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EFFECTS OF ULTRAVIOLET RADIATION AND CALCIUM AND THEIR INTERACTION ON SALT ABSORPTION BY EXCISED MUNG BEAN ROOTS [1,2]

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During the last few years, investigators studying salt absorption by plant roots have interpreted the selective uptake of ions by roots as being due to ion-binding carrier compounds with more or less specific binding sites. Overstreet and Jacobsen (11) have reviewed the various binding compounds proposed and have listed several characteristics which these compounds should have to fit into any working model of ion uptake. The recent proposal of Lansing and Rosenthal (7) identifying ribonucleic acid as a binding compound in Elodea cells has opened up several possible lines of investigation to test the role of nucleic acids in salt absorption by roots. One of these is the effect on salt absorption of ultraviolet radiation in the wavelength region of 2600 Å where there is strong absorption by nucleic acids. Several investigators have inferred that genetical effects of ultraviolet are due to absorption by nucleoproteins (8). Greenstein (6) has suggested that high sensitivity at 2600 Å be used to determine whether a biologically active material has nucleic acid associated with it. This line of investigation was carried out by studying the effect of ultraviolet radiation of 2537 Å on salt absorption by excised mung bean roots (*Phaseolus aureus*).

One of the salient features of salt absorption by roots is the stimulatory effect of polyvalent cations, which was first reported by Viets (13) for barley roots. Preliminary results have shown that salt absorption by mung bean roots is also greatly increased in the presence of Ca and Mg ions. The nature of this stimulation has been investigated. In addition, the suggestion of Viets (13) that the action of polyvalent cations is localized on or near cytoplasmic surfaces of root cells and the demonstration of ribonucleic acid on cell surfaces by Lansing and Rosenthal (7) have pointed to the desirability of looking for an interaction between radiation-induced effects and calcium ions on salt absorption.

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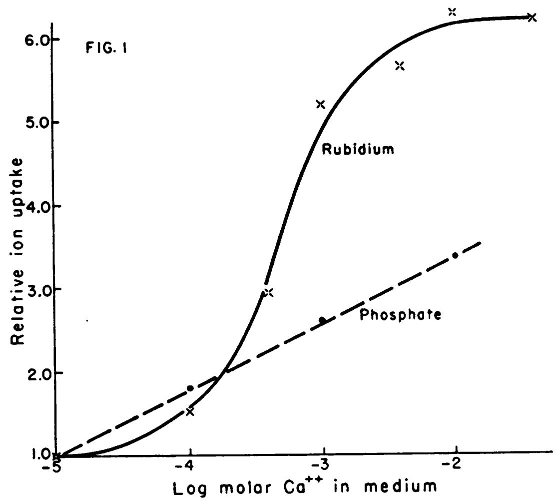

FIG. 1. Absorption of Rb and phosphate by excised mung bean roots as influenced by Ca concentration.

Materials and Methods

Mung beans were germinated and grown at 25° C in contact with aerated 10^{-4} M $CaCl_2$ solution which was changed daily. Root tips 1 cm in length were excised from three-day-old seedlings. Fifty root tips were placed in a beaker, washed twice with deionized water, and placed in 50 ml solution of either RbCl or KH_2PO_4 of 10^{-4} M concentration containing tracer amounts of Rb^{86} or P^{32} (5 to 10 μc/l). The solution was vigorously aerated during the absorption period of 30 min at 25° C. After absorption, the radioactive solution was siphoned off and the roots rapidly washed 3 times with 10^{-2} M concentration of inactive carrier in 10^{-2} *N* HCl and 8 times in deionized water. The root segments were then placed in an aluminum dish, dried at 100° C, and the radioactivity assayed in the conventional manner.

In the ultraviolet studies, the root tips were irradiated with a General Electric 30 watt germicidal lamp at a distance of 30 cm. During the exposure period, the roots were in 50 ml of water and agitated by a magnetic stirrer. Absorption of rubidium and phosphate by control and irradiated roots was then determined in the absence and presence of 10^{-3} M $Ca(NO_3)_2$ or 10^{-3} M $MgSO_4$.

Experimental Results

Before reporting the results, it might be pertinent to mention that preliminary studies have shown that with the concentration of salts used both Rb and phosphate uptake by excised mung bean roots were directly proportional to time up to two hours of absorption. Consequently, with an absorbing period of 30 min lack of substrates can be ruled out as a factor in these results. In order to conserve space, the results obtained with Mg have not been reported since they were essentially similar to those obtained with Ca.

Effect of Calcium: Typical results showing Ca stimulation of Rb and phosphate uptake are presented in figure 1. Rubidium uptake was greatly enhanced by Ca and increased with increasing Ca concentration up to about 10^{-2} M Ca^{++}. Phosphate absorption was also stimulated markedly by Ca and increased with increasing Ca concentration. Incidentally, neither KNO_3 up to 10^{-2} M nor NaCl up to 10^{-2} M had any significant effect on phosphate uptake. The effects of high concentrations of K and Na on Rb absorption were not investigated because of the competitive nature of these ions on Rb absorption (4).

With the object of determining the locus of Ca stimulation, the experimental procedure was modified by reducing the time of absorption and increasing the specific activity of radiophosphorus. The shortest period of absorption employed was one minute. The remainder of the procedure was the same. Only phosphate uptake was studied in this manner. The findings obtained in these short-time studies are shown in figure 2. The results of phosphate uptake, with or without Ca, show a linear increase with time which appear to extrapolate to zero at zero time.

Because the preceding results suggested that mild treatments to remove Ca from roots might alter the course of salt absorption, excised roots were pretreated for one minute with dilute HCl and NaCl solutions and thoroughly washed with deionized water. Absorption studies were then carried out with these pretreated roots in the usual manner. Some of the results from these studies are presented in table I. Contrary to expectation, Rb uptake by pretreated roots was markedly increased by the HCl and NaCl treatments. On the other hand, phosphate uptake was decreased by the treatments. It can also be seen

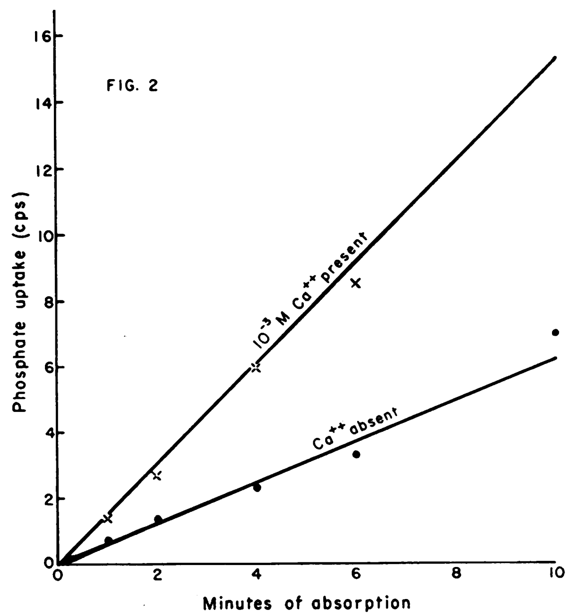

FIG. 2. Time course of phosphate absorption in the absence and presence of 0.001 M $Ca(NO_3)_2$.

that the Ca concentration employed reversed the effects of HCl and NaCl so that the treated roots behaved nearly like Ca-stimulated control roots. Magnesium also reversed the effects of HCl and NaCl. Other solutions of citric acid, ethylene diaminetetraacetate, and alkaline phosphate also increased Rb or decreased phosphate absorption.

ULTRAVIOLET EFFECTS: The effects of ultraviolet radiation on Rb and phosphate uptake are shown in figure 3. It can be seen from figure 3 that the response of excised mung bean roots to ultraviolet radiation is somewhat complicated. Irradiation increased the Rb uptake in the absence of Ca, but the presence of Ca brought about a severe reduction in Rb absorption. In contrast, phosphate absorption was reduced by irradiation, with Ca slightly enhancing the radiation effect. Somewhat similar results were obtained with Mg for both Rb and phosphate uptake. Potassium at a concentration of 10^{-2} M KNO_3, however, had no effect on phosphate absorption by irradiated roots.

A more detailed study of the effect of Ca on Rb uptake by irradiated roots was made by varying the concentration of Ca from 10^{-5} to 10^{-2} M as $Ca(NO_3)_2$. In this experiment, ultraviolet exposure was for two minutes. The results are shown in figure 4. Each point represents the relative Rb uptake by irradiated roots as compared to unirradiated roots in a medium of the same Ca concentration. According to figure 4, the relative ion uptake by irradiated roots appears to decrease linearly with increasing log Ca concentration.

DISCUSSION

Interpretation of the results reported here is made somewhat difficult by the complex nature of the influence of Ca and Mg ions on ion uptake by roots treated with ultraviolet radiation, HCl and NaCl

TABLE I

EFFECT OF PRETREATMENT WITH HCL AND NACL SOLUTIONS ON SUBSEQUENT RUBIDIUM AND PHOSPHATE UPTAKE BY EXCISED MUNG BEAN ROOTS *

PRETREATMENT *	MOLAR CA CONC IN ABSORPTION MEDIUM	RELATIVE ABSORPTION	
		RUBIDIUM	PHOSPHATE
None	0	100	100
"	0.0001	194	165
"	0.001	...	234
0.001 *N* HCl	0	468	74
"	0.0001	280	172
0.005 *N* HCl	0	...	47
"	0.001	...	225
None	0	100	100
"	0.0001	230	157
0.05 M NaCl	0	441	72
"	0.0001	307	158

* Excised roots were pretreated for one min and washed with water. Absorption was carried out in 0.0001 M RbCl or 0.0001 M KH_2PO_4 in the presence or absence of $Ca(NO_3)_2$.

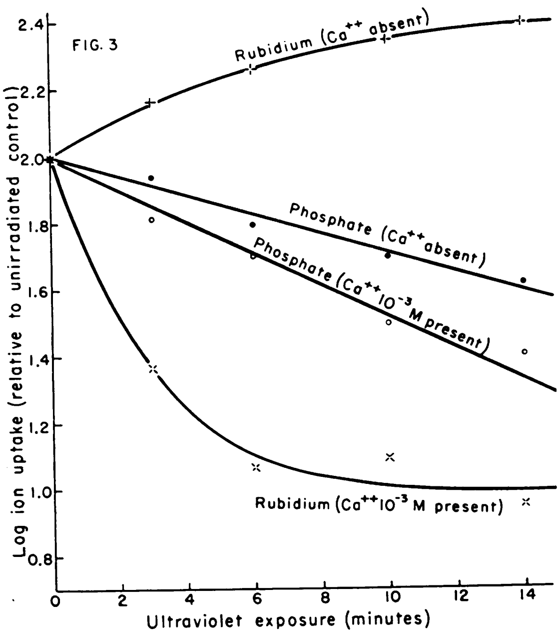

FIG. 3. Effect of ultraviolet radiation on Rb and phosphate uptake by excised mung bean roots. After irradiation, absorption was conducted in the absence and presence of $Ca(NO_3)_2$.

solutions. The rates of both Rb and phosphate absorption by mung bean roots are greatly increased in the presence of Ca and Mg (fig 1). The magnitude of stimulation is much greater than similar Ca-stimulated K uptake reported earlier by Viets (13) for barley roots. Overstreet et al (12) have postulated that Ca enhances K uptake by acting as a cofactor in the breakdown of a K complex, KR, where R is a binding compound.

From the results presented in figure 2, which show the absence of any noticeable lag in the Ca-mediated stimulation of P uptake, and in view of the known slow uptake of Ca, it appears that the locus of action of Ca ions might be located on or near the external surface of the cytoplasm of epidermal cells. Viets (13) has made the same suggestion. Further evidence in support of a surface reaction was obtained in an experiment in which the results indicated that Ca stimulation occurred only when it was present in the medium during absorption. In this experiment excised mung bean roots were pretreated for 30 min in a 10^{-3} M $Ca(NO_3)_2$ solution and thoroughly washed with water. Absorption of radiorubidium and radiophosphorus in the absence of Ca by these roots amounted to 7.6 and 6.4 cps, respectively. On the other hand, roots not pretreated with Ca absorbed four times more radiorubidium and 2.7 times more radiophosphorus in the presence of 10^{-3} M $Ca(NO_3)_2$.

Besides its role in stimulating both anion and cation uptake, Ca appears to play an additional part during salt absorption by mung bean roots. The reversal by Ca of effects brought about by acid and

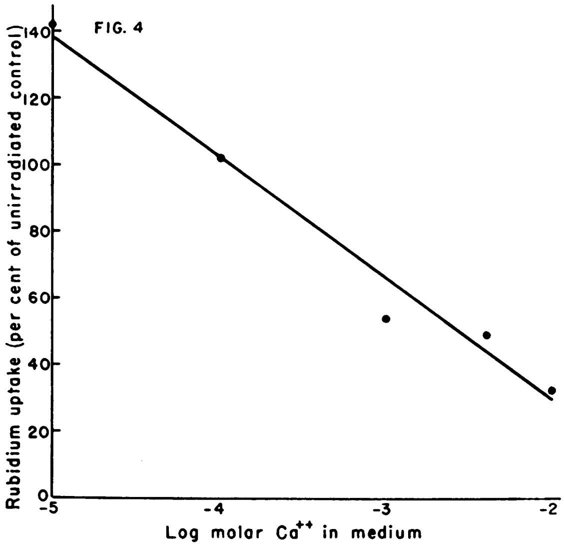

FIG. 4. Effect of Ca concentration on Rb uptake by excised mung bean roots irradiated with ultraviolet. Radiation exposure was for 2 min. Each point is the relative Rb absorption of irradiated roots as compared to control roots in a medium of the same Ca concentration.

NaCl treatments (table I) indicates that these treatments removed Ca ions and that Ca plays some part in regulating cation and anion uptake. At present no adequate explanation is proposed as to how Ca " regulates " salt absorption.

The action of Ca on salt absorption by irradiated roots (figs 3 and 4) points to a strong interaction between radiation effects and Ca ions. Here also, the response of irradiated roots to Ca in the external medium suggests that the site of ultraviolet-Ca interaction is located on or near the cytoplasmic surface of the epidermal cells. Such an interpretation leads one to consider the possibility of a close connection between the salt-absorbing system acted upon by polyvalent cations and the radiation-sensitive system.

Since relationships which show a linear dependence of " log survival fraction " with dose have been interpreted as due to " one-hit " inactivation (8), some of the results presented in figure 3 could also be interpreted in the same manner. The data of phosphate absorption by irradiated roots appear to show the operation of such a " one-hit " mechanism. Likewise, the reduction of rubidium absorption by irradiated roots in the presence of calcium at the lower ultraviolet exposures also suggests a " one-hit " mechanism. Rubidium absorption by roots exposed to the higher ultraviolet exposures, which amounts to about 10 % of the control, could be due to exchange reactions.

Obviously, if the identity of ion-binding carrier compounds involved in salt absorption were known, interpretation of the results reported here would be facilitated. However, in view of our ignorance as to the nature of such compounds, one can only speculate and propose some likely compounds. Owing to their strong absorption of radiation at 2537 Å, the purine- and pyrimidine-containing nucleosides, nucleotides, and polymers are the most likely to be affected by radiation of that wavelength. From the chemistry of the simple nucleosides and nucleotides, it is difficult to visualize how specificity of cation- and anion-absorption sites (3, 4) can be attributed to them. This tends to rule out the possibility of their being the ion-binding compounds. The complex polymers, such as the nucleic acids, offer greater possibility of having specific absorption sites. Moreover, action spectrum studies of biological effects of ultraviolet radiation have indicated that in many instances nucleic acids are the immediate receptors of the effective radiations in the neighborhood of 2600 Å (5). The observation of Lansing and Rosenthal (7) that Elodea cells treated with ribonuclease lose their capacity to absorb Ca strongly indicates that ribonucleic acid participates in some manner in salt absorption—probably as an ion-binding compound as they have suggested. If ribonucleic acids, or more likely ribonucleoproteins, are ion-binding compounds, the effect of ultraviolet on salt absorption could be due to changes brought about in them by the radiation. Some probable changes which might explain some of the reported results could be unfolding of coiled molecules and greater sensitivity to salt denaturation (5, 9). If ribonucleoproteins do take part in salt absorption, the negative charge of the nucleic acid would enable it to bind cations, while the positive charge of the protein moiety would bind anions.

Although the results do not show unequivocally the direct involvement of a ribonucleoprotein in salt uptake, other observations can be mentioned which support the proposal that a ribonucleoprotein could be a salt-binding carrier compound in the initial uptake of salts by plant cells. Besides their work with ribonuclease, Lansing and Rosenthal (7) have reported cytological observations which show a localization of ribonucleic acid on surfaces of cells. Claude (2) has pointed out that ribonucleic acids are often found associated with phospholipids, the latter probably forming the cell membrane. The hydration of nucleoproteins has been shown to be markedly affected by polyvalent cations (1). This property might be a clue leading to the explanation of the stimulation of salt absorption by polyvalent cations. Neuberg and Roberts (10) have shown that nucleic acids have remarkable properties to form complexes with many insoluble salts, and they have pointed out that the reactive groups on a nucleic acid molecule can form numerous combinations. The different ion-binding potentials of the reactive groups and their positions on nucleic acid and protein molecules could probably explain the specificity of cation- and anion-binding sites as reported by Epstein and Hagen (4) and Epstein (3). Since it is highly probable that ribonucleoproteins from various species of plants would show some differences in properties, some of the differences in the nature of salt absorption among plants could be due to variations in the ion-binding properties of their ribonucleoproteins.

SUMMARY

The effects of Ca, ultraviolet radiation, dilute HCl and NaCl solutions on salt absorption by excised mung bean roots (*Phaseolus aureus*) were investigated using Rb^{86} and P^{32} as tracers. Both Rb and phosphate uptake were greatly enhanced by the presence of Ca. Short-time studies indicated that the locus of Ca action is on or near the outer surface of the cytoplasm. Short periods of pretreatment of excised roots with dilute HCl and NaCl solutions increased subsequent Rb uptake but decreased phosphate absorption. The effects of HCl and NaCl were reversed by Ca.

Rubidium absorption was increased by ultraviolet radiation of 2537 Å, but the presence of Ca during absorption drastically decreased Rb uptake by irradiated roots. Phosphate absorption by irradiated roots was reduced by ultraviolet radiation to a slight degree, and was further decreased by the presence of Ca ions. Some of the absorption curves show a linear decrease of log ion uptake with ultraviolet dose.

The results obtained have been interpreted as being due to the involvement of a ribonucleoprotein in salt absorption. The suggestion has been made that the nucleic acid binds cations, while the protein moiety binds anions.

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ON THE NATURE OF THE ENZYMATICALLY CATALYZED OXIDATION PRODUCTS OF INDOLEACETIC ACID [1]

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The plant growth hormone, indoleacetic acid (IAA), is known to be oxidatively inactivated by enzyme preparations from various plant sources (6, 12, 16, 18, 19). It has usually been assumed that the oxidation product is 3-indolealdehyde (16, 19). The evidence for this belief is as follows: (a) Manometric and colorimetric measurements made during the oxidation show that one mole of O_2 is absorbed and one mole of CO_2 is released per mole of IAA disappearing (16, 19); (b) The product yields colors with Ehrlich's reagent (19) and with the Hopkins-Cole reagent (16) which could be due to the persistence of the indole ring; (c) The product apparently has a carbonyl group, since it forms an insoluble 2,4-dinitrophenylhydrazone (19).

Our reinvestigation of this question was prompted by several facts. In the first place, other products may be proposed, the properties of which reasonably fit all of the known data (fig 1). Secondly, the apparent similarity of the IAA oxidase system from etiolated peas to tryptophan oxidase from various sources (9, 10, 20) suggested a mechanism of oxidation involving cleavage of the indole ring. Thirdly, neither Ehrlich's reagent nor the Hopkins-Cole reagent provides a specific test for the indole ring, certain aromatic amines giving positive reactions with the former, and certain indolic compounds failing to give a pronounced color with the latter.

We therefore undertook to characterize the products of the oxidation of IAA by the IAA-oxidase of etiolated peas, using paper chromatography as a diag-

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