Membrane-Bound Proteins: A Study of Their Structure and Dynamics

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ABSTRACT

Integral membrane proteins are essential to cellular processes due to their ability to partially or completely span the cell membrane. The structure of these proteins is important because it mediates their function in the cell. Transmembrane proteins contain hydrophobic midsections, within the membrane, and hydrophilic ends, which are exposed to the aqueous cellular and extracellular environment. The objective of this research is to study the gas-phase structures of a series of integral membrane peptides.[1] These peptides will be studied by coupling electrospray ionization mass spectrometry with ion mobility studies to determine the size and structure of the peptides. The peptides are known to form a-helices in the membrane, but a gas-phase analysis using ion mobility has never been performed. The peptides will be introduced into the vacuum system from two types of aqueous solutions; one where the peptides are incorporated into phospholipid vesicles and one where the peptides are simply dissolved in solution. The phospholipid vesicles mimic the cellular membrane. Cell membrane conditions can be analyzed and structural differences due to the presence of the vesicles may be studied. Structural assignments based on mobility results are generated by comparing experimentally measured mobilities with candidate structures determined from molecular mechanics.

"Interfacial Positioning and Stability of the Transmembrane Peptides in Lipid Bilayers Studied by Combining Hydrogen/Deuterium Exchange and Mass Spectrometry" Heck et al., The Journal of Biological Chemistry, 2001, Vol. 276, No. 37, pp. 34501-34508.

BACKGROUND

FIGURE 1

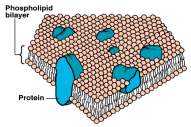
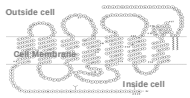


FIGURE 2



A living cell is separated from its environment by a discrete boundary called the cell membrane. In order to completely understand the function of the cell membrane its composition and structure must be analyzed. Electron microscopy, and physical studies of permeability and the motion of individual protein and lipid molecules within the membrane have led to the development of the fluid mosaic model for biological membranes **(Figure 1).** The fluid mosaic model states that the cellular membrane is composed of proteins, lipids, and carbohydrates incorporated in glycoproteins and glycolipids. Phospholipids form a bilayer, in which the hydrophobic phosphate head groups face outward. The proteins are incorporated irregularly through the bilayer and are held by hydrophobic interactions between membrane lipids and the hydrophobic domains of the protein. These proteins can function as highly selective pumps, channel, receptors, and energy transducer and are the focus of this research.

The way in which a membrane protein is imbedded in the lipid bilayer, and its interactions with the membrane is significant for membrane protein structure and function. Transmembrane peptides span the lipid bilayer, and protrude from the inner and outer membrane surfaces **(Figure 2).** Transmembrane proteins contain hydrophobic midsections within the membrane, and hydrophilic ends, which are exposed to the aqueous cellular and extracellular environment.

The objective of this research is to study the structures of peptide fragments of membrane-bound proteins by coupling electrospray ionization mass spectrometry with ion mobility studies. The peptides will be introduced into the vacuum system from two types of solutions; one where the peptides are incorporated into phospholipid vesicles to mimic the cellular membrane and one where the peptides are simply dissolved in solution. Electrospray ionization is a soft ionization technique that is ideal for proteins. When a sample is introduced the nanospray source forms a continuous beam of ions which are focused by an ion funnel into a drift cell containing ~5 torr of Helium buffer gas. The ions then pass through a scanning quadrupole for mass analysis. The quadrupole can also mass select an ion and obtain an arrival time distribution (ATD) for that ion. From the ATDs the ion's mobility and cross section can be found. Small compact ions travel faster than large extended ions and since cross section and ion mobility are inversely proportional the small compact ion will have a small cross section and the large extended ion will have a larger cross section. Thus measuring the ions mobility yields information about the ions structure. Structural assignments based on ion mobility results are generated by comparing experimentally measured ion mobilities with theoretically calculated candidate structures. The theoretically calculated candidate structures are generated using the AMBER suite of programs and performing molecular mechanics/dynamics on the ion of interest. This approach uses force field methods to determine the cross sections of low energy structures, which are compared with experimental cross sections. Through this research, we will be able to propose a structure of the peptide we are studying.

The peptides we studied were provided to us by Albert J. R. Heck at Utrecht University **(Table 1).** These peptides have a hydrophobic core and are known to form alpha-helices in the membrane. These peptides differ by the hydrophilicity of the near-terminal residues, and the insertion of a proline residue in the hydrophobic core.

Research thus far has focused on examining peptides dissolved in a trifluoroacetic acid (TFA) solution. These results will later be compared with peptides incorporated into phospholipid vesicles in order to observe any induced structural changes and interactions.

FIGURE 3

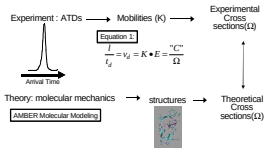


Figure 3:
a) The ion's arrival time is inversely proportional to its mobility and directly proportional to its cross section. Compact ions with smaller cross sections drift through the cell faster and have shorter arrival times. From the ATDs, the mobility of the ion can be obtained using Equation 1. From the mobility we can then calculate cross section.
b) To obtain conformation details about the ions measured in ion mobility experiments, cross sections extracted from ATDs are compared to calculated cross sections of theoretical models. The AMBER suite of MM/MD programs is used to provide theoretical structures.

TABLE 1

Peptide	Sequence	Mass (monoisotopic)
WALP23	Ac-GWWLALALALALALALALWWA-Etn	2561.49
WALP21Pro	Ac-GWWLALALALPLALALALWWA-Etn	2403.39
WALP23Pro	Ac-GWWLALALALAPALALALALWWA-Etn	2545.46
WALP23Pro18	Ac-GWWLALALALALALALAPALWWA-Etn	2545.46
RALP23	Ac-GRRLALALALALALALALRRA-Am	2397.55

EXPERIMENTAL TECHNIQUE

FIGURE 4

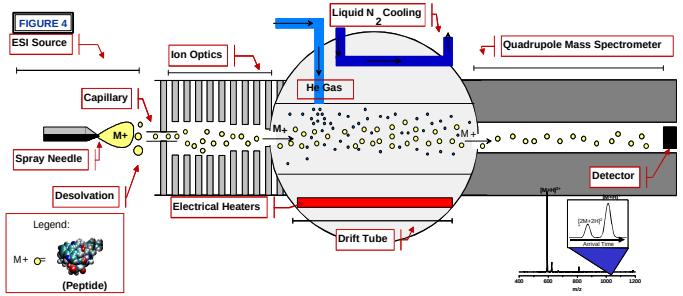


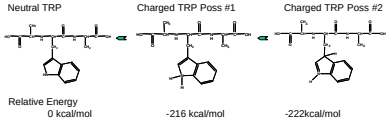
Figure 4: Schematic of the Electrospray Ionization Mass Spectrometer (ESI-MS).
Mass spectrometry has become an important tool to examine the conformations of proteins in the gas phase. This is not only due to advances in instrumentation, but also to the growing number of examples of non-covalent interactions that have found to be maintained in the gas phase. In our ESI technique, charged gas phase molecules are pulse-injected into a temperature and pressure regulated drift cell. The pulse of ions drifts through a helium buffer gas under the influence of a weak electric field. Upon exiting the cell, the ions pass through a quadrupole mass spectrometer to a detector where an arrival time distribution (ATD) of the mass-selected ion is collected.

RESULTS

ESI RESULTS

		Exp. Cross Section (Å²)	Theory Cross Section (Å²)
WALP23	+2	532	538 (BC)
WALP21Pro	+2	485	483 (BC)
	+3	517	
WALP23Pro	+2	511	516 (AC)
	+3	542	552 (ACD)
WALP23Pro18	+2	522	522(BD)
RALP23	+3	530	
	+4	576	576

Protonated TRP



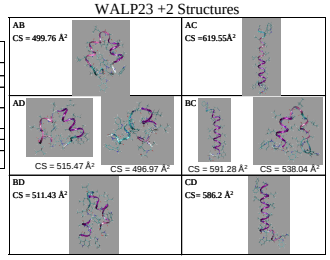
• Gaussian Optimization Energies B3LYP Theory

4 Charge Locations: ACE-GLY-TRP-TRP-(LEU ALA)$_6$-LEU-TRP-TRP-ALA-Etn
(A) (B) (C) (D)

MOLECULAR MODELING

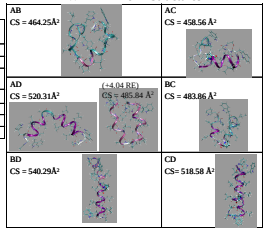
WALP23 +2 Structures

WALP23 +2	Lowest Energy (kcal/mol)	Theory Cross Section (Å²)
AB	-179.2	493.78
AC	-173.55	619.55
AD	-177.93	619.55 475.63 (+ 0.63 RE)
BC	-175.67	591.28 **538.04 (+ 1.33 RE)**
BD	-183.53	511.43
CD	-200.89	586.12
		Exp. = 532 Å²



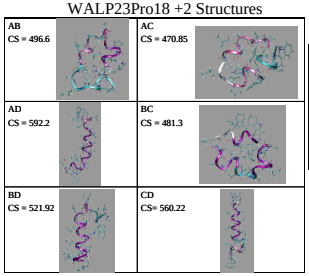
WALP21Pro +2	Lowest Energy (kcal/mol)	Theory Cross Section (Å²)
AB	-137.79	464.25
AC	-131.81	458.56
AD	-127.56	530.31 485.84 (+ 4.04 RE)
BC	-142.28	**483.86**
BD	-134.7	540.29
CD	-147.34	583.93
		Exp. = 485Å²

WALP21Pro+2 Structures



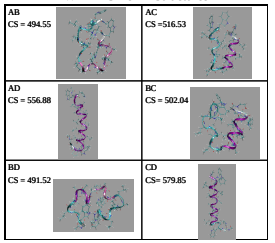
WALP23Pro18 +2 Structures

WALP23Pro18 +2	Lowest Energy (kcal/mol)	Theory Cross Section (Å²)
AB	-135.35	496.6
AC	-146.1	470.85
AD	-132.35	592.2
BC	-141.45	481.3
BD	-141.69	**521.9**
CD	-140.16	560.22
		Exp. = 522 Å²)



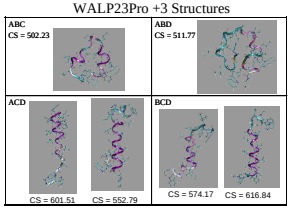
WALP23Pro18 +2	Lowest Energy (kcal/mol)	Theory Cross Section (Å²)
AB	-130.56	494.55
AC	-136.67	**516.53**
AD	-144.92	556.88
BC	-136.47	502.04
BD	-140.89	491.52
CD	-151.33	579.85
		Exp. = 511 Å²

WALP23Pro +2 Structures

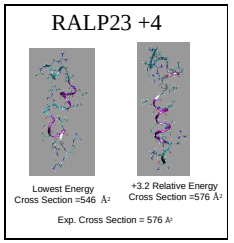


WALP23Pro +3 Structures

WALP23Pro +3	Lowest Energy (kcal/mol)	Theory Cross Section (Å²)
ABC	-44.835	502.23
ABD	-37.493	511.77
ACD	-61.008	601.51 **552.79 (+ 9.9 RE)**
BCD	-57.554	574.17 616.84 (+ 4.046 RE)
		Exp. = 542 Å²



RALP23 +4



FUTURE WORK

The way in which a membrane protein is imbedded in the lipid bilayer, and its interactions with the membrane are significant for membrane protein structure and function. Future work will focus on the peptides incorporated into phospholipid vessicles. These results will be compared with the bare peptide results in order to analyze the structural effect of peptide-membrane interactions. We will also attempt tp determine the position of the peptide within the membrane.

ACKNOWLEDMENTS

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