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Effects of Kinetin on the Permeability of *Allium cepa* Cells

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ABSTRACT

Permeability changes of *Allium cepa* cells were studied by a plasmometric method. Onion epidermis was floated in phosphate buffer solution with kinetin (2.5 milligrams/liter), or buffer without kinetin as a control, for 10 hours. The treated and control tissues were then transferred to one of the following four permeants: thiourea, urea, maloamide, dimethylurea.

Kinetin treatment increased the permeability of onion epidermal cells to thiourea and urea. The kinetin effect on permeability to malonamide and dimethylurea was not significant. It is suggested that kinetin might affect the protein component of the cell membrane.

It has been suggested that changes of membrane permeability to water and solutes could be one of the principal effects of growth substances (25). Permeability effects due to growth substances have frequently been reported, but little quantitative data are available on these compounds as they relate to cell permeability (1). It is known, for instance, that IAA increases the release of anthocyanin from *Beta vulgaris* root tissue (18) and changes the permeability of the epidermal cells of *Rhoeo discolor* to water (26). It is further known that 2,4-D decreases the uptake of ^{32}P (23) and glucose (13). The action of kinetin on the permeability of plant cells is not clear, although Feng and Unger (5) have reported a change of permeability of kinetin-treated onion epidermal cells to glycerol. Some studies indicate that kinetin causes reduced capacity of beet root tissue disks to absorb phosphate (16); kinetin is also reported to alter the uptake of monovalent cations by sunflower cotyledons (14); further, the half-time for tritiated water efflux in both cylinders of carrot root tissue and of pelargonium stem pith is influenced by kinetin (6). This paper is a report of a study of kinetin effects on the permeability of onion epidermal cell membrane to four nonelectrolytes.

MATERIALS AND METHODS

The inner epidermis of onion (*Allium cepa*) bulb scale was used. Epidermal tissue was cut into 12- × 15-mm strips. After floating the tissue on buffer solution for 10 hr in the dark at room temperature with or without kinetin, it was then transferred into a perfusion chamber. The flow of permeating solution through the chamber was kept constant by gravity.

The phosphate buffer solution (2.5 mM, pH 6) for pretreatment contained 1% sucrose with 2.5 mg/liter kinetin. The same buffer solution, less kinetin, was used for the pretreatment of the control tissue. All chemicals used were practical grade from Fisher Scientific Products, except kinetin (Calbiochem), and 1,3-dimethylurea (Eastman Kodak).

Permeability determinations were made by the plasmometric method (12, 21). After transferring the tissue to the perfusion chamber with buffer solution, the chamber was covered with a glass slip sealed with Vaseline and placed on the stage of a microscope. The permeating solutions were 1 M thiourea, 1 M urea, 0.9 M malonamide, and 0.8 M 1,3-dimethylurea. For the experiments with urea and malonamide, the hypertonic solutions were introduced directly into the chamber containing pretreated tissues. The solutions, at first, caused plasmolysis of the cells. As the permeants entered the cell, the protoplast expanded. The deplasmolysis is assumed to result from a permeation of the "plasmolyticum" into the treated cell. For experiments with the other two compounds (thiourea, dimethylurea) pretreated tissues were first plasmolyzed with 1 M mannitol and were then subjected to each of the permeants for observation of deplasmolysis. As the permeant was introduced into the perfusion chamber, a time lapse camera mounted on the microscope photographed the onion cells at given intervals. The camera was equipped with an auxiliary lens system to photograph a watch, automatically recording the time for each frame.

Cells nearly cylindrical in shape were selected for study. The lengths of the protoplasts were measured on the photographic negative by means of a low magnification dissecting microscope and an eyepiece micrometer. This was done as soon as the protoplast was perfectly plasmolyzed, at which time both ends were clearly rounded off. Measurements were made for each frame, and the time was recorded.

Using these measurements, a time-protoplast length diagram was plotted. Since the dilation of a protoplast proceeds linearly with time, a straight line was drawn and extrapolated to the time (t_0) when the permeating solution was introduced into the perfusion chamber. The protoplast length L_0 at time t_0, length L_1 at t_1, L_2 at t_2, and the width of the cell (b) were used to determine the absolute permeability constant (20):

$$K_s = \frac{b}{4} \times \frac{(L_2 - L_1) - \left(\frac{b}{3} \times \ln \frac{L_2}{L_1}\right)}{\left(L_0 - \frac{b}{3}\right) \times (t_2 - t_1)}$$

The appropriate conversion factors from min to sec, and micrometric units to cm were also applied, permitting the permeability constant K_s to be read in $cm \cdot sec^{-1}$.

The osmotic ground value (O_g) was calculated according to equation $O_g = C \cdot (L - b/3)/h$ (12, 21), wherein h is the length of the cell, b is the diameter of the cell, L is the length of the protoplast after the completion of plasmolysis, and C is the concentration (M) of the plasmolyticum.

RESULTS

Figure 1 shows the changes in length of the protoplast (L) in thiourea solution (1 M). Figure 2 is a similar graph for cells in 1 M urea solution. Both figures show the general tendency

of an increased rate of deplasmolysis due to kinetin treatment. Although cells in thiourea were preplasmolyzed in 1 M mannitol solution and cells in urea were plasmolyzed in the same (urea) solution, the kinetin effects were similar. However, in either malonamide and dimethylurea solutions, the rate of deplasmolysis of the kinetin-treated cells was almost the same as that of the control (Figs. 3 and 4).

About 20 cells were measured from each treated, or control, epidermis. Each experiment was repeated a minimum of five times. Ten cells were picked at random for each statistical evaluation. The absolute permeability constant (K_s) of the cells in the control and kinetin-treated groups that were tested with thiourea and urea were all distributed in a normal curve. The means and standard errors are presented in Table I for the t tests (2) and appear to be statistically significant. It is clear that the K_s values for thiourea and urea of the treated cells were greater than those of the controls. The mean K_s values for malonamide and dimethylurea are also presented in Table I. There is no significant difference in permeability to these permeants between the treated and control cells.

Since variation in osmotic values may change the permeability constant of a cell, all such values during incipient plasmolysis (O_g) were calculated. The mean O_g of the controls was

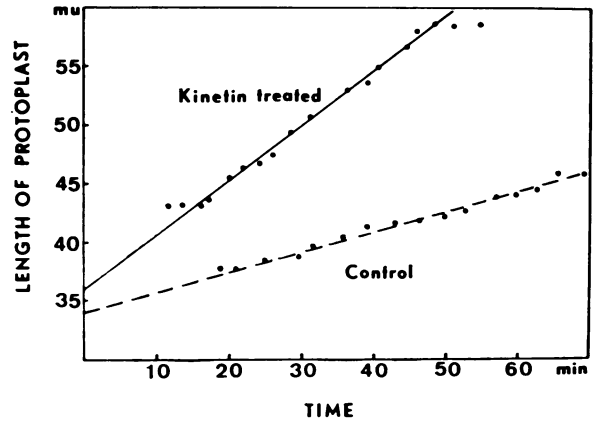

FIG. 1. Effects of kinetin on the rate of deplasmolysis of onion epidermal cells in thiourea (1 M). All cells had been preplasmolyzed in mannitol solution (1 M). Kinetin-treated cell (——): 10 hr in buffer with kinetin (2.5 mg/liter). Control cell (– – –): 10 hr in buffer solution. MU: micrometric unit.

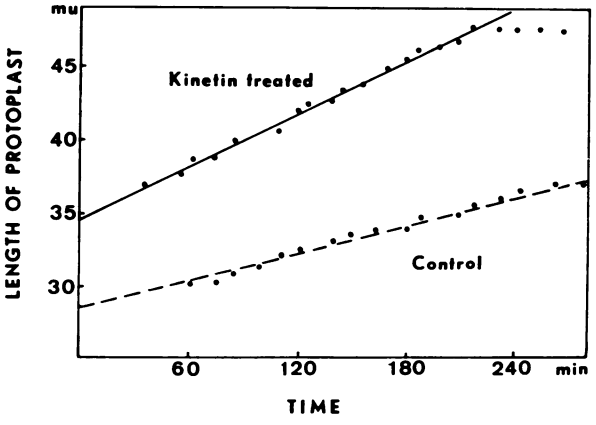

FIG. 2. Effects of kinetin on the rate of deplasmolysis of onion epidermal cells in urea (1 M). Kinetin-treated cell (——): 10 hr in buffer with kinetin (2.5 mg/liter). Control cell (– – –): 10 hr in buffer solution. MU: micrometric unit.

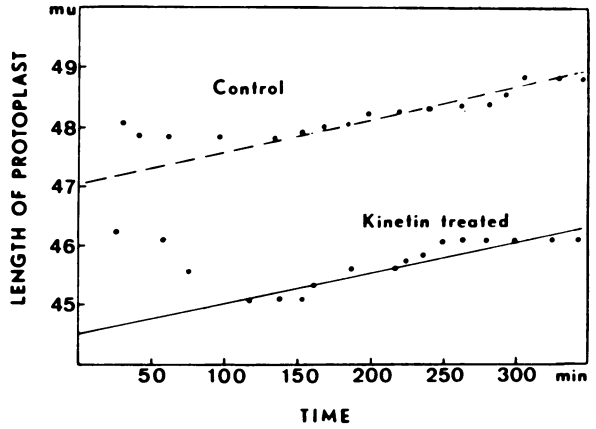

FIG. 3. Effects of kinetin on the rate of deplasmolysis of onion epidermal cells in malonamide (0.9 M). Kinetin-treated cell (——): 10 hr in buffer with kinetin (2.5 mg/liter). Control cell (– – –): 10 hr in buffer solution. MU: micrometric unit.

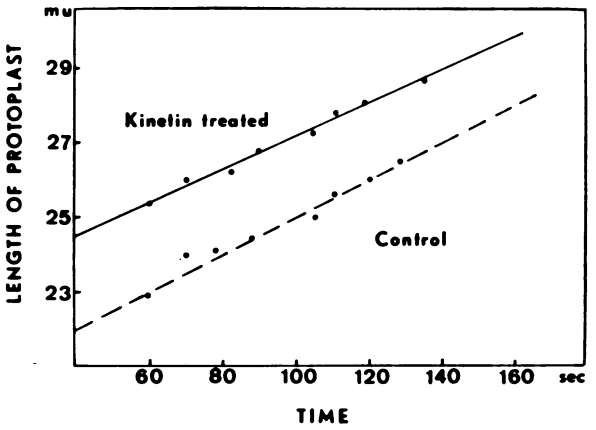

FIG. 4. Effects of kinetin on the rate of deplasmolysis of onion epidermal cells in 1,3-dimethylurea (0.8 M). All cells had been preplasmolyzed in mannitol solution (1 M). Kinetin-treated cell (——): 10 hr in buffer with kinetin (2.5 mg/liter). Control cell (– – –): 10 hr in buffer. MU: micrometric unit.

Table I. *Permeability of Onion Epidermal Cells Treated with Kinetin (2.5 mg/liter) Compared with Controls*

Ten cells were picked at random for each statistical evaluation.

Permeants	Control Cells	Kinetic-treated Cells
	K_s = cm·sec^{-1}	
Thiourea	$8.23 \pm 0.30 \times 10^{-8}$	$2.58 \pm 0.19 \times 10^{-7}$*
Urea	$3.93 \pm 0.12 \times 10^{-8}$	$6.05 \pm 0.13 \times 10^{-8}$*
Malonamide	$3.44 \pm 0.55 \times 10^{-9}$	$2.32 \pm 0.33 \times 10^{-9}$
Dimethylurea	$3.95 \pm 0.71 \times 10^{-6}$	$3.92 \pm 0.78 \times 10^{-6}$

* Significantly different from control at 1% level.

527 ± 62 mM, and that of the treated cells 536 ± 15 mM. Hence, the difference is not significant.

DISCUSSION

It is generally assumed that one or both of two principles can be used to explain the permeation of nonelectrolytes through a membrane: (*a*) lipid solubility and (*b*) sieve effect principle (4). These principles are referred to as the passing

of permeants through the lipid phase or the aqueous channel (3, 10, 22). The sieve effect may be visualized as transmission of molecules via pores in the lipid layer (8, 17). Lipid solubility is accomplished by an orientation effect which enhances the penetration of small molecules through the lipid layer (4). While malonamide does not penetrate via a sieve effect (24), urea, and also thiourea, may do so (27).

Both urea and thiourea are frequently used as test substances in permeability research, although it has been reported that they are possibly damaging to membranes (11, 9). However, in the experiments presented here, no damaging effects were observed; the time course of deplasmolysis remained linear (Figs. 1 and 2), indicating that no change in permeability occurred during deplasmolysis of the control cells.

Among the four permeants, both malonamide and dimethylurea have high oil-water partition coefficients and molar refraction compared to thiourea and urea. Of the four, dimethylurea has the fastest permeating rate and malonamide, the slowest, as shown in Table I. The same table also shows that the effects of kinetin on permeability to the more polar molecules urea and thiourea are significant in contrast to the nonsignificant effects of the less polar compounds. It might be assumed that the kinetin treatment does not affect the lipid phase of the membrane system.

The results show that kinetin is effective in changing the permeability to substances passing, at least partially, through the "aqueous channel." Such a "channel" does not appear to be in the lipid bilayer of the membrane, since the lipid phase of the treated cells does not appear to be affected. Therefore, the aqueous channel could be via the protein phase of the membrane. In the "protein crystal model" (7), or the "fluid mosaic model" (19), the aqueous channel can be thought to be linked to protein reaching through the membrane. Changes in these proteins by kinetin would need to be assumed to explain the above findings. Involvement of kinetin in changes of proteins has been suggested (15).

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