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Effect of Poly-L-Lysine on Potassium Fluxes in Red Beet Tissue

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Summary. Poly-L-lysine concentrations (10^{-6} M) which cause slight leakage of pigment from beet cells completely disrupt the kinetics of *K (labeled) absorption at 25 °C in the range 0.01 to 50 mM KCl. Lower concentrations of polylysine (10^{-7} to 10^{-9} M) interfere with potassium fluxes at both cell membranes, initially increasing efflux across the plasma membrane and decreasing the capacity of the cytoplasm to retain ions during flux experiments at 2 °C. At 25 °C, these concentrations of polylysine increase *K (labeled) absorption from 0.2 mM KCl, but not from 10 mM KCl. These responses are discussed in relation to ion transport via the three-compartment in-series model proposed for plant cells. Particular emphasis is placed on the role of the plasma membrane in K transport from solutions of low concentration.

The toxic effects of L-lysine homopolymers in bacterial and fungal systems [1, 2] are presumably due to the disruption of membrane integrity by this strongly basic ion. A recent report shows poly-L-lysine (polylysine) causes leakage of pigment from the vacuoles of cells of thin slices of red beet [16]. The experiments suggest that polylysine displaces Ca^+ from cell membranes, thereby increasing membrane permeability. Many publications have demonstrated the membrane activity of polylysine and other basic polymers in mitochondrial systems [7, 15]. Many bases stimulate an energy-dependent K^+ efflux from the mitochondrion [7]. Similar concentrations of polylysine (10^{-5} to 10^{-7} M) increase chloroplast membrane permeability to H^+ and K^+, and inhibit light-induced volume changes [4]. These responses may reflect nonspecific membrane-active properties of polybases.

Plant cells are characterized by a peripheral thin layer of cytoplasm bounded externally by the plasma membrane, and internally by the tonoplast which separates the cytoplasm from a large watery vacuole. Much evidence indicates that at low external concentrations (0.01 to 0.5 mM) transport of ions across the plasma membrane limits the rate of ion uptake to the vacuole of higher plant cells [3, 10, 12]. At higher concentrations

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(above 1 mM), it is proposed that the plasma membrane no longer limits ion uptake to the vacuole, which is then determined by processes at the tonoplast. It is the change from plasma membrane-limited to tonoplast-limited absorption of ions which, in our view, gives rise to the characteristic dual isotherm describing the carrier properties of ion influx vs. concentration in plant cells [5, 8, 12]. This hypothesis could be examined further if it were possible to remove the barrier of the plasma membrane, thereby exposing the tonoplast more directly to the outside solution.

This paper describes preliminary attempts to expose the tonoplast by using polylysine to modify the ion-transporting properties of the plasma membrane and cytoplasm. In so doing, we have endeavored to determine the effects of polylysine on individual K fluxes at the cell membranes of beet tissue.

Methods

Discs (10 × 1 mm) of red beets (*Beta vulgaris L.*) were prepared and aged as described earlier [12]. KCl solutions of different concentrations containing 0.5 mM $CaSO_4$ were labeled with ^{86}Rb as tracer for potassium [6, 12] (designated *K). Polylysine hydrochloride (usually of mol wt 51,000) was added to pretreatment and to labeled experimental solutions as specified. Samples of radioactive solution or discs were dried on aluminium planchets and counted in a gas flow counter. The loss of pigment from discs was assayed colorimetrically at 500 nm.

The effect of polylysine on the exchange of ions from the cytoplasm and vacuole of beet cells was examined by comparing the rate constants for *K loss from preloaded tissue during washing in successive changes of unlabeled solution [3, 13, 14]. These rate constants are determined by the fluxes into and out of each compartment and by the content of that compartment. Many features of the exchange of ions in storage tissues such as beet and carrot are satisfactorily explained by a simple three-compartment in-series model, comprising outside solution (*o*), cytoplasm (*c*) and vacuole (*v*) [3, 14]. The rate constant for exchange from the cytoplasm (k_c) is much greater than that from the vacuole (k_v), and is determined by the relationship:

$$k_c = \frac{\phi_{co} + \phi_{cv}}{Q_c}. \tag{1}$$

The direction of the fluxes (ϕ) is shown by the subscript sequence (i.e., ϕ_{co} = flux from cytoplasm to outside), and Q_c is the content of the cytoplasmic compartment [3]. Similarly k_v, the rate constant for exchange from the vacuole, is determined by:

$$k_v = \frac{\phi_{vc} \cdot \phi_{co}}{\phi_{co} + \phi_{cv}} \cdot \frac{1}{Q_v}, \tag{2}$$

where direction of fluxes (ϕ) is indicated as before, and Q_v is the content of the vacuole. Rate constants k_v and k_c were determined by the graphical methods already described [14]. These methods also provide estimates of apparent content of labeled ions in each compartment at the commencement of exchange (I_c, I_v). The K^+ content of the tissue was determined by extraction and spectrophotometry at the end of the experiment.

From these quantities and with use of the expressions derived by Cram [3], individual fluxes and an estimate of Q_c were obtained. All exchange experiments were done at 1 to 2 °C to facilitate separation of cytoplasmic and free space compartments for *K [14].

In order to relate the effects of polylysine on fluxes at individual membranes to the kinetic parameters used to study the transport of ions in plant cells (dual isotherms of ion uptake [6, 12]), additional short-term isotope influx experiments were done. Previous experience had shown the rate of isotope influx to be linear during the first hour and to extrapolate to zero [12]. This was confirmed in some experiments, and in others the rate was computed from replicate samples taken after 30-min uptake. Discs were placed in labeled solutions of the prescribed concentration at 25 °C, with or without polylysine, and, after sampling, were washed in three changes of unlabeled solution during 30 min. This treatment removed at least 50 % of the cytoplasmic label, and the remainder was transported to the vacuole during the washing period [12].

Results and Discussion

Aged beet discs treated with polylysine solutions of high concentration (10^{-4} to 10^{-6} M) lose pigment from the vacuole at a steady rate for several hours. Fig. 1 shows that polylysine of mol wt 51,000 is more effective than high or low molecular weight polymers, and that increasing the concentration of polymers of each molecular weight increases the rate of pigment loss. These data are similar to those reported earlier [16].

The initial rate of *K absorption in aged beet discs, and in many other tissues, displays a dual hyperbolic isotherm when isotope influx is plotted against external concentration [5, 12]. Fig. 2 shows such an isotherm for aged beet discs and the effect of polylysine (10^{-6} M). This isotherm reflects

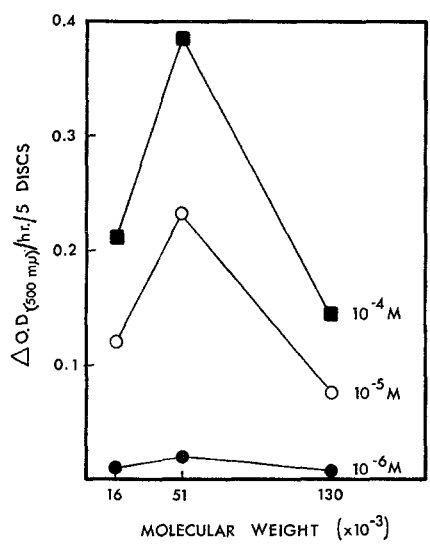

Fig. 1. Initial rate of pigment leakage from beet discs as a function of polylysine concentration and mol wt. Five discs shaken in 10 ml of solution at 20 °C

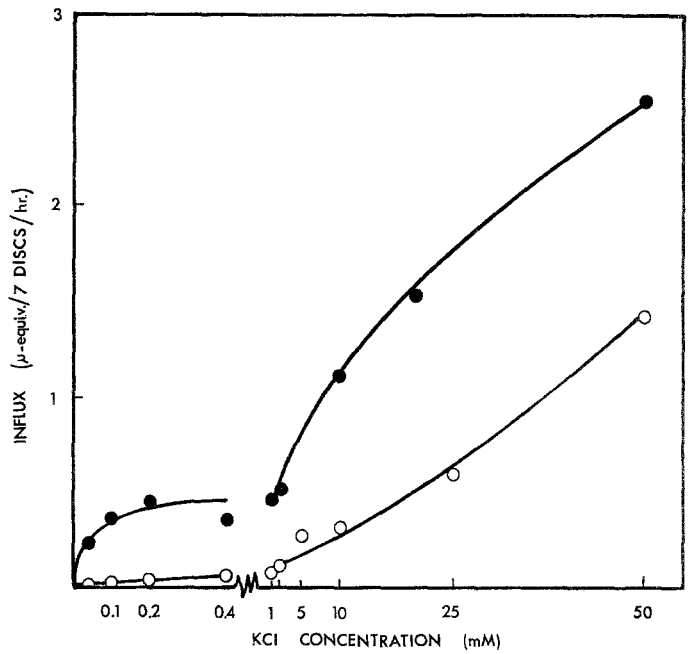

Fig. 2. Initial rate of *K influx into beet discs as a function of external concentration. Control discs (•) and discs pretreated for 3 hr in 10^{-6} M polylysine before uptake (○)

the active transport of ions across membranes, and the inhibition of active transport by low temperature converts the hyperbolic isotherm to an upwards concave form, identical to that predicted for diffusive entry of ions [9]. Fig. 2 shows that treatment of beet discs for 3 hr, at 25 °C with 10^{-6} M polylysine (which causes little pigment leakage) drastically alters the ion absorption isotherm for uptake at 25 °C, converting it to a form suggestive of a diffusion-dependent process. These data suggest that high concentrations of polylysine (10^{-6} M) inhibit the metabolically dependent transfer of ions in beet discs. However, slight pigment leakage suggests, and subsequent exchange experiments confirm, that this is associated with severe membrane damage.

Beet discs preloaded with *KCl (0.2 mM, 8 hr at 25 °C) exchange radioactivity to unlabeled solutions as shown in Fig. 3. In control discs (closed symbols), the successive semi-log plots permit clear separation of vacuolar and cytoplasmic components and the estimation of k_v and k_c from the linear portions of the graph. When 10^{-6} M polylysine was added to the unlabeled solution at the commencement of exchange, this analysis was no longer possible. The time course of exchange did not yield smooth curves of the type shown in Fig. 3, and the estimated k_v from such curves was increased

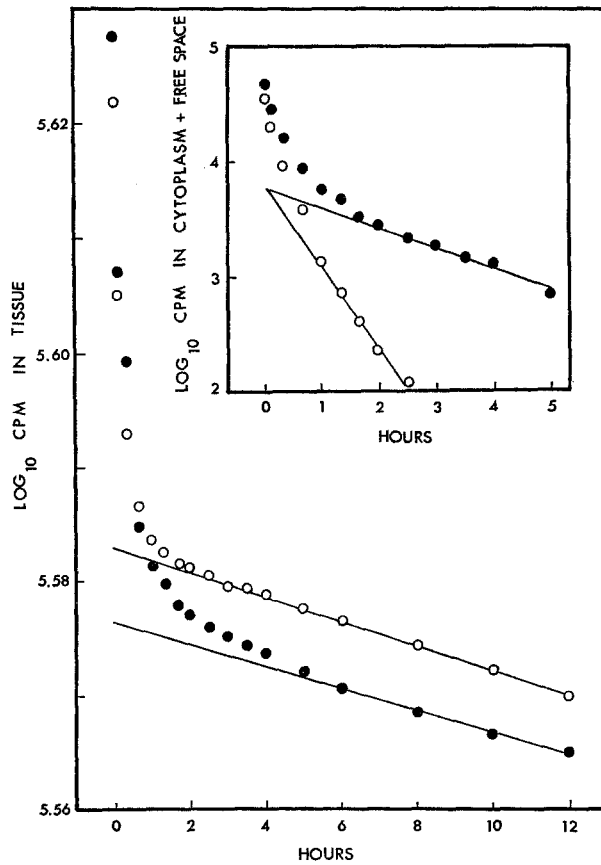

Fig. 3. Time course of exchange of *K from preloaded beet discs into 0.2 mM KCl at 2 °C. Control (•) and control + 2×10^{-7} M polylysine added to unlabeled solution during efflux (○). (Discs preloaded for 8 hr at 25 °C in 0.2 mM *KCl solution.) Insert, prepared by subtraction of extrapolated vacuolar content, shows exchange from cytoplasmic and free space compartments

about 10-fold over that of the control. Pigment leakage was also detected. These observations are consistent with the drastic effects shown in Figs. 1 and 2. By including treatments with progressively lower polylysine concentrations, it was possible to obtain a situation in which k_v was unaltered by the presence of polylysine. Fig. 3 shows this effect for 2×10^{-7} M polylysine solution (open symbols). Although k_v is unaltered by this treatment, k_c is accelerated about fivefold. The apparent isotope content of the cytoplasmic compartment of discs in 2×10^{-7} M polylysine and of control discs is identical (shown by extrapolation). When discs were preloaded for 12 hr in the same solution but at 2 °C, exchange in the presence of 10^{-7} M polylysine gave a twofold stimulation of k_c, and curves again extrapolated to

the same apparent isotope content in the cytoplasm. Polylysine above 5×10^{-7} M consistently gave irregular exchange curves, markedly increased k_v and resulted in pigment leakage.

These observations allow some predictions as to the location of polylysine action. The apparent isotope content of the cytoplasm (the fraction $\frac{\phi_{co}}{\phi_{co}+\phi_{cv}}$ of cytoplasmic isotope lost to the external solution) was unaltered by polylysine treatment. At the same time, there was no change in k_v; therefore, from Eq. (2), ϕ_{vc}/Q_v must be unchanged. Now, from Eqs. (1) and (2),

$$k_c = \frac{\phi_{co}+\phi_{cv}}{Q_c} = \frac{1}{Q_c} \cdot \frac{\phi_{co} \cdot \phi_{cv}}{k_v \cdot Q_v}. \tag{3}$$

Polylysine had little effect on Q_v (Table 1) so that, with Q_v, ϕ_{cv} and k_v constant, an increase in k_c must be the result of an increase in ϕ_{co}. This may be due to a change in ϕ_{co} or in Q_c, or in both. If ϕ_{co} increases, either ϕ_{cv} must increase proportionally or ϕ_{co} must be much larger than ϕ_{cv}. In 0.2 mM KCl, plasma membrane fluxes are small compared to tonoplast fluxes [3, 12]. Thus, in experiments of the type shown in Fig. 3, increased k_c implies an increase in ϕ_{co}, ϕ_{vc} and/or a decrease in Q_c.

Some support for this interpretation is obtained from an experiment in which discs were loaded ($t = 12$ hr) in labeled solution containing polylysine at different concentrations and then exchanged in unlabeled solutions of the same concentration. The primary data and fluxes calculated for this experiment are shown in Table 1. These calculations must remain suspect, for the equations used were derived for a steady state with zero net flux [3, 13]. At 2 °C and with 0.2 mM KCl, there is a net efflux in beet and this efflux is altered by experimental treatments. Q_v may have changed by as much as 10% during the experiment. However, the estimates show that polylysine increases net efflux at the plasma membrane up to twofold, and this is associated with a drop in Q_c of about 50%. These are among the conditions predicted to lead to the large increase in k_c relative to k_v found in experiments of the type described in Fig. 3.

It is evident from these experiments that polylysine at 10^{-6} M causes severe damage to cell membranes in beet discs. In the region of 10^{-7} to 10^{-8} M, polylysine applied for a short period of time (2 to 4 hr) seems to have little effect on tonoplast fluxes but increases efflux across the plasma membrane and appears to modify the "capacity" of the cytoplasm to retain ions. These may be manifestations of a nonspecific membrane-active effect of polylysine, first on the plasma membrane and subsequently on

Table 1. *Effect of polylysine on K fluxes in beet tissue at 2 °C*[a]

Property measured	Polylysine concn (M)			
	0	5×10^{-8}	10^{-7}	2×10^{-7}
Q_v (μequiv · g^{-1})	0.67	0.64	0.58	0.59
k_c (hr^{-1})	0.37	0.42	0.47	0.48
k_v (hr^{-1}) $\times 10^2$	0.90	1.09	1.18	1.32
I_v (μequiv · g^{-1})	3.20	2.34	1.98	1.43
I_c (μequiv · g^{-1})	0.24	0.23	0.22	0.20
$\phi_{oc}=\frac{I_v}{t}+k_c \cdot I_c$	0.36	0.29	0.27	0.22
$\phi_{co}=k_v \cdot Q_v+k_c \cdot I_c$	0.69	0.79	0.79	0.88
$\phi_{cv}=\phi_{co} \cdot \frac{I_v}{t} \cdot \frac{1}{k_c \cdot I_c}$	2.06	1.60	1.24	1.08
$\phi_{vc}=\phi_{cv}-(\phi_{oc}-\phi_{co})$	2.39	2.10	1.78	1.74
$Q_c=\frac{\phi_{co}+\phi_{cv}}{k_c}$	7.5	5.7	4.3	4.1

[a] Tissue was loaded in 0.2 mM *KCl + 0.5 mM $CaSO_4$ with polylysine for 12 hr at 2 °C, and then washed in frequent changes of the same but unlabeled solution for 12 hr at 2 °C.

cytoplasmic or organelle membranes. Alternatively, polylysine may lower the plasma membrane potential, leading to a decrease in ϕ_{oc} and an increase in ϕ_{co} (Table 1). This too would result in a decrease in Q_c, and, in the initial exposure type of experiment (Fig. 3), would cause a large increase in k_c relative to k_v.

It is difficult to extrapolate from these low-temperature flux experiments to the initial rate of isotope influx at 25 °C which is used to construct isotherms (Fig. 2). However, it is interesting to note that 4-hr treatment in polylysine in the range 5×10^{-9} to 10^{-7} M stimulates the subsequent uptake of labeled K to the vacuole of beet discs at 25 °C (Fig. 4). This response corresponds, in terms of effective polylysine concentration, to the modification of plasma membrane and cytoplasmic ionic relations at low temperature. The response to polylysine was found in 0.2 mM KCl where the plasma membrane appears to limit ion flux to the vacuole [3, 10, 12], but it was not found in 10 mM KCl (Table 2). At these concentrations (10 mM), ions may negotiate the plasma membrane more freely by diffusive processes [8, 12].

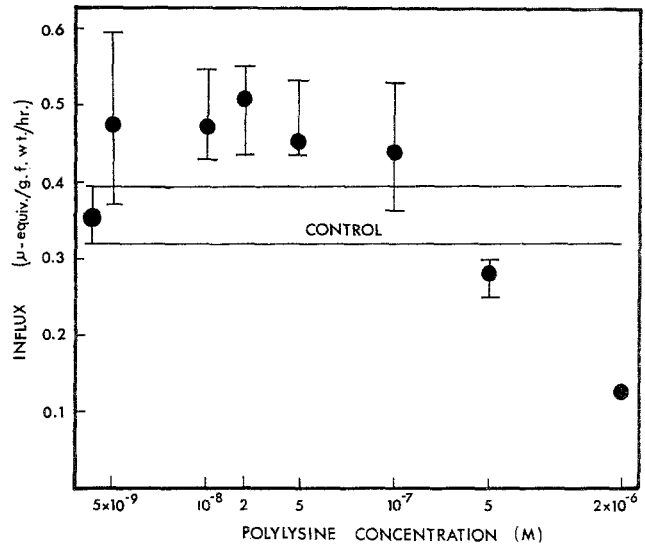

Fig. 4. Effect of polylysine concentration on initial rate of *K influx into beet discs from 0.2 mM *KCl. Discs pretreated 4 hr in polylysine solution prior to uptake from KCl solutions also containing polylysine (range of variability indicated by vertical bars)

Table 2. *Effect of polylysine on the initial influx of *K to the vacuole of beet discs at 25 °C*[a]

Polylysine concn (M)	Initial influx (μequiv/7 discs/hr)	
[External concn	0.2 mM KCl	10.0 mM KCl]
0 (control)	0.30	0.91
2×10^{-7}	0.48	0.96
10^{-6}	0.14	0.62

[a] Discs pretreated for 3 hr in polylysine solutions. Uptake solutions also contained polylysine.

Conclusions

These experiments have shown that polylysine has profound effects on the ion-transporting properties of cell membranes in beet. Polylysine may be selectively active in the concentration range 10^{-9} to 10^{-7} M, but at 10^{-6} M and above it causes severe damage to all membranes and results in pigment leakage [16]. The effective range for modification of ion fluxes in beet corresponds to the range found to interfere with membrane-dependent processes in organelles [4, 7, 15], perhaps suggesting a relatively nonspecific response to polylysine. The preliminary experiments described here do not permit distinction between direct effects of polylysine on cell membranes

and an indirect effect on ion movements via dependence on energy supply, particularly at 25 °C [7, 15].

On the basis of a three-compartment in-series model, these experiments suggest that polylysine at about 10^{-7} to 10^{-8} M (at 2 °C) may rapidly modify the movement of K^+ across the plasma membrane and decrease the cytoplasmic capacity to hold this ion. Further refinement of experimental techniques and mathematical analysis are required to establish these indications. For example, k_v may have been grossly overestimated in these 12-hr experiments [13], and the inadequacy of a simple three-compartment in-series model to describe ionic relations of plant cells is becoming increasingly apparent [11].

There is good reason to believe that ion uptake to the vacuole at 25 °C from 0.2 mM KCl is limited by transport of ions across the plasma membrane [3, 10, 12]. At higher ionic concentrations (e.g., 10 mM KCl), electrical and other evidence (cited in [12]) shows that the passive flux of ions across the plasma membrane permits more rapid uptake to the vacuole. The above data suggest that 10^{-7} to 10^{-8} M polylysine may mimic the effect of high ion concentrations on the plasma membrane, thus permitting more rapid access of ions to the tonoplast and vacuole from 0.2 mM KCl. These experiments support the notion that the plasma membrane limits ion uptake to the vacuole of plant cells at low but not at high external ion concentrations [8].

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