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THE EFFECT OF DIVALENT CATIONS ON THE LEAKAGE OF SUCROSE FROM CORN SCUTELLUM SLICES*

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Abstract—During the incubation of corn scutellum slices in fructose, the presence of Ca^{2+}, Mn^{2+} or Mg^{2+} in the bathing medium reduced the leakage of sucrose (leakage A). Ca^{2+} was particularly effective in this respect. Following a period of incubation in fructose, the subsequent leakage of sucrose into water (leakage B) was greatly reduced by the addition of either Ca^{2+} or Mn^{2+} to the aqueous bathing medium, whereas the addition of Mg^{2+} to the bathing medium only slightly decreased this leakage. In order for Ca^{2+} to be effective in inhibiting the leakage of sucrose, it had to be present in the medium into which leakage was occurring. During Ca^{2+}-inhibition of leakage B, the sucrose of the synthesis compartment was stored in an area of the cell from which it would not leak into water or EDTA. Possible mechanisms for the effect of Ca^{2+} on sucrose leakage are discussed.

INTRODUCTION

INCUBATION of corn scutellum slices in fructose (0·2–0·9 M) resulted in the synthesis of sucrose (25–30 μmoles/hr/g fresh wt.).[1] Some sucrose leaked from the tissue during incubation in fructose (leakage A) and a further leakage occurred when the fructose solution was replaced by water (leakage B). The rate of sucrose synthesis was independent of the fructose concentration of the surrounding medium within the range 0·2–0·9 M, and the amount of sucrose leakage A was independent of the fructose concentration within the range 0·2–0·7 M. However, the amount of leakage B increased as the fructose content of the incubation medium was increased from 0·2 to 0·9 M. This increase in leakage B was accompanied by a decrease in the amount of sucrose stored. These data indicate that high concentrations of fructose inhibit sucrose storage in the tissue slices. The results of experiments utilizing ^{14}C-fructose suggested that leakage B sucrose was newly synthesized sucrose which was not stored by the tissue, while leakage A came from a sucrose storage pool which existed prior to incubation in fructose and was released during incubation to the cell exterior by an exchange reaction at a storage compartment membrane.

In this previous study, the rate of sucrose leakage B was found to be inhibited by fructose, galactose, polyhydric alcohols, and 2,4-dinitrophenol. The present study is concerned with the effects of certain divalent cations on sucrose leakage from tissue slices with particular attention to the fate of newly synthesized sucrose under conditions which inhibit leakage B.

RESULTS

Effect of Divalent Cations on Sucrose Leakage A

The leakage of sucrose from the scutellum slices during a 3-hr period of incubation in 0·5 M fructose (leakage A) was considerably reduced when Ca^{2+} (0·04 M) or Mn^{2+} (0·04 M)

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[1] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **5**, 653 (1966).

were present in the bathing medium (Table 1). In addition, the presence of Mg^{2+} (0·04 M) in the fructose bathing medium caused a reduction in the amount of leakage A; however, this cation was not as effective in this regard as either Ca^{2+} or Mn^{2+}. At the lowest level tested (0·001 M), Ca^{2+} was still effective in reducing the amount of sucrose leakage A whereas Mg^{2+} and Mn^{2+} did not show significant effects.

TABLE 1. THE EFFECT OF DIVALENT CATIONS ON SUCROSE LEAKAGE A*

Additions to incubation solution containing 0·5 M fructose	No. determinations	Sucrose leakage A (μmoles from 1 g fresh wt.)
0	9	18·7 ± 2·09
0·001 M $CaCl_2$	7	13·3 ± 1·76
0·04 M $CaCl_2$	9	8·1 ± 0·76
0	12	18·6 ± 2·12
0·001 M $MgCl_2$	10	18·7 ± 1·69
0·04 M $MgCl_2$	10	15·8 ± 1·36†
0	6	17·5 ± 0·70
0·001 M $MnCl_2$	6	16·3 ± 1·22
0·04 M $MnCl_2$	6	9·2 ± 1·00

* The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 10 ml of the various solutions after which time samples of the bathing solutions were taken for sucrose analysis.

† Significantly different at the 1 per cent level from the control value as shown by the *t*-test.

Effect of Divalent Cations on Sucrose Leakage B

Inasmuch as Ca^{2+}, Mn^{2+} and to some extent Mg^{2+} retarded the leakage of sucrose from the scutellum slices during their incubation in fructose (leakage A), experiments were conducted to determine the amounts of sucrose leakage B in the presence of these cations. The tissue slices were usually incubated at 30° for a period of 3 hr in 1·0 M fructose during which time they accumulated sucrose (both stored and leakage B sucrose). A 1·0 M fructose concentration was used in these experiments to increase the amount of leakage B sucrose thus facilitating its measurement. The amount of sucrose in the tissue following incubation in fructose (140–160μmoles/g fresh wt.) varied among samples from different crops of seedlings, as did the tissue levels of sucrose prior to incubation in fructose. However, such variations were not observed among samples taken from a single crop of seedlings. Immediately following the incubation period in fructose, the bathing medium was removed and the slices were then allowed to leak sucrose (leakage B) into water or various concentrations of the different cations. The time course of sucrose leakage B was then followed for 60 min. The effect of Ca^{2+} on sucrose leakage B is shown in Fig. 1 and that of Mg^{2+} is shown in Fig. 2. It is apparent that Ca^{2+} strongly inhibited leakage B and that the degree of inhibition depended on the concentration of the cation in the surrounding medium. The presence of Mg^{2+} in the bathing solution during leakage B only slightly inhibited sucrose leakage from the slices, and the pronounced effect of concentration of cation on leakage which was observed with Ca^{2+} was not apparent in this case. The effect of Mn^{2+} on sucrose leakage B (data not given in this paper) was almost identical to that observed for Ca^{2+}. From the results shown in Table 2, it is apparent that the reduction, due to the presence of the cations, in the amount of

sucrose which leaked into the bathing medium could be accounted for by an increase in the amount of sucrose remaining in the tissue. There is no evidence that varying the concentration of either cation in the bathing solution had any effect on sucrose catabolism.

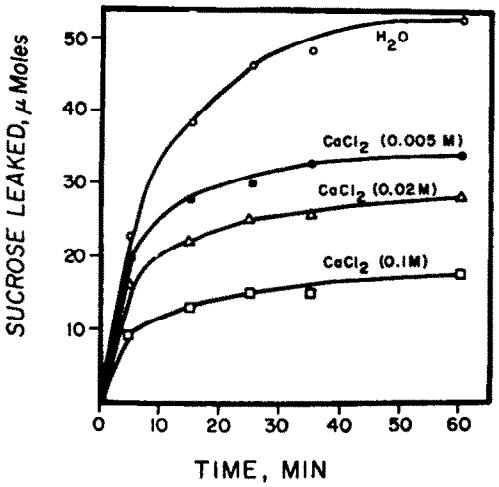

FIG. 1. CALCIUM INHIBITION OF SUCROSE LEAKAGE B.

The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 1·0 M fructose. At the end of this period, the bathing solution was removed from the flask by suction, and the slices were washed by the rapid addition and removal of 10 ml of water or $CaCl_2$ solution. Then 10 ml of water or $CaCl_2$ were added to the flasks (time zero on the graph). Portions of the bathing solution were removed for sucrose analysis at the times shown.

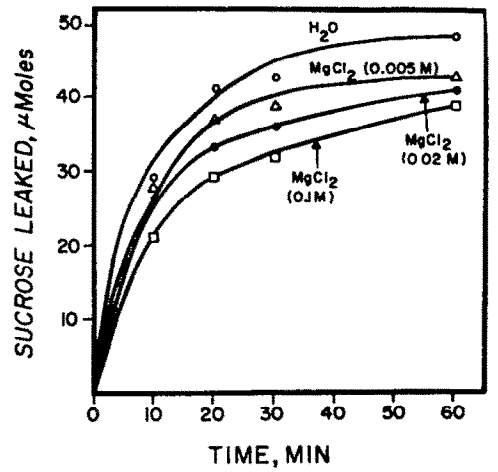

FIG. 2. MAGNESIUM INHIBITION OF SUCROSE LEAKAGE B.

The experimental procedure was the same as that shown in Fig. 1 except that $MgCl_2$ was substituted for $CaCl_2$.

In order for Ca^{2+} to be effective as an inhibitor of sucrose leakage, it must be present in the medium during leakage. As can be seen in Table 3, the presence of Ca^{2+} during incubation in fructose had little effect on the amount of sucrose subsequently leaked into water. In

addition, the presence of Ca^{2+} both during incubation in fructose and during leakage was no more effective in reducing sucrose loss from the tissue than when Ca^{2+} was present only during the leakage period.

TABLE 2. THE EFFECT OF Ca^{2+} AND Mg^{2+} ON SUCROSE LEAKAGE B AND TISSUE SUCROSE*

Bathing solution	Sucrose (μmoles from 1·0 g fresh wt.)				
	Tissue level after leakage B	+	Leakage B	=	Total
Water (control)	87·8	+	53·0	=	140·8
$CaCl_2$ (0·1 M)	124·0	+	18·0	=	142·0
$CaCl_2$ (0·02 M)	112·2	+	28·4	=	140·6
$CaCl_2$ (0·005 M)	105·0	+	34·5	=	139·5
Water (control)	102·8	+	48·8	=	151·6
$MgCl_2$ (0·1 M)	116·7	+	39·3	=	156·0
$MgCl_2$ (0·02 M)	111·1	+	41·5	=	152·0
$MgCl_2$ (0·005 M)	114·0	+	43·3	=	157·3

* The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of 1·0 M fructose. At the end of this period, the bathing solution was removed from the flask by suction, and the slices were washed by the rapid addition and removal of 10 ml of water or cation solution. Then 10 ml of water or cation solution were added to the flasks. After sucrose leakage B was completed (60 min), the slices were quickly washed with 50 ml of water and then killed and extracted for the determination of tissue sucrose.

TABLE 3. THE EFFECT OF THE ADDITION OF $CaCl_2$ TO THE FRUCTOSE BATHING SOLUTION OR TO THE LEAKAGE B BATHING SOLUTION ON THE AMOUNT OF SUCROSE LEAKAGE B*

Addition to bathing solution during initial incubation containing 1·0 M fructose	Bathing solution into which sucrose leaked	Sucrose leakage B (μmoles from 1·0 g fresh wt.)
0	Water	49·3
$CaCl_2$ (0·02 M)	Water	41·4
0	$CaCl_2$ (0·02 M)	28·0
$CaCl_2$ (0·02 M)	$CaCl_2$ (0·02 M)	28·8

* The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of the initial bathing solution. At the end of this period, the bathing solution was removed from the flask by suction and the slices were washed with 10 ml of water or $CaCl_2$. After washing, 10 ml of water or $CaCl_2$ were added to the flasks. The slices were incubated for an additional 1 hr after which a portion of each bathing solution was removed for determination of sucrose leakage B.

The effects of the duration of incubation in Ca^{2+} on sucrose leakage B were investigated. Scutellum slices which had been allowed to synthesize sucrose for 3 hr in the presence of 1·0 M fructose were placed in 0·02 M $CaCl_2$ for periods of 10, 20 and 30 min after which the Ca^{2+} solutions were replaced by water. Incubation was then continued for periods sufficient to give a total of 60 min leakage. Additional groups of slices were incubated in water and in 0·02 M $CaCl_2$ for the entire 60-min incubation period. The time course of sucrose leakage B was followed. These results are shown in Fig. 3. When the slices which were pretreated

in fructose were placed in water, sucrose leakage B was pronounced (50 μmoles/g fresh wt.) and essentially complete within 60 min. If the slices were placed in 0·02 M Ca^{2+} for the entire 60-min incubation period, there was a marked reduction (48 per cent) in the amount of sucrose that leaked from the slices. However, exposure of the slices to 0·02 M Ca^{2+} for the first 10 min of the 60-min leakage period resulted in only a 12 per cent reduction in the total amount of leakage B. Increasing the exposure of the slices to Ca^{2+} to 20 min further reduced (32 per cent) the total amount of leakage B. After 30 min in Ca^{2+}, little additional leakage of sucrose occurred after removal of the cation, and the time-course curve for leakage B was almost identical to that observed when the slices were incubated in Ca^{2+} for the entire 60-min period. It is apparent that as the period of exposure to Ca^{2+} (or the length of time during which leakage B was inhibited) was increased, there was a progressive reduction in the tissue

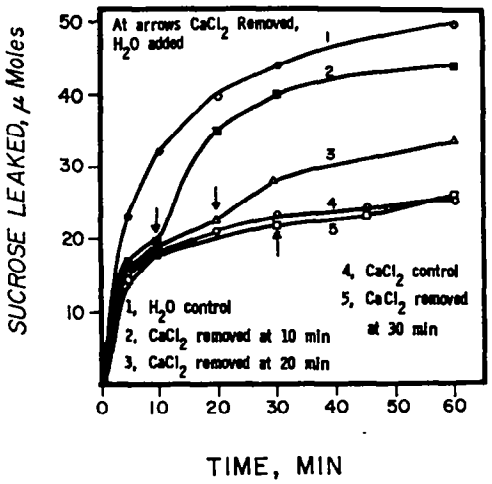

FIG. 3. EFFECT OF DURATION OF $CaCl_2$ TREATMENT ON $CaCl_2$ INHIBITION OF SUCROSE LEAKAGE B. The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of 1·0 M fructose. At the end of this period the bathing solution was removed and the slices were washed with 10 ml of water or $CaCl_2$ (0·02 M). Then 10 ml of water or $CaCl_2$ (0·02 M) were added to the flasks (zero time on the graph). Portions of the solutions were removed for sucrose analysis at the times shown. At the times indicated by the arrows, the $CaCl_2$ solutions were removed from the appropriate flasks, the slices were washed with 10 ml of water, and 10 ml of water were added to each flask for additional sucrose leakage.

of leakable sucrose. It is probable that during the inhibition of leakage B by Ca^{2+} some of the sucrose which would be free to leave the tissue in the absence of the cation is stored in an area of the cell from which it cannot leak.

It has been postulated that sucrose is synthesized and stored in different compartments within corn scutellum cells, and that there is no obligatory coupling between the two processes.[1] It is assumed that the origin of leakage B is the sucrose synthesis compartment, and that the leakage itself represents an amount of sucrose produced above that stored by the tissue. This accumulation of leakable sucrose is believed to result from the inhibition of the storage process by high concentrations of fructose. Since Ca^{2+} inhibits the leakage of sucrose from the B compartment, and leakable sucrose entirely disappears from the tissue during incubation in Ca^{2+} (see Fig. 3), the question arises: does the sucrose which fails to leak into Ca^{2+} solutions remain in the synthesis compartment or is it transported to a storage area of the cell? To provide an answer to this question, the following experiment was conducted (Table 4).

Scutellum slices were incubated in 1·0 M fructose for 3 hr during which time they synthesized sucrose (leakable and stored). Following this incubation, the slices were placed either in water or 0·1 M $CaCl_2$ and leakage B was determined after 1 hr. The slices were again placed in fructose and allowed to synthesize sucrose for an additional 1-hr period after which the tissue sucrose and leakable sucrose (leakage B) were determined. Following the initial incubation in fructose, the presence of Ca^{2+} in the leakage medium reduced the amount of leakage B by approximately 36 μmoles. This amount of sucrose remained in the tissue. That this sucrose was actually stored by the tissue during incubation in Ca^{2+} is shown by the results of the second incubation in fructose. Following this second fructose incubation, the total tissue sucrose of the Ca^{2+}-treated slices remained above that of the water controls by an

TABLE 4. TISSUE SUCROSE AND LEAKAGE B SUCROSE FOLLOWING A SECOND FRUCTOSE INCUBATION OF SCUTELLUM SLICES PREVIOUSLY TREATED WITH WATER OR $CaCl_2$

Treatment*	Sucrose (μmoles from 1·0 g fresh wt.)	
First incubation: 3 hr in 1·0 M fructose followed by determination of leakage B from:		
Groups 1 and 2 into water	50·5	50·5
Groups 3 and 4 into 0·1 M $CaCl_2$	14·0	15·0
Difference	36·5	35·5
Second incubation: 1 hr in 1·0 M fructose followed by determination of leakage B from:		
Group 1 into water	22·7	
Group 3 into water	26·6	
or by determination of tissue sucrose in:		
Group 2	116·7	
Group 4	155·5	
Difference	38·8	

* Following each fructose incubation the slices were washed with 10 ml of water (or $CaCl_2$) and then 10 ml of water (or $CaCl_2$) were added to the flasks. After the first leakage B period the slices were washed with 10 ml of water and then 10 ml of 1·0 M fructose were added. Leakage B sucrose was determined after 1-hr leakage period.

amount equivalent to the initial reduction in leakable sucrose attributable to Ca^{2+} (38·8 μmoles compared to 36 μmoles). Because the leakable sucrose following the second incubation in fructose was essentially the same for both groups of slices, the excess sucrose in the Ca^{2+}-treated tissue must be regarded as stored sucrose. The obvious interpretation of these data is that following the initial incubation in fructose the synthesis compartment could readily be emptied of sucrose by allowing leakage to take place into water. If, however, this leakage was inhibited by the presence of Ca^{2+} in the surrounding medium, depletion of sucrose in the synthesis compartment was primarily the result of sucrose storage in a cellular compartment from which leakage of sucrose would not occur. The Ca^{2+} in the leakage medium following the first incubation in fructose apparently had no residual effect on the synthesis and storage of sucrose during the second period in fructose. This is apparent because (1) the amount of leakable sucrose produced during the second incubation in fructose was essentially the same for both groups of slices, and (2) the difference in tissue sucrose

between Ca^{2+}-treated and non-treated slices could be explained as increased stored sucrose prior to the second incubation period in fructose.

In order to characterize further the sucrose distribution patterns in corn scutellum tissue, several preliminary experiments were conducted during which the tissue was exposed to solutions of ethylenediaminetetraacetic acid (disodium salt, Na_2EDTA). The effect of EDTA on sucrose leakage B is shown in Fig. 4. The slices were allowed to synthesize sucrose for 3 hr while being incubated in 1·0 M fructose. Following this incubation, the slices were placed in various solutions (zero time of the graph) and the time course of sucrose leakage B was followed. If, following their incubation in fructose, the slices were placed in 0·02 M Na_2EDTA

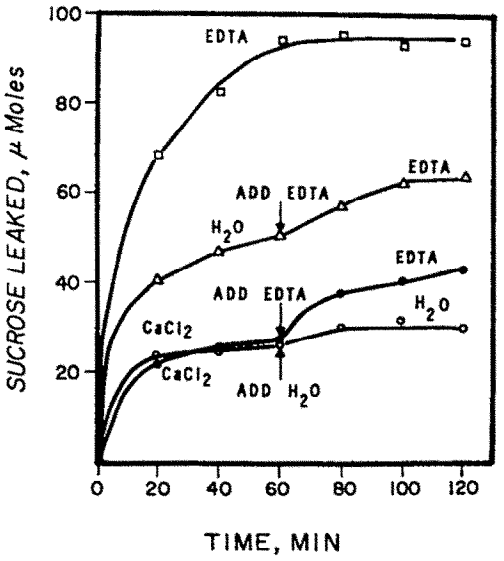

FIG. 4. EFFECT OF EDTA ON SUCROSE LEAKAGE.

The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of 1·0 M fructose. At the end of this period, the bathing solution was removed and the slices were washed with 10 ml of water, 10 ml of Na_2EDTA (0·02 M) or 10 ml of $CaCl_2$ (0·02 M). After the slices had been washed, 10 ml of water, Na_2EDTA or $CaCl_2$ were again added (zero time on the graph). At the times indicated, portions of these solutions were removed for sucrose analysis. As indicated on the graph, after 60 min the solutions in some of the flasks were replaced with 10 ml of Na_2EDTA (0·02 M) or with 10 ml of water after first washing the slices with 10 ml of Na_2EDTA or water.

for 120 min, sucrose leakage was rapid and complete within 60 min. The amount of sucrose leaked into EDTA represented 70–75 per cent of the total tissue sucrose at the end of the initial incubation period in fructose. Under similar conditions, the amount of sucrose leaked into water was only about 35 per cent of the total sucrose found in the tissue following incubation in fructose. These data suggest that EDTA treatment altered the tissue in such a manner as to allow the leakage of a large portion of the sucrose from the storage area(s) of the cells. It is interesting to note that when the slices were first placed in water for a period of 60 min, only a small increase in sucrose leakage occurred when the slices were subsequently incubated in Na_2EDTA. Inasmuch as a 60-min period of leakage into water is sufficient to deplete the slices of leakable sucrose (Figs. 1, 2 and 3), it may be concluded that, in this case, EDTA had little if any effect in causing the leakage of stored sucrose. This is in sharp contrast with results obtained when EDTA was added immediately following the initial incubation in fructose.

If, immediately following incubation in fructose, the slices were placed in 0·02 M $CaCl_2$ (see Fig. 4) there was a marked decrease in sucrose leakage, as was noted previously (Fig. 1). Following this period of inhibited leakage into Ca^{2+}, incubation in EDTA produced no greater amount of sucrose leakage than when EDTA treatment was preceded by 60 min incubation in water. These data suggest that the sucrose which was held in the tissue by Ca^{2+} was transported to an area of the cell from which it was not released by treatment with EDTA or by further incubation in water.

The effect of the fructose concentration of the bathing medium on the subsequent leakage of sucrose into EDTA is given in Table 5. If the slices were incubated in 0·5 M fructose for 3 hr and then were placed in either water or 0·02 M EDTA, there was little difference in the amounts of sucrose that leaked from the tissue. In contrast, when the slices were incubated in 1·0 M fructose the amount of sucrose which leaked into EDTA was almost double the amount which leaked into water. In addition, when EDTA was added to the fructose incuba-

TABLE 5. THE EFFECT OF Na_2EDTA ON SUCROSE LEAKAGE

Conditions*	Sucrose (μmoles from 1·0g fresh wt.) leaked into: Water	Na_2EDTA (0·02 M)
Prior 3-hr incubation in:		
Fructose (0·5 M)	26·7	32·7
Fructose (1·0 M)	59·8	105·1
Mannitol (0·9 M)	10·0	14·4
Prior 1·5-hr incubation in:		
Fructose (1·0 M)	32·5	76·2
Fructose (1·0 M)+ Na_2EDTA (0·02 M)	87·8	81·5

* The slices (1·0 g fresh wt.) were incubated at 30° in 9·0 ml of the solution indicated. At the end of the incubation period, the slices were washed with 10 ml of water or Na_2EDTA (0·02 M) after which 10 ml of water or Na_2EDTA were added to the flasks. The slices were incubated for an additional 90 min and then portions of the bathing solutions were removed for sucrose determination.

tion medium, subsequent sucrose leakage into water was of the same magnitude as that into EDTA. These data indicate that the action of EDTA in increasing sucrose leakage from scutellum tissue is in some manner dependent on the presence of high concentrations of fructose (or possibly sucrose) in the synthesis compartment, or at least, some prior "conditioning" of the cell membranes by high concentrations of these sugars. That this effect is not a simple osmotic phenomenon is demonstrated by the fact that sucrose leakage into EDTA following incubation of the slices in 0·9 M mannitol was of the same magnitude as sucrose leakage into water. Based on the above evidence, it is postulated that EDTA has its effect at the storage compartment membrane, and that this effect depends on the concentration of either fructose of sucrose (or both) in the synthesis compartment. If tissue slices are placed in EDTA immediately following incubation in fructose (or when the sucrose and fructose contents of the synthesis compartment are high), the storage compartment membrane is affected in such a manner as to allow the loss of stored sucrose. When the period of incubation in EDTA is preceded by incubation in water (synthesis compartment depleted by leakage of both sugars) or Ca^{2+} solutions (synthesis compartment depleted by storage of sucrose and

leakage of both fructose and sucrose) EDTA has little effect in increasing the loss of sucrose from the storage area of the cell.

DISCUSSION

It has long been recognized that polyvalent cations may exert a marked influence on the absorption and retention of monovalent cations by the roots of higher plants.[2, 3, 4, 5] That Ca^{2+} has a fundamental role in the maintenance of cell membrane structure and function has been convincingly demonstrated. Hanson[6] and Foote and Hanson[7] found that the ability of root tissue to absorb and retain ions was impaired if the tissue was deprived of Ca^{2+} by treatment with EDTA. In addition, evidence has been presented that Ca^{2+} is essential for the maintenance of specificity of the K^+/Na^+ (K^+/Rb^+) absorption mechanism in barley root.[8, 9] Additional evidence that Ca^{2+} is necessary to maintain functional membraneous systems in cells comes from the electron microscope studies of Marinos.[10] When barley seedlings were grown in the absence of Ca^{2+}, cells of the shoot apex showed a deterioration and general disorganization of membraneous components along with the appearance of structureless areas in the cytoplasm.

The present investigation of the effects of various polyvalent cations on sucrose leakage from corn scutellum slices further supports the contention that at least one function of polyvalent cations, particularly Ca^{2+}, is to regulate the permeability of cell membranes. As observed in a previous study, during incubation of corn scutellum slices in 0·5 M fructose, sucrose leaked from the slices at a constant rate of 6–7 μmoles/hr/g fresh wt. for at least 7 hr.[1] During these investigations it was shown that the addition of Ca^{2+} (0·001–0·04 M), Mn^{2+}(0·04 M) or Mg^{2+}(0·04 M) to the fructose bathing medium reduced the leakage of sucrose during the incubation period. The effect of Ca^{2+} in inhibiting sucrose leakage required that the cation be present in the medium at the time leakage was occurring (see Table 3, and Fig. 3). This fact and the observed rapid response of the tissue to the addition or removal of Ca^{2+} (Fig. 1 and 3) suggest that the locus of activity of the cation is the surface of the protoplast. This is in agreement with conclusions reached during studies of the effect of Ca^{2+} on the absorption of monovalent cations.[2, 5, 11, 12] While the present investigation was confined for the most part to the action of Ca^{2+}, it is assumed that Mn^{2+} (and perhaps Mg^{2+}) act in the same manner in reducing sucrose leakage.

While any attempt to define the mechanism of action of Ca^{2+} in controlling membrane permeability would at best be speculative, several interesting possiblities exist. The passive movement of non-lipid-soluble non-electrolytes may be assumed to take place through aqueous pores which traverse cellular membranes. The permeability properties of membranes toward these solutes would be determined in a large part by the size of the pore (generally expressed as equivalent pore radius) and the size of the traversing molecule. It is possible

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that Ca^{2+} exerts its influence by affecting the equivalent pore radius. In this regard, Whittembury *et al.*[13] found that Ca^{2+} exerted a control of the equivalent pore radius of membranes in cells of *Necturus* kidney. It was demonstrated that increasing the Ca^{2+} concentration from 1 mM to 10 mM had no effect in increasing the permeability of the kidney slices to sucrose (molecular radius 4·5 Å). However, removal of the Ca^{2+} from the bathing medium caused a sharp increase in the permeability of the tissue to sucrose while it remained impermeable to raffinose (molecular radius 6·0 Å). The ability of Ca^{2+} to reduce pore size is perhaps related to its ability to bind certain anionic groups of the membrane substructure and to form bridges between such groups of adjacent structural components. Solomon[14] has proposed a possible mechanism for the effect of Ca^{2+} on the permeability of the red blood cell membrane which entails the binding of the cation within negatively charged pores in such a manner as to provide the net positive charge required for impermeability of the membrane to other cations.

During inhibition of leakage by Ca^{2+}, the sucrose of the B compartment (synthesis compartment) was rapidly depleted of sucrose as a result of sucrose storage (see Table 4 and Fig. 3). This was in sharp contrast with results obtained when sucrose leakage B was inhibited by fructose and polyhydric alcohols.[1] In the latter instances, the sucrose remained in the B compartment from which it leaked only after removal of the inhibitors. These observations suggest that fructose and polyhydric alcohols reversibly influence both the sucrose storage mechanism and the permeability of the plasma membrane while the influence of Ca^{2+} is restricted to the surface of the protoplast. An earlier investigation showed that both fructose and mannitol could penetrate the plasma membrane, and that these compounds occupied an intracellular space similar in size to the glucose space.[15] It is posulated that the glucose space of the corn scutellum is the area of sucrose synthesis and the area of the tissue from which leakage B originates. In addition, it is believed that this compartment is not readily accessible to Ca^{2+}. This assumption is supported by the observation that even a brief washing with water of tissues treated with Ca^{2+} was sufficient to remove the Ca^{2+} effect (see Table 3).

The effect of EDTA on sucrose leakage is of interest. Incubation of freshly prepared tissue slices in EDTA resulted in no greater sucrose leakage than when the slices were incubated in water. Since freshly prepared slices contain only stored sucrose, it may be concluded that EDTA had no effect on the storage compartment membrane. This absence of EDTA effect may be attributed either to the exclusion of the agent from the cell by the plasma membrane or a resistance of the storage compartment membrane to the chemical. If, however, EDTA was added to the slices immediately following a 3-hr incubation in 0·5 M and 1·0 M fructose, approximately 5 per cent and 50 per cent of the stored sucrose leaked, respectively. In contrast, placing the slices in either water or 0·02 M $CaCl_2$ for 60 min following the initial incubation period in fructose (during which time the synthesis compartment is depleted of sugars) sharply reduced the subsequent leakage of stored sucrose into EDTA. These data suggest that high concentrations of fructose or sucrose (or both) in the synthesis compartment are necessary for the action of EDTA in increasing the leakage of stored sucrose. These observations, however, do not allow definite conclusions as to the site of action of EDTA (tonoplast or plasmalemma) or whether this action involved chelation of the cation components of membraneous structures.

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It has been suggested that sucrose phosphate is an intermediate in the sucrose accumulation process in sugar cane, and that accumulation involves the hydrolysis to free sucrose during or after transport into the storage compartment.[16, 17] If sucrose phosphate is the form in which sucrose is actively stored in corn scutellum tissue, then the sucrose stored during the inhibition of leakage B by Ca^{2+} must either be directly phosphorylated by a proposed sucrose kinase or broken down to hexose and resynthesized by UDP-glucose-fructose-6-phosphate glucosyltransferase following the phosphorylation of fructose by hexokinase.

EXPERIMENTAL

Plant Materials

Corn grains (*Zea mays* L., var. Funks G-76) were soaked in running tap-water for 24 hr and then placed on moist filter paper in the dark at 24–25° for 72 hr. The scutella were excised from the germinating grains and cut transversely into slices 0·5 mm or less in thickness. The slices were washed in distilled water until the wash water remained clear, and then were blotted on filter paper and weighed in groups of 1 g.

Experimental Procedure

While the detailed procedures for these experiments are given in the Tables and Figures in the Results section of this paper, certain methods remained the same throughout these investigations. Each group of slices (1 g fresh wt.) was placed in a 25 ml Erlenmeyer flask containing 9·0 or 10·0 ml of the appropriate bathing medium. Incubation of the slices was conducted at 30° in a "Gyrotory" water bath (New Brunswick Scientific Company, New Brunswick, N.J.) set to provide approximately 180 rev/min. In experiments requiring the determination of leakage A sucrose (sucrose leakage during incubation in fructose or fructose+cations) 5·0 ml portions of the bathing medium were removed at the end of the incubation period and placed in centrifuge tubes. These samples were centrifuged at 1000 *g* for 5 min after which they were decanted into clean tubes and placed in a boiling water bath for 2 min. After cooling, the samples were poured into plastic tubes and frozen. Usually the samples were analyzed for sucrose within 24 hr of their preparation. In experiments requiring the determination of the time course of sucrose leakage during incubation in various media (leakage B), samples of 0·1 or 0·2 ml were taken from the bathing solutions and placed directly into the colorimeter tubes used during sucrose analysis. These samples were then frozen and held until they could be analyzed.

The tissue levels of sucrose at zero time and after various treatments were determined following the preparation of scutellum extracts. One gram of scutellum slices was placed in a 50-ml beaker and killed by the addition of 20 ml of boiling 80% ethanol, boiling was continued for 30 sec, and after 1 hr at room temperature the ethanol solution decanted into a 150-ml beaker. This was repeated and the slices were then washed with three 5-ml portions of 80% ethanol. The combined extracts were evaporated on a steam bath until the volume was reduced to approximately 2 ml. The remaining aqueous solution was quantitatively washed into a 50-ml volumetric flask, the pH adjusted to 7·0–7·2, and water added to the mark. Five milliliter portions of the tissue extracts were placed in plastic tubes and frozen until they could be analyzed. Prior to analysis, the tissue extracts were centrifuged at 1000 *g* for 10 min. Aliquots of 0·1 ml were used for the sucrose determination.

Carbohydrate Analysis

The sucrose contents of the bathing solutions and tissue extracts were determined by analyzing these solutions for glucose before and after invertase treatment. Glucose was determined by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N.J.).

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[17] M. D. Hatch, *Biochem. J.* **93**, 521 (1964).