STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XV[1]

THE MONOVALENT CATION SELECTIVE IONOPHOROUS ACTIVITIES OF CARRIOMYCIN, LONOMYCIN AND ETHEROMYCIN

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The cation selectivity profiles of the carboxylic ionophores, carriomycin, lonomycin and etheromycin, have been investigated by measuring the complexation affinities for metal cations and the cation transport activity through an organic barrier. In a two-phase partition study, carriomycin and lonomycin formed complexes more readily with K^+ than with NH_4^+, Rb^+ or Na^+, but not with Li^+ or Cs^+. On the other hand, etheromycin exhibited a great preference for K^+ or NH_4^+ over Na^+, Li^+ or Rb^+, but displayed no binding affinity for Cs^+. The alkaline degradation product of lonomycin exhibited a preference for K^+ or Na^+, but its complexation affinities were much lower than those of the parent compound. Carriomycin, lonomycin and etheromycin efficiently transported K^+, Rb^+ and Na^+ through a CCl_4 barrier. But did not carry Ca^{2+}. These antibiotics caused a massive release of K^+, Rb^+ or Na^+, but not of Li^+ and Cs^+, from mitochondria previously loaded with these cations by valinomycin or monazomycin. Thus, it is concluded that carriomycin, lonomycin and etheromycin are monovalent cation selective ionophores.

Carriomycin,[2] lonomycin[3] and etheromycin[4,5] (the identity with CP-38295[6] and T-40517[2] has been confirmed by direct comparison by N. ŌTAKE) are members of monocarboxylic polyether antibiotics produced by *Streptomyces*. These compounds are active against gram-positive bacteria, fungi and mycoplasma and also exhibit a potent protective effect against coccidial infection in poultry. Carriomycin is an unique antibiotic with a marked low toxicity among the polyether family.[2] The structures of these antibiotics have been established by a X-ray analysis, as illustrated in Fig. 1.[7~9]

The ability of ionophorous antibiotics to transport cations across both biological and artificial membranes as lipid-soluble cation complexes has been well established.[10~12] These antibiotics, in particular A 23187, lasalocid A and lysocellin which exhibit a significant preference for divalent metal cations, have served as useful tools to study the regulatory mechanism of either cation transport systems or cation-dependent biological processes.[5,10,13~16]

In the present study, we show the ability of carriomycin, lonomycin and etheromycin to transport alkali metal cations into or across an organic solvent barrier. We also describe the antibiotic-induced release of alkali metal cations from mitochondria, which indicates a function of these compounds as mobile carriers of cations across the mitochondrial inner membrane.

Materials and Methods

All antibiotics used in the present study were stock samples in our laboratory. ^{22}Na, ^{86}Rb and ^{137}Cs were purchased from Radiochemical Centre, Ammersham, England, ^{45}Ca and ^{133}Ba from New England Nuclear, U.S.A., ^{42}K from the Japan Atomic Energy Research Institute, Tokyo, Japan.

The association constants of the antibiotics for various metal cations were determined by a two-

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phase partition experiment on the assumptions of 1 : 1 complexes with monovalent cations and 2 : 1 complexes with divalent cations, respectively, as described in the previous report.[17]

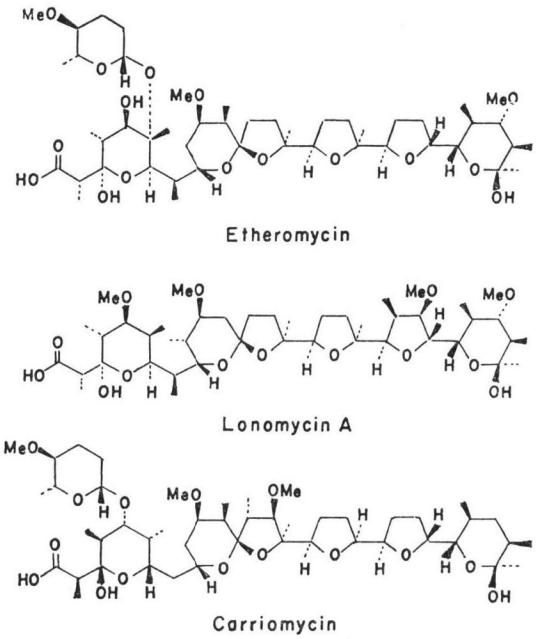
The cation selectivity profiles of the ionophores were determined in a ^{86}Rb competition experiment as described previously.[17] In this experiment, the calculation of relative complexation affinity was based on ^{86}Rb displacement by the test cations from ^{86}Rb-ionophore complexes.

Bulk cation transport mediated by the antibiotics was measured by use of a glass reaction vessel in which two parts of an aqueous phase were completely separated by a CCl_4 barrier as described previously.[17]

Rat liver mitochondria were isolated in a solution containing 225 mM mannitol, 75 mM sucrose and 2 mM Tris-chloride (pH 7.4) as described by JOHNSON and LARDY.[18] Mitochondrial swelling and contraction were monitored spectrophotometrically by measuring the changes in absorbance at 520 nm.

The concentration of K^+ was monitored with an Orion K^+-specific electrode 92-19.

Fig. 1. Structures of carriomycin, lonomycin and etheromycin.



Results

The equilibrium affinity between the antibiotics and the cations in a two-phase distribution system are shown in Table 1. Carriomycin, lonomycin and etheromycin exhibited a great preference for K^+

Table 1. K_A of various ionophore complexes

Cation	Carriomycin	Lonomycin	Lonomycin-alkali degradation product	Etheromycin
Na^+	0.13	0.24	0.05	0.46
K^+	1.16	1.42	0.12	0.85
Rb^+	0.17	0.15	0.02	0.32
Cs^+	0.04	0.003	0.001	0.01
Mg^{2+}	0.2	0.1	0.01	0.1
Ca^{2+}	0.7	0.3	0.06	0.1
Ba^{2+}	1.8	1.7	0.6	1.2

The complex formation was determined in a two-phase distribution system consisting of aqueous Tricine-TMAH buffer (0.5 ml, pH 8.3) and 30% *n*-butanol - 70% toluene mixture (1 ml). The antibiotics were used at a concentration of 5×10^{-4} M and the concentration of the test cations varied from 0.1 to 20 mM. The association constants were calculated from the following equations:

For monovalent cation, $$K_A=\frac{[\text{Ionophore}^-\cdot\text{Cation}^+_{org}]}{[\text{Ionophore}^-_{org}]\cdot[\text{Cation}^+_{H_2O}]}$$

For divalent cation, $$K_A=\frac{[\text{Ionophore}^-_2\cdot\text{Cation}^{2+}_{org}]}{[\text{Ionophore}^-_{org}]^2\cdot[\text{Cation}^{2+}_{H_2O}]}$$

For K_A multiply above values by 10^3.

over the other monovalent cations. Since the constants were calculated on the basis of different equations, direct comparison of these data as indices of complexation affinity for monovalent and divalent cations seems not compatible. It was observed that only a weak activity was retained in the alkaline degradation product of lonomycin.

Fig. 2 shows the cation selectivity profiles of carriomycin, lonomycin and etheromycin determined in a ^{86}Rb competition study. Carriomycin and lonomycin displayed a greater preference for K^+ than for NH_4^+, Rb^+ or Na^+, but exhibited weak binding affinity for Li^+ or Cs^+. On the other hand, etheromycin formed complexes more efficiently with NH_4^+ or K^+ than with Na^+, Li^+ or Rb^+, but failed to form complex with Cs^+. Cation selectivity sequences of these antibiotics are summarized as follows:

Carriomycin: $K^+ > NH_4^+ > Rb^+ > Na^+ > Li^+, Cs^+$

Lonomycin: $K^+ > NH_4^+ > Na^+ > Rb^+ > Li^+, Cs^+$

Etheromycin: $K^+, NH_4^+ > Na^+ > Li^+ > Rb^+ > Cs^+$

Fig. 3 shows the time course of the ionophore-mediated bulk transport of cations across a CCl_4 barrier. Carriomycin, lonomycin and etheromycin efficiently mediated the net transport of K^+, Rb^+ and Na^+ through an organic-solvent barrier but did not transport Ca^{2+}. In agreement with results obtained from the complexation affinity series, these data indicate the monovalent cation selective activity of carriomycin, lonomycin and etheromycin.

Fig. 2. Ion selectivity spectra of carriomycin, lonomycin and etheromycin determined by a ^{86}Rb displacement study.

Relative affinities for various cations were determined by the ability of the test cation to displace ^{86}Rb from ^{86}Rb-ionophore complexes under conditions similar to those shown in Table 1.

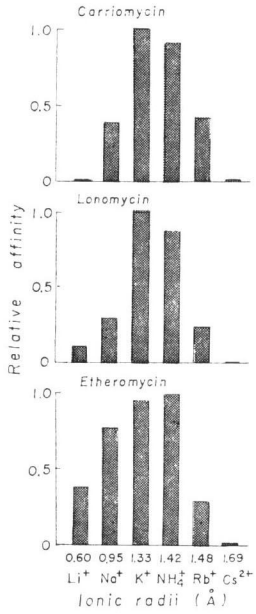


Fig. 3. The ionophore-mediated bulk transport of metal cations across a CCl_4 barrier layer.

The antibiotics were added to a CCl_4 phase at a concentrations of 2×10^{-4} M. The isotopically labeled ions (10 mM) were added to one part of the aqueous phase containing 25 mM glycine-tetramethylammonium hydroxide buffer (pH 9.8) and the other part of the aqueous phase were filled with the same buffer containing non-isotopical ions. The time required for appearance of radioactivity in this part of aqueous phase was monitored.

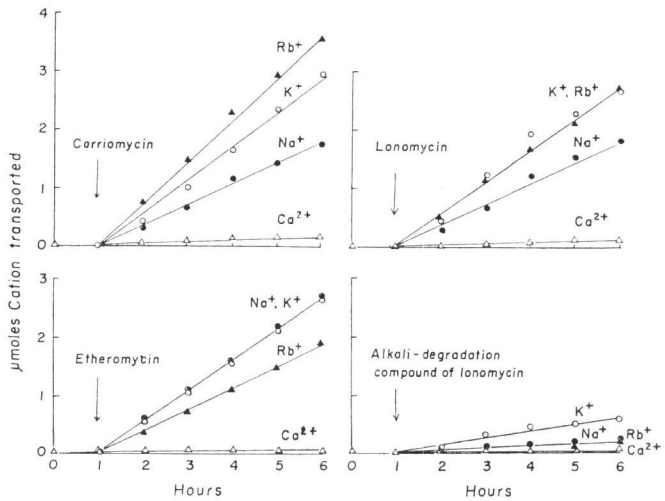
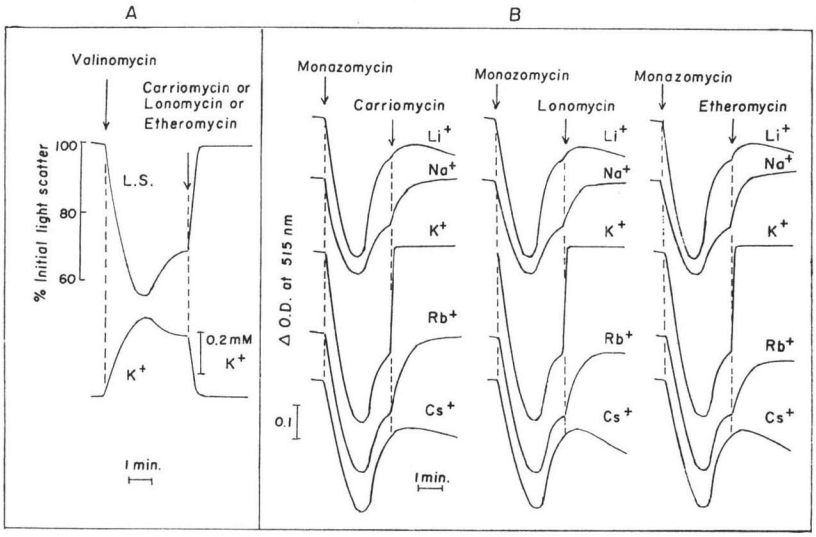


Fig. 4. Effects of carriomycin, lonomycin and etheromycin on alkali metal cation transport and light-scattering changes induced by valinomycin or monazomycin.

(A) A downward deflection of the light-scattering trace (L.S.) is associated with swelling of mitochondria. An upward deflection in K^+ trace represents a decrease of its concentrations in the medium or uptake of K^+ by mitochondria. The reaction system contained 20 mM triethanolamine-chloride (pH 7.3), 10 mM acetate-triethanolamine (pH 7.3), 5 mM $MgCl_2$, 5 mM KCl, 5 mM Tris-ATP, 180 mM sucrose and 4 mg of mitochondrial protein. Final volume, 5 ml; temperature, 27°C. Antibiotic additions at indicated point: 10^{-7} M valinomycin, 0.2 μM carriomycin, lonomycin or etheromycin.

(B) The reaction system contained 20 mM Tris-acetate (pH 7.3), 5 mM $MgCl_2$, 30 mM alkali metal cation (Cl salt), 3 mM Tris-ATP, 150 mM sucrose and 0.2~0.3 mg of mitochondrial protein. Final volume, 3 ml; temperature, 27°C. Where indicated, further additions were made: 0.1 μM monazomycin, 0.2 μM carriomycin, lonomycin or etheromycin.



To ascertain this activity in a biological system, we measured the effects of these compounds on alkali metal cation translocation in mitochondria as shown in Fig. 4. Valinomycin and monazomycin are ionophores known to stimulate the uptake of alkali metal cations by respiring mitochondria, which is accompanied by oscillatory swelling of mitochondria.[19,20] When potassium uptake was stimulated by valinomycin in the presence of ATP, the additions of carriomycin, lonomycin and etheromycin caused rapid release of K^+ and mitochondrial contraction (Fig. 4A). These antibiotics induced contraction of mitochondria preloaded with K^+, Rb^+ or Na^+ by monazomycin, but did not with Li^+ or Cs^+ (Fig. 4B). These results suggest that the antibiotics reverse mitochondrial swelling through loss of accumulated alkali metal cations.

Discussion

The cation selectivity profiles determined by measuring the complexation affinity and the bulk cation transport activity indicate that carriomycin, lonomycin and etheromycin are monovalent cation selective ionophores. The findings that these antibiotics induced strong release of K^+, Rb^+ and Na^+ from previously loaded mitochondria suggest that these compounds may also act as mobile carriers of K^+, Rb^+ or Na^+ in biological systems.

In a previous study,[5] we have reported that etheromycin as well as the divalent cation ionophores lysocellin and lasalocid A induced the platelet secretion reaction and aggregation by mobilizing intra-

cellular calcium. Preliminary results with other monovalent cation selective ionophores indicated that high levels of these compounds induced platelet aggregation. PRESSMAN[10] has reported that the monovalent cation ionophores as well as lasalocid A produced cardiovascular stimulation in the intact dog and has proposed that this inotropic activity may be due to their sodium transport activity which indirectly increases intracellular calcium levels and thereby induces exocytotic release of catecholamines. In studies with the isolated mitochondria,[21,22] we found that monovalent and divalent cation ionophores produced a significant effect on divalent cation translocation in mitochondria. These findings indicate the possibility that the monovalent cation ionophores would be able to trigger the calcium-dependent biological processes through increasing monovalent cation movement across membranes. It has been suggested[23] that the increased intracellular sodium produces an increase in cytoplasmic calcium levels by liberating calcium from the intracellular pools. As reported by PRESSMAN,[10] the equilibrium affinity of an ionophore for cation in a given *in vitro* system may not provide a definitive index to its transport activity in a biological system, but may only suggest a relative trend of cation selectivity. In spite of some complexity of mode of action of the ionophores, these compounds are powerful tools in studying the bioregulation mechanism of cation-dependent biological processes.

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