TI-205 NUCLEAR MAGNETIC RESONANCE DETERMINATION OF THE THERMODYNAMIC PARAMETERS FOR THE BINDING OF MONOVALENT CATIONS TO GRAMICIDINS A AND C

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ABSTRACT Thermodynamic parameters for the binding of the monovalent cations, Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+, Tl^+, and Ag^+, to gramicidin A and for the binding of Tl^+ to gramicidin C, incorporated into lysophosphatidylcholine, have been determined using a combination of Tl-205 nuclear magnetic resonance spectroscopy and competition binding. The thermodynamic parameters, enthalpy and entropy, are discussed in terms of a process involving the transfer of cations from an aqueous to amide environment.

INTRODUCTION

The gramicidin family of linear polypeptides represents a biologically viable channel system of related peptides in which specific changes in structure can be correlated with cation binding selectivity and transport. The interaction between the transported ions and the gramicidin channel can be studied using analogs in which individual amino acids of the parent molecule, gramicidin A, are substituted. Gramicidin A, from *Bacillus brevis*, has the amino acid sequence

formyl—L(1)—Val(2)—Gly(3)—L(4)—Ala(5)—D(6)—Leu(7)—L(8)—Ala(9)—D(10)—Val(11)—L(12)—Val(13)—D(14)—Val(15)—L—Trp(16)—D(17)—Leu(18)—L(19)—Trp(20)—D(21)—Leu(22)—L(23)—Trp(24)—D(25)—Leu(26)—L(27)—Trp—ethanolamine(28)

It has been shown that the polarity and the position of a particular amino acid side chain in the gramicidin sequence can have a pronounced effect on the channel transport and selectivity (Morrow et al., 1979; Mazet et al., 1984; Russell et al., 1986). With the use of analogs, cation binding and transport by the gramicidin channel can be studied at the molecular level in more detail than is currently possible for other transmembrane channel systems. Two analogs, available from *B. brevis*, that have single-amino acid differences at position 11 are gramicidins A and C. Gramicidins A and C have Trp and Tyr, respectively, at position 11 (Sarges and Witkop, 1965). Position 11 is a critically located site for cation binding at the channel entrance (Urry, 1982). The $\beta^{6.3}$ helical dimer of Urry (1971), as presented by Etchebest and Pullman (1985), is shown in Fig. 1 for gramicidins A and C. In this figure, an ion diffuses through water to the channel entrance in step 1, binds to the channel entrance in step 2 and then is transported through the channel in step 3, with steps 4 and 5 being the reverse of steps 2 and 1. Although the side chains of gramicidin do not come into direct contact with the transported ions, an aromatic side chain at position 11 extends over an area of ~5–10 Å away from a cation bound to the carbonyl residue and can dramatically influence the transport properties of the channel. Therefore, analogs with single-amino acid substitution at position 11 provide models for investigating the relationship of cation binding and transport to channel structure.

In order to directly compare the cation binding or transport properties of analogues, it is important that the analogs form the same type of channel in a lipid environment. Circular dichroism spectroscopy has proven to be an appropriate technique for interrogating the channel forms of gramicidin analogs in various lipid systems (Wallace et al., 1981; Wallace, 1986; Spisni et al., 1979; Prasad et al., 1983; Spisni et al., 1983). As shown in Fig. 2, the circular dichroism spectra of gramicidins A and C incorporated into lyso-PC dispersions are very similar. Consequently, it appears to be appropriate to assume that the channel conformation of the two analogues is similar enough to make legitimate comparisons of their cation binding and transport properties.

This paper reports the results of a Tl-205 nuclear magnetic resonance (NMR) spectroscopic determination of the thermodynamic parameters for the binding of the

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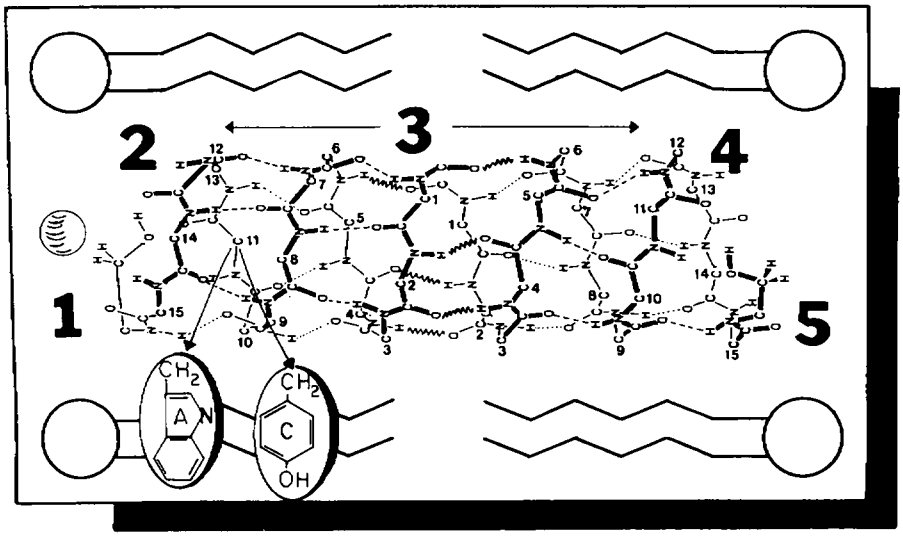

FIGURE 1 A schematic drawing of a gramicidin dimer within a lipid environment showing an approaching cation and the positions of the five steps involved in the transport process. The difference in gramicidins A and C at position 11 in a monomer is also indicated. The gramicidin dimer is that of Etchebest and Pullman (1985).

Tl^+ cation by gramicidins A and C incorporated into lyso-PC dispersions. The enthalpy and entropy for the binding process will be discussed with respect to the effect of single-site substitution on cation binding and transport. Through the combination of Tl-205 NMR spectroscopy and competition binding effects, the thermodynamic parameters for the binding of other monovalent cations (i.e., Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+, and Ag^+) have been determined for gramicidin A. The enthalpy and entropy of binding these cations will be discussed with respect to their similarity to the enthalpy and entropy of transfer of cations from one solvent to another. The relationship between the thermodynamic parameters for binding and transport through the channel will be considered.

MATERIALS AND METHODS

Mixtures of gramicidins A, B, and C were purchased as gramicidin D from Sigma Chemical Company, St. Louis, MO and from U.S. Biochemical Corporation Cleveland, OH. The individual A, B, and C components were separated on a 0.78 × 240 cm column of Chromosorb LC-5, a phenyl reversed phase 37–44 μm pellicular packing (Johns-Manville, Denver, CO), using a multi-step 70 h water-methanol gradient, as previously described (Koeppe et al., 1985). The gramicidins were further purified on Sephadex LH-20 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ). Gramicidin concentrations were determined by vacuum drying to constant weight and verified by UV spectroscopy. The relative amounts of the A and C components were assayed by analytical HPLC (Koeppe and Weiss, 1981). The samples used for the NMR experiments consisted of (92% A, 8% C) and (81% C, 19% A).

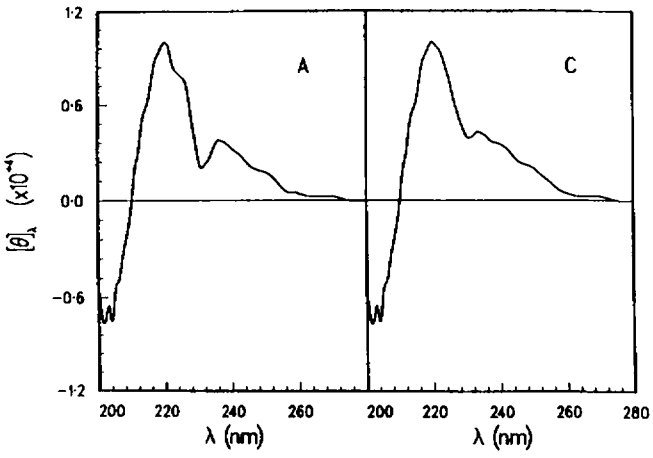

FIGURE 2 CD spectra of gramicidins A and C at 35°C.

Lyso-PC from egg yolk, containing primarily palmitic and stearic acids at position one, was obtained from Sigma Chemical Co. and used without further purification. Gramicidin was incorporated into lyso-PC dispersions using the procedure of Urry (Urry et al., 1979; Spisni et al., 1979). This procedure, as employed in our laboratory, has been described previously in detail (Hinton et al., 1982, 1986*a*).

Thallous nitrate, from Alfa Products, Morton Thiokol Inc., (Danvers, MA), was recrystallized twice from water and dried to a constant weight at 90°C, 40 mm Hg. All other chemicals were reagent grade.

The Tl-205 NMR spectra were obtained as a function of temperature with a JEOL FX90Q spectrometer (JEOL USA, Electronoptics Div., Peabody, MA). The technique used for obtaining the thermodynamic parameters for the binding process involves measuring the $Tl^+ - 205$ chemical shift as a function of the concentration of the thallous ion concentration in the presence of a constant concentration of gramicidin. The equilibrium constant for the binding of the thallous ion to the gramicidin channel is obtained from a theoretical analysis of the relationship between the chemical shift and the cation concentration. The calculated equilibrium constants are corrected for the effective ionic and gramicidin concentration. A correction is also made for the concentration of the minor gramicidin component in each sample so that the equilibrium constant values obtained are for the specific gramicidin analog and not the mixture of A and C. Details of this technique are in the literature (Hinton et al., 1986*a*).

The enthalpy for the equilibrium binding process is obtained from the slope of the line resulting from a plot of R ln K as a function of the reciprocal of the absolute temperature, see Fig. 3. The entropy at a given temperature is then obtained from the thermodynamic relationship: $-RT \ln K = \Delta G = \Delta H - T\Delta S$.

An inspection of Fig. 3 reveals that the data points for each analog at the temperature extremes appear to not correlate very well with the data obtained at the other temperatures. This may indicate a difference in the lipoprotein system at these temperatures. Additional experimental evidence for this is provided by the fact that the effective gramicidin concentration and the chemical shift of the thallium-gramicidin complex, both factors that are obtained in the analysis of the data, (see Hinton et al., 1986*a* for a description of these factors) at these temperatures are

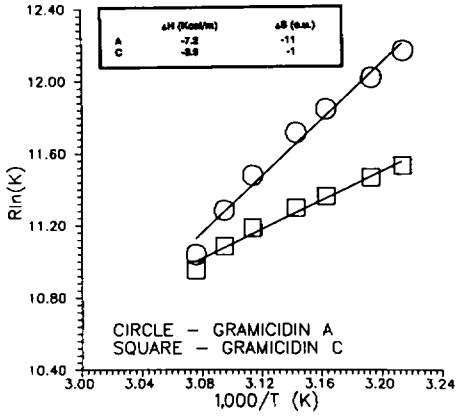

FIGURE 3 Plots of RlnK as a function of $1/T$ for gramicidins A and C with Tl.

different from those at the intermediate temperatures. Consequently, in obtaining the thermodynamic parameters, the data at these two temperatures were not used.

The competition binding, Tl-205 NMR technique used to determine the equilibrium binding constants for other monovalent cations has been described in detail in a previous paper (Hinton et al., 1986*b*). This method involves measuring the Tl-205 NMR chemical shift in the gramicidin-lipid system as a function of the concentration of the competing monovalent cation. The observed Tl^+ chemical shift is related to the equilibrium binding constant of the added monovalent cation through the equation (Steinhart and Reynolds, 1969; Forsen and Lindman, 1982)

$$\delta_{obs} = (n[Gr_2]\delta_{Tl}K_{Tl})/(1 + K_{Tl}[Tl] + K_c[C]). \quad (1)$$

In this equation, δ_{obs} is the Tl-205 NMR chemical shift, n is the number of binding sites on gramicidin, $[Gr_2]$ is the effective gramicidin dimer concentration. The parameters associated with Tl^+ binding are δ_{Tl}, the chemical shift of the bound Tl^+ ion in the gramicidin complex, K_{Tl}, the equilibrium binding constant and [Tl], the activity of the Tl^+ ion. Finally, [C] and K_c are the activity and equilibrium binding constant of the added monovalent cation, respectively.

Circular dichroism spectra were obtained over the 200–300 nm range using a Jasco J-500A spectrometer (Jasco Inc., Easton, MD). The spectra were digitized and drawn by a computer.

RESULTS AND DISCUSSION

From the data presented in Fig. 3, the ΔH for the Tl^+ ion binding process for gramicidins A and C is -7.2 kcal/mol and -3.9 kcal/mol, respectively. The value for ΔS at 34°C for gramicidins A and C is -11 eu and -1.1 eu, respectively. From the signs of the thermodynamic parameters, it is obvious that the binding process is favorably driven by the enthalpy and not the entropy. The difference in enthalpy change between gramicidins A and C shows that there is stronger binding of the Tl^+ ion at the channel entrance with Trp at position 11 than with Tyr at the same position. Eisenman and Horn (1983) suggest that a high channel binding affinity enhances the permeability through the channel. If that is correct, one would predict the permeability of the Tl^+ ion through the gramicidin A channel to be greater than through the gramicidin C channel. Unfortunately, such data for the Tl^+ ion do not appear to be available in the literature. However, it has been shown (Bamberg et al., 1976; Mazet et al., 1984) that the single-channel conductance of the Na^+ ion is about the same for gramicidins A and C. This is in agreement with preliminary results concerning the determination of the equilibrium binding constants for the Na^+ ion with gramicidins A and C using a combination of Tl-205 NMR spectroscopy and competition binding developed in this laboratory (Hinton et al., 1986*b*). The equilibrium binding constants at 34°C are 31.6 M^{-1} and 30.2 M^{-1} for the binding of the Na^+ ion to gramicidins A and C, respectively.

The change in entropy during the binding process involves a number of factors that include: (*a*) the partial dehydration of the cation at the channel entrance, (*b*) coordination of the cation with the carbonyl oxygen atoms at the channel entrance, and (*c*) constraint of the normal motion of the amino acid groups at the channel entrance. Assuming the dehydration and hydration processes to be thermodynamically equivalent for gramicidins A and C, then factors 2 and 3 are most important in determining the difference in entropy for the two systems. The fact that the change in entropy is more positive for gramicidin C than for gramicidin A suggests that the bound Tl^+ ion has more freedom to move and/or there is less constraint of motion of the amino acid groups at the channel entrance with gramicidin C ion than with gramicidin A. Since the enthalpy change is more negative for gramicidin A than for gramicidin C, indicating stronger cation binding, one would predict a larger negative entropy change with gramicidin A than with gramicidin C (i.e., the ion is more tightly bound by A and, consequently, the motional constraint on the binding groups at the channel entrance is larger).

Perhaps the most important use of the thermodynamic parameters obtained for the binding of the Tl^+ ion to the gramicidin channel is in the determination of the same parameters for the binding of any other cation using a combination of Tl-205 NMR spectroscopy and competition binding (Hinton et al., 1986*b*). Using this procedure, described in the previous section, equilibrium binding constants were determined for the monovalent cations, Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+, and Ag^+ at four temperatures, 34, 40, 45, and 50°C. Table I contains the thermodynamic parameters for the binding of these cations to gramicidin A. The binding enthalpies were obtained from the slopes of the lines obtained from plots of R ln K versus $1/T$, as shown in Fig. 4 for several of the cations. Again, the change in entropy associated with the binding process was calculated at 34°C using the thermodynamic relationship: $\Delta S = (\Delta H/T) + R \ln K$.

Although a comparison of equilibrium binding constants, at a given temperature, for a group of cations is informative, the results may be misleading. This can be illustrated by considering two cations with different binding properties (e.g., different binding enthalpies). Plots of $-R \ln K$ versus $1/T$ for the two cations would produce

TABLE I
THERMODYNAMIC PARAMETERS FOR CATION BINDING

Cation	T	K	ΔH	ΔS
	°C	M^{-1}	Kcal/M	cal/degM
Li	34	29.6	−3.070	−3.27
	40	26.8		
	45	25.2		
	50	22.8		
Na	34	31.6	−3.537	−4.66
	40	29.4		
	45	27.2		
	50	23.4		
K	34	58.4	−4.876	−7.80
	40	52.2		
	45	43.4		
	50	39.9		
Rb	34	49.0	−4.00	−5.30
	40	42.5		
	45	37.9		
	50	35.8		
Cs	34	48.3	−4.100	−5.65
	40	42.8		
	45	38.2		
	50	33.8		
NH_4	34	82.4	−6.100	−11.1
	40	73.0		
	45	61.2		
	50	50.4		
Ag	34	1163	−7.700	−11.1
	40	947		
	45	810		
	50	609		
Tl	34	506	−7.200	−11.1
	38	457		
	40	424		
	43	389		
	45	364		
	48	323		
	52	259		
$(CH_3)_4N$	34	25.0		

two lines of different slope. If, fortuitously, one only determined equilibrium binding constants at the temperature at which lines intersect, the equilibrium binding constants would be the same. This point can be illustrated with the equilibrium binding constant (K) data obtained for the monovalent cations. As shown in Fig. 4, a comparison of the data for the K^+ and NH_4^+ cations indicates that at the highest temperature the equilibrium binding constants are quite similar, however, they are significantly different at the lowest temperature. The high temperature result would certainly not represent the true binding difference for the two cations. Consequently, the use of the thermodynamic parameters, enthalpy and entropy, provides a better opportunity for obtaining a true comparison of the binding difference between the monovalent cations.

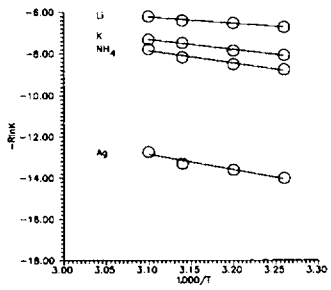

FIGURE 4 Plots of $-R \ln K$ as a function of $1/T$ for gramicidin A with the Li, K, NH_4, and Ag cations.

To understand the differences in the thermodynamic parameters associated with the binding of the monovalent cations, it is logical to try to relate them to some physical property of the cations, since only the cation changes in these systems. The enthalpy of binding reflects the strength of the interaction between the cation and the binding ligand. If one assumes that this interaction is predominately electrostatic in nature (i.e., ion-dipole) and that the cations are very electropositive, as is the case with the Group I cations, then the interaction energy is related to the reciprocal of the radius of the cation. Fig. 5 illustrates this relationship in which the enthalpy of solvation for the Group I cations in water and dimethylformamide (DMF) is plotted as a function of the reciprocal of the cation radius. DMF was chosen because the carbonyl group is involved in cation binding in much the same manner as the carbonyl groups at the channel entrance of gramicidin are involved in cation binding. As predicted, the solvation enthalpy decreases with increasing cation radius in both solvents. However, this simple relationship does not seem to exist for the equilibrium binding enthalpies determined for the gramicidin ligand. In fact, quite the opposite is found. The data contained in Table I show that, in general, the binding enthalpy increases as the size of the cation increases.

The reason the enthalpies for the binding of the Group I cations to gramicidin do not correlate with the cation radius, as one might predict, lies in the fact that the binding enthalpy is associated with a change in solvation environment. The cation is transferred from an aqueous to an amide solvation environment during the binding process. The binding enthalpy measured is, therefore, quite similar to the enthalpy of transfer of a cation from one

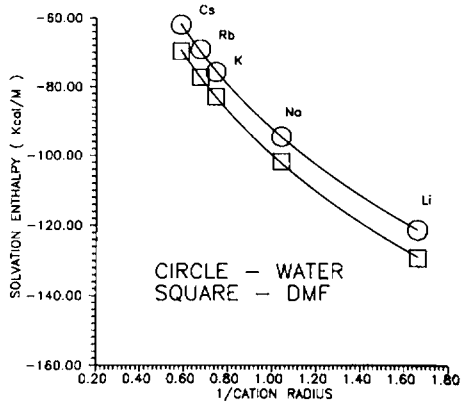

FIGURE 5 Plots of the solvation enthalpy as a function of the reciprocal of cation radius for water and DMF.

solvent to another. Fig. 6 contains a plot of the single-ion enthalpies of transfer of the Group I cations from water to DMF at 25°C (Cox, et al., 1974) as a function of the reciprocal of the cation radius. In general, the relationship is one of increasing enthalpy of transfer with increasing cation radius. There are several very important characteristics of this relationship: (*a*) The enthalpy of transfer for the Li^+ and Na^+ cations is almost the same but quite different from those of the other Group I cations; (*b*) The K^+ cation has the largest enthalpy of transfer among the Group I cations and; (*c*) The Rb^+ and Cs^+ cations have enthalpies of transfer that are almost the same but smaller than that of K^+ and greater than those of Li^+ and Na^+. Several factors influence the enthalpy of transfer but it is often very difficult to isolate one mechanism as being responsible for a particular value. The factors which influence the enthalpy of transfer are: (*a*) born-type ion-dipole electrostatic interactions; (*b*) specific interactions between the ion and solvent, such as hydrogen-bonding and Lewis acid-base interactions; (*c*) the breaking of solvent-solvent hydrogen bonds; (*d*) the strengthening of hydrogen bonds in the surface about a hydrophobic ion; (*e*) the formation of hydrogen bonds between the water molecules in the first and second hydration sphere of cations and; (*f*) mutual polarizability or dispersion forces between ion and solvent. The enthalpy of transfer for the Group I cations is certainly not a smooth function of a cation property, such as the radius. It appears obvious that competition among a number of contributing factors is responsible for determining the value for a specific cation.

Because the binding enthalpies determined for interaction of the Group I cations with gramicidin can be perceived as being similar to an enthalpy of transfer from an aqueous to amide environment, a comparison of this data with that for the water-DMF system is of interest. Fig. 6 shows a comparison of the two sets of enthalpies as a function of the reciprocal of the cation radius. The similarity between the two data sets is striking. It provides confidence that the binding enthalpies determined for the gramicidin system are reasonable in value and consistent with the complex behavior of the solvation and/or complexation of the Group I cations.

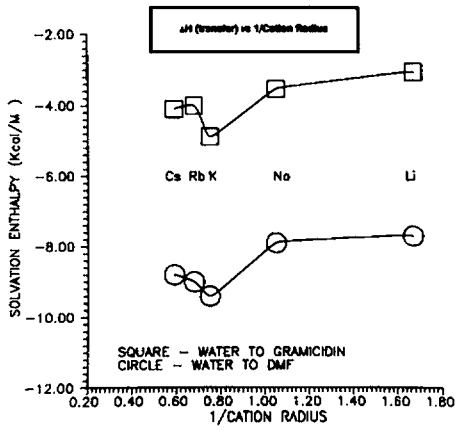

FIGURE 6 Enthalpy of transfer of the Group I cations from water to DMF and enthalpy of binding of the Group I cations to gramicidin. The enthalpy of binding is assumed to be similar to an enthalpy of transfer of the cations from water to an amide environment.

Consequently, the experimental observations that: (*a*) the K^+ cation has the largest binding enthalpy; (*b*) the Rb^+ and Cs^+ cations have binding enthalpies that are the same, within experimental error, while being smaller than that for K^+ but greater than those for Li^+ and Na^+ and; (*c*) the binding enthalpies of Li^+ and Na^+ are very similar in magnitude but significantly smaller than those of the other cations appear to accurately represent the intricate balance of factors that determine the strength of interaction between gramicidin and these monovalent cations. Assuming the binding enthalpy to be similar in character to an enthalpy of transfer provides an explanation for why the values determined for the gramicidin system are relatively small. Since the solvation enthalpies of the Group I cations with water and amides, such as DMF, are very similar in magnitude and since the enthalpies of transfer from water to DMF are quite small, one would, therefore, anticipate the binding enthalpies to be rather small. Furthermore, the cations bound at the gramicidin entrance still have some water molecules in the solvation sphere. Therefore, it is suggested that the binding enthalpy should be smaller than the transfer enthalpy, which represents the change in enthalpy in the transference of a cation from one pure solvent to another. Studies of single-ion enthalpies of transfer of cations from water to mixed aqueous organic solutions provide support for this suggestion (Fuchs and Hagan, 1973).

The NH_4^+, Tl^+ and Ag^+ cations were found to have significantly larger binding enthalpies than the Group I cations. The larger binding enthalpies are a manifestation of the greater electron polarizability of these cations. This tighter binding to the channel entrance is the reason that the Tl^+, Ag^+, and, to some extent, NH_4^+ cations have the ability to block the transport of the Group I cations through the gramicidin channel. The experimentally determined binding enthalpies for the monovalent cations provide supporting evidence for the theoretical predictions of Eisenman indicating that the permeability of the gramicidin channel increases as the magnitude of the channel binding affinity for a cation increases (Eisenman and Horn, 1983). The higher permeability of the NH_4^+, Tl^+, and Ag^+ cations, relative to the Group I cations, would then be associated with their larger binding enthalpies.

It is interesting to note that the $(CH_3)_4N^+$ cation has the smallest equilibrium binding constant of all of the monovalent cations investigated. According to the permeability argument of Eisenman and Horn (1983), the $(CH_3)_4N^+$ cation should be relatively impermeable. The size of the cation (i.e., the cation radius is 3.47 Å) would also tend to prohibit it being transported even if the binding constant were larger. It has been shown experimentally that the

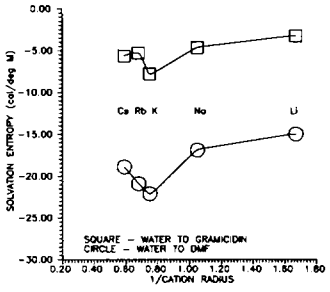

FIGURE 7 Entropy of transfer of the Group I cations from water to DMF and the entropy of binding of the Group I cations to gramicidin. The entropy of binding is assumed to be similar to an entropy of transfer of the cations from water to an amide environment.

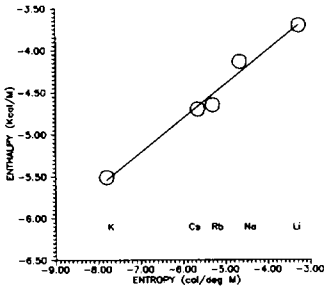

FIGURE 8 Plot of the enthalpy of binding versus the entropy of binding for the Group I cations.

permeability of this cation is very low (Meyers and Haydon, 1972).

The change in entropy associated with the binding of monovalent cations to gramicidin can be related to the absolute single-ion entropy of transfer of cations from water to DMF (Abraham, 1972) in a manner similar to that used for the binding enthalpy change. Fig. 7 shows the relationship between the transfer entropy, the entropy change for binding and the reciprocal of the cation radius. The analogous nature of both relationships suggests that the processes related to cation transfer and binding are similar. The relatively small values of the change in entropy with cation binding are consistent with the values for the entropy change related to the transfer of the cations from water to DMF.

The experimental thermodynamic data obtained for the binding of monovalent cations to the gramicidin A channel indicate that the driving force for binding is enthalpic and not entropic. The relationship between the change in enthalpy and entropy for the Group I cations, as shown in Fig. 8, implies that the stronger the binding, the greater the entropic force opposing it. It is clear that the binding of monovalent cations to the gramicidin channel is complex, involving a number of competing factors and cannot be related in a simple manner to a single property of the cation.

Insufficient quantities of gramicidin C have prevented similar cation binding studies to be made with this analogue of gramicidin.

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REFERENCES

Abraham, M. H. 1972. Entropies of Transfer of Ions from Water to Methanol, Dimethylformamide, and Acetonitrile. *J. Chem. Soc. Chem. Commun.* 888.

Bamberg, E., K. Noda, E. Gross, and P. Lauger. 1976. Single-channel parameters of gramicidin A, B, and C. *Biochim. Biophys. Acta.* 419:223–228.

Cox, B. G., G. R. Hedwig, A. J. Parker, and D. W. Watts. 1974. Solvation of ions. Thermodynamic properties for the transfer of single ions between protic and dipolar aprotic solvents. *Aust. J. Chem.* 27:477–501.

Eisenman, G., and R. Horn. 1983. Ion selectivity revisited: the role of kinetic and equilibrium processes in ionic permeation through channels. *J. Membr. Biol.* 76:197–225.

Etchebest, C., and A. Pullman. 1985. The effect of the amino-acid side chains on the energy profiles for ion transport in the gramicidin A channel. *J. Biomol. Struct. & Dyn.* 2:859–870.

Forsen, S., and B. Lindman. 1982. Ion binding in biological systems measured by nuclear magnetic resonance. *Methods Biochem. Anal.* 27:289–486.

Fuchs, R., and C. P. Hagan. 1973. Single Ion Enthalpies of Transfer from Water to Aqueous Dimethyl Sulfoxide Solutions. *J. Phys. Chem.* 77:1797–1799.

Hinton, J. F., G. Young, and F. S. Millett. 1982. Thallous ion interaction with gramicidin incorporated in micelles studied by thallium-205 NMR. *Biochemistry.* 21:651–654.

Hinton, J. F., R. E. Koeppe II, D. Shungu, W. L. Whaley, J. A. Paczkowski, and F. S. Millett. 1986*a*. Equilibrium binding constants for Tl^+ with gramicidins A, B, and C in a lysophosphatidylcholine environment determined by Tl-205 nuclear magnetic resonance spectroscopy. *Biophy. J.* 49:571–577.

Hinton, J. F., W. L. Whaley, D. Shungu, R. E. Koeppe II, and F. S. Millett. 1986*b*. Equilibrium binding constants of the Group I metal cations with gramicidin A determined by competition studies and Tl-205 nuclear magnetic resonance spectroscopy. *Biophys. J.* 50:539–544.

Koeppe, R. E. II, and L. B. Weiss. 1981. Resolution of linear gramicidins by preparative reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 208:414–418.

Koeppe, R. E. II, J. A. Packowski, and W. L. Whaley. 1985. Gramicidin K, a new linear channel forming gramicidin from *Bacillus brevis*. *Biochemistry.* 24:2822–2826.

Mazet, J.-L., O. S. Andersen, and R. E. Koeppe II. 1984. Single-channel studies on linear gramicidins with altered amino acid side sequence: a comparison of phenylalanine, tryptophan, and tyrosine substitution at positions 1 and 11. *Biophys. J.* 45:263–276.

Meyers, V. B., and D. A. Haydon. 1972. Ion Transfer Across Lipid Membranes in the Presence of Gramicidin A. *Biochim. Biophys. Acta.* 274:313–317.

Morrow, J. S., W. R. Veatch, and L. Stryer. 1979. Transmembrane channel activity of gramicidin A analogs: effects of modification and deletion of the amino-terminal residue. *J. Mol. Biol.* 132:733–738.

Prasad, K. U., T. L. Trapane, G. Szabo, and D. W. Urry. 1983. Synthesis and characterization of (1-13C)Phe gramicidin A. *Int. J. Pept. Protein Res.* 22:341–347.

Russell, E. B., L. B. Weiss, F. I. Navetta, R. E. Koeppe II, and O. S. Andersen. 1986. Single-channel studies on linear gramicidins with altered amino acid side chains. *Biophys. J.* 49:673–686.

Sarges, R., and B. Witkop. 1965. Gramicidin VIII. The structure of valine- and isoleucine-gramicidin C. *Biochemistry.* 4:2491–2494.

Spisni, A., M. A. Khaled, and D. W. Urry. 1979. Temperature induced incorporation of gramicidin A into lysolecithin micelles demonstrated by C-13 NMR. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 102:321–324.

Spisni, A., I. Pasquali-Ronchetti, E. Casali, L. Linder, P. Cavatorta, L. Masotti, and D. W. Urry. 1983. Supramolecular organization of lyso-phosphatidylcholine-packaged gramicidin A. *Biochim. Biophys. Acta.* 732:58–68.

Steinhardt, J., and J. A. Reynolds. 1969. Multiple Equilibria in Proteins. Academic Press, Inc., New York. 391.

Urry, D. W. 1971. The gramicidin A transmembrane channel: a proposed n(L,D) helix. *Proc. Natl. Acad. Sci. USA.* 68:672–676.

Urry, D. W., A. Spisni, and M. A. Khaled. 1979. Characterization of micellar-packaged gramicidin A channels. *Biochem. Biophys. Res. Commun.* 88:940–949.

Urry, D. W., K. U. Prasad, and T. L. Trapane. 1982. Location of monovalent cation binding sites in the gramicidin channel. *Proc. Natl. Acad. Sci. USA.* 79:390–394.

Wallace, B. A., W. R. Veatch, and E. Blout. 1981. Conformation of gramicidin A in phospholipid vesicles: circular dichroism studies of the effects of binding, chemical modification, and lipid structure. *Biochemistry.* 20:5754–5760.

Wallace, B. A. 1986. Structure of gramicidin. *Biophys. J.* 49:295–303.