Table III: Composition of Transcarboxylase and Its Subunits

| component | $M_r$ | AA residues per molecule | fraction of 26S TC[a] |
|---|---|---|---|
| $1.3S_E$ | 12 000 | 123 | 0.130 |
| $5S_E$ | 120 000 | 1094[b] | 0.576 |
| $6S_E$ | 144 000 | 1340 | 0.706 |
| $12S_H$ | 360 000 | 3342[b] | 0.294 |
| 26S TC | 1 224 000 | 11382 | 1.000 |

[a] Transcarboxylase is abbreviated TC. [b] Zwolinski et al. (1977).

during binding, the change in the total $\alpha$ helix of transcarboxylase would not be sufficient to be detected. Likewise, if $\alpha$-helix formation were involved in the $1.3S_E$ subunit when the carboxyl transfer occurs between the substrate sites on the $5S_E$ and $12S_H$ subunits, it might not occur in the $1.3S_E$ in the static situation when transfer is not occurring (Table III).

It is clear from our results that there is a very significant change in the secondary structure of the $2.5S_H$ monomer when they associate and form the hexameric $12S_H$ subunit. There is a 19% increase in $\alpha$ helix, an 8% decrease in antiparallel $\beta$ sheet, and 7% decrease in other or random structure (no. 8 compared to no. 9 of Table II). It is interesting that Zwolinski et al. (1977), in considering models for the arrangement of the six polypeptides of the $12S_H$ subunit, suggested that in forming the isologous binding of the $12S_H$ subunit distortion would be necessary. Perhaps the presently observed changes in secondary structure are a reflection of this distortion. However, they also postulated distortion of the $5S_E$ subunit during assembly of the enzyme, and such a change has not been detected by our CD measurements. Again it is possible, though perhaps unlikely, that there are compensating changes in secondary structure between the respective subunits that balance so that the sum of the secondary structures of the complete enzyme does not differ greatly from the sum in the individual subunits.

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## References

Berger, M., & Wood, H. G. (1975) *J. Biol. Chem. 250*, 927–933.

Chen, G. C., & Yang, J. T. (1977) *Anal. Lett. 10*, 1195–1207.

Chou, P. Y., & Fasman, G. D. (1977) *J. Mol. Biol. 115*, 135–175.

Chuang, M., Ahmad, F., Jacobson, B., & Wood, H. G. (1975) *Biochemistry 14*, 1611–1619.

Fall, R. F., Glaser, M., & Vagelos, P. R. (1976) *J. Biol. Chem. 251*, 2063–2069.

Hennessey, J. P., Jr., & Johnson, W. C., Jr. (1981) *Biochemistry 20*, 1085–1094.

Johnson, W. C., Jr. (1971) *Rev. Sci. Instrum. 42*, 1283–1286.

Kumar, K. G., & Beegen, H. (1981) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 40*, 1676.

Maloy, W. L., Bowien, B. U., Zwolinski, G. K., Kumar, K. G., & Wood, H. G. (1979) *J. Biol. Chem. 254*, 11615–11622.

Poto, E. M., & Wood, H. G. (1977) *Biochemistry 16*, 1949–1955.

Rylatt, D. B., Keech, D. B., & Wallace, J. C. (1977) *Arch. Biochem. Biophys. 183*, 113–122.

Sutton, M. R., Fall, R. R., Nervi, A. M., Alberts, A. W., Vagelos, P. R., & Bradshaw, R. A. (1977) *J. Biol. Chem. 252*, 3934–3940.

Wood, H. G. (1979) *CRC Crit. Rev. Biochem. 7*, 143–160.

Wood, H. G., & Zwolinski, G. K. (1976) *CRC Crit. Rev. Biochem. 4*, 47–122.

Wood, H. G., Ahmad, F., Jacobson, B., Chuang, M., & Brattin, W. (1975) *J. Biol. Chem. 250*, 918–926.

Wood, H. G., Chiao, J. P., & Poto, E. W. (1977) *J. Biol. Chem. 252*, 1490–1497.

Wrigley, N. G., Chiao, J. P., & Wood, H. G. (1977) *J. Biol. Chem. 252*, 1500–1504.

Zwolinski, G. K., Bowien, B. U., Harmon, F., & Wood, H. G. (1977) *Biochemistry 16*, 4627–4637.

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# Thallium-205 Nuclear Magnetic Resonance Study of the Thallium(I)–Gramicidin A Association in Trifluoroethanol[†]

G. L. Turner, J. F. Hinton,* and F. S. Millett

ABSTRACT: Association of the thallous ion with gramicidin in 2,2,2-trifluoroethanol has been investigated by thallium-205 NMR spectroscopy. The data obtained suggest that the gramicidin dimer has two strong binding sites and one or more weak binding sites. Association constants for the strong binding sites were found to have the same value. From the temperature dependence of the strong binding site association constants, values for the association enthalpy and entropy of −2.13 ± 0.12 kcal/mol and +5.45 ± 0.04 eu, respectively, were obtained.

Gramicidin A, a pentadecapeptide isolated from *Bacillus brevis*, is known to form channels which assist the passive transport of cations across membranes (Hladky & Haydon, 1972; Krasne et al., 1971). Significant effort has been directed at the study of ion association of gramicidin A in membrane systems (Myers & Haydon, 1972; Bamberg, & Lauger, 1974; Neher, 1975; Sandblom et al., 1977; McBride & Szabo, 1978; Urry et al., 1979, 1980; Veatch & Darkin, 1980; Venkatachalam & Urry, 1980). Work has also been performed on the solution conformations of gramicidin A (Glickson et al., 1972; Urry et al., 1972, 1975; Fossel et al., 1974; Veatch et al., 1974; Veatch & Blout, 1974; Heitz et al., 1979; Iqbal & Weidekamm, 1980; Sychev et al., 1980) and ion–gramicidin

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complexes (Byrn, 1974; Urry, 1978; Cornelis & Laszlo, 1979; Kowalsky, 1979).

As part of a general research program aimed at using $^{205}$Tl NMR spectroscopy as a probe for studying ion-transport across membranes, we report here the results of an investigation of the complexation of the $Tl^+$ ion by gramicidin A in trifluoroethanol solution. Trifluoroethanol was chosen as the solvent because the conformation of gramicidin in this solvent is thought to be more nearly like that of the channel state of gramicidin in membranes and model membrane systems (Urry et al., 1972). It was of interest to determine the extent of complexation of $Tl^+$ by gramicidin in these solutions since previous work with $Na^+$ and $K^+$ showed almost no interaction with gramicidin in trifluoroethanol solution (Urry, 1978). It was also important to explore the possible differences between the complexation of $Tl^+$ by gramicidin in solution as opposed to complexation of $Tl^+$ by gramicidin incorporated into a membrane system.

Thallium is a very good NMR probe for studies of the role of potassium ions in biological systems. The chemical properties of the two ions are sufficiently alike so that $Tl^+$ can replace $K^+$ in several enzymes. The utility of $Tl^+$ as a probe in biological systems and in complexation studies has been described elsewhere (Hinton & Briggs, 1979; Briggs et al., 1980; Hinton & Metz, 1980).

## Materials and Methods

Thallium acetate (TlOAc) was obtained from Alfa and was multiply recrystallized from water and then dried at 90 °C and 40 mmHg for 24 h in a vacuum oven. 2,2,2-Trifluoroethanol was obtained from Aldrich Chemical Co. A commercial preparation of gramicidin was obtained from ICN Pharmaceuticals containing approximately 85% of gramicidin A. Veatch & Blout (1974) investigated the commercial preparation as well as the pure form of gramicidin A and found the dimer equilibrium parameters were essentially identical. On the basis of this study, we have assumed that our results are not compromised by using the commercial preparation. All samples were prepared in a glove box under dry nitrogen and sealed in 5-mm NMR tubes under about 0.8 atm of dry nitrogen.

The NMR spectrometer used to obtain the $^{205}$Tl resonance frequencies has been previously described (Hinton & Briggs, 1979; Hinton & Metz, 1980). All chemical shifts are referenced to the resonance frequency of the $Tl^+$ ion in water at infinite dilution. Downfield shifts are assigned positive values. The chemical shift of the $^{205}$Tl nucleus was determined as a function of gramicidin concentration. The total concentration of TlOAc was 0.012 M for all but one sample where the TlOAc concentration was 0.005 M. Only one resonance signal was observed in every solution, indicating fast exchange between all of the possible chemical environments for the $Tl^+$ ion.

All of the calculations were performed on a Commodore Pet Series 2001 microprocessor equipped with 32 KB RAM.

## Results

With the $^{205}$Tl chemical shift data obtained from the "titration" of the $Tl^+$ ion with gramicidin, we hoped to be able to answer a number of questions: (1) Is there a strong complexation of the $Tl^+$ ion by gramicidin. (2) How many strong binding sites are there in gramicidin. (3) What are the thermodynamic parameters for the complexation?

Figure 1 contains a summary of all of the data obtained at 298 °C with the $^{205}$Tl chemical shift plotted as a function of the mole ratio of the total available $Tl^+$ concentration to the total gramicidin dimer concentration. The dimer concentration was used instead of total gramicidin because it has been shown that at the concentrations of gramicidin used in nonpolar solvents similar to trifluoroethanol, the dimer form of gramicidin is the dominant species (Urry et al., 1975; Veatch et al., 1974; Veatch & Blout, 1974). A cursory examination of the data reveals a number of very important features of this system. First, it is seen that the chemical shift changes by about 360 ppm in going from a solution containing no gramicidin to one containing a mole ratio of $Tl^+$ to gramicidin dimer of about 0.2. This enormous chemical shift change clearly indicates a strong interaction between the $Tl^+$ ion and gramicidin. There also appears to be three distinct regions in the data: (1) from pure TlOAc solution to a mole ratio of 8, there appears to be only a small effect on the chemical shift due to the presence of gramicidin, (2) from a mole ratio of 8 to about 3, the chemical shift changes more with the addition of increasing amounts of gramicidin, and finally, (3) at a mole ratio of below 3 to the smallest ratio studied, 0.2, there is a precipitious change in the chemical shift.

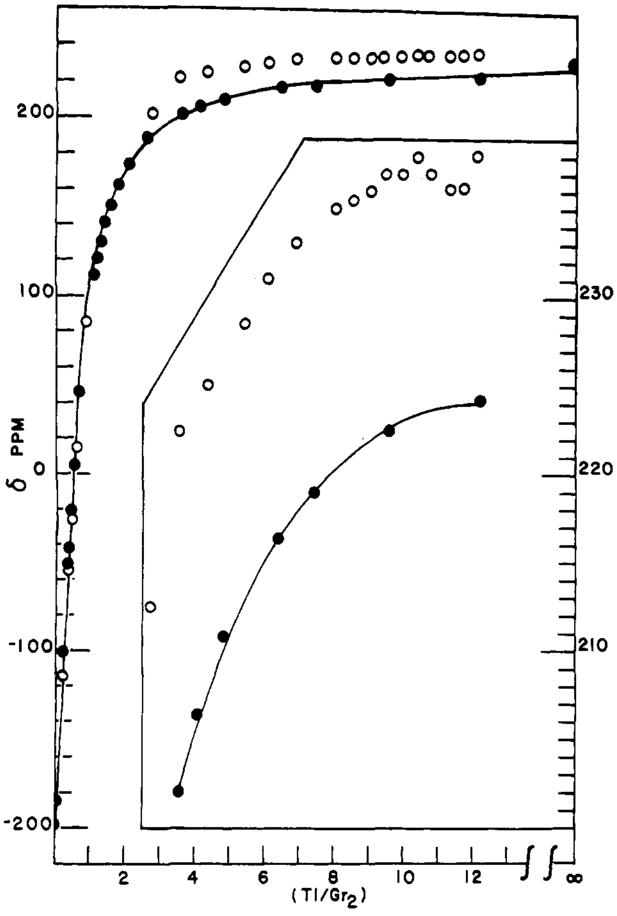

FIGURE 1: Plot of the thallium chemical shift as a function of the mole ratio of total available thallium concentration (i.e., total thallium concentration minus the $Tl^+OAc^-$ ion pair concentration) to the gramicidin dimer concentration. The inset is the same plot but with an expanded parts per million scale. The same mole ratio scale is used for both plots. The temperature is 298 K. The open circles are experimental data, and the solid line with filled circles represents the theoretical curve of the strong binding sites only.

Having determined that there was strong interaction between $Tl^+$ ions and gramicidin dimers at ratios lower than 3:1 of available $Tl^+$ ion to gramicidin dimer, it was then necessary to determine what type of complexation model would fit the data and to determine the thermodynamic parameters for the process. The experimental data used for the model fitting

Table I: Binding Constants as a Function of Temperature

| temp (K) | $K_2$ ($M^{-1}$) | temp (K) | $K_2$ ($M^{-1}$) |
|---|---|---|---|
| 298 | 566 ± 29 | 314 | 453 ± 20 |
| 303 | 544 ± 31 | 316 | 452 ± 21 |
| 309 | 504 ± 41 | 322 | 449 ± 27 |

procedure were for ratios less than 1 to ensure that an excess of gramicidin was present.

Since the gramicidin dimer presents identical ends to the solution for complexing ions (Urry et al., 1971; Urry, 1971; Weinstein et al., 1979), we chose a two-site binding model for each dimer compatible with models suggested by Sandblom et al. (1977) and Laszlo (Cornelis & Laszlo, 1979), for example. The pertinent equilibrium expressions are as follows:

$$Tl^+ + OAc^- \rightleftarrows Tl^+OAc^-$$

$$K_1 = \frac{[Tl^+OAc]\gamma_{Tl^+OAc^-}}{[Tl^+]\gamma_{Tl^+}[OAc^-]\gamma_{OAc^-}} \tag{1}$$

$$Tl^+ + Gr_2 \rightleftarrows (Tl^+Gr_2)$$

$$K_2 = \frac{[Tl^+Gr_2]\gamma_{(Tl^+Gr_2)}}{[Tl^+]\gamma_{Tl^+}[Gr_2]\gamma_{Gr_2}} \tag{2}$$

$$Tl^+ + (Tl^+Gr_2) \rightleftarrows (Tl_2{}^{2+}Gr_2)$$

$$K_3 = \frac{[Tl_2{}^{2+}Gr_2]\gamma_{(Tl_2{}^{2+}Gr_2)}}{[Tl^+]\gamma_{Tl^+}[Tl^+Gr_2]\gamma_{(Tl^+Gr_2)}} \tag{3}$$

where the $\gamma$'s represent the activity coefficients of the species involved in the equilibria.

Equations 1–3 may be combined to yield

$$K_1 = \frac{1 - X_1 - X_3 - X_4}{X_1 C_T \gamma_\pm{}^2 (X_1 + X_3 + X_4)} \tag{4}$$

$$K_2 = \frac{X_3}{X_1\gamma_\pm[Gr_2 - (X_3 + X_4/2)C_T]} \tag{5}$$

$$K_3 = \frac{X_4}{2X_1X_3C_T\gamma_\pm} \tag{6}$$

where $C_T$ = stoichiometric TlOAc concentration, $Gr_2$ = stoichiometric gramicidin dimer concentration, and $X_1$, $X_3$, and $X_4$ refer to the mole fractions of $Tl^+$, $(Tl^+Gr_2)$, and $(Tl_2{}^{2+}Gr_2)$, respectively. It was also assumed that the activity coefficients of $Gr_2$, $Tl^+Gr_2$, $Tl_2{}^{2+}Gr_2$, and $Tl^+OAc^-$ were unity because of partial charge cancellation or because the species was not charged. The activity coefficients for $Tl^+$ and $OAc^-$ were calculated from the Debye–Hueckel equation (Bockris & Reddy, 1970).

The observed chemical shift of the $^{205}Tl$ nucleus is given by

$$\delta_{obsd} = X_1\delta_1 + X_2\delta_2 + X_3\delta_3 + X_4\delta_4 \tag{7}$$

where $\delta_1$, $\delta_2$, $\delta_3$, and $\delta_4$ represent the chemical shifts of the $Tl^+$, $Tl^+OAc^-$, $(Tl^+Gr_2)$, and $(Tl_2{}^{2+}Gr_2)$ species, respectively. If one now assumes that since both ends of the dimer are the same, $\delta_3$ is equal to $\delta_4$, then the chemical shift expression becomes

$$\delta_{obsd} = X_1\delta_1 + (1 - X_1 - X_3 - X_4)\delta_2 + (X_3 + X_4)\delta_3 \tag{8}$$

Letting

$$X_3 + X_4 = (A + BX_1)/C \tag{9}$$

where

$$A = \delta_{obsd} - \delta_2 \tag{10}$$

$$B = \delta_2 - \delta_1 \tag{11}$$

$$C = \delta_3 - \delta_2 \tag{12}$$

one can write $K_1$ as

$$K_1 = \frac{1 - X_1 - [(A + BX_1)/C]}{[X_1{}^2 + (X_1A + BX_1{}^2)/C]C_T\gamma_\pm{}^2} \tag{13}$$

or

$$X_1 = \frac{-D \pm \sqrt{D^2 - 4E(A - C)}}{2E} \tag{14}$$

where

$$D = K_1C_T\gamma_\pm{}^2A + C + B \tag{15}$$

$$E = K_1C_T\gamma_\pm{}^2(C + B) \tag{16}$$

The values of $K_1$, $\delta_1$, and $\delta_2$ have been determined as a function of temperature in a manner described elsewhere (J. F. Hinton, G. L. Turner, and F. S. Millett, unpublished results); hence, $X_1$ can be calculated by assuming a value for $\delta_3$ based upon an extrapolation of the experimental data.

Another equation containing only the mole fraction, $X_1$, may be obtained as (see Appendix)

$$\begin{aligned}&[K_1C_T{}^3\gamma_\pm{}^4K_2K_3]X_1{}^4 + \\ &[K_1C_T{}^2\gamma_\pm{}^3K_2 + 2C_T{}^2\gamma_\pm{}^4K_1K_2K_3Gr_2 + K_2\gamma_\pm{}^2K_3C_T{}^2]X_1{}^3 + \\ &[K_1C_T\gamma_\pm{}^2 + C_T\gamma_\pm{}^3K_1K_2Gr_2 - K_2\gamma_\pm{}^2K_3C_T{}^2 + K_2\gamma_\pm C_T + \\ &2K_2K_3C_T\gamma_\pm{}^2Gr_2]X_1{}^2 + [K_2\gamma_\pm Gr_2 - K_2\gamma_\pm C_T + 1]X_1 - 1 = \\ &0\end{aligned} \tag{17}$$

Values of $K_2$ and $K_3$ are varied to solve eq 17 by the Newton–Ralphson iterative method (Dence, 1975) until the $X_1$ obtained is in agreement with that obtained from eq 14. When the average values for $K_2$ and $K_3$ are used, mole fractions $X_3$ and $X_4$ may then be calculated. The value of $\delta_{obsd}$ is then calculated for each data point [i.e., $(Tl^+/Gr_2)$ ratio]. The whole process is then repeated (i.e., a new value for $\delta_3$ is chosen for use in eq 14, and new values for $K_2$ and $K_3$ are obtained) until the $\delta$ minimum in the standard deviation between experimentally and theoretically determined data points is obtained.

The fit of the calculated observed chemical shift as a function of the $(Tl^+/Gr_2)$ ratio to the experimental points is shown in Figure 1. The solid line with filled circles is the theoretical curve, and the open circles are the experimental data. The fit of the model (i.e., two strong binding sites with equal $K$ values) appears to be quite good for the region where no weak association occurs. There is one other criteria used to check the model calculations, and that is the relationship between the theoretically determined chemical shift value for $\delta_3$ and temperature. Other studies (Hinton & Briggs, 1979; Hinton & Metz, 1980) have shown that the chemical shift of thallium complexes is a linear function of the temperature. Figure 2 is a plot of the theoretically determined complex shift, $\delta_3$, as a function of temperature, and the linearity is apparent.

The values obtained for $K_2$ ($K_2 = K_3$) as a function of temperature are found in Table I. A plot of ln $K$ vs. $1/T$ is shown in Figure 3. From this plot the thermodynamic parameters $\Delta H = -2.13 \pm 0.12$ kcal/mol and $\Delta S = +5.45 \pm 0.04$ eu were obtained. The magnitude and sign of these thermodynamic parameters appear to be consistent with similar types of ion–ionophore complexes (Kauffmann et al., 1976; Lamb et al., 1979; Popov & Lehn, 1979; Shamsipur et al., 1980).

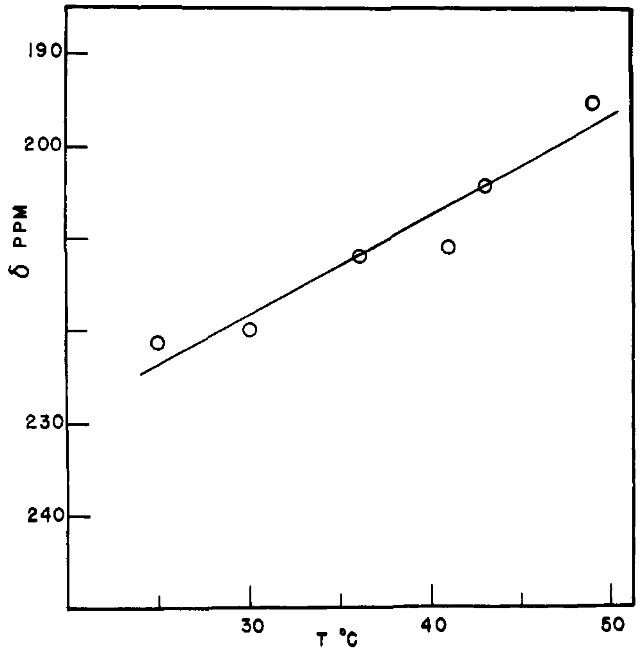

FIGURE 2: Plot of the theoretically calculated $Tl^+Gr_2$ complex chemical shift as a function of temperature.

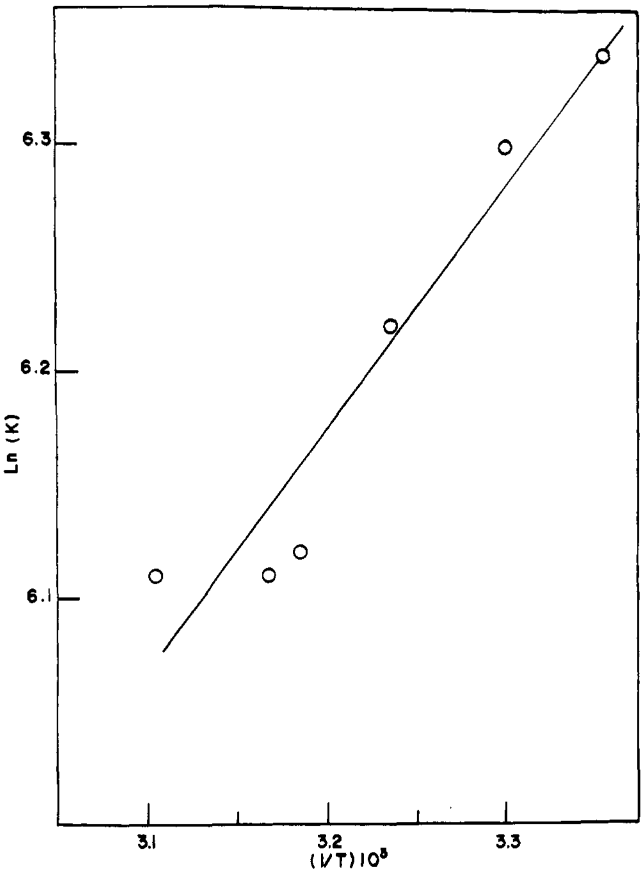

FIGURE 3: Plot of ln $K$ as a function of the reciprocal of the absolute temperature.

The positive value obtained for $\Delta S$ suggests the liberation of solvent molecules during the complexation process. However, the data obtained provide evidence that suggest that some trifluoroethanol remains associated with the $Tl^+$ ion in the gramicidin channel, as suggested previously (Urry et al., 1975). The calculated resonance frequency of the complexed $Tl^+$ ion at 49 °C in trifluoroethanol is 51 905 300 Hz compared to a value of the experimentally determined resonance frequency of the intact $Tl^+$–gramicidin dimer complex in dioxane at the same temperature of 51 880 400 Hz (Hinton et al., 1981). The higher resonance frequency in trifluoroethanol strongly suggests that the $Tl^+$ ion is still partially solvated at the binding site of gramicidin.

Although the location of the binding sites in the dimer channel was not determined in this work, $^{23}Na$ (Cornelis & Laszlo, 1979) and $^{205}Tl$ (Hinton et al., 1981) NMR studies indicate that the strong binding sites are near the mouth of each end of the channel. The X-ray studies of Koeppe et al. (1979) also lend support to this proposed location. Theoretical calculations (Eisenman et al., 1978; Levitt, 1978) on the gramicidin system gave results in agreement with this model, although they differ on the number of weak binding sites.

The presence of weak binding sites in the channel is indicated by the results shown in Figure 1. In the mole ratio range of about 2–8, there is strong evidence of multiple occupancy of the channel (i.e., more than two ions per dimer). It appears that the binding of ions in the channel under these conditions undergoes a general weakening.

Caution should be observed in trying to extrapolate too far the results obtained on solutions to the gramicidin contained in membranes since it has been shown that the binding of cations to gramicidin A in solution (Urry, 1978) is not consistent with the passage of cations through the gramicidin A channel in membranes (Bamberg & Lauger, 1977). Indeed, it appears that the dimer that forms the membrane channel may be a minor species in solution (Sychev et al., 1980).

The accuracy of the results presented here could suffer because the conformation of gramicidin A is temperature and concentration dependent (Urry et al., 1975). However, we have attempted to minimize this influence by studying a relatively narrow temperature range and only calculating equilibrium constants for the higher concentration region. Circular dichroism patterns have indicated that with these temperature and concentration ranges the conformational changes of gramicidin A are less drastic.

Finally, it should be mentioned that we examined several other theoretical systems to try to reproduce the experimental data in the low mole ratio range, but none gave nearly as good agreement as the two strong binding site model.

## Conclusion

The gramicidin A dimer in trifluoroethanol appears to have two strong binding sites accessible to the $Tl^+$ ion near the ends of the channel dimer. The thermodynamic parameters obtained for this binding process are $\Delta H = -2.13 \pm 0.12$ kcal/mol and $\Delta S = +5.45 \pm 0.04$ eu. The thermodynamic parameters would also suggest partial solvation of the $Tl^+$ ion being bound in the channel. Evidence for weak binding in the channel has also been obtained at high ratios of $Tl^+$ to gramicidin dimer.

## Appendix

The equilibrium expressions for the two binding site model of the gramicidin dimer along with the coupled ion pairing of TlOAc are given as

$$Tl^+ + OAc^- \rightleftarrows Tl^+OAc^-$$

$$K_1 = \frac{[Tl^+OAc^-]\gamma_{Tl^+OAc^-}}{[Tl^+]\gamma_{Tl^+}[OAc^-]\gamma_{OAc^-}} \quad \text{(A1)}$$

$$Tl^+ + Gr_2 \rightleftarrows (Tl^+Gr_2)$$

$$K_2 = \frac{[Tl^+Gr_2]\gamma_{(Tl^+Gr_2)}}{[Tl^+]\gamma_{Tl^+}[Gr_2]\gamma_{Gr_2}} \quad \text{(A2)}$$

$$\mathrm{Tl^+ + (Tl^+Gr_2) \rightleftarrows (Tl_2{}^{2+}Gr_2)}$$

$$K_3 = \frac{[\mathrm{Tl_2{}^{2+}Gr_2}]\gamma_{(\mathrm{Tl_2{}^{2+}Gr_2})}}{[\mathrm{Tl^+}]\gamma_{\mathrm{Tl^+}}[\mathrm{Tl^+Gr_2}]\gamma_{(\mathrm{Tl^+Gr_2})}} \tag{A3}$$

where the $\gamma$'s represent the activity coefficients of the species involved in the equilibria. Letting $C_\mathrm{T}$ = stoichiometric TlOAc concentration, $\mathrm{Gr_2}$ = stoichiometric gramicidin dimer concentration, and $X_1$, $X_3$, and $X_4$ refer to the mole fractions of $\mathrm{Tl^+}$, $\mathrm{(Tl^+Gr_2)}$, and $\mathrm{(Tl_2{}^{2+}Gr_2)}$, respectively, A1–A3 can be expressed as

$$K_1 = \frac{1 - X_1 - X_3 - X_4}{X_1 C_\mathrm{T}\gamma_\pm(X_1 + X_3 + X_4)} \tag{A4}$$

$$K_2 = \frac{X_3}{X_1 C_\mathrm{T}\gamma_\pm[\mathrm{Cr_2} - (X_3 + X_4/2)C_\mathrm{T}]} \tag{A5}$$

$$K_3 = \frac{X_4}{2X_1X_3C_\mathrm{T}\gamma_\pm} \tag{A6}$$

where it has been assumed that the activity coefficients of $\mathrm{Gr_2}$, $\mathrm{Tl^+Gr_2}$, $\mathrm{Tl_2{}^{2+}Gr_2}$, and $\mathrm{Tl^+OAc^-}$ are equal to unity and the activity coefficients of $\mathrm{Tl^+}$ and $\mathrm{OAc^-}$ are calculated from the Debye–Hueckel equation (Bockris & Reddy, 1970).

Under the assumption that both ends of the dimer are the same, the chemical shift of both sites should then be equal. The expression for the observed chemical shift is then given as

$$\delta_\mathrm{obsd} = X_1\delta_1 + (1 - X_1 - X_3 - X_4)\delta_2 + (X_3 + X_4)\delta_3 \tag{A7}$$

where $\delta_1$, $\delta_2$, and $\delta_3$ represent the chemical shifts of the $\mathrm{Tl^+}$, $\mathrm{Tl^+OAc^-}$, and $\mathrm{(Tl^{\mathit{n}+}Gr_2)}$ ($n$ = 1 and 2) species, respectively.

Solving eq A7 for $X_3 + X_4$ yields

$$X_3 + X_4 = \frac{A + BX_1}{C} \tag{A8}$$

where

$$A = \delta_\mathrm{obsd} - \delta_2 \tag{A9}$$

$$B = \delta_2 - \delta_1 \tag{A10}$$

$$C = \delta_3 - \delta_2 \tag{A11}$$

Substituting eq A8 into eq A4 yields

$$K_1 = \frac{1 - X_1 - [(A + BX_1)/C]}{[X_1{}^2 + (X_1A + BX_1{}^2)/C]C_\mathrm{T}\gamma_\pm{}^2} \tag{A12}$$

Solving eq A12 for $X_1$ gives

$$X_1 = \frac{-D \pm \sqrt{D^2 - 4E(A - C)}}{2E} \tag{A13}$$

where

$$D = K_1C_\mathrm{T}\gamma_\pm{}^2A + C + B \tag{A14}$$

$$E = K_1C_\mathrm{T}\gamma_\pm{}^2(C + B) \tag{A15}$$

To derive an expression for $X_1$ using just the equilibrium expression is accomplished in the following method. Equation A6 is expressed as

$$X_4 = 2K_3X_1X_3C_\mathrm{T}\gamma_\pm \tag{A16}$$

Substituting eq A16 into eq A5 and solving for $X_3$ yields

$$X_3 = \frac{K_2\gamma_\pm X_1\mathrm{Gr_2}}{K_2\gamma_\pm{}^2X_1{}^2K_3C_\mathrm{T}{}^2 + K_2\gamma_\pm X_1C_\mathrm{T} + 1} \tag{A17}$$

Substituting eq A16 and A17 into eq A4 and solving for $X_1$ gives the final result:

$$\begin{gathered}[K_1C_\mathrm{T}{}^3\gamma_\pm{}^4K_2K_3]X_1{}^4 + \\ [K_1C_\mathrm{T}{}^2\gamma_\pm{}^3K_2 + 2C_\mathrm{T}{}^2\gamma_\pm{}^4K_1K_2K_3\mathrm{Gr_2} + K_2\gamma_\pm{}^2K_3C_\mathrm{T}{}^2]X_1{}^3 + \\ [K_1C_\mathrm{T}\gamma_\pm{}^2 + C_\mathrm{T}\gamma_\pm{}^3K_1K_2\mathrm{Gr_2} - K_2\gamma_\pm{}^2K_3C_\mathrm{T}{}^2 + K_2\gamma_\pm C_\mathrm{T} + \\ 2K_2K_3C_\mathrm{T}\gamma_\pm{}^2\mathrm{Gr_2}]X_1{}^2 + [K_2\gamma_\pm\mathrm{Gr_2} - K_2\gamma_\pm C_\mathrm{T} + 1]X_1 - 1 = \\ 0\end{gathered} \tag{A18}$$

Equation A18 can readily be solved for $X_1$ by using the Newton–Ralphson iterative method (Dence, 1975).

## References

Bamberg, E., & Lauger, P. (1974) *Biochim. Biophys. Acta 367*, 127.

Bamberg, E., & Lauger, P. (1977) *J. Membr. Biol. 35*, 351.

Bockris, J. O'M., & Reddy, A. K. (1970) *Mod. Electrochem. 1.*

Briggs, R. W., Etzkorn, F. A., & Hinton, J. F. (1980) *J. Magn. Reson. 37*, 523.

Byrn, S. R. (1974) *Biochemistry 13*, 5186.

Cornelis, A., & Laszlo, P. (1979) *Biochemistry 18*, 2004.

Dence, J. B. (1975) *Mathematical Techniques in Chemistry*, p 54, Wiley, New York.

Eisenman, G., Sandblom, J., & Neher, E. (1978) *Biophys. J. 22*, 307.

Fossel, E. T., Veatch, W. R., Ovchinnikov, Y. A., & Blout, E. R. (1974) *Biochemistry 13*, 5264.

Glickson, J. D., Mayers, D. F., Settine, J. M., & Urry, D. W. (1972) *Biochemistry 11*, 477.

Heitz, F., Spach, G., Cary, P. D., & Crane-Robinson, C. (1979) *Eur. J. Biochem. 93*, 135.

Hinton, J. F., & Briggs, R. W. (1979) *J. Solution Chem. 8*, 479.

Hinton, J. F., & Metz, K. R. (1980) *J. Solution Chem. 9*, 197.

Hinton, J. F., Turner, G. L., & Millett, F. S. (1981) *J. Magn. Reson. 45*, 42.

Hladky, S. B., & Haydon, D. A. (1972) *Biochim. Biophys. Acta 274*, 294.

Iqbal, Z., & Weidekamm, E. (1980) *Arch. Biochem. Biophys. 202*, 639.

Kauffmann, E., Lehn, J. M., & Sauvage, J. P. (1976) *Helv. Chim. Acta 59*, 1099.

Koeppe, R. E., Berg, J. M., Hodgson, K. E., & Stryer, L. (1979) *Nature (London) 279*, 723.

Kowalsky, A. (1979) in *NMR and Biochemistry* (Opella, S. J., & Lu, P., Eds.) p 125, Marcel Dekker, New York.

Krasne, S., Eisenman, G., & Szabo, G. (1971) *Science (Washington, D.C.) 174*, 412.

Lamb, J. D., Izatt, R. M., Christensen, J. J., & Eatough, D. J. (1979) in *Coordination Chemistry of Macrocyclic Compounds* (Melson, G. A., Ed.) p 145, Plenum Press, New York.

Levitt, D. G. (1978) *Biophys. J. 22*, 209.

McBride, D., & Szabo, G. (1978) *Biophys. J. 21*, A25.

Myers, U. B., & Haydon, D. A. (1972) *Biochim. Biophys. Acta 274*, 313.

Neher, E. (1975) *Biochim. Biophys. Acta 401*, 540.

Popov, A. I., & Lehn, J. M. (1979) in *Coordination Chemistry and Macrocyclic Compounds* (Melson, G. A., Ed.) p 537, Plenum Press, New York.

Sandblom, J., Eisenman, G., & Neher, E. (1977) *J. Membr. Biol. 31*, 383.

Shamsipur, M., Rounaghi, G., & Popov, A. I. (1980) *J. Solution Chem. 9*, 701.

Sychev, S. V., Nevskaya, N. A., Jordanov, S. T., Shepel, E. N., Miroshnikov, A. I., & Ivanov, V. T. (1980) *Bioorg. Chem. 9*, 121.

Urry, D. W. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 672.

Urry, D. W. (1978) *Ann. N.Y. Acad. Sci. 307*, 3.

Urry, D. W., Goodall, M. C., Glickson, J. D., & Meyers, D. F. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 1907.

Urry, D. W., Glickson, J. D., Mayers, D. F., & Haider, J. (1972) *Biochemistry 11*, 487.

Urry, D. W., Long, M. M., Jacobs, M., & Harris, R. D. (1975) *Ann. N.Y. Acad. Sci. 264*, 203.

Urry, D. W., Spisni, A., & Klaled, M. A. (1979) *Biochem. Biophys. Res. Commun. 88*, 940.

Urry, D. W., Venkatachalam, C. M., Spisni, A., Bradley, R. J., Trapane, T. L., & Drasad, K. U. (1980) *J. Membr. Biol. 55*, 29.

Veatch, W. R., & Blout, E. R. (1974) *Biochemistry 13*, 5257.

Veatch, W. R., & Darkin, J. T. (1980) *J. Mol. Biol. 143*, 411.

Veatch, W. R., Fossel, E. T., & Blout, E. R. (1974) *Biochemistry 13*, 5249.

Venkatachalam, C. M., & Urry, D. W. (1980) *J. Magn. Reson. 41*, 313.

Weinstein, S., Wallace, B. A., Blout, E. R., Morrow, J. S., & Veatch, W. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4230.

# Thallous Ion Interaction with Gramicidin Incorporated in Micelles Studied by Thallium-205 Nuclear Magnetic Resonance[†]

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ABSTRACT: A study has been made of the interaction between the thallous ion and gramicidin dimers incorporated into micelles by using $^{205}$Tl NMR spectroscopy. The chemical shift data obtained are interpreted in terms of a model in which the dimer has only one tight binding site. The binding constant for this site was determined to be 900 M$^{-1}$ at 30 °C. The thallous ion also appears to be partially solvated in the channel.

Thallium-205 NMR spectroscopy has been shown to be a very sensitive probe for studying the interaction between antibiotics and the thallous ion in solution (Hinton & Briggs, 1978; Briggs et al., 1980; Briggs & Hinton, 1978a,b) and in the solid state (Hinton et al., 1981a). Recently, we have investigated the complexation of the thallous ion by gramicidin A in dioxane (Hinton et al., 1981b) and in trifluoroethanol solutions (Turner et al., 1981) using $^{205}$Tl NMR. Gramicidin A is a linear polypeptide antibiotic that has been very well characterized for its ability to form ion-selective membrane channels (Hladky & Haydon, 1970, 1972; Krause et al., 1971; Mueller & Rudin, 1967; Myers & Haydon, 1972; Neher, 1975; Sandblom et al., 1977). In membranes the channel is formed by gramicidin dimers (Tosteson et al., 1968; Goodall, 1970; Urry et al., 1971; Bamberg & Lauger, 1973; Zingsheim & Neher, 1974; Veatch et al., 1975; Veatch & Stryer, 1977) connected by N-terminal to N-terminal interaction (Goodall, 1971; Urry et al., 1971; Bamberg et al., 1977; Bamberg & Janko, 1977; Bradley et al., 1978; Szabo & Urry, 1979; Apell et al., 1977; Morrow et al., 1979; Weinstein et al., 1979, 1980).

It was of interest to study the interaction between the thallous ion and gramicidin incorporated into micelles by using the $^{205}$Tl NMR technique for several reasons: (1) to compare the results with those obtained previously in trifluoroethanol and dioxane solutions; (2) to compare the NMR results with those obtained by equilibrium dialysis (Veatch & Durkin, 1980) and electrical measurements (Eisenman et al., 1978; Levitt, 1978; Hladky et al., 1979) where gramicidin was incorporated in a membrane; (3) to obtain the number of strong binding sites per dimer channel; (4) to determine the binding constant; (5) to try to determine the extent of hydration of the ion in the channel.

## Materials and Methods

Gramicidin was incorporated into lysolecithin micelles by using the procedures of Urry (Urry et al., 1979; Spisni et al., 1979). L-$\alpha$-Lysolecithin from egg yolk, containing primarily palmitic and stearic acids at position one, was obtained from Sigma Chemical Co., St. Louis, MO, and was used without further purification. Gramicidin was obtained from ICN and was a mixture composed of approximately 85% gramicidin A, 10% gramicidin B, and 5% gramicidin C. Thallium nitrate from Alfa was twice recrystallized before use.

Lysolecithin was weighed under N$_2$ atmosphere and suspended in 3 mL of degassed water. This suspension was sonicated in an ice bath under N$_2$ flow for 3 min with a Branson W-185 cell disruptor fitted with a microtip. Gramicidin was added under N$_2$ atmosphere to give a lysolecithin:gramicidin ratio of approximately 10:1. The suspension was shaken until all of the gramicidin was dispersed and then sonicated for 6 min in an ice bath under N$_2$ flow. Samples were then heated in a water bath, with agitation, for 15 h at 68 °C.

Samples for $^{205}$Tl NMR spectroscopy were prepared by diluting aliquots of the lysolecithin–gramicidin suspension with 0.2 M TlNO$_3$ degassed aqueous solution to give the desired TlNO$_3$ and gramicidin concentrations. Samples were stored at 68 °C until placed in the spectrometer.

For each gramicidin-containing sample an aqueous TlNO$_3$ solution at the same Tl$^+$ concentration was prepared. The resonance frequency of this samples was used as the "free" ion resonance frequency for that TlNO$_3$ concentration in calculating the gramicidin induced shift.

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