Ionophore Properties of Cationomycin in Large Unilamellar Vesicles Studied by ^{23}Na- and ^{39}K-NMR

Anne-Marie DELORT,*[,a] Georges JEMINET,[a] Sina SARETH,[b] and Frank G. RIDDLE[b]

Laboratoire de Synthèse, Electrosynthèse et Etude de Systèmes à Intérêt Biologique, Université Blaise Pascal-CNRS,[a] UMR 6504, 63177 Aubière Cedex, France and School of Chemistry, The University of St Andrews,[b] KY169ST, U.K.
Recieved May 11, 1998; accepted July 7, 1998

Cationomycin, isolated from *Actinomadura azurea* belongs to a large family of carboxylic polyether antibiotics, transporting monovalent cations through membranes by a mobile carrier mechanism, leading globally to an H^+, M^+ exchange.

In this report the cation transporting properties of cationomycin were characterized in large unilamellar vesicles (LUVs) by ^{23}Na- and ^{39}K-NMR. Kinetic studies showed that cationomycin transported potassium more rapidly than sodium, and the more stable complex was formed with potassium at the water/membrane interface. The transport rate constants measured for cationomycin were compared with those obtained for monensin. Cationomycin transports Na^+ more slowly than monensin and has a lower stability complex with Na^+ because of the lower formation rate for the complex on the membrane surface. Our results show that transport selectivity of cationomycin is in favour of K^+ *versus* Na^+ while the reverse situation is observed for monensin.

The relationships between the ionophore properties of cationomycin and monensin with their biological activities are discussed.

Key words cationomycin; NMR; large unilamellar vesicle

Cationomycin (Fig. 1A) isolated from *Actinomadura azurea*[1—3] belongs to a large family of carboxylic polyether antibiotics, with a structure designed for the transport of monovalent cations through membranes, by a mobile carrier mechanism, leading globally to an H^+, M^+ exchange (Fig. 1C). Transport experiments in a bulk model system[4,5] gave the following order: $K^+ > NH_4^+ > Rb^+ > Na^+ > Li^+ > Cs^+$. Structural studies carried out in the solid state by X diffractometry for the thallium salt[6] or in solution by NMR for the acid form and the neutral complexes with K^+, Rb^+, Na^+, Li^+, Cs^+ and NMe_4^+[7] all indicated a globular conformation, with head-to-tail chelating interactions and the aromatic part pointing away from the rigid cavity (Fig. 1B). Also, only slight structural differences were observed between the acid and neutral complexes in solution, and it was not possible to identify any preferential conformation linked to the ionic preference.

Cationomycin presents high antibacterial and anticoccidial activities, with a surprisingly low toxicity in mice in comparison with other polyether antibiotics.[1,4] This feature makes this compound especially interesting. Structure–activity studies performed on cationomycin derivatives demonstrated the specific role of the aromatic side chain attached to C3.[5,8]

However, in the literature, the transporting abilities of cationomycin and derivatives are not well documented. The only data available concerned $^{22}Na^+$ transport in a bulk CCl_4 layer.[5] K^+ transport was not explored and we have previously shown that it is necessary to examine both Na^+ and K^+ transport to fully characterize a carboxylic ionophore antibiotic, especially to investigate the relationship between induced cation movements and biological properties.[9] It was thus of interest to study cationomycin Na^+ and K^+ transport in a relevant membrane model such as large unilamellar vesicles (LUVs).[10—15]

LUVs are good models for studying the transport of metal ions through phospholipid bilayers. In a typical experiment LUVs are prepared by the dialytic detergent removal technique introduced by Reynolds and co-workers[16] with equal concentrations of metal ions inside and outside. A chemical shift difference for the alkali metal ion is established by means of an aqueous shift reagent and a dynamic line broadening or magnetization transfer technique is used to obtain transport rates as the ionophore is added. We have also demonstrated that the classical mobile carrier system presented by Painter and Pressman[17] accounts satisfactorily for the observed kinetics and we have developed a series of equations to allow dissection of the rate data into the formation rate of the metal ion at the membrane surface (k'_f), the dissociation rate (k'_d), and the diffusion coefficient (k_{diff}).[12] We used these same methods for cationomycin in this paper. Comparison of rate constants is made with those obtained with monensin, a well characterized polyether antibiotic[9,10,13,18—24] under the same experimental conditions. The relationships between the ionophore properties of cationomycin and monensin with their biological activities are discussed.

Experimental

Ionophores Monensin was produced in our laboratory from *Streptomyces cinnamonensis* ATCC 15413 strain. Cationomycin was obtained from Kaken Pharmaceutical (Japan) through a joint venture of Sanofi and our laboratory.

Experiments with LUVs LUVs were prepared from egg-yolk PC by a modification of the dialytic detergent removal technique introduced by Reynolds and co-workers described in our previous papers.[12,13] A typical preparation would have approx. 30×10^{-3} moles of lipid in 1.5 cm^3 of salt solution. Three dialyses (>2 l each; >12 h each) at 40 °C against the chloride of the appropriate metal produced large, detergent-free, unilamellar vesicles with the same concentration of metal ion inside and out. A final dialysis introduced tripolyphosphate into the external medium as described previously.[12] Sufficient $DyCl_3$ (Na^+) or $TbNO_3$ (K^+), typically a few microliters of a 1 M solution was then added to generate a chemical shift difference of approximately 4 ppm (Na^+) and 7 ppm (K^+).

Metal ion transport was studied by dynamic line broadening on either a Bruker AM 300 or Varian Unity 500 for Na^+ at 308 K or with a Bruker MSL 500 (K^+) at 303 K. In all cases the spectrometer was field/frequency locked on the ^2H resonance of ^2H_2O in a capillary tube. Spectra were line broadened

* To whom correspondence should be addressed.

by typically 1 Hz (Na^+) and 5 Hz (K^+) to improve the signal to noise ratio. Approximately 90 degree pulse widths were employed with recycle delays of at least 3 times T_1 in all cases.

Addition of small aliquots (microliter amounts of a standard solution of cationomycin in methanol) gave rise to dynamic line broadening effects which were then analysed as previously to extract the rate constants.[12] All lipids were purchased from Lipid Products.

Results and Discussion

The transport ability of cationomycin was studied in egg PC vesicules using ^{23}Na and ^{39}K NMR spectroscopy as previously described for other ionophoric antibiotics.[12,13]

The graphs of the reciprocal of the rate constants *versus* metal ion concentration for sodium and potassium transport in LUVs are shown in Fig. 2. As expected for the Painter and Pressman model,[17] and as always found previously[12,13] for other ionophoric antibiotics, the graphs were linear. The line for K^+ is lower than that for Na^+ showing more rapid transport of K^+ at all the concentrations studied. These graphs allow the extraction of the rate constants for complex formation on the membrane surface (k'_f=1/intercept) and for dissociation (k'_d=1/slope) (see Table 1). The slopes of the two graphs are very similar, indicating similar dissociation rates, and the main difference is in the intercepts, which show the K^+ complex has a more rapid formation rate. The difference in transport rates for the Na^+ and K^+ complexes is only consistent with diffusion not being the rate determining step, as the size of the Na^+ and K^+ complexes should be almost identical, giving almost identical diffusion coefficients and hence almost identical transport rates. The ratio of slope to intercept gives the stability constant of the metal/ionophore complexes on the membrane surface (Table 1) and it is the K^+ complex that is the more stable. The values obtained are typical of the other ionophoric antibiotics we have studied previously.[12,13]

In the model lipid membranes of the LUVs with similar ionic contents inside and outside, potassium is transported more rapidly and forms the more stable complex. The difference in transport rates arises because of a more rapid formation for the K^+ complex (k'_f), while the dissociation rates (k'_d) for the complexes of both metals are comparable. These results are consistent with the selectivity of complexation K^+/Na^+ previously measured in liquid phases.[4,5,8]

Interestingly, cationomycin transports Na^+ more slowly than monensin and has a lower stability complex with Na^+ because of the lower formation rate for the complex on the membrane surface (Table 1). In LUVs, transport selectivity of cationomycin is on favour of K^+/Na^+ while the reverse sit-

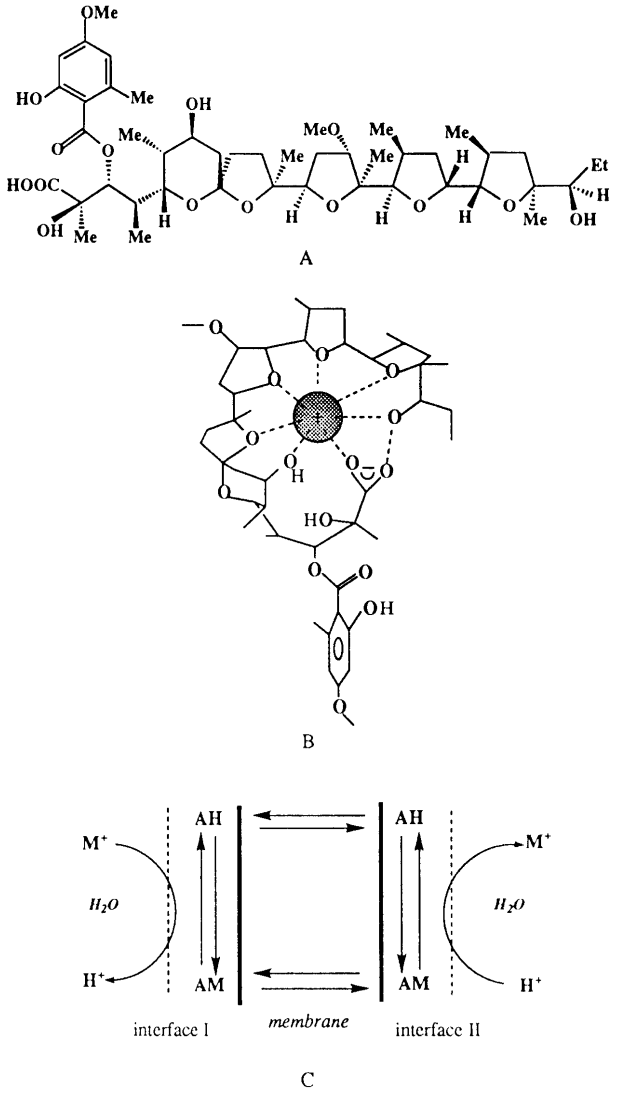

Fig. 1. A) Cationomycin, B) Schematic Representation of X-Ray Structure for Tl^+–Cationomycin Complex, C) M^+, H^+ Induced Transport Exchange by a Carboxylic Polyether AH

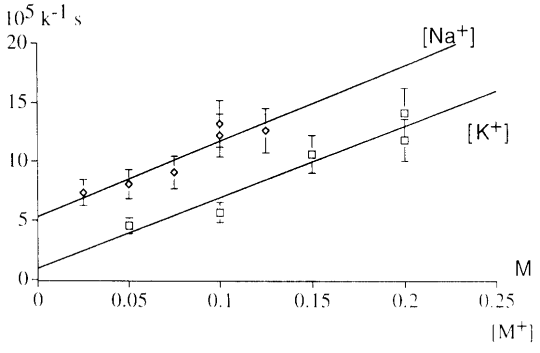

Fig. 2. Cationomycin-Mediated Na^+ and K^+ Transport

Graph showing the linear relationships between k^{-1} and [M^+] from which the values of k'_f (1/intercept) and k'_d (1/slope) are obtained on the assumption that diffusion is not the rate-limiting step. The error bars indicate ±15% of the measured rates.

Table 1. Comparison of Rate and Stability Constants for Cationomycin and Monensin in Egg PC Vesicles

Ionophore	Sodium			Potassium		
	k'_f ($10^4 s^{-1}$)	k'_d ($10^4 M s^{-1}$)	k_s (M^{-1})	k'_f ($10^4 s^{-1}$)	k'_d ($10^4 M s^{-1}$)	k_s (M^{-1})
Cationomycin	1.88±0.37	0.155±0.003	12.12±2.17	10.50±6.0	0.167±0.023	63.0±40
Monensin	4.878±0.694	0.150±0.007	32.6±8.3	2.30±0.35	0.433±0.097	5.3±2.1

uation is observed for monensin.

In previous works we showed that the antibiotic properties of monensin derivatives were correlated with their selectivity of transport in favour of K^+/Na^+.[9,23] In this work this relationship is confirmed as cationomycin is much more active on $Gram^+$ bacteria than monensin : for example the minimum inhibitory concentration (MIC) obtained on *Bacillus cereus* ($mg \cdot ml^{-1}$) are for monensin: 1.56; for cationomycin: 0.05 (unpublished results).

Opposite to antimicrobial properties, antimalarial properties of carboxylic polyether antibiotics are not correlated with Na^+/K^+ selectivity.[9,25] The most important factor for antimalarial activity is the selectivity for monovalent *versus* divalent cations, for instance calcimycin (selective for Ca^{2+}) showed weak antimalarial activity (150 ng/ml) whereas monensin and nigericin (selective for Na^+ and K^+, respectively) were both equally active on *Plasmodium* (around 1 ng/ml).[25] Surprisingly, though cationomycin and monensin are both monovalent cations transporters, IC_{50} for *in vitro* growth inhibition of *P. falciparum* was higher for cationomycin (35 ng/ml) than for monensin (1.5 ng/ml).[25]

Antibacterial and antimalarial assays notably differ in the amounts of serum present in the assay medium (0 and 10%, respectively), and we showed that increasing serum amounts in the growth medium decreased the *in vitro* antimalarial activity against *P. falciparum*.[26] Work is in progress to study a possible differential interaction of some seric factor(s) with cationomycin and monensin that could modify their apparent antimalarial activity.

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