PROTEIN-MEMBRANE ORGANIZATION AND FUNCTION STUDIED BY METAL-ION NMR: THE GRAMICIDIN CHANNEL

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INTRODUCTION

The transport of matter through membranes is a basic phenomenon of life. All organisms are separated from their environment by special membrane barriers which control the exchange of matter with their surroundings. In addition, nearly all life processes are intimately involved with membrane function. Thus, membrane function plays a central role in the transformation of metabolic energy into osmotic, electrical and to some extent mechanical work, in the acquisition and processing of information, and also, to a lesser extent, in reproduction. In living cells an electrical potential difference exists between the cytoplasma and the extracellular medium due to the unequal distribution of ions on both sides of the plasma membrane surrounding the cell. The electrical resistance of cell membranes is several orders of magnitude smaller than the resistance of a lipid bilayer. This fact suggests that cell membranes are equipped with special transport mechanisms which accelerate the passage of ions across the lipid barrier. An understanding of these transport mechanisms is a vital link to the ultimate goal of the interpretation of some types of membrane function.

The transport of monovalent cations through cell membranes plays a vital role in many different physiological processes. The transport of cations through membranes normally involves a molecular channel system. The description of such a system must be based upon a comprehension of the relationship between channel structure and function at the molecular level. An understanding of how the channel structure-function relationship operates at the molecular level in the selective transport of cations requires the determination of the structure of the channel in the membrane environment, the activation energy parameters for channel formation, and the activation energy parameters for cation binding and transport.

The gramicidin family of linear polypeptides represents a biologically viable channel system of related peptides for which specific changes in structure can be correlated with differences in incorporation into membranes and transport. The effect of structural change on the incorporation process and transport can be studied using analogs of the parent molecule, gramicidin-A, in which a single amino-acid is changed. Because the primary structures of gramicidins are known, these polypeptides are ideal for studying the relationship between the structure and function in a cation transporting channel system.

Gramicidin is a 15 amino-acid linear polypeptide which forms monovalent cation conducting channels in lipid membranes (1). The amino-acid sequence of gramicidin-A is: formyl-L-Val^{1}-Gly^{2}-L-Ala^{3}-D-Leu^{4}-L-Ala^{5}-D-Val^{6}-L-Val^{7}-D-Val^{8}-L-Trp^{9}-D-Leu^{10}-L-Trp^{11}-D-Leu^{12}-L-Trp^{13}-D-Leu^{14}-L-Trp^{15}-ethanolamine. The channel consists of two monomeric beta helices joined by hydrogen bonds at their NH_2 terminal ends (2,3,4,5,6). The dimer spans the membrane bilayer. The channel has a lipophilic exterior and a hydrophilic interior. Gramicidin-A was the first ion-selective transmembrane channel of known molecular structure. It is particularly impermeable to anions and multivalent cations and displays appreciable selectivity between monovalent

cations (7). Gramicidin has a number of characteristics similar to those of physiological channels such as monovalent cation selectivity and blocking, saturation effects, concentration dependence of permeability ratios (8,9), multivalent anion screening effects (10) and divalent cation damping of single channel currents (11). Investigations of the ion binding and ion transport properties of gramicidin have been aided by the availability of either naturally occurring or chemically synthesized analogs which differ from the parent molecule by only a single amino acid substitution (12-17). Because these analogs provide subtly and well-defined perturbations of the chemical composition of gramicidin, they represent powerful tools for deciphering the mechanisms which govern ion-peptide interactions.

A metal-ion (e.g., Na-23) NMR technique has been developed that permits one to determine the activation energy parameters (enthalpy and entropy) for the incorporation of gramicidin, as a functional cation transporting channel, into membranes and the activation enery parameters for the transport of the cations through the gramicidin channel (18,19). This technique involves the separation of the NMR signals of metal ions (Na^+-23) on the inside and outside of vesicles with the use of a chemical shift reagent [$Dy(PPP)_2^{-7}$]. From measurements of the change in linewidth of the inside signal as a function of gramicidin channel concentration and temperature the rate constants and activation parameters for the incorporation and channel formation and for the transport process can be obtained.

A number of gramicidin analogs have been synthesized that exhibit significant differences in activation energy parameters for the incorporation process. Differences in the activation energy parameters for the transport of the monovalent cations have been found with a single analog, as well as differences between gramicidin analogs. The results obtained with four gramicidin analogs having phenylalanine at position 9, 11, 13 or 15 are presented and compared to those for gramicidin-A which has tryptophan at all of these positions. The phenylalanine analogs (Phe-9, Phe-13 and Phe-15) were obtained using the Merrifield solid phase peptide synthesis technique (20).

RESULTS AND DISCUSSION

Single channel conductance studies of gramicidin-A and the phenylalanine substituted gramicidin analogs have shown the following order of increasing transport rate for the Na^+ ion: Phe-9 < Phe-11 < Phe-13 < Phe-15 < Gr-A (21). Table 1 contains the activation energy parameters for the transport of the Na^+ ion across vesicles membranes by the same gramicidin analogs. The activation enthalpy for transport is, in fact, linearly related to the single channel conductance for these gramicidin analogs. It has been shown that changes in single channel conductance result from the side chain substitution rather than changes in peptide structure (22,23). In addition, the size of the amino acid chains does not influence the conductance to any appreciable extent (24). Therefore, this leads to ion-side chain interactions as the predominant influence upon conductance or transport by the amino acid side chains. There are several possible types of interactions that could affect the enthalpy of transport. Previous work suggests that inductive effects and hydrogen bonding probably do not play a major role in modifying channel conductance or transport (25). Dipole-dipole interactions are relatively short-range interactions and would not be expected to contribute significantly to the enthalpy of activation of ion transport. It appears that ion-dipole electrostatic interactions are the predominant type of interaction responsible for the changes observed in

Table 1. Enthalpy and entropy of activation for the transport of Na^+ by phenylalanine analogs of gramicidin across a vesicle membrane

Gramicidin Analog	ΔH (Kcal/mol)	ΔS (e.u. at 25°C)
phe^{9}-GA	10.6	7.5
phe^{11}-GA	10.1	6.5
phe^{13}-GA	9.6	5.5
phe^{15}-GA	9.2	5.0
GA	8.5	4.1

transport. Thus, replacing tryptophan with less polar phenylalanine results in a decrease in ion-dipole interaction and, consequently, a lowering of the channel transport ability. The sequence position and orientation of the side chains are also important in determining channel conductance (24,26,27). The effect of sequence position on the activation enthalpy of transport is shown in Table 1. The replacement of tryptophan in gramicidin-A by the less polar phenylalanine at positions 15, 13, 11 and 9 produces a significant increase in activation enthalpy of transport. This may signify that a dipolar side chain is needed to provide a favorable electronic environment for the ion, either in the binding and/or translocation of the ion through the channel to compensate for the hydrophobic nature of the membrane.

Tryptophan-tryptophan and tryptophan-lipid interactions play an important role in gramicidin-lipid organization and gramicidin's ability to self-associate. The tryptophans are known to be essential for gramicidin's ability to modulate lipid structure (28), to incorporate into micelles (29) and to induce the formation of lipid bilayers (30). Table 2 contains the activation energy parameters for the incorporation and channel formation of gramicidin analogs into vesicle membranes. It is obvious from this data that single amino acid substitution can have a significant effect on the rate of incorporation and channel formation of gramicidin. The four analogs with a tryptophan being replaced by a phenylalanine all show a slower rate of incorporation and channel formation than gramicidin-A which has all four tryptophans that positions 9, 11, 13, and 15. Clearly, the polarity and position of the side chains have a significant effect upon the incorporation of gramicidin into a membrane environment and the subsequent formation of an active transport channel.

Table 2. Enthalpy and entropy of activation for the incorporation and channel formation of the gramicidin analogs in PC/PG vesicle membranes.

Gramicidin Analog	ΔH (Kcal/mol)	ΔS (e.u. at 25°C)
GA	11.8	-11
phe^{9}-GA	13.1	-8
phe^{11}-GA	14.6	-4
phe^{13}-GA	15.5	-1
phe^{15}-GA	17.0	4

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