Synthesis of Artificial Protein Nanopores via Metal-mediated Self-assembly

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Statement of Purpose. Template-assembled synthetic proteins (1) offer the potential for the construction of three-dimensional artificial proteins without addressing the challenges of protein folding often encountered in native systems. Prior work has focused on the use of porphyrins, cyclodextrins, and cyclic peptides as the templates to spatially position peptides via covalent bonds. This approach, however, requires harsh reaction conditions and often results in low product yield. Herein we describe the first steps towards developing an alternative strategy for the construction of artificial membrane proteins. Specifically, we outline our efforts to construct a membrane-spanning, three-dimensional nanopore via the self-assembly of four peptide chains to a metal-coordination complex (Figure 1). The approach uses supramolecular assembly of a metal-binding peptide that chelates to Pt, which is constrained in a square-planar configuration via bipyridyl (2). This work offers a possible route for the engineering of artificial proteins that may serve as functional components in a wide range of nanoscale devices.

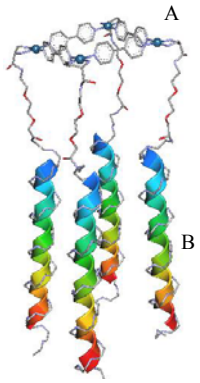

Figure 1. Schematic representation of synthetic target. (metal complex (A) and peptides (B)).

Methods: Peptides were made on an ABI 431 Solid-phase synthesizer (Foster City, CA) using standard Fmoc / tBu protection strategy on a Rink amide MBHA resin from EMD Biosciences, Inc. (San Diego, CA). Purification was carried out using water / acetonitrile gradients on a C18 reverse-phase HPLC. The identity of the peptides was confirmed using an ABI QSTAR XL mass spectrometer. Lipid vesicles were formed by extrusion using an Avanti Mini-Extruder (Alabaster, AL). Fluorescence spectra were recorded on a Shimadzu RF-1501 Fluorescence Spectrophotometer (Columbia, MD) using a 10 mm rectangular cell and a λ_{EX} = 280 nm.

Results/Discussion: In these studies, we describe the synthesis and characterization of two peptides designed to constitute the walls of a membrane-spanning, synthetic channel-mimic. The peptide sequence was designed in four segments: an *N*-terminal, metal-chelating diamine moiety; a linker region; a membrane-spanning peptide known to form ion channels (3); and a *C*-terminal tryptophan residue that functions as an optical reporter to monitor membrane insertion. Two peptides were synthesized that differed in their linker region. The first incorporated a flexible, hydrophobic trisglycyl linker. The second possessed a hydrophilic linker, 8-amino-3, 6-dioxaoctanoic acid. UV-visible absorption spectroscopy (λ_{max} = 280 nm) confirmed that substitution of the trisglycine with the dioxaoctanate linker increased aqueous solubility (0.25 vs. 0.60 mg/mL). Next, the insertion of the two peptides into model lipid bilayers was examined using fluorescence spectroscopy. Specifically, the tryptophan fluorescence emission, which is known to depend upon local environment of the amino acid residue, was monitored after reconstitution into large unilamellar vesicles (LUVs) comprising dimyristoylphosphotidyl-choline (DMPC). The observed blue shift of the tryptophan emission peak and the increase in its intensity signaled successful peptide insertion into the hydrophobic lipid bilayer. Fluorescence titrations conducted on each of the peptides with DMPC LUVs were used to determine an equilibrium partition coefficient (K_χ) between the lipid bilayer and aqueous phase (Figure 2). Figure 2 shows that the nature of the linker significantly alters the titration curves, and thus influences the insertion of the peptide into the bilayer. The linker region altered the thermodynamics by nearly a factor of two (K_χ=97000 vs. 54000).

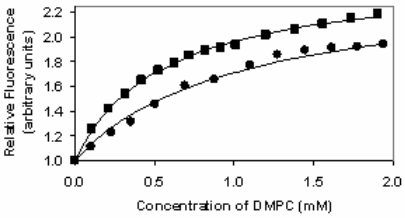

Figure 2: Fluorescence titration of trisglycyl (squares) and dioxaoctanate (circles) peptides with DMPC LUVs.

Conclusions: We have taken the first step toward devising a synthetic approach for the preparation of an artificial, channel-forming protein. Specifically, we have designed and synthesized channel wall-defining peptides and have demonstrated that they readily insert into a model lipid bilayer. Future work will be directed at determining synthetic conditions that will promote supramolecular assembly formation.

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References:

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