Thermodynamic parameters for the binding of divalent cations to gramicidin A incorporated into a lipid environment by Tl-205 nuclear magnetic resonance

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ABSTRACT Thermodynamic parameters, enthalpy and entropy, for the binding of the divalent cations, Mg^{+2}, Ca^{+2}, Sr^{+2}, Ba^{+2}, and Cd^{+2}, to gramicidin A, incorporated into lysophosphatidylcholine, have been determined using a combination of Tl-205 nuclear magnetic resonance spectroscopy and competition binding. The binding process is thermodynamically driven by the enthalpy and not the entropy. The enthalpy values are related to the process involving the transfer of cations from an aqueous environment to an amide environment. A comparison is made between the thermodynamic parameters for the binding of monovalent and divalent cations to gramicidin A to illustrate the channel blocking ability of the divalent cations with respect to monovalent cation transport.

INTRODUCTION

The transport of ions across lipid bilayer membranes requires the presence of either "carrier or channel-forming" molecules, which facilitate the passage of ions through the hydrophobic interior of the membrane. Gramicidin A is a linear polypeptide, channel-former which has the ability to induce monovalent cation permeability in both natural and artificial lipid membranes. The gramicidin A channel exhibits monovalent cation transport and selectivity but it is practically impermeable to divalent cations and anions (Meyers and Haydon, 1972; Mueller and Rudin, 1967; Hladky and Haydon, 1972; Bamberg et al., 1976; Urry et al., 1982*a*). The divalent cations block the channel to monovalent cation transport by binding to the channel entrance (Bamberg and Lauger, 1977; Urry et al., 1982*b*). The conductance of the gramicidin channel in the presence of alkali cations is strongly reduced when divalent cations, such as Ca^{+2} and Ba^{+2}, are added to the aqueous solution in concentrations between 0.1 and 1.0 M. The divalent cations differ considerably in their blocking action on the gramicidin channel; the effect of Mg^{+2} being smaller than that of Ca^{+2} and Ba^{+2}. Besides reducing the single channel conductance, the blocking ions also change the current–voltage characteristics of the channel from a nearly linear to a strongly saturating behavior. These observations suggest that Ca^{+2} and Ba^{+2}, which are not permeable themselves, bind to the channel, thereby reducing the rate by which permeable ions enter and leave the channel (Bamberg and Lauger, 1977).

Recently, we showed that a combination of Tl-205 nuclear magnetic resonance (NMR) spectroscopy and competition binding could be used to determine the thermodynamic parameters for the binding of cations to the gramicidin channel (Hinton et al., 1986*a*, 1988). This technique has now been applied to the determination of the thermodynamic parameters for the binding of the divalent cations (Mg^{+2}, Ca^{+2}, Sr^{+2}, Ba^{+2}, and Cd^{+2}) to the gramicidin A channel. These thermodynamic parameters for divalent cations are compared to those obtained for monovalent cations. This comparison illustrates, in a quantitative manner, the differential blocking capacity of divalent cations for monovalent cation transport by gramicidin A. The enthalpies for binding the divalent cations to gramicidin A are also discussed with respect to their similarity to the enthalpies of transfer of the cations from one type of solvation environment to another.

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MATERIALS AND METHODS

Lysophosphatidylcholine (lyso-PC) from egg yolk, was obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Gramicidin was incorporated into lyso-PC dispersions using the method of Urry (Urry et al., 1979, Spisni et al., 1983). This procedure, as employed in our laboratory, has been described in detail (Hinton et al., 1982, 1986*b*).

Thallous nitrate, from Alfa Products (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.

Mixtures of gramicidins A, B, and C were purchased as gramicidin D from Sigma Chemical Co. The individual A, B, and C components were separated and analytically assayed using established techniques (Koeppe et al., 1985; Koeppe and Weiss, 1981).

The competition binding, Tl-205 NMR technique used to determine the equilibrium binding constants for cations other than Tl^{+} has been described in detail (Hinton et al., 1986*a*) and the application to monovalent cations has now been successfully achieved (Hinton et al., 1988). This method involves measuring the Tl-205 NMR chemical shift in the gramicidin–lipid system as a function of the concentration of the

competitive cation, monovalent, or divalent. The observed Tl^+ chemical shift is related to the equilibrium binding constant of the competitive cation through the equation (Steinhart and Reynolds, 1969; Forsen and Lindman, 1982)

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

In this equation, δ(obs) is the Tl-205 NMR chemical shift, n is the number of binding sites on gramicidin, and $[Gr_2]$ is the effective gramicidin concentration. The parameters associated with Tl^+ binding are δ_{Tl}, the chemical shift of the bound Tl^+ 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 competitive cation, respectively. The equilibrium binding constant of the competing cation is determined as a function of temperature to determine the binding enthalpy and entropy. The temperature dependence of the equilibrium binding constant of Tl^+ has been determined (Hinton et al., 1988). The enthalpy for the binding process is obtained from the slope of the line resulting from a plot of $R\ln K$ as a function of temperature. The entropy at a given temperature is then obtained from the thermodynamic relationship $-RT\ln K = \Delta H - T\Delta S$.

Fig. 1 shows the $R\ln K$ versus $1/T$ relationship for a typical data set from one experiment. The final thermodynamic parameters were obtained from a number of repeated experiments.

A modified JEOL FX90Q NMR spectrometer was used to obtain the Tl-205 NMR spectra and chemical shifts.

RESULTS AND DISCUSSION

A summary of the thermodynamic parameters obtained for the divalent cations is contained in Table 1, along with those for monovalent cations previously published (Hinton et al., 1988). For the alkaline earth (divalent) cations, the enthalpy of binding to the gramicidin A channel changes smoothly with increasing cation size. The change in enthalpy determined for each divalent cation is related to a change in solvation environment. The cation is transferred from an aqueous to an amide-type solvation environment at the binding site of the gramicidin A channel. The binding enthalpy is, therefore, quite similar to the enthalpy of transfer of the cation from one solvent to another. Fig. 2 shows a plot of the single ion enthalpies of transfer for Ca^{+2}, Sr^{+2}, and Ba^{+2} from water to dimethylformamide (DMF) and from water to *N*-methylformamide (NMF) at 25°C (Finch et al., 1967, 1971) as a function of the reciprocal of the cation radius (similar data for Mg^{+2} were not available). The relationship of enthalpy to the reciprocal of the cation radius follows the Born equation, which represents the interaction of an ion with a binding ligand. The data obtained for the enthalpy of binding the same divalent cations to gramicidin A are also shown in Fig. 2 as a function of the reciprocal of the cation radius.

A comparison of the two sets of enthalpy data shown in Fig. 2 indicates the similarity in behavior of the two processes. The cation order is the same in each case and the binding enthalpies are significantly less than the

Table 1 Thermodynamic parameters for cation binding by gramicidin A

Cation	ΔH	ΔS
	kcal/mol	*e.u. 34°C*
Li	−3.07	−3.27
Na	−3.54	−4.66
K	−4.88	−7.80
Rb	−4.00	−5.30
Cs	−4.10	−5.65
Mg	−4.90	−8.5
Ca	−5.10	−8.8
Sr	−6.10	−11.1
Ba	−6.90	−12.4
Cd	−4.10	−6.2

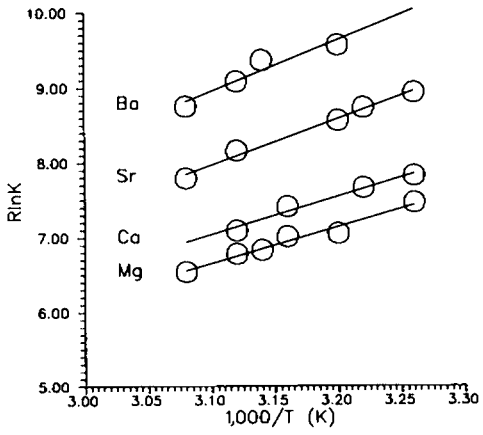

FIGURE 1 Plot of $R\ln K$ as a function of $1{,}000/T$ (K) for the Group II cations.

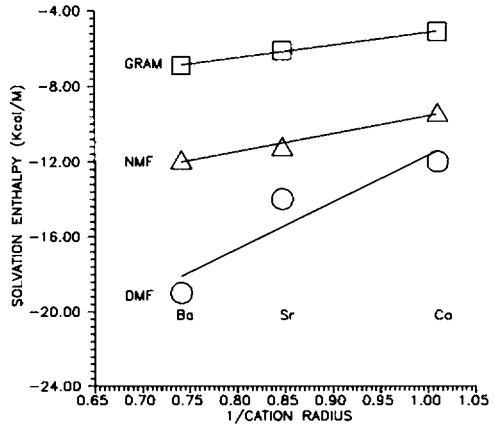

FIGURE 2 Plot of the enthalpy of transfer from water to DMF and to NMF and the binding enthalpy for gramicidin as a function of the reciprocal of the cation radius.

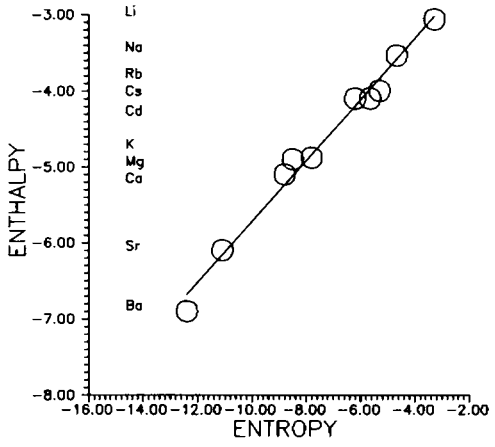

FIGURE 3 Plot of the binding enthalpy as a function of the binding entropy for monovalent and divalent cations.

transfer enthalpies because the cations remain partially hydrated at the gramicidin A binding site. These results provide confidence that the binding enthalpies determined for the gramicidin system are reasonable in magnitude and consistent with the complex behavior of the solvation process of the divalent cations. Furthermore, the binding enthalpies obtained for the divalent cations of Group II are significantly larger than those for the Group I (monovalent) cations. This result is consistent with the conductance experiments (Bamberg and Lauger, 1977), which showed that the divalent cations, Ca^{+2} and Ba^{+2}, block the channel to the transport of a monovalent cation, such as Na^+, of Group I. Theoretical calculations also indicate that the ion Ca^{+2} binds stronger to the gramicidin A channel than does the cation Na^+ (Pullman, 1987; Pullman and Etchebest, 1987; Etchebest et al., 1985). The binding enthalpy for the cation Cd^{+2} was found to be less than for any of the Group II cations and intermediate in magnitude with respect to the Group I cations. Experimentally, it has been found that the enthalpy of transfer of the ion Cd^{+2} from water to DMF is much less than that for the transfer of the ion Ba^{+2} (Finch et al., 1967).

The data in Table 1 show that the binding process is thermodynamically driven by the enthalpy and opposed by the entropy terms. The change in entropy is seen to become more negative as the enthalpy becomes increasingly negative. This strongly indicates that as the binding interaction becomes stronger, the cation mobility, as well as that of the amide-type binding moieties of the amino acids at the gramicidin channel, are diminished. The relationship between the change in enthalpy and entropy for the binding of monovalent and divalent cations is shown in Fig. 3. It is clear that the stronger the binding, the greater the entropic force opposing it.

In summary, the Tl-205 NMR-competition binding technique allows one to determine the thermodynamic parameters for the binding of monovalent and divalent cations to the gramidicin A channel. These results provide a quantitative thermodynamic description of channel selectivity and blocking.

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