STUDIES ON IONOPHOROUS ANTIBIOTICS VII. A BROAD CATION SELECTIVE IONOPHORE, LYSOCELLIN

Sir:

Lysocellin is a monocarboxylic polyether antibiotic produced by *Streptomyces cacaoi* var. *asoensis* and exhibited both antibacterial and anticoccidial activities.[1] The entire chemical structure including absolute configuration of lysocellin has been established by a three-dimentional X-ray analysis.[2] The structure of lysocellin is shown in Fig. 1.

The function of ionophorous antibiotics as mobile carriers of cations across natural and artifact membranes has been recently investigated.[3~5] These ionophores form hydrophobic complexes with cations and catalyze transmembranous exchanges thereof for protons. Having the ability to alter ionic permeability, they should be served as models for understanding molecular mechanism of membrane functions.[6~9]

In this communication, a broad range cation selectivity of lysocellin in complexing and transporting activities is reported.

The association constants of lysocellin for various cations were determined in two-phase distribution experiments on the assumption of 1 : 1 complexes with monovalent cations or amines, and 2 : 1 complexes with divalent cations according to the following equations:

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

or

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

Lysocellin exhibited a broad range of selectivity in cation complexation (Table 1). The antibiotic displayed preferences not only for alkali metal cations but also for divalent metal cations. It is noteworthy that lysocellin formed lipid-soluble complexes with biogenic amines such as serotonin, *l*-norepinephrine and histamine (Table 1). The cation-complexing property of lysocellin was lost almost completely by chemical modifications of both the terminal carboxylic acid and C_{21} hydroxyl groups, respectively, by methylation or acylation (Table 1). For the sake of comparison on cation-selectivity with other polyether antibiotics, the complexation affinity of salinomycin, a known monovalent ionophore,[11,12] is referred in Table 1.

The cation-selectivity pattern of lysocellin was determined by measuring the amount of

Fig. 1. The structure of lysocellin.

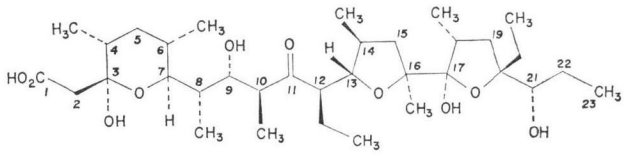


Table 1. K_A of various ionophore complexes

Cations and amines	Lysocellin	21-O-Acetyl lysocellin	21-O-Acetyl lysocellin methyl ester	Lysocellin methyl ester	Lysocellin hexa-ol	Salinomycin
Na^+	0.21	0.0001	0.0001	0.0001	0.0001	0.10
K^+	0.51	0.0004	0.0001	0.0001	0.0001	2.32
Rb^+	0.52	0.0006	0.0002	0.0001	0.0001	1.12
Mg^{2+}	50.8	0.1	0.1	0.1	0.1	0.1
Ca^{2+}	49.2	0.1	0.1	0.1	0.1	0.1
Serotonin	4.56	0.001	0.001	0.001	0.001	0.24
l-Norepinephrine	0.13	0.001	0.001	0.001	0.001	0.017
Histamine	0.31	0.001	0.001	0.001	0.001	0.012

Complex formation was determined from the transport of the test cation from aqueous Tricine-TMAH buffer (0.5 ml, pH 8.3) into 70 % toluene-30 % *n*-butanol (1 ml), two-phase distribution system. The antibiotics were used at a concentration of 5×10^{-4} M.

For K_A, multiply above the values by 10^3.

Fig. 2. Ion selectivity spectrum of lysocellin determined by a $^{86}Rb^+$ or $^{45}Ca^{2+}$ competition study.

Relative affinities for various metal cations were determined by the ability of the test cation to displace ^{86}Rb or $^{45}Ca^{2+}$ from the respective ionophore complexes under conditions similar to those shown in Table 1.

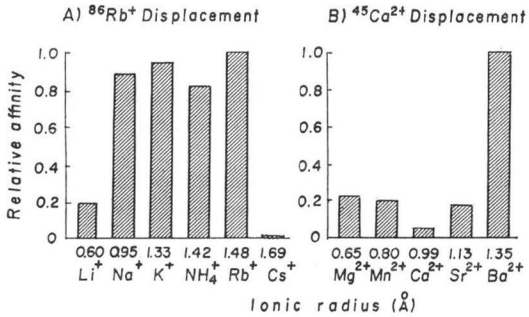
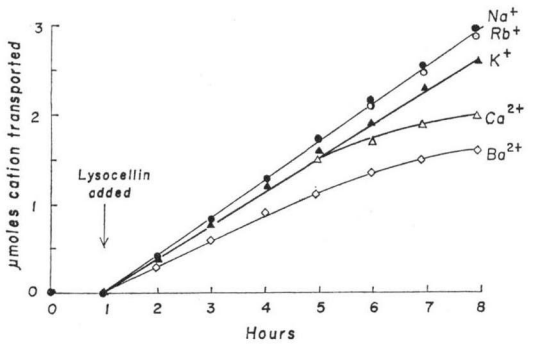


Fig. 3. Lysocellin mediated bulk transport of metal cations across a CCl_4 barrier layer.

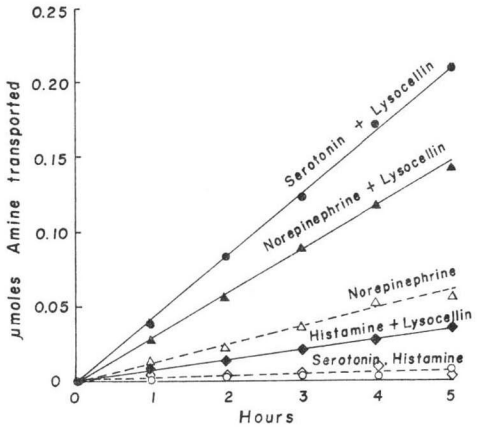
Lysocellin was added to a CCl_4 phase at a concentration of 2×10^{-4} M. The isotopically labeled ions (10 mM) were added to one part of the aqueous phase containing 25 mM glycine tetramethylammonium hydroxide buffer, pH 9.8, and the time required for the appearance of radioactivity in the other part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



$^{86}Rb^+$ or $^{45}Ca^{2+}$ complexes in the presence of the various test cations. Complexation affinity was given as the value of the changes in apparent K_A for $^{86}Rb^+$ or $^{45}Ca^{2+}$ induced by competition with the test cation relative to that caused by Rb^+ or Ca^{2+} competition. In $^{86}Rb^+$ displacement study, lysocellin complexed more efficiently with Na^+, K^+, NH_4^+, or Rb^+ than with Li^+ but failed to complex with Cs^+ (Fig. 2). On the other hand, in $^{45}Ca^{2+}$ competition experiment, the antibiotic exhibited more affinity for Ba^{2+}

Fig. 4. Lysocellin mediated net transport of biological amines through a CCl_4 barrier phase.

The reaction system was the same as Fig. 3 except for isotopically labeled amines were used instead of metal ions. The pH or aqueous phase was adjusted to 8.3 by 30 mM Tricine-tetramethylammonium hydroxide buffer.



than the other divalent cations (Fig. 2). The cation-selectivity pattern of lysocellin is summarized as follows:

$Rb^+ > K^+ > Na^+ > NH_4^+ > Li^+ > Cs^+$ and
$Ba^{2+} \gg Mg^{2+}$, Mn^{2+}, $Sr^{2+} > Ca^{2+}$

Fig. 3 shows the time course of lysocellin-mediated bulk transport of metal cations through a CCl_4 barrier phase. The system consisted of two parts of aqueous phase (3 ml each) which were completely separated by a CCl_4 barrier phase (12 ml), and the time lapse between the cations appearance in one aqueous part and its disappearance in another part was measured. Lysocellin carried Na^+, Rb^+, and K^+ efficiently, as well as Ca^{2+} and Ba^{2+}. The antibiotic was also able to transport the biogenic amines across an organic barrier layer, and the transporting velocity series are summarized as follows (Fig. 4):

serotonin > *l*-norepinephrine > histamine

With respect to a wide ion selectivity spectrum, lysocellin therefore resembles the antibiotic lasalocid A which have been reported as a broad-spectrum ionophore, capable of complexing and transporting not only alkali ions but also the polyvalent inorganic ions and complicated organic amines as well.[5,7] In two-phase partition studies, lasalocid A exhibits more preference

for Cs^+ than the other alkali cations, while no lipid-soluble lysocellin-complex with Cs^+ was detected. On the other hand, lasalocid A shows more preference for Ba^{2+}, Sr^{2+}, and Ca^{2+} than Mg^{2+}, whereas lysocellin displayed a great affinity for Ba^{2+} but less for the other divalent metals, and no significant discrimination was observed between Mg^{2+}, Mn^{2+}, Ca^{2+} and Sr^{2+} (Fig. 4).

The data obtained with bulk transport tests suggest the possible effects of lysocellin as an ionophore in altering the membrane permeability to metal cations and biogenic amines, thereby perturbing its function. Preliminary results with rat liver mitochondria and rabbit blood platelets showed that lysocellin was remarkably active in releasing both metal cations and biogenic amines from the organelles. These results will be published in the following report.

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MITSUAKI MITANI
TADASHI YAMANISHI
EIICHI EBATA
Research Laboratory
Kaken Chemical Co., Ltd.
Bunkyo ku, Tokyo 113, Japan
NOBORU ŌTAKE
MITSUO KOENUMA
Institute of Applied Microbiology
University of Tokyo
Bunkyo ku, Tokyo 113, Japan

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