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VALINOMYCIN-INDUCED POTASSIUM AND RUBIDIUM PERMEABILITY OF INTACT CELLS OF *ACHOLEPLASMA LAIDLAWII* B

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SUMMARY

The valinomycin-induced K^+ and Rb^+ permeability in cells of *Acholeplasma laidlawii* B differing in fatty acid and cholesterol content was studied using three different techniques: (i) by following the swelling of the cells in potassium acetate optically; (ii) by recording the efflux of K^+ using a potassium-selective glass electrode; and (iii) by measuring the efflux of Rb^+ (after preincubation of the cells with $^{86}Rb^+$) with a filter technique.

If unsaturation of the membrane lipids was increased, the permeability was found to increase. Cholesterol appeared to cause a slight decrease in permeability.

The valinomycin-induced efflux of K^+ is gradually reduced when the temperature is lowered and becomes zero below the gel–liquid crystalline phase transition.

INTRODUCTION

In this communication we report on valinomycin-induced K^+ and Rb^+ permeabilities in *Acholeplasma laidlawii* B cells with different lipid compositions. The fatty acid chains and the cholesterol content of the plasma membrane of this organism can be altered within certain limits by varying the fatty acid and cholesterol content of the growth medium [1–3]. Studies on non-electrolyte permeability [3–7] have demonstrated already that these *A. laidlawii* cells are very useful to study the relationships between permeability properties and membrane lipids in natural membranes.

Studies on membrane model systems indicated that valinomycin-induced ion permeability is not only determined by the polar groups [8–10] but that also the composition of the apolar interior of the membrane is of importance for the rate of the mediated ion diffusion processes [11, 12]. The present results will show that the conclusions taken for the model systems are also valid for a natural membrane.

MATERIALS AND METHODS

Materials

Valinomycin A grade was obtained from Calbiochem, Los Angeles, Calif.,

U.S.A. It was added as an ethanolic solution in a total volume of 5 μl. All other chemicals were commercial and of Analytical Reagent Grade.

Organism and growth conditions

A. laidlawii strain B cells were grown in a lipid-poor tryptose medium (0.1–0.5 l quantities) as described [4, 13]. Fatty acid and cholesterol additions are indicated in the legends to the figures.

Measurement of the release of intracellular K^+

A. laidlawii cells were washed once with 100 mM calcium acetate at room temperature. The cell pellet was resuspended in 100 mM calcium acetate and stored at room temperature. Appropriate amounts of cells were dispersed in 10 ml 100 mM calcium acetate at various temperatures and the change in K^+ activity of the solution followed [12] with a Scott and Gen. K^+ glass electrode (Jena Glass Werk, Mainz, Germany) connected with a Radiometer type P.H.M. 26 pH meter (Radiometer, Copenhagen, Denmark) and a recorder. Valinomycin was added after at least 5 min equilibration. Total K^+ trap of the cells was determined after lysis of the cells with Triton X-100.

Measurement of $^{86}Rb^+$ leakage from the cells

The cells were washed once with a solution of 150 mM NaCl–10 mM Tris–HCl, pH 7.5. The thick cell pellet of a 250-ml culture was incubated (1–3.5 h) with 0.1 ml 150 mM ^{86}RbCl solution (0.5 Ci/l) at room temperature. 50–100 μl of this thick cell suspension was brought into 25 ml of a carefully stirred 150 mM NaCl solution, buffered with 10 mM Tris–HCl at pH 7.5 and at experimental temperature. After appropriate time intervals, cells in 1 ml of this solution were collected by filtration through membrane filters (Sartorius, effective diameter 0.45 μm) and washed with 5 ml of the buffered NaCl solution [7]. After 15 min valinomycin was added to the incubation mixture and the sampling was continued. Residual radioactivity on the filters was measured as described earlier [8].

Osmotic volume changes of A. laidlawii cells

Swelling or shrinking of the cells, when dispersed in appropriate amounts in K^+ (150 mM), Na^+ (150 mM) or Ca^{2+} (100 mM) acetate, before and after addition of valinomycin, was followed by absorption at 450 nm using a recording spectrometer (Vitatron U.F.D.). For the swelling experiments, the cultured cells were washed in 150 mM choline chloride and resuspended in 200 mM sucrose. Shrinkage of the cells was assessed after washing and resuspending in 100 mM calcium acetate.

All experiments were performed at least in triplicate; typical results of individual experiments are given.

RESULTS AND DISCUSSION

Internal K^+ content of A. laidlawii B cells

K^+ is needed for the growth of *A. laidlawii* B cells and is accumulated by the cells [14, 15], whereas there appears to be no absolute requirement for Na^+ [15].

Using a solid state Na^+ electrode (Orion model 96-11), which is highly selective

for Na^+ over K^+, it was ascertained that under the conditions used in our experiments, the cells contained no detectable concentrations of Na^+. In subsequent experiments therefore, a K^+ glass electrode, which is not very selective in discrimination between Na^+ and K^+, could be used for measuring leakage of K^+ out of the cells.

From our experiments it appeared that the internal K^+ concentration of the cells depended on the medium used. If the cells were washed and equilibrated in a Ca^{2+} solution (100 mM, pH 7.0–8.0), the cells were found to contain a considerable amount of K^+. A slow but significant leak of K^+ into the Ca^{2+}-containing medium could be observed (usually less than 1% min). When the pH of the solution was lowered to less than pH 7.0, leakage of K^+ increased.

When the cells were suspended in 200 mM sucrose, however, it appeared that all the K^+ was released. It can be concluded that the K^+ retainment is strongly dependent on the ionic strength of the medium. In addition, there may be specific interactions of Ca^{2+} with the polar headgroups of the lipids [16] in the calcium acetate media that diminish the K^+ leak.

Swelling of cells in potassium acetate

Cells suspended in the sucrose medium demonstrate a linear proportionality between cell volume and osmolarity [4,6]. Cells grown with different fatty acids did not show differences in size and shape [6], and are suitable for comparing swelling rates.

The results demonstrated in Figs 1 and 2 show changes in the swelling rate of *A. laidlawii* cells of different lipid composition when dispersed into potassium acetate.

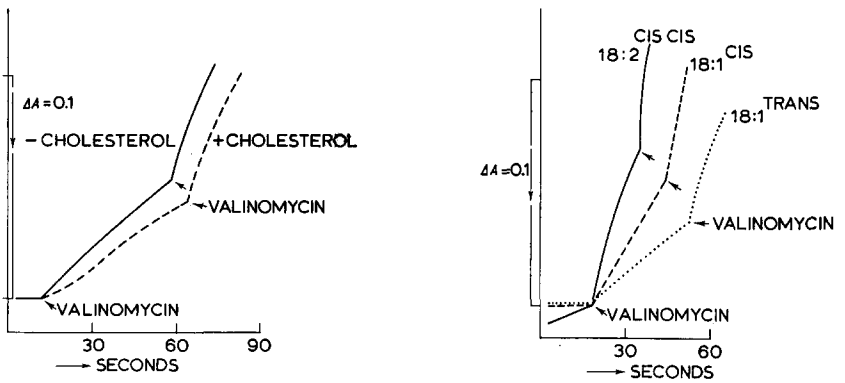

Fig. 1. Osmotic swelling of *A. laidlawii* cells in potassium acetate. Cells were grown on 0.03 mM palmitic acid (16:0) and 0.03 mM oleic acid ($18:1_{cis}$), with or without 25 mg cholesterol/l medium. Cells were added to 10 ml of 150 mM potassium acetate to an initial absorbance of 0.85 (450 nm). After equilibration valinomycin was added to a concentration of 5.10^{-6} mg/ml and a second time to a concentration of $55 \cdot 10^{-6}$ mg/ml. Temperature, 18 °C.

Fig. 2. Osmotic swelling of *A. laidlawii* cells in potassium acetate. Cells were grown on 0.06 mM palmitic acid (16:0) plus either 0.06 mM oleic acid ($18:1_{cis}$), 0.06 mM elaidic acid ($18:1_{trans}$) or 0.06 mM linoleic acid (18:2). Cells were added to 10 ml of 150 mM potassium acetate to an initial absorbance of 1.00 (450 nm). After equilibration valinomycin was added to a concentration of $5 \cdot 10^{-6}$ mg/ml and a second time to a concentration of $55 \cdot 10^{-6}$ mg/ml. Temperature, 18 °C.

Fig. 1 shows that when K^+ permeability was induced in cells with and without cholesterol different permeability rates were observed at low concentrations of valinomycin. The presence of cholesterol decreased the swelling rates. Cholesterol is incorporated as such in the membrane lipids up to 8% (by wt) [3,4]. In Fig. 2 the fatty acid effect is shown. Under the given growth conditions both oleic acid and elaidic acid are incorporated to a relative concentration of about 45% whereas linoleic acid is incorporated only to 30% [5]. The results show that despite the lower incorporation of linoleic acid compared to oleic acid and elaidic acid, the swelling rate induced by low concentrations of valinomycin in cells grown on linoleic acid is higher. This holds true after correction for the small spontaneous swelling of linoleic acid-grown cells before addition of valinomycin. The swelling rate of cells grown on oleic acid is significantly higher than of those grown on elaidic acid. At high concentrations of valinomycin all swelling rates attained a maximum value and became independent of valinomycin concentration. When 150 mM sodium acetate was used (instead of potassium acetate), no swelling could be observed, consistent with the mentioned specificity [16,17] of valinomycin for K^+ over Na^+.

Considering the mechanism of valinomycin-induced swelling, we can deduce from the requirement of electroneutrality that the membrane of *A. laidlawii* is permeable to H^+ or to anions such as acetate [17]. In this respect cells of *A. laidlawii* and liposomes behave in a quite different manner. The lipid bilayers of liposomes appeared to be impermeable to acetate ions, but permeability could be induced indirectly by combined addition of valinomycin and uncouplers [12]. Uncouplers appeared to have no effect on the valinomycin-induced swelling of *A. laidlawii* cells.

Leakage of $^{86}Rb^+$ and K^+ out of A. laidlawii cells

When a thick cell pellet of *A. laidlawii* was preincubated with ^{86}RbCl, it appeared that the cells were rapidly loaded with $^{86}Rb^+$. Upon dilution of the cell pellets in buffered NaCl solution, there was only a very limited leak of radioactivity, but as soon as valinomycin was added to the solution, radioactivity was lost from the cells. From the results given in Fig. 3 it can be seen that the rate of leakage is dependent on the composition of the paraffin chains. Furthermore, cholesterol incorporation (not shown) reduced the leak.

In comparable experiments the leak of endogenous K^+ out of the cells was followed directly with a K^+-sensitive electrode, or indirectly as shrinking measured

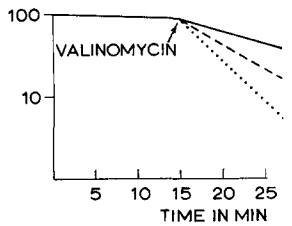

Fig. 3. Leakage of $^{86}Rb^+$ out of *A. laidlawii* cells as a function of membrane lipid composition. Cells were grown on 0.06 mM palmitic acid (16:0) plus either 0.06 mM oleic acid ($18:1_{cis}$) (– – – –), 0.06 mM elaidic acid ($18:1_{trans}$) (———) or 0.06 mM linoleic acid (18:2) (· · · · · ·). Cells were harvested, washed and preincubated with $^{86}Rb^+$ as described in Materials and Methods. The leak of $^{86}Rb^+$ from the cells into a NaCl–Tris–HCl solution (pH 7.5) was measured at 18 °C. After 15 min valinomycin was added to a concentration of $1 \cdot 10^{-6}$ mg/ml.

as an increase in optical density. The results are illustrated in Figs 4 and 5. Comparable amounts of cell protein were present in each set of experiments. Under these conditions initial absorbances of the cell dispersions and the amount of K^+ inside and outside the cells were also comparable, as can be seen in Figs 4b and 5b and Table Ia and b.

Summarizing the results from the present experiments we can conclude that the valinomycin-induced Rb^+ and K^+ permeability of *A. laidlawii* B is at least in part determined by the structure of the hydrophobic core of the membrane lipids in the same way as was found before for non-electrolyte permeabilities [3–7].

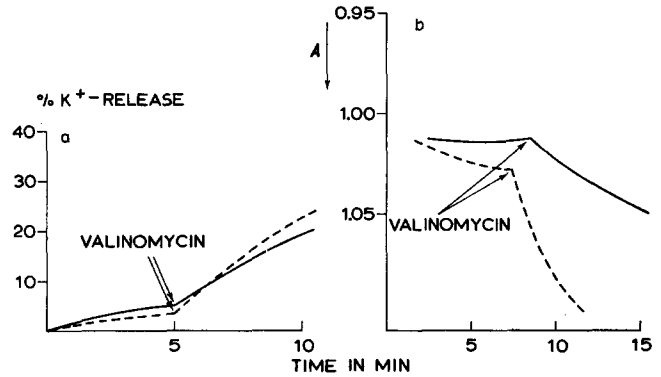

Fig. 4. Leakage of K^+ out of cells of *A. laidlawii* B as a function of membrane lipid composition. Cells were grown on 0.03 mM palmitic acid (16:0) plus 0.003 mM oleic acid (18:1_{cis}) with (———) and without (– – – – –) cholesterol to a concentration of 25 mg/ml. Equal amounts of cells were added to 10 ml of calcium acetate. After 5 min valinomycin was added to a concentration of $5 \cdot 10^{-6}$ mg/ml. After another 5 min 0.2 ml Triton X-100 (10% solution) was added. Absorbances were measured at 450 nm. Temperature, 18 °C.

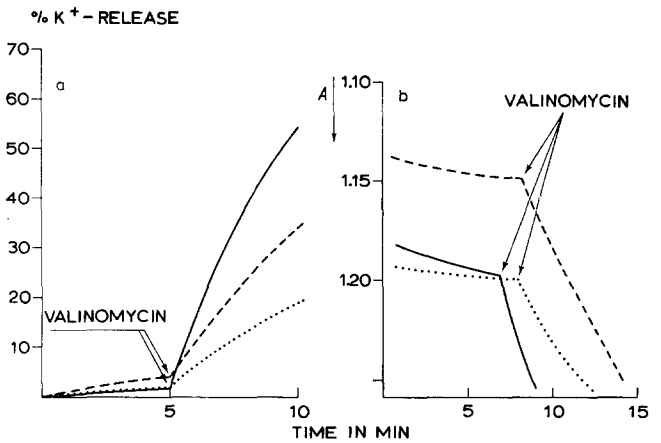

Fig. 5. Leakage of K^+ out of cells of *A. laidlawii* B. Cells were grown on 0.06 mM palmitic acid (16:0) plus either 0.06 mM loeic acid (18:1_{cis}) (– – – – –), 0.06 mM elaidic acid (18:1_{trans}) (· · · · · ·), or 0.06 mM linoleic acid (18:2) (———). Equal amounts of cells were added to 10 ml of 100 mM calcium acetate. After 5 min valinomycin was added to a concentration of $5 \cdot 10^{-6}$ mg/ml. After another 5 min 0.2 ml Triton X-100 (10% solution) was added to determine the total content of K^+. Absorbances were measured at 450 nm. Temperature, 18 °C.

TABLE I

K^+ CONTENT OF CELLS

K^+ content of the cells represented in Figs 4a and 4b (a) and 5a and 5b (b). Total amount of K^+ represents the amount of K^+ in the test solution after lysis of the cells with Triton X-100. From this value and the amount of K^+ outside the cells as measured within 1 min after addition of the cells to the calcium acetate solution, the original K^+ content of the cells was calculated.

Cells grown with	Total K^+ (nmoles)	K^+ inside (nmoles)	Fraction K^+ inside
(a). Oleic acid	800	589	0.74
Oleic acid + cholesterol	750	563	0.75
(b). Linoleic acid	1150	875	0.76
Oleic acid	1025	835	0.81
Elaidic acid	1175	825	0.70

The dependence of the valinomycin-mediated ion transport on the degree of unsaturation and the presence or absence of cholesterol in the membrane is in agreement with results found in liposome studies for the valinomycin-mediated $^{86}Rb^+$ leak [11]. These results may be explained in two manners. Either the mobility of the valinomycin–K^+ complex in the bilayer or the solubility of the valinomycin in the hydrophobic core and subsequent complexation with the K^+ at the border of the bilayer may be the rate-limiting factor of the transport process [18].

The spontaneous leak from the cells, which we consider to arise mainly from lysis of a minor part of the cells and which exhibited no correlation with the lipid composition, demonstrates only small increase with temperature as can be seen in Fig. 6. From this figure it also can be concluded that starting from 15 °C valinomycin-induced leakage is strongly increased with temperature, below this temperature valinomycin-induced K^+ transport is zero.

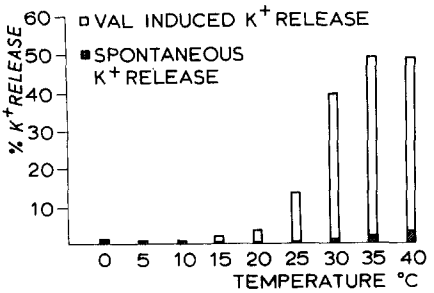

Fig. 6. Leakage of K^+ from cells of *A. laidlawii* B as a function of temperature. Cells were grown on 0.06 mM palmitic acid (16:0) and 0.06 mM elaidic acid ($18:1_{trans}$). Equal amounts of cells were added to 10 ml of 100 mM calcium acetate and the spontaneous leak of K^+ was recorded for 5 min. Then valinomycin was added to a concentration of $5 \cdot 10^{-6}$ mg/ml. The valinomycin-induced K^+ leak in the first minute after addition of the valinomycin and the extrapolated spontaneous leak in that time were calculated as a percentage of the total amount of K^+ in the cells on starting each experiment. The total amount of K^+ was calculated from the K^+ activity after lysis of the cells with Triton X-100.

From differential scanning calorimetry [4, 6] and abnormal swelling behaviour in isotonic glycerol [4, 6] of intact cells (grown on an equimolar mixture of palmitic and elaidic acid) it is known that a gel to liquid–crystalline phase transition occurs at about 15–20 °C. This temperature is not influenced by the presence of Ca^{2+} (de Kruijff, B., personal communication). Therefore we conclude that below the phase transition valinomycin is unable to bring about any ion transport. Such a conclusion was also reported from black-film experiments [19], showing that valinomycin was unable to bring about an increase in conductivity of the film below the transition temperature. The explanation for these effects can be an immobilization of the carrier or a complete exclusion of the valinomycin from the paraffin core.

From earlier studies in this laboratory it was reported [20] that cells of *Escherichia coli* and also liposomes prepared from synthetic lecithins, became fragile below the gel to liquid–crystalline phase transition. Rapid cooling of both the cells and the liposomes caused rapid release of intracellular K^+. In contrast with these results cells of *A. laidlawii* retain their K^+ content below the phase transition and there is no sudden release of K^+ as a result of a cold shock.

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