peak at ~25. These belong to residues with rigid internal motions and are assigned to the helices. The global tumbling time is estimated from these peaks to be ~25 ns. 12% of the NMR peaks have similarly high R_2/R_1 but lower hetNOE (0.35–0.5), and are assigned to helices with relatively flexible internal motions. 11–12% peaks are associated with R_2/R_1~8–14 and hetNOE~0.35–0.5, belonging to transition residues from helices to loops. The remaining 30–31% showed high flexibility with low hetNOE and R_2/R_1 values. The NMR-determined α helical content (58%) agrees with the CD results (60%), but is significantly lower than predicted (73%) based on the homology modeling using the cryo-EM nAChR structure as a template. All pore-lining TM2 domain peaks are assigned, and their hetNOE, R_2/R_1, and chemical shift index all showed that the extracellular 1/3 of TM2 are intrinsically non-helical within the framework of the entire TM domains. Our results suggest that the TM domain structures of the members in the Cys-loop receptor superfamily are not identical and should be determined individually.

(Funded by NIH R37GM049202 & R01GM069766)

42-Plat Lipids, Water, And Protein Interactions Of Intramembrane Rhomboid Protease

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Rhomboid proteases are membrane-embedded proteins whose catalytic efficiency depends on the lipid environment. To understand the structure and dynamics of lipid:rhomboid interactions and how lipid molecules influence rhomboid protease function, we performed an extensive set of molecular dynamics simulations of the E. Coli GlpG rhomboid in different lipid environments. Two additional sets of simulations of mutant phenotypes with activities markedly different from the wild-type allowed us to assess the role of structural elements identified by experiments. The results indicate that the irregular shape of the E. Coli rhomboid has a significant impact on the geometry of the lipids interacting with the protein, leading to thinning of the lipid bilayer in the vicinity of the protein. The lipid:protein hydrogen bonds depend on the nature of the lipid membrane environment, and influence the details of the protein: water interactions. These changes in lipid:protein interactions likely contribute to the influence of the lipid membrane environment on the catalytic efficiency of rhomboid proteases.

This work was supported by research grants from the National Institute for General Medical Sciences and the NIH National Center for Research Resources.

43-Plat Inhibitor Complexed Structures of the Cytochrome bc1 from the Photosynthetic Bacterium Rhodobacter sphaeroides

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The cytochrome bc1 complex (bc1) is a major contributor to the proton motive force across the membrane by coupling electron transfer to proton translocation. The crystal structures of wild type and mutant bc1 complexes from the photosynthetic purple bacterium Rhodobacter sphaeroides (Rsbc1), stabilized with the quinol oxidation (QP) site inhibitor stigmatellin alone or in combination of with the quinone reduction (QN) site inhibitor antimycin, were determined. The high quality electron density permitted assignments of a new metal-binding site to the cytochrome c1 subunit and a number of lipid and detergent molecules. Structural differences between Rsbc1 and its mitochondrial counterparts are mostly extra membranous and provide a basis for understanding the function of the predominantly longer sequences in the bacterial subunits. Functional implications for the bc1 complex are derived from analyses of 10 independent molecules in various crystal forms and from comparisons with mitochondrial complexes.

44-Plat Curvature-Inducing Behavior of Light Harvesting Complex II

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The photosynthetic apparatus of purple bacteria is contained within organelles called chromatophores, which form as extensions of the cytoplasmic membrane. The shape of these chromatophores can be spherical (as in Rb. sphaeroides and Rb. capsulatus), lamellar (as in Rps. acidophila and Rs. molischianum), or tubular (as in certain Rb. sphaeroides mutants). Chromatophore shape is thought to be induced and maintained by the integral membrane proteins Light Harvesting Complexes I and II (LH1 and LH2), which pack tightly together in the chromatophore. LH2 is a ring-shaped oligomer whose function is to absorb light via scaffolded bacteriochlorophylls and to pass the excitation energy on to LH1. In addition, LH2 has been implicated in the creation of the spherical shape of Rb. sphaeroides chromatophores. It has been suggested that the shape of LH2, together with its close packing in the membrane, induces membrane curvature. However, the mechanism by which LH2 and its close-packing produces curvature is not known. This question

was explored via molecular dynamics simulations of multiple LH2 complexes in a membrane patch. LH2s from two species - Rb. sphaeroides and Rps. acidophila - were simulated to also allow investigation into whether the LH2s from species with spherical chromatophores have different curvature properties than those from species with lamellar chromatophores. We found little dependence of curvature on species, suggesting that the packing of the complexes, as well as the distribution of LH1s, may be the largest factors in determining membrane shape.

45-Plat Structural and Energetic Aspects of Membrane Binding by Ras GTPases:

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Ras GTPases become functionally active when anchored to membranes by inserting their lipid modified side chains. However, the mechanism of membrane insertion and the structure of the resulting complex remain elusive. Recently, the structure of the full-length H-ras protein in a DMPC bilayer has been characterized through modeling and molecular dynamics simulations. It was found that ras binding to membrane involves, in addition to the anchor, a direct interaction with the membrane phosphates of basic residues from either the linker or the catalytic domain. Two nucleotide-dependent modes of membrane binding were obtained. In the GTP-bound active state, Ras predominantly binds the bilayer via helix 4 and the anchor, whereas in the GDP-bound form the role of helix 4 is replaced by the linker. These results were confirmed by mutagenesis experiments. Furthermore, the local structure and dynamics of the bilayer was perturbed upon protein insertion and this perturbation is dependent on the insertion depth and backbone localization of the anchor, which in turn is modulated by the catalytic domain and the linker. Computation of the potential of mean force (PMF) for the transfer of the anchor into the bilayer resulted in a downhill profile. The insertion into the hydrophobic core produces ~30 kcal/mol gain in free energy. Combining these results, a mechanistic model for the activation state-dependent membrane-reorganizing effect of Ras, and a novel "balance model" for the regulation of H-ras membrane orientation and signal output, are proposed.

46-Plat OmpG: A Quiet Pore?

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Bacterial outer membrane proteins are structurally robust molecules with a β-barrel architecture. Thus they are good candidates for applications in biotechnology: for example, as components of biosensors. OmpG from E. coli is a non-specific monomeric pore that undergoes pH-dependent, voltage-driven and spontaneous gating. Single channel recording studies have shown that gating of OmpG causes transient current blockades and results in numerous spikes in the single channel recording that would interfere with the analyte signal. Thus these intrinsic activities of OmpG must be eliminated if it is to be effective as a biosensor.

The spontaneous gating of OmpG is particularly challenging to understand. We have focused our attention on trying to eliminate the spontaneous gating activity of OmpG. We have used Molecular Dynamics (MD) simulations of OmpG to identify regions of the protein implicated in spontaneous gating to design mutants intended to be resistant to this type of gating. Two approaches were adopted to enhance the stability of the open conformation; optimization of the inter-strand hydrogen bonding of the barrel and reducing the mobility of the loop (L6) implicated in gating. Characterization of the mutants by single channel electrical recording has shown that they are substantially quieter than wildtype OmpG. In the quietest mutant, gating activity is reduced by ~90 %.

Platform E: Micro and Nanotechnology Nanopores

47-Plat Mica, Bioenergetics, and the Origin of Life

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Life may have originated between mica sheets, which would have provided many many confined spaces with surprising similarities to cells, as well as a possible source of energy for the synthesis of biomolecules. Mica is a layered mineral with flexible sheets, 2nm thick. Mechanical energy is produced by the movement of mica sheets, in response to forces such as ocean currents. The energy of a carbon-carbon bond at room temperature is comparable to a mechanical force of 6 nN moving a distance of 1 Angstrom. Mica movements may have facilitated mechanochemistry. Mica is also a rich source of potassium ions, which bridge anionic sites between adjacent mica sheets. With a hexagonal grid of anionic sites spaced 0.5 nm apart on each mica sheet, the K^+ concentration between mica sheets is 100 mM when the mica sheets are ~0.7 nm apart; this is comparable to the K^+ concentrations in cell cytoplasms. Low entropy is characteristic of life; entropy is much lower between mica sheets than on a mineral surface or in a 'prebiotic soup.' The sketch shows 22 mica sheets (44 nm thick) populated by a variety of types of 'prebiotic molecules.'