Cation Complexation by Valinomycin- and Nigericin-Type Ionophores Registered by the Fluorescence Signal of Tl^+ †

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ABSTRACT: The fluorescent signal of Tl^+ was used to study cation complexation by valinomycin- and nigericin-type ionophores which act as mobile cation carriers in biological membranes. The use of the Tl^+ fluorescent signal is a convenient method for study of ionophore complexation of cations in organic solvents. In methanol Tl^+ has an absorption maximum at 214 mμ, an emission maximum at 358 mμ, and a quantum yield of 0.029. Complexation with the ionophores valinomycin, *nonactin*, *monactin*, dinactin, dibenzo-30-crown-10, monensin$^-$, and X537A$^-$ occurs with stability constants in the range 10^4–10^5 M^{-1}. Complexation with these ionophores results in a decrease in the quantum yield to less than 10% of its original value. Complexation with dicyclohexyl-18-crown-6 results in disappearance of the emission signal at 358 mμ and the appearance of a new signal with an emission maximum at 305 mμ with a quantum yield of 0.048. Ionophore- and cation-substitution titrations were performed to determine the stability constants of the Na^+, K^+, Rb^+, Cs^+, and Tl^+ complexes of these ionophores. Acetone, $CHCl_3$, and Mn^{2+} quench Tl^+ fluorescence, and the mechanism of this quenching and its relevance to the interaction between the complexing oxygen groups of the ionophores and the complexed Tl^+ are discussed. The usefulness of Tl^+ as a fluorescent probe for K^+ in solvent and membrane systems is considered.

The neutral valinomycin-type and negatively charged nigericin-type ionophores, which act as cation carriers in artificial and biological membranes (Pressman *et al.*, 1967; Pressman, 1968; Pressman and Haynes, 1970), have been subjected to a large amount of study by physical-chemical methods. The finding that the ion selectivity of a number of ionophores for complexation of cations in organic solvents is paralleled by the ion selectivity for ion transport through the mitochondrial membrane induced by these carriers (Pressman *et al.*, 1967; Pressman, 1968; Pressman and Haynes, 1970) serves to emphasize the relevance of the determination of the complexation reactions in solution.

The present study reports the use of the fluorescent signal of Tl^+ to report its complexation by the ionophores in organic solvent, and shows how the binding of other cations can be reported by their competition with Tl^+. Previously, the complexation constants for the ionophores in alcohol solvents have been determined using electrical conductivity (Shemyakin *et al.*, 1969), circular dichroism (Shemyakin *et al.*, 1969; Grell *et al.*, 1972), optical absorption (Chock, 1972), and ion specific electrodes (Pioda *et al.*, 1967; Frensdorff, 1971). The Tl^+ method to be reported here has the advantage over the conductivity and circular dichroism methods in that it can report complexation within the concentration range 10^{-3}–10^{-7} M, and can thus measure quantitatively dissociation constants within that range.

The monovalent cation Tl^+ has been suggested as a probe for K^+ in biological systems (Williams, 1970; Kayne and Reuben, 1970). It substitutes for K^+ in activation of (Na^+–K^+)-activated ATPase (Britten and Blank, 1968) and pyruvate kinase (Kayne, 1971). Kayne and Reuben (1970) have used the nuclear magnetic resonance (nmr) signal to Tl^+ bound to the latter enzyme to gain information about the proximity of the bound Tl^+ to bound Mn^{2+} which acts as an activator.

The fluorescent properties of Tl^+ in aqueous media have been well characterized by Steffen and Sommermeyer (1968). Their findings can be summarized as follows. In water, Tl^+ absorbs at 214 mμ, with a quantum yield of 0.17. The fluorescence is pH independent for values between 3 and 9. Tl^+ reacts with Cl^- in both the ground and excited states to give TlCl and $TlCl_2^-$ complexes with a red shift in the emission maximum. The dissociation constants for TlCl in the ground and excited (*) states are 0.26 and 10^{-3} M, respectively, and the equilibration between Tl^{+*} and Tl^*Cl is completed within the lifetime of the excited state. The latter was estimated as $\geq 10^{-7}$ sec.

Experimental Section

Materials. Valinomycin and monensin (Na^+ salt) were obtained from Eli Lilly and Co., Indianapolis, Ind.; enniatin B and X537A from Hoffman-La Roche, Basel; the macrolide actins from CIBA, Basel; dicyclohexyl-18-crown-6, a mixture of two isomers (*cf.* Frensdorf, 1971), and dibenzo-30-crown-10 (Pedersen, 1967) from E. I. du Pont de Nemours and Co. through Drs. B. C. Pressman and P. C. Chock; beauvericin was a gift from Dr. R. Roeske. The organic solvents were reagent grade with less than 0.2% water, purchased from Merck. Atomic absorption analysis showed less than 10^{-6} M alkali or alkali earth cations. TlCl was converted to $TlClO_4$ by recrystallizing several times in $HClO_4$ and then in water until there were no indications of acid or Cl^- impurity.

Fluorescent Spectra. Routine measurements were made on an Aminco-Bowman SPF spectrofluorimeter, using 1-mm entrance slits and 3-mm exit slits. Inner filter effect corrections for solvent or ionophore absorption at the exciting wavelength were made by a comparison with the fluorescence concentration characteristic of standard fluorescent compounds, with a knowledge of the absorption of the solution at the exciting wavelength. The latter was determined on a Cary 14 spectrophotometer. Statements regarding the quantum yield of the Tl^+ species derive from experiments performed on a FICA 55 spectrofluorimeter, which gives quantum-corrected excitation

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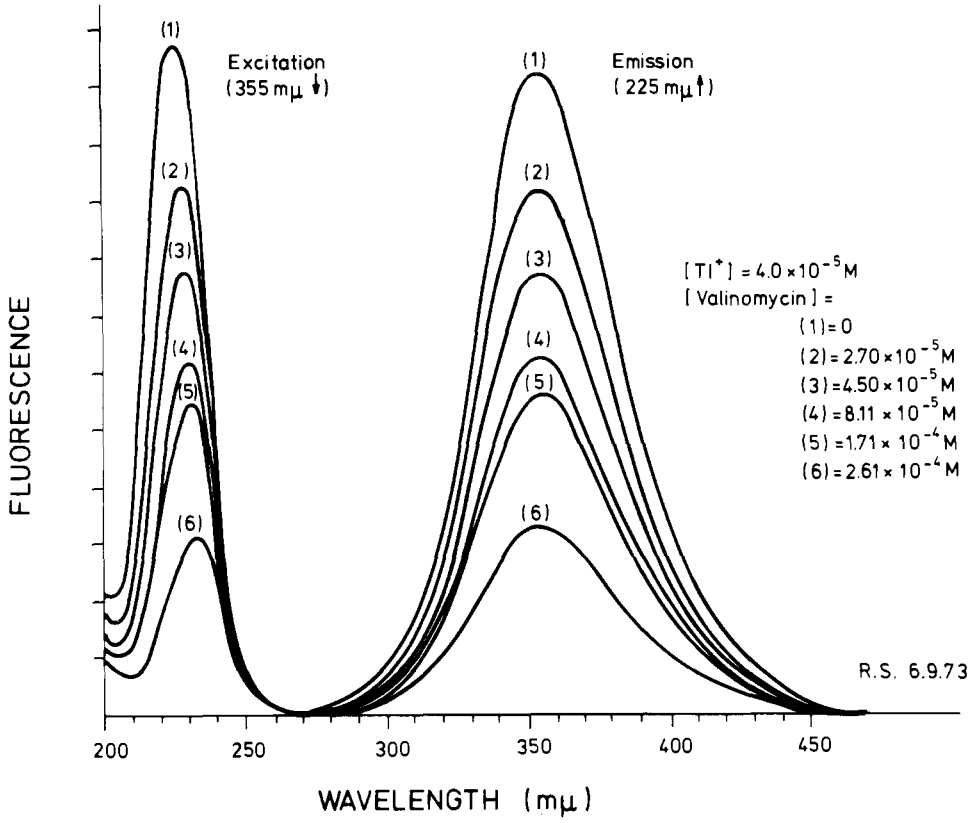

FIGURE 1: Quenching of fluorescence of a 4.0×10^{-5} M $TlClO_4$ solution in methanol by valinomycin. The experiment was performed on the Aminco instrument.

(220–550 mμ) and emission (200–800 mμ) spectra. All measurements were made in 5 × 5 mm quartz cuvets.

Calculations. The binding constants K_s for Tl^+ were calculated according to

$$K_s = \frac{(1 - x)}{x([I]_t - (1 - x)[Tl]_t^+)} \quad (1)$$

where I represents the ionophore, and where x is the ratio of free Tl^+ to total Tl^+, measured as

$$x = \left| \frac{Fl - Fl_\infty}{Fl_0 - Fl_\infty} \right| \quad (2)$$

Here, Fl_0, Fl, and Fl_∞ are the fluorescence amplitudes at the maximum for solvated Tl^+, measured before, during, and after the titration with the ionophore to a saturating concentration. In the case where the appearance of a fluorescence signal at a second wavelength was measured (with 18-crown-6), x was determined as

$$x = \left| \frac{Fl - Fl_0}{Fl_\infty - Fl_0} \right| \quad (3)$$

For the case of competition of a second cation M^+ for the ionophore, with a binding constant $K_{s(M^+)}$, the ratio of the two binding constants was calculated as

$$K_{s(M^+)}/K_{s(Tl^+)} = \frac{x([I]_t - (1 - x)[Tl]_t(1 + 1/(xK_{s(M^+)}[Tl]_T))}{(1 - x)[M]_t} \quad (4)$$

which obtains when the cation M^+ is in excess. For the case of competition between ionophores, the ratio of binding constants of Tl^+ for ionophore a and b is

$$K_{s(b)}/K_{s(a)} = \frac{(1 - x)([I_a]_t - x[Tl]_t^+) - (x/K_{s(a)})}{[I_b]_t x} \quad (5)$$

where x is measured at the wavelength characteristic for I_a–Tl^+ according to eq 3, and were $K_{s(a)}$ has been measured in a titration of Tl^+ with I_a.

Results

The fluorescence of $TlClO_4$ at concentrations up to 10^{-4} M in water had the spectral characteristics of Tl^+ given by Steffen and Sommermeyer (1968), and the effects of added Cl^- reported in this study have been confirmed here. Acetate and phosphate in millimolar concentrations were observed to quench the Tl^+ fluorescence. The spectral properties of Tl^+ in alcohol solutions (methanol, ethanol, 1-propanol) are similar to aqueous solutions except that the fluorescent yields are reduced and the emission maximum is shifted slightly to lower wavelengths (*cf.* Table III). The fluorescent yield and emission maxima are independent of ClO_4^- and Cl^- concentrations between 10^{-6} and 5×10^{-4} M, indicating that ion pairing probably does not occur in this concentration range.

Figure 1 shows that titration of Tl^+ in methanol with valinomycin decreases the fluorescent signal. The fluorescent amplitude extrapolated to infinite valinomycin concentration is less than 10% of that for free Tl^+. Assuming that this effect is due to direct complexation according to

$$Tl^+ + I \overset{K_s}{\longleftrightarrow} Tl^+\text{–}I \quad (6)$$

where I represents the ionophore, and analysis using eq 1 and 2 yielded $K_s = (2.3 \pm 0.5) \times 10^4$ M^{-1}. Experiments at higher Tl^+ concentrations showed no significant variation in the calculated binding constant. The effect of valinomycin addition shown in Figure 1 is reversed by subsequent additions of KCl which increase the fluorescent signal, approaching the original value at high KCl concentrations. Similar behavior was observed for the ionophores enniatin B, nonactin, monactin, dinactin, monensin, beauvericin, and dibenzo-30-crown-6. That

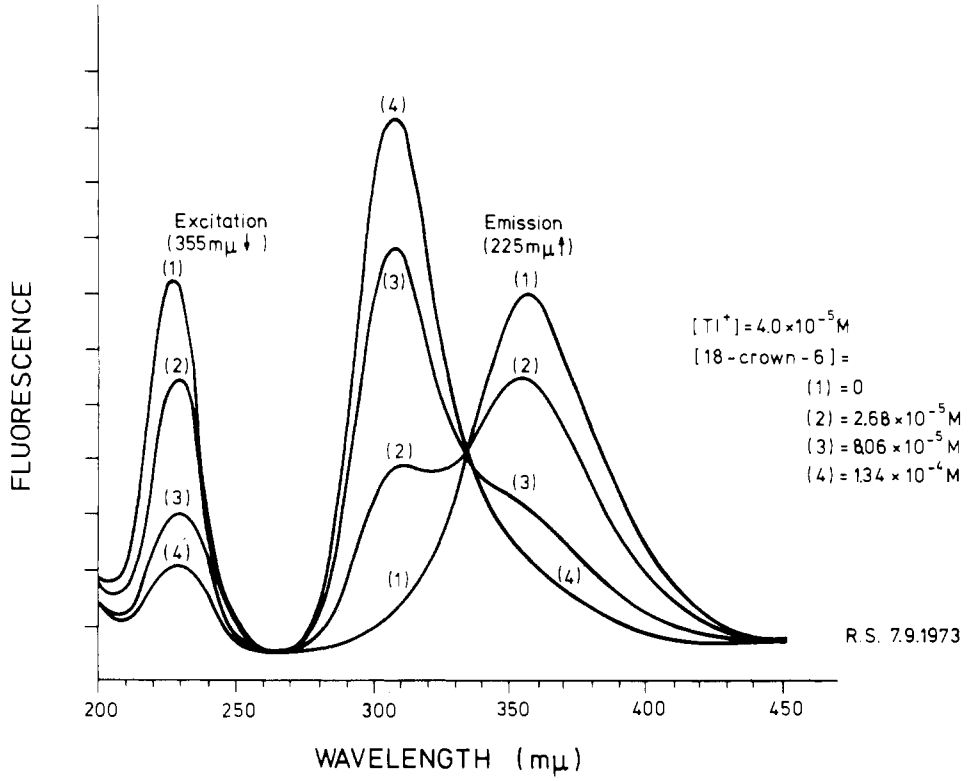

FIGURE 2: Complexation of Tl^+ in methanol at 25° by dicyclohexyl-18-crown-6. The experiment was performed on the Aminco instrument.

these effects are truly due to Tl^+ and K^+ complexation, respectively, is shown by experiments with 18-crown-6.

Figure 2 shows the effect of titration with 18-crown-6 on the Tl^+ fluorescent signal. Increasing the ionophore concentration results in a decrease in the fluorescent amplitude at the 355 mμ and the appearance of a signal at 308 mμ, indicating the formation of a new Tl^+ species. The degree of fluorescence increase at 308 mμ is proportional to the degree of decrease at 355 mμ (*cf.* eq 2 and 3), giving direct evidence that the observed effect is the result of direct complexation. Analysis according to eq 1 and 3 demonstrated that the behavior shown in Figure 2 is adequately described by the process of eq 6, with $K_s = (4.2 \pm 0.8) \times 10^4$. The effect shown in Figure 2 is reversed by the addition of either KCl or other ionophores. The latter result proves that the Tl^+ quenching effect of these other ionophores was also due to direct complexation according to eq 6. Table I gives the stability constants of Tl^+ with the ionophores determined according to the procedure of Figures 1 and 2. Identical values of K_s were obtained by competition experiments in which these ionophores competed with 18-crown-6 (*cf.* eq 5).

TABLE I: Tl^+ Binding Constants for the Ionophores.[a]

Ionophore	K_s Values (M^{-1})	
	Methanol	Ethanol
Valinomycin	$(2.3 \pm 0.5) \times 10^4$	$(4.2 \pm 0.2) \times 10^4$
18-Crown-6	$(3.7 \pm 0.7) \times 10^4$ (1.22)[b]	$(7.1 \pm 0.5) \times 10^4$ (0.94)[b]
Nonactin	$(1.4 \pm 0.2) \times 10^4$	
Monactin	$(3.7 \pm 0.6) \times 10^4$	
Dinactin	$(7.1 \pm 1.1) \times 10^4$	
Monensin$^-$	$(2.6 \pm 0.6) \times 10^4$	
Dibenzo-30-crown-10	$(2.5 \pm 0.6) \times 10^4$[c]	
Enniatin B	$<1 \times 10^3$	
X537A$^-$	$(2.4 \pm 0.3) \times 10^4$[d] (1.5)[e]	$(2.5 \pm 0.4) \times 10^6$[d] (1.3)[e]

[a] Where not otherwise noted, the fluorescence decreases to less than 10% of its original value upon complexation. The K_s value given for valinomycin is somewhat larger than that reported by Grell *et al.* (1972). [b] Enhancement, defined as the ratio of total fluorescence for the complexed and uncomplexed species. As noted in the text, the fluorescent maximum undergoes a blue shift upon complexation. [c] In agreement with value of Chock (1972) within the experimental error. [d] Based on the enhancement of the ionophore fluorescence. [e] Enhancement of total ionophore fluorescence.

The competition between Tl^+ and the cations Na^+, K^+, Rb^+, and Cs^+ for ionophore complexation was analyzed according to eq 4 to determine the ratio of the stability constants $K_{s(M^+)}/K_{s(Tl^+)}$. The results are shown in Table II. The values of $K_{s(M^+)}$ calculated for valinomycin for K^+, Rb^+, and Cs^+ are identical with those determined by Grell *et al.* (1972) for methanol at 25° using changes in the circular dichroism of the ionophore as a measure of complexation.

In the solvent systems studied here, the Tl^+ fluorescence was quenched by $CHCl_3$, acetone, and $MnCl_2$, but not by diethyl ether. The concentration dependence of the quenching process obeyed the following relationship

$$Fl_0/Fl = 1 + [A]K_q \qquad (7)$$

where Fl_0 and Fl are the total fluorescence signals in absence and presence of the added quencher, A. The values of the quenching constants, K_q, for these three species are given in Table III. It is noted that $CHCl_3$ quenches the excited state of Tl^+ complexed to 18-crown-6 just as efficiently as it quenches the excited state of free Tl^+.

The fluorescent signal of the ionophore X537A has been shown to be sensitive to both cation complexation (Degani *et*

TABLE II: Ion Specificity Values Normalized to Tl^+ for Methanol at 25°.

Ionophore	$K_{s(M^+)}/K_{s(Tl^+)}$ Where M^+ =			
	Na^+	K^+	Rb^+	Cs^+
Valinomycin	<0.1	2.7 ± 0.8[a]	5.7 ± 0.8[a]	0.9 ± 0.2[a]
Nonactin	0.04 ± 0.01	0.55 ± 0.10	0.46 ± 0.08	0.12 ± 0.02
Monactin	0.013 ± 0.003	0.69 ± 0.15	0.27 ± 0.08	0.11 ± 0.03
Dinactin	0.012 ± 0.003	0.77 ± 0.08	0.22 ± 0.02	0.04 ± 0.01
Monensin$^-$	2.8 ± 0.2	1.2 ± 0.2	0.60 ± 0.15	0.20 ± 0.05

[a] Calculated $K_{s(M^+)}$ values are in agreement with the results of Grell *et al.* (1972).

TABLE III: Effect of Solvent on Spectral Parameters and Quenching Constants.[a]

Species	Solvent	ϵ ($\times 10^{-3}$ M^{-1} cm^{-1})	Q	Abs Max. (mμ)	Emission Max. (mμ)	Quencher	K_q (M^{-1})
Tl^+	H_2O	3.8	0.17	214	368	$CHCl_3$	>500
Tl^+	Methanol	4.4	0.029	218	358	$CHCl_3$	188 ± 20
Tl^+-(18-crown-6)	Methanol	3.2[b]	0.048[b]	218	305[b]	$CHCl_3$	160 ± 15
Tl^+	Methanol	4.4	0.029	218	358	Acetone	27 ± 1
Tl^+	Ethanol	3.9	0.059	222	353	$CHCl_3$	24 ± 2
Tl^+	1-Propanol	4.2	0.032	222	333	$CHCl_3$	170 ± 20
TlCl	H_2O	3.8	0.13	225	395	Mn^{2+}	210 ± 30

[a] Values of the emission maximum and the total fluorescence integrated over the emission spectrum were obtained on the FICA instrument. Quantum yield values, Q, were calculated from the total fluorescence and ϵ values, using $Q(H_2O) = 0.17$ (Steffen and Sommermeyer, 1968) as the standard. [b] Complexation resulted in the appearance of a second absorption centered at 214 mμ with a half-width of *ca.* 7 mμ and $\epsilon \sim 3 \times 10^3$, indicating possible interaction between the C–O–C absorption of 18-crown-6 (210 mμ) and the Tl^+ absorption. The experiments reported here are based on excitation of the latter. Complexation with the ionophores of this study resulted in no change in the Tl^+ absorption spectrum.

al., 1973; Haynes and Pressman, 1974a) and to the polarity of the medium (Haynes and Pressman, 1974a). The binding constants of several monovalent and divalent cations for X537A have recently been reported (Degani *et al.*, 1973) and these values have been confirmed in the present study. The corresponding values for the solvent ethanol are given in Table IV. Competition experiments with X537A and other ionophores using the data of Table IV gave an independent confirmation of the data of Tables I and II.

TABLE IV: Binding Constants for $X537A^-$ in Ethanol at 25°.[a]

Cation	K_s (M^{-1})	Fl_∞/Fl_0
Na^+	$(1.5 \pm 0.7) \times 10^6$	1.6
K^+	$(1.4 \pm 0.2) \times 10^6$	1.2
Rb^+	$(1.2 \pm 0.2) \times 10^6$	1.3
Cs^+	$(0.95 \pm 0.15) \times 10^6$	1.5
Tl^+	$(2.5 \pm 0.4) \times 10^6$	1.3
Mg^{2+}	$(3.4 \pm 1.0) \times 10^5$	1.2
Ca^{2+}	$(2.7 \pm 1.5) \times 10^5$	0.8
Sr^{2+}	$(1.4 \pm 0.1) \times 10^5$	1.5
Ba^{2+}	$(4.6 \pm 0.2) \times 10^5$	1.1

[a] The determinations were made at an ionophore concentration of 2×10^{-6} M, under which condition the ionophore is in the −1 charged form. Excitation and emission were at 310 and 415 mμ, respectively. The cations were added as chloride salts.

Discussion

The present study has shown that the fluorescent signal of Tl^+ can be used as a convenient and sensitive indicator for ionophore complexation of Tl^+ and other cations in alcohol solvents. Furthermore, the K_s values for K^+ and Tl^+ for the various ionophores are quite similar, indicating that Tl^+ acts as a "probe for K^+."

Ion Specificity. The comparisons of Table II show that for the ionophores enniatin B, nonactin, monactin, dinactin, and monensin the Tl^+ binding consants are considerably larger than those for Rb^+, although these ions have identical radius (1.47 Å). For the ionophores valinomycin, the Rb^+ values are considerably larger than the Tl^+ values. These differences can not be rationalized in terms of the number of ligands or type of ligands involved in complexation: Valinomycin and enniatin contains six carbonyl ligands with a similar disposition about the complexed ions (*cf.* Haynes *et al.* 1971) but show different Tl^+/Rb^+ specificities (*cf.* Grell *et al.*, 1972). It seems that considerations of the size of the cavity produced by the ionophore and on the radius of the complex can predict the complexation energy only within ±1 kcal and can predict the binding constant only within a factor of 5. This point has been treated in more detail elsewhere (Haynes and Pressman, 1974b). Table II shows that the Tl^+/K^+ specificity ratios for all the ionophores between 0.55 and 2.7, and Tl^+ and K^+ (ionic radius, 1.33 Å) are similar where complexation with the membrane carriers is concerned.

Mechanism of Quenching. The quenching resulting from ionophore complexation could then result (a) from changes in the energy position contours in the excited state as the result of complexation, (b) from direct interaction between Tl^{+*} and the complexing ligands during the lifetime of the excited state, re-

sulting in changes in the energy contours, or (c) from radiationless energy transfer (Förster, 1951). These three alternatives are considered in terms of the information in Table II below.

Possibility (a) is difficult to evaluate. A $^{23}Na^+$ nuclear magnetic resonance study (Haynes *et al.*, 1971) of Na^+ complexation with the same ionophores used in the present study showed that the complexes are characterized by low quadrupole coupling constances, indicating that the Na^+ nucleus is subjected to small electric field gradients and that the distribution of complexing oxygens about the Na^+ is very symmetrical. This study also showed that complexation with all ionophores studied except valinomycin shifted the Na^+ resonance signal upfield, indicating that the degree of covalent interaction between the Na^+ and the ionophores is smaller than that between the uncomplexed Na^+ and the methanol molecules in its solvation shell. Both these observations, extrapolated to Tl^+, would indicate that the atomic orbital interaction between the complexed Tl^+ and the complexing oxygens in the ground state is minimal.

The data of Table III were collected to evaluate the possibility (b) that direct interaction between Tl^{+*} and the complexing ligands is responsible for the quenching. The fluorescence lifetime of Tl^{+*} in water has been determined as *ca.* 10^{-7} sec (D. Haynes and H. Staerk, unpublished data). The observation of rapid equilibration between Tl^{+*} and its chloride complexes (Steffen and Sommermeyer, 1968) indicates that ligand (Cl^- for H_2O) substitution occurs within the lifetime of the excited state. This would also be expected if Tl^+ could be classed with the cations Na^+, K^+, Rb^+, and Cs, which have first-order rate constants of the order of 10^9 sec^{-1} for water (or methanol) substitution in the inner sphere solvation (Diebler *et al.*, 1969). Evidence for this has been given by Chock (1972) in a study of the complexation kinetics of dibenzo-30-crown-10. The high quantum yield of Tl^+ in water and its small sensitivity to substitution of l-alcohols in the inner solvation sphere (*cf.* Table III) indicate that ligand substitution *per se* produces no substantial quenching.

Table III shows that $CHCl_3$, acetone and Mn^{2+} are effective in quenching Tl^+ fluorescence. The independence of this quenching reaction on the total Tl^+ concentration indicates that this reaction proceeds according to

$$Tl^{+*} + A \xrightarrow{k_1} Tl^+ + A \qquad (8)$$

and that complexation in the ground state is not involved. No quenching was found with diethyl ether which was considered as a model for the crown compounds. The quenching reaction with $CHCl_3$ probably involves formation of an encounter complex. For acetone, with a broad absorption band centered at 270 mμ, either encounter complex formation or energy transfer (Forster, 1951) to the carbonyl chromophoric group could be responsible for the quenching process. With Mn^{2+}, the quenching is probably the result of paramagnetic interaction. Using $k_f \sim 10^7$, and the K_q ($= k_1/k_f$) values of Table III, the k_1 values between 10^8 and 10^9 M^{-1} sec^{-1} are estimated. Since these values are in the range of diffusion control, these species must be extremely effective in quenching the excited state of Tl^+ when they are in the immediate vicinity of the cation.

That the quenching of Tl^+ fluorescence in the complexed state is attributed to the influence of the carbonyl groups is concluded by combining the observations of quenching with acetone and no quenching with diethyl ether and the observation that all the ionophores which quench contain carbonyl groups.[1] No quenching was observed with 18-crown-6 which contains only ether linkages. Quenching could be due to energy transfer from the higher excited states of Tl^+ to the complexing carbonyl oxygens which have a considerable optical absorption in the region of 230 mμ and which lie within *ca.* 3 Å of the complexed cation (*cf.* Haynes *et al.*, 1971). In absence of more specific information supporting other mechanisms the quenching mechanism (c) is thus preferred.

Upon complexation with 18-crown-6, a blue shift in the fluorescence emission and a small increase in the quantum yield are observed. Quenching would not be expected since the optical absorption of the ether groups at 230 mμ is smaller and since diethyl ether does not act as a quencher. The blue shift observed upon complexation with 18-crown-6 correlates with the blue shift observed upon substitution of 1-propanol for water in the hydration sphere of Tl^+. The observation that Tl^{+*}-(18-crown-6) is quenched as readily as Tl^{+*} by added $CHCl_3$ indicates that the complexed cation is fairly exposed to the solvent.

Conclusions

The present study has shown that Tl^+ acts as a "probe for K^+" regarding its complexation reactions with the ionophores in organic solvents. Furthermore, the probe offers a high sensitivity, allowing complexation to be registered unambiguously in the concentration range 10^{-3}–10^{-7} M. This property may be useful in characterizing the complexation reactions of new ionophores or suspected cation carriers.

The desire to use Tl^+ fluorescence in the characterization of cation–membrane interaction in biological membrane systems contributed a significant portion of the motivation for this study. The following properties of Tl^+ make such an application inauspicious. (a) The excitation maximum of Tl^+ lies at low wavelengths, rendering the fluorescent signal sensitive to inner filter effects from other components of the system. (b) The fluorescence emission has considerable overlap with the absorption bands of other chromophoric groups prevalent in biological membranes. (c) The cation complexes with many ligands such as chloride, phosphate and acetate. (d) The long lifetime of the excited state makes the fluorescence sensitive to quenchers in millimolar concentrations. On the other hand, careful use of the fluorescent signal may be useful in (b) establishing distances between Tl^+ and specific chromophoric groups and in (c and d) gaining information about compartmentalization of ions in biological membranes. Furthermore, the lifetime of Tl^+ in aqueous media (*ca.* 10^{-7} sec) is about 100 times greater than the time resolution of commercially available fluorescence lifetime instrumentation, allowing even severely quenched Tl^+ fluorescence affected by factors (b, c, and d) to be isolated and studied in the time domain.

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[1] Complexation with dibenzo-30-crown-10 resulted in fluorescence quenching at 355 mμ, but there was evidence for the appearance of a new band at 290 mμ, as found with 18-crown-6. However, the intensity of this bad represents only *ca.* 5% of the fluorescent intensity before complexation.

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Evidence for a Microviscosity Increase in the *Escherichia coli* Cell Envelope Caused by Colicin E1†

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ABSTRACT: Colicin E1 added at a low multiplicity to sensitive cells causes an increase in the polarization of fluorescence of the cell-bound probe *N*-phenyl-1-naphthylamine. The dye seems localized in the hydrocarbon regions of the cell envelope as inferred from its known properties and from the measurement of an abrupt change in fluorescence polarization at approximately 16°. An order-disorder transition in the lipid of cells of similar fatty acid composition has been documented by others to occur in this temperature range. Because the fluorescence lifetime of the cell-bound dye also increases in the presence of colicin E1, it is concluded that colicin causes an increase in the rotational relaxation time of the dye. Colicin E1 added at high multiplicities (10 μg/ml) causes a similar increase in fluorescence polarization from 8 to 26° and does not alter the magnitude of the order-disorder transition or change its midpoint by more than 1°. It is calculated from the Perrin equation that colicin causes the rotational relaxation time to increase from 3.6 to 6.5 nsec and from 6.6 to 10.5 nsec when added at 21 and 12.5°, respectively. The increase in rotational relaxation time could be explained by (1) a colicin-induced increase in microviscosity in the cell envelope or (2) a redistribution of the dye to regions of the envelope with higher microviscosity. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), which causes a decrease in intracellular ATP under the conditions of the fluorescence experiment, also causes an increase in fluorescence polarization and rotational relaxation time when added in the absence of colicin. The question of whether a colicin-induced increase in microviscosity in the cell envelope could be a physical mechanism responsible for the inhibitory effects of colicin E1 is discussed.

There is very little known about the physical and chemical details of colicin-induced structural changes in the cell envelope which are associated with or are the direct cause of specific biochemical inhibitory events. One approach to gaining information on this problem has been through the use of fluorescence probes. Colicin E1, which has been purified as a protein of mol wt 56,000 (Schwartz and Helinski, 1971), causes an increase in fluorescence intensity and a blue shift in emission maxima of the probes 8-anilino-1-naphthalenesulfonate and *N*-phenyl-1-naphthylamine (Cramer and Phillips, 1970; Phillips and Cramer, 1973; Cramer *et al.*, 1973). The basic properties of the colicin E1 induced fluorescence changes discussed in the above references are as follows. (1) The existence of a probe fluorescence increase caused by colicin E1 correlates very well with the circumstances under which the adsorbed colicin is lethal. In particular, colicin E1 does not cause a probe fluorescence change when added to *colicinogenic* or *tolerant* strains. (2) The fluorescence intensity increase arises from cell-bound dye which in the case of the uncharged *N*-phenyl-1-naphthylamine is very likely localized in the cell envelope. (3) Dye binding and emission spectra studies show that the increase in probe fluorescence caused by colicin E1 is mostly due to a change in the environment of the bound dye and not to an increase in dye uptake. (4) The rate of the fluorescence increase is similar to the rate of the colicin E1 induced decrease in intracellular ATP and potassium levels. (5) The fluorescence

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