Pure & Appl. Chem., Vol. 65, No. 3, pp. 579–584, 1993.
Printed in Great Britain.

Complexation chemistry of ionophores A23187 and ionomycin

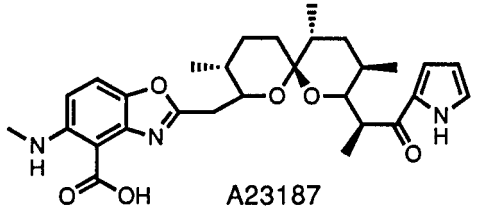
Richard W. Taylor[1], Douglas R. Pfeiffer[2], Clifford J. Chapman[2], Mark E. Craig[1], and Timothy P. Thomas[1]

[1] Department of Chemistry, University of Oklahoma, Norman, OK 73019 USA.
[2] The Hormel Institute, University of Minnesota, Austin, MN 55912 USA.

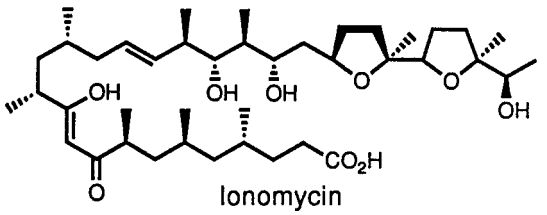
Abstract - A23187 and ionomycin are naturally occurring polyether carboxylic acid ionophores capable of transporting divalent cations across biological membranes. The equilibrium constants for protonation of A23187 and formation of 1:1 complexes with monovalent and divalent cations have been determined in methanol-water mixtures and in suspensions of phospholipid vesicles. With ionomycin, equilibrium constants for protonation and formation of 1:1 complexes with divalent cations have been determined in methanol-water mixtures.

INTRODUCTION

Ionophores A23187 and ionomycin are members of the polyether carboxylic acid class of antibiotics (ref. 1). These two compounds show a high degree of selectivity for the transport of divalent cations across artificial and biological membranes (refs. 2,3). The solid-state structures of these ionophores and their complexes with several divalent cations have been reported (refs. 4-8).



A23187



Ionomycin

A23187 and ionomycin have one and two ionizable protons, respectively, and form neutral $M(A23187)_2$ and M(ionomycin) complexes with divalent cations. The primary mode of cation transport for these two ionophores is by electroneutral exchange, i.e., $M^{2+}/2H^+$ or $M^{2+}/2M^+$ (refs. 9,10) The overall transport cycle may be considered to consist of a number of component reactions. In addition to the translocation of the protonated and complexed forms of the ionophore, steps involving protonation and metal-ion complexation reactions at each membrane-water interface are required to complete the cycle (ref 9). In order to gain insight into the transport mechanism(s) and the basis of the transport selectivity, the equilibrium and kinetic parameters of the individual steps

should be measured. This report provides a summary of equilibrium constant data obtained in our laboratories. The relevant equilibria involving protonation and metal-ion complexation are given in equations 1-6.

$$H^+ + L^{x-} \rightleftharpoons HL^{1-x} \quad (1) \qquad H^+ + HL^{1-x} \rightleftharpoons H_2L^{2-x} \quad (2)$$

$$M^{2+} + L^{x-} \rightleftharpoons ML^{2-x} \quad (3) \qquad ML^{2-x} + L^{x-} \rightleftharpoons ML_2^{2-2x} \quad (4)$$

$$ML^{2-x} + H^+ \rightleftharpoons MLH^{3-x} \quad (5) \qquad ML^{2-x} + OH^- \rightleftharpoons ML(OH)^{1-x} \quad (6)$$

where M is the metal ion and L is the unprotonated, uncomplexed ionophore (A23187, x = 1; ionomycin, x = 2). The equilibrium constants for these reactions were determined in methanol-water mixtures and, in some cases, in aqueous suspensions of phospholipid vesicles. Methanol-water (M/W) solvent mixtures ranging from 35-95 wt% methanol were employed because of; (i) the limited solubility of the ionophores in pure water, (ii) the existence of operational pH scales (pH*) for these solvent mixtures (refs. 11,12), and (iii) the availability of data for other ligands in these solvents for comparison (ref. 13). In order to characterize the reactions of the membrane-associated ionophore, aqueous suspensions of unilamellar phospholipid vesicles were used. Vesicles composed of a single phospholipid, dimyristoylphosphatidylcholine (DMPC) or palmitoyloleoylphosphatidylcholine (POPC), were used as a model system. Comparison of the results obtained in methanol-water solutions with those from vesicle suspensions will help to reveal any effects due to specific interactions between the ionophore and/or ionophore complexes with the phospholipid membrane. Metal ions from the alkaline-earth (Mg, Ca, Sr, Ba), transition-metal ion (Mn, Co, Ni, Cu), and post-transition-metal (Zn) families were chosen to include those of biological importance and a wide range of cation sizes, coordination numbers and geometries, and solvent exchange rates.

METHODS

The experimental procedures used to measure the equilibrium constants have been reported. Spectrometric titrations employed fluorescence, absorbance, and circular dichroism techniques (refs. 14-17). Procedures for pH* measurements in methanol-water solvents have been described (refs. 11-14). Small (SUV) and large (LUV) unilamellar vesicles were prepared by sonication (DMPC) and extrusion (POPC) techniques, respectively (refs. 14,18-20). Titration data was analyzed using non-linear least-squares techniques (refs. 14-17,21).

PROTONATION CONSTANTS

The protonation constants of A23187 have been measured in methanol-water mixtures ranging from 0-100% M/W (refs. 14,22) and DMPC (ref. 14) and POPC vesicle suspensions using fluorescence, UV-Vis absorbance and circular dichroism spectroscopic techniques. The protonation constants of ionomycin have been measured using UV-Vis spectrometric and pH* titration techniques (ref. 17). Selected values of the protonation constants are listed in Table 1. The protonation constants are defined by the expression $K_{Hi} = [\ H_iL\ ]/[\ H_{i-1}L\ ]a_H$, where a_H is the hydrogen ion activity obtained from the experimental pH* reading, i.e., $a_H = 10^{-pH^*}$. For A23187 the constants K_{H1} and K_{H2} refer to the stepwise protonation of the carboxylic acid and N-methylamino groups, respectively (ref. 14). With regard to ionomycin, K_{H1} and K_{H2} refer to the stepwise protonation of the β-diketone and carboxylic acid groups, respectively

Table 1. Protonation constants (log K_{Hi}) of ionophores A23187 and ionomycin[a].

	medium	A23187	ionomycin[b]
K_{H1}	H_2O	5.69[c]	~10.0
	80% M/W	7.84[c]	11.94
	100% M/W	10.7[d]	
	DMPC	7.85[c]	
	DMPC	7.67[e] (7.55)[f]	
	POPC	7.86	
K_{H2}	80% M/W	1.7[c]	6.80
	100% M/W	3.3[d]	

[a] 25.0 °C, μ = 0.050 (Et_4NClO_4).
[b] ref. 17. [c] ref. 14. [d] μ = 0.10, ref. 22.
[e] 35.0 °C, ref. 14. [f] 34.0 °C, ref. 23.

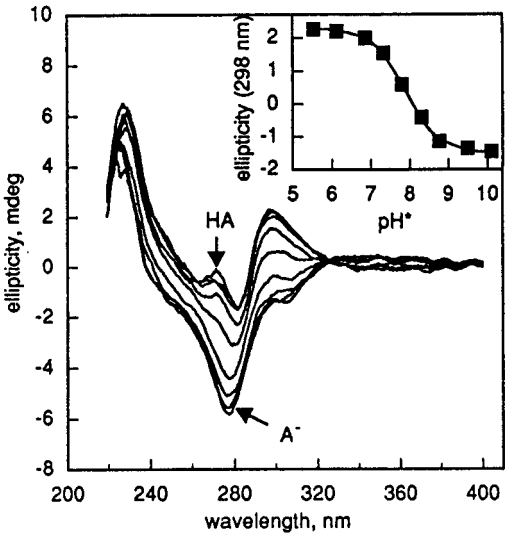


Fig. 1. CD spectra of 50 μM A23187 in 80% M/W at various pH* values. Inset; plot of ellipticity at 298 nm.

(ref. 17). Note the similarity of K_{H1} values for A23187 in 80% M/W with those for DMPC and POPC vesicle suspensions. Results from previous studies indicate that the benzoxazole portion of the HL and L^- forms of A23187 have an interfacial location in phosphatidylcholine-type membranes (ref. 24). Circular dichroism studies on A23187 also reveal information about the environment, and conformation(s) of A23187 (ref. 25). Figure 1 shows that changes in the ellipticity as a function of pH for A23187 can be used to determine the value of K_{H1} in 80% M/W. Similar titrations with POPC vesicles give the analogous value for K_{H1} (see Table 1). In contrast to the relatively small differences in the CD spectra of the neutral (HL) and anionic (L^-) forms of A23187, Fig. 2 reveals that solvent polarity has a much more pronounced effect of the spectra of HL. NMR studies in a non-polar solvent ($CDCl_3$) indicate that A23187 adopts a predominantly closed conformation similar to that found in the solid state (refs. 5,26-28). Consideration of the CD spectra shown in Fig. 2. suggests that

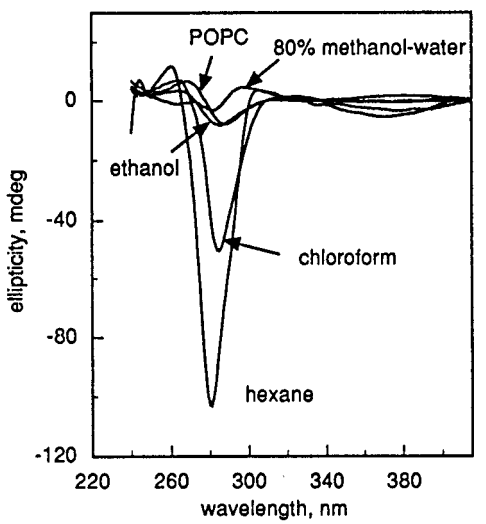


Fig. 2. CD spectra of 50 μM A23187 in. various solvents and in POPC vesicles.

the conformation(s) of A23187 in POPC vesicles is closer to that found in the more polar solvents, 80% M/W and ethanol, than the closed conformation found in nonpolar environments. These findings are also consistent with an interfacial location for membrane-bound A23187 (ref. 24).

COMPLEXATION WITH MONOVALENT CATIONS

Although A23187 and ionomycin are usually described as divalent (calcium) cation ionophores, they also form stable 1:1 complexes with monovalent cations such as Li^+, Na^+ and K^+ (refs. 10, 15, 22). For A23187, values of K_{ML} with Li^+ and Na^+ were determined in 65-95% M/W and in suspensions of DMPC vesicles (ref. 15). The latter values, along with those in 80% M/W and 100% methanol (ref. 22) are listed in Table 2. The values of K_{ML} decrease as the charge density of the alkali cation decreases. Furthermore, the Li^+/Na^+ selectivity (K_{LiL}/K_{NaL}) increases from ~4 in 80% M/W to ~80 in DMPC vesicles. This change in selectivity indicates that interactions of the ML and/or M^+ species with the membrane may play a significant role in establishing the overall transport activity of A23187 (ref. 15) Although no quantitative data are currently available for the equilibrium constants of alkali cations with ionomycin, spectroscopic and transport studies provide qualitative evidence for complexation with Li^+, Na^+ and K^+ (ref. 10).

Table 2. Stability constants of A23187 with monovalent cations[a].

M^+	medium	log K_{ML}
Li^+	100% M/W	4.1[b]
	80% M/W	3.08
	DMPC	3.38
Na^+	100% M/W	3.4[b]
	80% M/W	2.36
	DMPC	1.32
K^+	100% M/W	2.4[b]

[a] 25.0 °C, μ = 0.050 (Et_4NClO_4), ref. 15. [b] $\mu \simeq 0.0$, ref. 22.

COMPLEXATION WITH DIVALENT CATIONS

The equilibrium constants, K_{ML}, for 1:1 complex formation (eq 3) between divalent cations and A23187 and ionomycin are listed in Tables 3 and 4, respectively. The pattern of K_{ML} values, shown in Fig. 3, is similar to that found for many simple chelating ligands (refs. 30,31). For each ionophore, the K_{ML} values for Ca^{2+} and Mg^{2+} are toward the lower end of the observed range, a situation often found with ion carriers. An unusual feature of A23187 complexation chemistry is that the equilibrium constant, K_{ML2}, for the addition of the second A23187 ligand (eq 4) is equal to or larger than K_{ML} (ref. 22). This may be due to the presence of hydrogen bonds between the two molecules of A23187 in the ML_2 complex (refs. 6,7,26-28). With transition metal- and lanthanide ions, M^{n+}, A23187 forms ternary complexes with the stoichiometry $ML(OH)^{n-2}$ (ref. 29). With Zn^{2+}, the value of K_{MLOH}, defined by eq 6, is 4.5×10^7

in 80% M/W (ref. 16). Ionomycin forms ternary, protonated, complexes (eq 5) of the form MLH^+ with the cations listed in Table 4. The presence of these species must be taken into account in any consideration of the mechanism of cation transport and the factors affecting transport efficiency and selectivity.

Table 3. Stability Constants of A23187 with divalent cations at 25.0 °C.

	log K_{ML}		
M^{2+}	80% M/W[a]	DMPC vesicles[b]	
Ba^{2+}	3.60		(3.68)[c]
Sr^{2+}	3.86		(3.90)[c]
Ca^{2+}	4.50	5.59	(5.19)[c]
Mg^{2+}	4.55	5.32	(4.82)[c]
Mn^{2+}	6.08	6.59	
Co^{2+}	7.07	7.23	
Ni^{2+}	7.54	7.35, 7.59[d]	
Cu^{2+}	9.77		
Zn^{2+}	6.79	~6.0	

[a] 25.0 °C, ref. 16. [b] 30.0 °C, SUV.
[c] 34.0 °C, SUV, ref. 23.
[d] 35.0 °C, LUV (DMPC).

Table 4. Stability Constants of ionomycin with divalent cations in 80% M/W at 25.0 °C[a].

M^{2+}	log K_{ML}	log $K_{ML(H)}$[b]
Sr^{2+}	5.30	8.40
Ca^{2+}	6.27	8.32
Mg^{2+}	6.95	7.33
Mn^{2+}	8.60	6.34
Co^{2+}	9.59	6.08
Ni^{2+}	10.25	5.95
Cu^{2+}	(4.3)[c]	
Zn^{2+}	9.73	6.47

[a] μ = 0.050 (Et_4NClO_4), ref. 17.
[b] For $ML + H^+ \rightleftharpoons MLH^+$; eq 5.
[c] Conditional constant at pH* = 3.5.

Table 3 lists the formation constants, K_{ML}, for several divalent cations with A23187 in DMPC vesicle suspensions. The pattern of the values, shown in Fig. 3, is qualitatively the same as that found for the corresponding values in 80% M/W. With Ni^{2+}, the values of K_{ML} obtained using small and large unilamellar vesicles are within a factor of two of each other. This indicates that the nature of the acyl chains has little effect on ionophore equilibria, a conclusion consistent with an interfacial location for the ionophore.

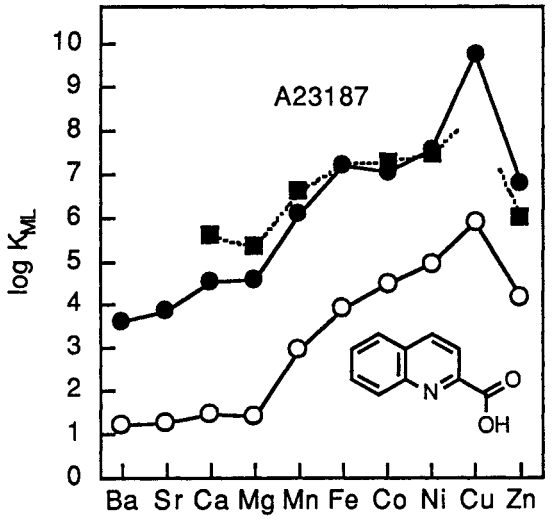


Fig. 3. Plots of the values of K_{ML} for divalent cations with A23187 in 80% M/W (●,●,●) and DMPC vesicles (■,■,■) and for the simple chelator , quinaldic acid in water (O,O,O). The values of K_{ML} for quinaldic acid were taken from Critical Stability Constants, A. E. Martell and R. M. Smith , Eds., Vol. 1, p. 372, Plenum, New York (1974).

Acknowledgement

This research was supported by U. S. Public Health Services Grants GM-24701, HL-08214 and HL-49181, the Hormel Foundation, and the OU Research Council.

REFERENCES

1. J. W. Westley, The Polyether Antibiotics: Naturally Occurring Acid Ionophores, J. W. Westley, Ed., Vol. 1, pp. 1-41, Marcel Dekker, New York (1982).
2. R. W. Taylor, R. F. Kauffman and D. R. Pfeiffer, The Polyether Antibiotics: Naturally Occurring Acid Ionophores, J. W. Westley, Ed., Vol. 1, pp. 103-184, Marcel Dekker, New York (1982).
3.. C. M. Liu and T. E. Hermann, J. Biol. Chem. 253, 5892-5894 (1978).
4. B. K. Toeplitz, A. I. Cohen, P. T. Funke, W. L. Parker and J. Z. Gougoutas, J. Am. Chem. Soc. 101, 3344-3353 (1979).
5. M. O. Chaney, P. V. Demarco, N. D. Jones and J. L. Occolowitz, J. Am. Chem. Soc. 96, 1932-1933 (1974).
6. G. D. Smith and W. L. Duax, J Am. Chem. Soc. 98, 1578-1580 (1976).
7. M. Alleaume and Y. Barrans, Can. J. Chem. 63, 3482-3485 (1985).
8. E. Baker, E. N. Maslen, K. J. Watson and A. H. White, J. Am. Chem. Soc. 106, 2860-2864 (1984).
9. D. R. Pfeiffer, R. W. Taylor and H. A. Lardy, N. Y. Acad. Sci. 307, 402-23 (1978).
10. R. F. Kauffman, R. W. Taylor and D. R. Pfeiffer, J. Biol. Chem. 255, 2735-2739 (1980).
11. C. L. de Ligny, P. F. M. Luykx, M. Rehback and A. A. Weineke, Recl. Trav. Chim. Pays-Bas 79, 699-712, 713-726 (1960).
12. W. J. Gelsema, C. L. de Ligny, A G. Remijnse and H. A. Blijleven, Recl. Trav. Chim. Pays-Bas 84, 647-660 (1966).
13. D. B. Rorabacher, W. J. Mackellar, F. R. Shu and Sr. M. Bonavita, Anal. Chem. 43, 561-573 (1971).
14. R. F. Kauffman, R. W. Taylor and D. R. Pfeiffer, Biochemistry 21, 2426-2435 (1982).
15. R. W. Taylor, C. J. Chapman and D. R. Pfeiffer, Biochemistry 24, 4852-4859 (1985).
16. C. J. Chapman, A. K. Puri, R. W. Taylor and D. R. Pfeiffer, Biochemistry 26, 5009-5018 (1987).
17. M. K. Stiles, M. E. Craig, S. L. N. Gunnell, D. R. Pfeiffer and R. W. Taylor, J. Biol. Chem. 266, 8336-8342 (1991).
18. C. Huang and T. E. Thompson, Methods Enzymol. 32, 485-489 (1974).
19. L. D. Mayer, M. J. Hope and P. R. Cullis, Biochim. Biophys. Acta 858, 161-168 (1986).
20. C. J. Chapman, W. L. Erdahl, R. W. Taylor and D. R. Pfeiffer, Chem. Phys. Lipids 55, 73-83 (1990).
21. S. D. Christian and E. E. Tucker, Am. Lab. 14(8), 36-41; 14(9), 31-34 (1982).
22. C. Tissier, J. Juillard, D. W. Boyd, A. M. Albrecht-Gary, J. Chim. Phys. 82, 899-906 (1985).
23. M. A. Kolber and D. H. Haynes, Biophys. J. 36, 369-391 (1981).
24. R. F. Kauffman, C. J. Chapman and D. R. Pfeiffer, Biochemistry 22, 3985-3992 (1983).
25. S. Divikar and K. R. K. Easwaran, Biophys. Chem. 27, 139-147 (1987).
26. C. M. Deber and D. R. Pfeiffer, Biochemistry 15, 132-141 (1976).
27. M. J. O. Anteunis, Bioorg. Chem. 6, 1-11 (1977).
28. A. M. Albrecht-Gary, S. Blanc-Parasote, D. W. Boyd, G. Dauphin, G. Jeminet, J. Juillard, M. Prudhomme, C. Tissier, J. Am. Chem. Soc. 111, 8598-8609 (1989).
29. C. J. Chapman, A. K. Puri, R. W. Taylor and D. R. Pfeiffer, Arch. Biochem. Biopys. 281, 44-57 (1990).
30. H. Irving and R. J. P. Williams, J. Chem. Soc. 156, 3192-3210 (1953).
31. B. L. Vallee and J. E. Coleman, Compr. Biochem. 12, 165-177 (1953).