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Monovalent Cation–A23187 Equilibria in MeOH–H_2O Solutions and on Phospholipid Vesicles

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Although the value of ionophore A23187 as a research tool arises from its transport specificity for divalent cations, the source and limits of this specificity have not been thoroughly studied. Previous work has shown that A23187 can complex monovalent cations [1], extract them to a bulk organic phase [2] and produce monovalent cation transport across certain biological membranes [3]. To elucidate the basis of the normal divalent cation specificity of this ionophore, detailed studies of the mechanism of monovalent cation transport will be necessary. In this communication we report equilibrium constants for complexation reactions involved in the transport of monovalent cations by this compound.

Experimental

Complexation constants in MeOH–H_2O mixtures were determined from absorbance measurements whereas fluorescence methods were employed for suspensions of small, unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC). Nonaqueous pH* values in MeOH–H_2O mixtures were established and measured as described previously [4]. DMPC vesicles were prepared by sonication [5] and purified by ultracentrifugation [6].

Results

Table I shows 1:1 complexation constants of the ionophore with Li^+ and Na^+. The values in MeOH–H_2O mixtures were determined as conditional constants, K'_{MA}, by titration of A23187 with excess metal ion over the pH* range of 6–10. The equilibrium constants, K^*_{MA}, for the reaction $M^+ + A^- \rightleftarrows MA$ were obtained from the conditional values utilizing the relationship $K'_{MA} = K^*_{MA}(1 + K^*_{H1}a^*_H)$, where K^*_{H1} and a^*_H are the mixed-mode protonation constant and hydrogen ion activity in a given solvent, respectively. The complexation constant in suspensions of DMPC vesicles, K^b_{MA}, is defined by the reaction $M^+_{aq} + A^-_b \rightleftarrows MA_b$ where the subscripts aq and b denote solution and membrane-bound species, respectively. Constants for the bound compound were determined under conditions where the fraction of ionophore in the aqueous phase is small. The species A^-_b was generated by utilizing a high aqueous phase pH and then titrated with excess metal ion.

TABLE I. Complex Formation Constants of Ionophore A23187 with Li^+ and Na^+ at 25 °C.[a]

Medium	log K_{MA}	
	M = Li^+	M = Na^+
65% MeOH–H_2O[b]	2.53 ± 0.04[c]	1.95 ± 0.08
80% MeOH–H_2O	3.08 ± 0.05	2.36 ± 0.07
95% MeOH–H_2O	3.54 ± 0.03	–
100% MeOH[d]	4.1	3.4
DMPC vesicles	3.22 ± 0.04	–

[a] Ionic strength maintained at 0.05 *M* with $(C_2H_5)_4NClO_4$, buffer composition similar to that cited in Ref. 4. [b] Weight % MeOH. [c] Error given as 1 std. dev. [d] Taken from Ref. 1, $\mu \sim 0$.

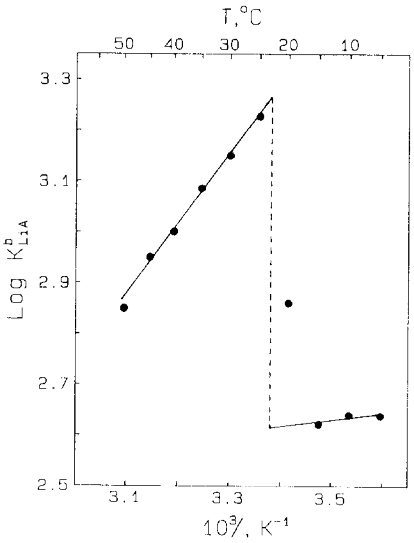

Fig. 1. The effect of temperature on log K_{LiA} values was determined as described in the text. The dashed line shows the location of the gel to liquid phase transition temperature of DMPC vesicles.

The effect of temperature on K^b_{LiA} is shown in Fig. 1 as a Vant'Hoff plot. The pronounced discontinuity in these data is located near the gel to liquid phase transition temperature (T_c) of DMPC vesicles (23 °C). The ΔH and ΔS values obtained from these data above T_c, are equal to –6.4 Kcal/mol and –6.7 cal/degree mol, respectively.

Discussion

The smooth increase in log K^{*}_{MA} with decreasing solvent polarity is similar to trends observed for other polyether ligands [7] and analogous to the effect of solvent polarity on K^{*}_{Hl} [4]. The stability constants of the LiA complex are 4–5 fold greater than the NaA complex, in contrast to other carboxylic acid ionophores [1]. The stability of the Li^+ complex on vesicle membranes is similar to the solution value in 80–85% MeOH–H_2O, as is the case with the protonation constant [4]. This finding suggests a predominantly interfacial location of the complex. However, the discontinuity in log K^{b}_{LiA} indicates that portions of complex penetrate the membrane acyl group region [8].

The present conditions do not allow observation of the 2:1, ionophore:cation, complexes with monovalent cations which were observed in bulk solvent extraction experiments [2]. These higher order complexes may be of greater interest for the transport of monovalent cations by A23187 since preliminary experiments show the rate of A23187-dependent Li^+ transport into mitochondria is a second order function of the ionophore level.

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Complex Formation in the Al^{3+}–Citrate System

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TABLE I. Formation Constants Defined According to the General Equilibria: $pH^+ + qAl^{3+} + rH_3L \Leftrightarrow (H^+)_p(Al^{3+})_q(H_3L)_r$; β_{pqr}. The errors given correspond to $3\sigma(\log \beta_{pqr})$.

Complex	p	q	r	log($\beta_{pqr} \pm 3\sigma$)
H_2L^-	−1	0	1	−2.769 ± 0.003
H_2L^{2-}	−2	0	1	−6.850 ± 0.003
L^{3-}	−3	0	1	−12.067 ± 0.004
$AlHL^+$	−2	1	1	−2.68 ± 0.024
AlL	−3	1	1	−4.925 ± 0.008
AlL_2^{3-}	−6	1	2	−12.53 ± 0.12
$Al_3(OH)_4)_3^{4-}$	−13	3	3	−21.77 ± 0.019

Aluminium is regarded as a non-essential element for living organisms and has been considered as non-toxic in biological systems. This situation has changed, however, with the suspicion that aluminium is the agent causing dialysis encephalopathy in patients undergoing haemodyalysis with domestic water supplies high in aluminium [1]. Furthermore, the characteristic pathological finding in Alzheimer's disease is an abnormal build-up of aluminium in some regions of the Alzheimer brains [2]. Also, aluminium is implicated as a metal toxic to aquatic life, possibly as the $Al(OH)_2^+$ species [3], in acidified lakes and streams.

As the toxicity of a metal is closely related to its speciation, a way to suppress the toxic effect would be to complexate the metal ion into non-toxic, stable chelate species. The aim of the present study is to show this upon stable Al complexes formed with citrate (L^{3-}) ions.

Experimental

The measurements were performed as a series of potentiometric (glass electrode) titrations in 0.6 *M* Na(Cl) at 25 °C. The following concentration ranges were investigated: $0.00025 \leqslant B, C \leqslant 0.016$ *M* (B and C stand for the total concentration of Al^{3+} and H_3L resp.). $-\lg[H^+]$ was varied within the limits $2 \lesssim -\lg[H^+] \lesssim 8$.

Results and Discussion

An equilibrium analysis of the present system showed that besides a series of mononuclear complexes, *viz.* $AlHL^+$, AlL and AlL_2^{3-} a very stable trinuclear complex is formed. Calculations based upon graphical ('core + links' mechanism [4]) as well as least squares computer calculations (LETAGROPVRID [5–7]) showed the composition of this complex to be $Al_3H_{-4}L_3^{4-}$) (tentative composition $Al_3(OH)_4L_3^{4-}$).

Formation constants for the different species are given in Table I.

The stability of $Al_3(OH)_4L_3^{4-}$ implies that with $4 \leqslant -\lg[H^+] \leqslant 7$, Al is almost quantitatively bound in this complex (provided $C/B \gtrsim 1$). As a conse-