Structural Biology of Transmembrane Domains: Efficient Production and Characterization of Transmembrane Peptides by NMR

J. Hu, H. Qin, C. Li, M. Sharma, T.A. Cross, F.P. Gao (NHMFL, FSU, Chemistry & Biochemistry, Institute of Biophysics)

Introduction

Structural characterization of transmembrane peptides (TMPs) is justified because transmembrane domains of membrane proteins appear to often function independently of the rest of the protein. However, the challenge in obtaining milligrams of isotopically labeled TMPs to study these highly hydrophobic peptides by nuclear magnetic resonance (NMR) is significant. In the present work, a protocol is developed to produce, isotopically label, and purify TMPs in high yield as well as to initially characterize the TMPs with CD and both solution and solid state NMR. Six TMPs from three integral membrane proteins, CorA, M2 and KdpF, were studied. CorA and KdpF are from *M. tuberculosis* while M2 is from Influenza A virus. Several milligrams of each of these TMPs ranging from 25 to 89 residues were obtained per liter of M9 culture. The initial structural characterization results showed that these peptides were well folded in both detergent micelles and lipid bilayer preparations. The high yield, the simplicity of purification and the convenient protocol represents a suitable approach for NMR studies and a starting point for characterizing the transmembrane domains of membrane proteins.

Experimental

PISEMA spectra were obtained from uniformly aligned samples between glass slides using 600 and 900 MHz NMR spectrometers at the NHMFL and Low-E NHMFL probes.

Results and Discussion

Here, PISEMA spectra of three transmembrane peptides are shown where the data can be used to provide initial structural results. The CorA peptide is just a portion of the CorA transmembrane domain which can be characterized as an intact domain by solution NMR spectroscopy in detergent micelles. The M2 peptide forms a functional H⁺ channel, while the KdpF 30 residue peptide is actually a full length protein associated with the Kdp K⁺ transport system. The calculated PISA wheels for helical tilt angles of 27, 32 and 34° for CorA-TM2, M2 (22-46) and KdpF are superimposed on the spectra.

Conclusions

While the transmembrane domain is the most difficult domain to characterize for membrane proteins by most technologies, solid state NMR can characterize this domain in a liquid crystalline lipid bilayer, a unique advantage for this technology.

Acknowledgment

This work was supported by NIGMS and NIAID grants. The NMR experiments were conducted at the National High Magnetic Field Laboratory supported by cooperative agreement DMR-0084173 with the NSF and the State of Florida.

References: This effort was recently published in Prot. Sci. 16:2153-2165.