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THALLIUM-205 NMR DETERMINATION OF THE THERMODYNAMICS OF THE INTERACTION BETWEEN THE THALLOUS ION AND GRAMICIDIN DIMERS INCORPORATED INTO MICELLES

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A study has been made of the thermodynamics of the interaction between the thallous ion and gramicidin dimers incorporated into micelles using thallium-205 NMR spectroscopy. The chemical shift data obtained are interpreted interms of a model in which the dimer has only one tight binding site. The variation of the binding constant over the temperature range 303–323 K is used to determine the changes in enthalpy and entropy of binding giving values of −11.3 kcal/mole and −16 e.u. at 303 K, respectively.

The properties of the channel-forming polypeptide, gramicidin, have been the subject of extensive investigation. This polypeptide has been used in studies of ion-transport across model membranes because gramicidin has a known molecular structure and a reasonable model of the channel which it forms in model membrane systems is available so that transport properties can be related to known structural features. Also, its cationic selectivity and its intercationic specificities are relevant to many channels in biological membranes.

Gramicidin is known to dimerize in model membrane systems forming ion-permeable channels [1–9]. The structure of the dimer proposed by Urry [10] and Ramachandran and Chandrasekaran [11] as being two single stranded β-helix monomers associated formyl end to formyl end by means of six intermolecular hydrogen bonds has been confirmed [12–14]. The mechanism of ion transport, ion occupancy of the channel, location of channel binding sites, binding constants, rate constants for binding and the rate constant for jumping between sites during single occupancy of the channel have been studied extensively [3,15–28]. X-ray crystallographic studies [14], ^{13}C-NMR [29], and ^{205}Tl-NMR studies [26], and current-voltage analysis [25] show that the cation binding site for the gramicidin channel are located at the channel entrance (i.e., in the first helical turn of the channel, close to the aqueous phase). The location of the cation attached to a gramicidin dimer is pictorally represented in Fig. 1. Two types of energy profiles have been proposed for ion movement through the gramicidin channel. Each energy profile has an energy minimum near the ends of the channel where cation binding occurs; however,

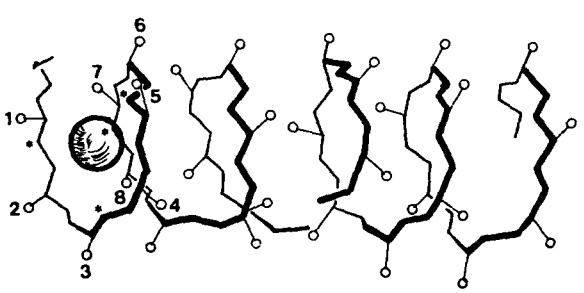

Fig. 1. The helical gramicidin dimer with a cation-bound at the channel entrance (the number refers to the carbonyl functional groups at the entrance and * locates the tryptophan moieties).

one model profile contains a central energy barrier to movement [24] and the other model profile has no significant energy barrier to ion movement [30]. These two models are shown in Fig. 2, where model A contains a channel barrier and model B does not have a significant energy barrier to ion movement through the channel. Using dielectric relaxation measurements for studying the interaction of the thallous ion with gramicidin (packaged in micelles) a central energy barrier to ion movement of $E_a < 6.7$ kcal/mol was obtained [24]. Finkelstein and Andersen [30], however, argue that there is no significant electrostatic barrier to ion movement between the energy wells at the two ends of the channel.

We wish to report the results of a ^{205}Tl-NMR study on the interaction between the thallous ion and gramicidin-A incorporated into micelles which permitted the calculation of the energy minimum (ΔH) for binding at the entrance to the channel. Gramicidin was incorporated into lysophosphatidyl-choline micelles by the method of Urry [28].

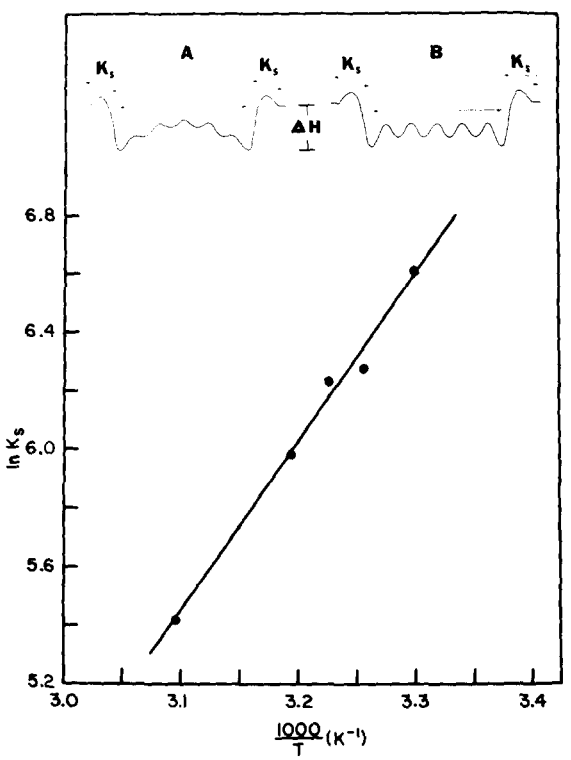

Fig. 2. (Top) Energy profile models of the channel (A, barrier model; B, no barrier model). (Bottom) Van 't Hoff plot.

Sample preparation and experimental procedures are described in detail in an earlier publication [27]. The gramicidin-induced shift in the $^{205}Tl^+$ resonance frequency was measured for samples of fixed gramicidin concentration as a function of thallous ion concentration over the temperature range 303–323 K. Using curve-fitting procedures described earlier [27], the shift vs. thallous ion concentration data were found to fit a model described by a single tight binding site per dimer. The binding constant (K_s), derived from the fitting procedure for each temperature, was used to obtain ΔH and ΔS for the association of the thallous ion with gramicidin.

Using the ^{205}Tl-NMR technique we have measured the depth of the thermodynamic energy well (ΔH) at the entrance of the channel and found it to be -11.3 kcal/mol. Fig. 2 shows a characteristic Van 't Hoff plot of the data from which the value of ΔH was obtained. The negative value for ΔH is consistent with the analysis of Meyers and Haydon [2] that univalent cations are favored in the channel relative to the free solution and with the fact that the thallous ion is weakly hydrated in aqueous solution [31].

It is difficult to rationalize in specific terms the value obtained for the entropy change upon binding ($\Delta S = -16$ e.u. at 303 K). However, the sign of ΔS would be consistent with stronger binding by gramicidin than by water and a possible conformation change in gramicidin produced by the binding of the cation.

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