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CHARACTERIZATION OF THE PLASMA MEMBRANE OF *MYCOPLASMA LAIDLAWII*

VIII. EFFECT OF TEMPERATURE SHIFT AND ANTIMETABOLITES ON K^+ TRANSPORT

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SUMMARY

The internal K^+ concentration in cells of *Mycoplasma laidlawii* was shown to be a function of the temperature at which the determinations were carried out rather than the initial growth temperature of the cells. This was shown in temperature shift experiments. The efflux of K^+ from cells at 15 °C was also insensitive to the initial growth temperature. The efflux rate when measured as a function of efflux temperature yields a straight Arrhenius plot corresponding to an energy of activiation of 17.8 kcal/mole. K^+ efflux is drastically reduced by metabolic inhibitors as well as by the absence of K^+ in the external medium. Gramicidin renders the membranes completely permeable to K^+. The temperature experiments indicate that K^+ transport in these cells is not strongly effected by the temperature induced lipid phase transition in the membrane.

INTRODUCTION

The lipid phase transition in *Mycoplasma* membranes has been studied primarily from a structural point of view[1-4]. In these investigations we have examined certain features of the temperature dependence of transport of K^+ to see if this function can be directly related to the phase state of the membrane lipids. In related studies on the possible lipid involvement in transport we have investigated the effects of a number of polypeptide antibiotics and metabolic inhibitors on the uptake and release of K^+. All the studies reported below were carried out on *Mycoplasma laidlawii* B.

MATERIALS AND METHODS

The growth and preparation of cultures were carried out using the same methods described in our previous paper on K^+ transport in *M. laidlawii*[5]. K^+ uptake using $^{42}K^+$ was also carried out as in the previous study[5].

The general procedure for studying temperature effects was to grow cultures at one temperature and then to study the K^+ uptake or efflux either at the growth temperature or at a series of higher or lower temperatures. A 200 ml sample of cells

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grown at temperature T_1 was thermally equilibrated at temperature T_2. Medium at temperature T_2 containing $^{42}K^+$ was added and 29 ml samples were periodically collected and assayed as previously described. In a related series of temperature shift experiments the $^{42}K^+$ uptake described above was allowed to come to a steady state and the culture was then shifted to another temperature after which samples were periodically removed to assay for intercellular K^+.

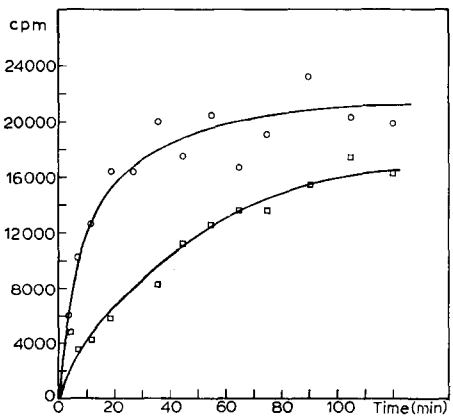
K^+ efflux experiments were generally carried out at 15 °C to slow the process to an easily measurably rate. Cultures were grown at some temperature T_1 and then pelleted and resuspended in a small volume of $^{42}K^+$ medium at 15 °C and incubated for 2 h to assure a steady state. To study leakage 2 ml samples with steady state $^{42}K^+$ levels were mixed with 50 ml of non-radioactive medium. At subsequent time intervals the 52 ml samples were filtered and assayed for intercellular K^+. Two types of non-radioactive diluting media were used: one the normal growth medium and the second normal growth medium lacking K^+.

The effect of polypeptide antibiotics on K^+ uptake was carried out on cells grown at 37 °C then incubated for 30 min with varying concentrations of antibiotic. Medium containing $^{42}K^+$ was then added and the entire mixture incubated for 30 min after which samples were withdrawn and assayed for intracellular $^{42}K^+$.

A series of experiments was also carried out in which cells were grown at temperature T_1 mixed with $^{42}K^+$ and incubated at temperature T_2. After 2 h to allow a steady state level of $^{42}K^+$, metabolic inhibitors were added and samples were periodically taken to measure the release of cation.

RESULTS

Uptake curves of $^{42}K^+$ at 25 °C are shown in Fig. 1. The cells were grown at 25 and 37 °C. The initial rate of uptake by 37 °C cells was about 2.5 times that of 25 °C cells. When the data is normalized on the basis of protein mass, the 37 °C



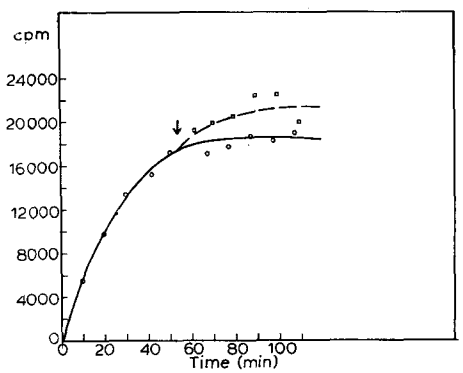


Fig. 1. Uptake of $^{42}K^+$ at 25 °C by *M. laidlawii* cells grown at 25 and 37 °C. ○—○, 37 °C grown cells; □—□, 25 °C grown cells.

Fig. 2. Effect of temperature lowering on the cellular level of $^{42}K^+$. Cells were grown at 25 °C and the uptake was carried out at that temperature. After the steady state was reached a portion was shifted to 15 °C. ○—○, uptake at 25 °C; □--□, uptake at 15 °C after temperature shift.

grown cells show a 13 % higher uptake of K^+. The times for half-maximal uptake were 7.5 min and 30 min for 37 and 25 °C cells, respectively.

The temperature shift experiments are shown in Figs 2–4. Shift to a lower temperature increases K^+ accumulation whereas shift to a higher temperature decreases this pool. The K^+ changes appear to be monotonically related to the tem-

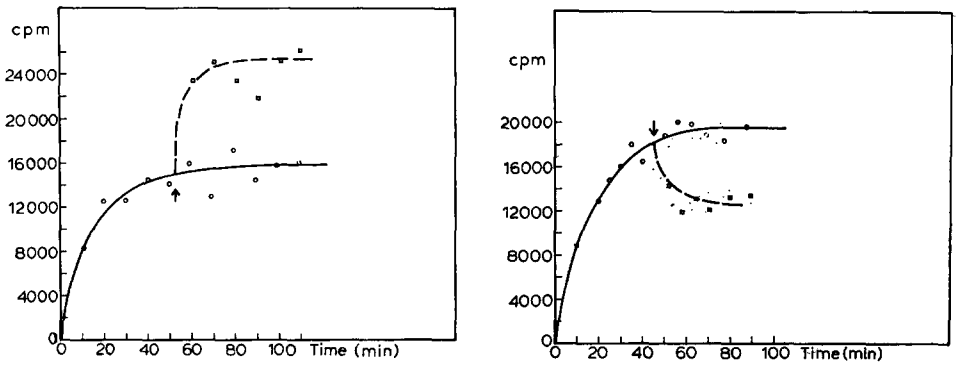


Fig. 3. Effect of temperature lowering on the cellular level of $^{42}K^+$. The growth temperature of the cells was 37 °C. After $^{42}K^+$ uptake has reached a steady state at 37 °C a portion was shifted to 15 °C. ○—○, uptake at 37 °C; □--□, uptake at 15 °C after temperature shift.

Fig. 4. Effect of temperature elevation on the cellular level of $^{42}K^+$. The growth temperature of the cells was 25 °C. After $^{42}K^+$ uptake has reached a steady state at 25 °C a portion was shifted to 37 °C. ○—○, uptake at 25 °C; □--□, uptake at 37 °C after temperature elevation.

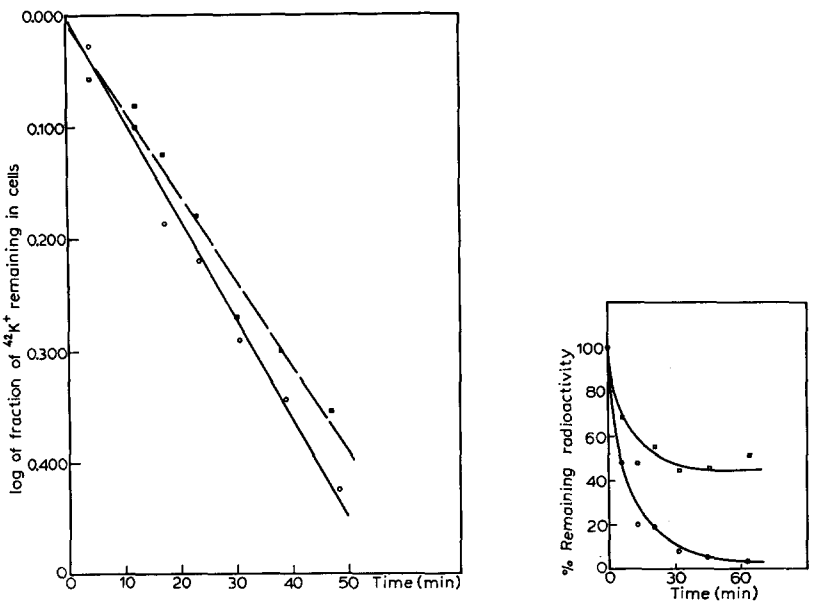


Fig. 5. Efflux of cellular $^{42}K^+$ at 15 °C from cells grown at 37 and 20 °C in the presence of $^{39}K^+$ in the medium. ○—○, 37 °C grown cells; □--□, 20 °C grown cells. Curves were fitted by the least square method.

Fig. 6. Efflux of cellular $^{42}K^+$ in the presence and absence of $^{39}K^+$ in the medium. Cells were grown at 37 °C. ○—○, $^{39}K^+$ containing diluting medium; □—□, diluting medium without K^+.

TABLE I

EFFECTS OF POLYPEPTIDE ANTIBIOTICS ON THE UPTAKE OF $^{42}K^+$ BY *M. laidlawii*

Based on the reproducibility of experiments the percent inhibition values are good to within better than ± 10%.

Polypeptide antibiotic	*Concn (μg/ml)*	*Inhibition of uptake at 30 min (%)*
Bacitracin	50	− 4
	300	−13
Polymixin B	200	34
	600	26
Tyrocidine	3	84
	1	23
Gramicidin S	3	98
	1	90
Valinomycin	0.5	91
	0.1	78
Gramicidin D	0.017	100
	0.0060	97
	0.0014	92
	0.0007	36

TABLE II

Inhibitor	*Concn (mM)*	*Average rate of efflux at 20° (s^{-1})*
p-Chloromercuriphenyl sulfonate	1.0	0.000286
Iodoacetate	1.0	0.000065
N-Ethylmaleimide	10.0	0.000097
Control	—	0.000990

perature shifts. The change in K^+ level occurred immediately after the temperature change.

The efflux rate at 15 °C was essentially independent of the growth temperature as shown in Fig. 5 where the loss of $^{42}K^+$ is shown for cells grown at 37 and 20 °C. The efflux curve was, however, drastically altered by the absence of K^+ in the diluting medium. This can be seen in Fig. 6 where aliquots of the same culture are diluted into media with and without exogenous potassium.

Efflux rate was also determined at three temperatures for cells grown at 37 °C. The average rate constants at 37, 20 and 15 °C were 0.00505, 0.000990 and 0.00033 s^{-1}, respectively.

The effects of polypeptide antibiotics on K^+ uptake is shown in Table I. The effect of various metabolic inhibitors on K^+ efflux is shown in Table II. Efflux experiments were also carried out in the presence of $1.75 \cdot 10^{-5}$ M gramicidin D. The loss of

intracellular $^{42}K^+$ was so fast that we were unable to measure a rate constant except to note that it is appreciably larger than 0.01 s^{-1}.

DISCUSSION

While the rate of uptake of K^+ appears to be influenced by the growth temperature of *Mycoplasma*, the final level appears to be a function of the actual temperature at the time of measurement and is virtually independent of the growth temperature. Thus the temperature shift experiments show the response of the steady state K^+ level to the temperature. If the phase state of the membrane lipids were principally responsible one would have anticipated strong effect of the growth temperatures on the temperature shift behavior since the transition temperature is related to the growth temperature[3]. For example, the curves shown in Figs 2 and 4 are for cells grown at 25 °C and combining the effect of a down shift to 15 °C and an up shift to 37 °C one would calculate a 63 % increase in cellular K^+ in going from 37 to 15 °C. When the 37 to 15 °C down shift experiment was actually carried out for cells grown at 37 °C (Fig. 3) the increase was 66 % which is within experimental error of the calculated value of 63 %. The change in steady state levels is thus independent of growth temperatures and indicates little effect of the phase behavior on this physiological property.

The independence of the 15 °C efflux rate on the growth temperature further argues against a strong correlation between this function and the phase state. The Arrhenius plot of logarithm of efflux rate *versus* the reciprocal of the absolute temperature yields an approximate straight line which again suggests little effect of the transition on K^+ exchanged. These data when viewed from the point of view of reaction rate theory yield a heat of activation of 17.8 kcal/mole and an entropy of activation of -14.1 cal·deg^{-1}·mole^{-1} for the process of K^+ efflux.

Very low concentrations of gramicidin D ($3 \cdot 10^{-9}$M) completely inhibit K^+-uptake in *M. laidlawii*. A cell concentration in these experiments is 10^9/ml. There are, therefore, only 1800 gramicidin molecules present per cell at the 100 % inhibition level. Since gramicidin D also causes the very rapid release of cellular K^+, it seems likely that the lipophilic compound acts to facilitate the diffusion of K^+ and therefore renders the membrane highly permeable to that ion. Similar effects of gramicidin have been demonstrated in other systems by Tosteson *et al.*[6] and by Harold and Baarda[7].

The fact that K^+ efflux is drastically reduced by the metabolic inhibitors, iodoacetate, *p*-chloromercuriphenyl sulphonate and *N*-ethyl-maleimide which are also inhibitors of uptake suggests that the efflux from these cells is basically an active process which has at most a small component of passive leakage. The residual flux in the presence of iodoacetate may represent this passive component. The dependence of efflux or external K^+ concentration further suggests that the efflux process cannot be one of leakage in the sense of passive diffusion of cation through the cell membrane.

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