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EFFECT OF SULFHYDRYL INHIBITORS ON RUBIDIUM ABSORPTION BY EXCISED MUNG BEAN ROOTS[1]

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In recent years several investigators have used metabolic inhibitors in their studies on salt absorption by plants. Ordin and Jacobson (9) have reviewed publications in this field. All of these workers have found that at appropriate concentrations the inhibitors reduced salt uptake. This paper reports unique effects of several sulfhydryl[2] inhibitors on the absorption of Rb by excised mung bean roots (*Phaseolus aureus*). Under certain conditions some -SH agents have been found to increase greatly the uptake of Rb. Other conditions have resulted in these inhibitors drastically decreasing the absorption of Rb.

Material and Methods

Mung beans were grown at 25° C in contact with an aerated 10^{-4} M $CaCl_2$ solution which was changed daily. Root tips were excised in 1-cm lengths from three-day-old seedlings. Fifty root tips were placed in 50 ml solution of 10^{-4} M RbCl containing tracer amounts of Rb^{86} (less than 5 μc/l) and various concentrations of a -SH inhibitor. The inhibitors used were phenylmercuric nitrate, $CuSO_4$, iodoacetic acid, and 3-amino-4-hydroxy-phenyldichloroarsine hydrochloride.[2] In one series of experiments the absorption medium contained 10^{-3} M $Ca(NO_3)_2$. The solution was vigorously aerated during the absorption period of 30 min at 25° C. After absorption, the radioactive solution was rapidly siphoned off and the roots washed several times with 10^{-2} M inactive salt solution and water. The root segments were then placed in an aluminum dish, dried at 100° C, and the radioactivity assayed in the conventional manner.

Experimental Results

Typical results of many experiments showing the effects of the -SH inhibitors on Rb absorption by mung bean roots in the absence of Ca are presented in figure 1. The strong inhibitors such as Cu and PMN brought about an unexpected and surprisingly large increase in Rb uptake at low concentrations (less than 2 ppm). At higher concentrations Cu tended to depress Rb uptake. High concentrations of PMN were not used because of its insolubility in water. On the other hand, the weaker -SH agent, AHP, brought about much smaller increases in Rb absorption; while the weak inhibitor, IDA, decreased Rb uptake as previously reported by others (9).

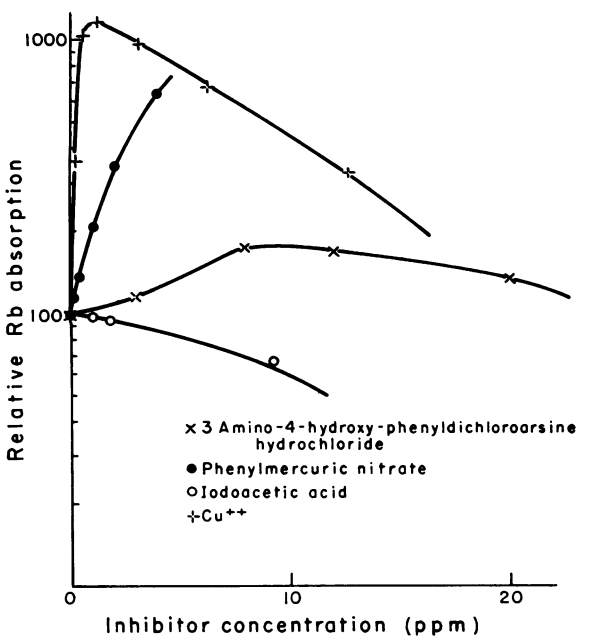

Fig. 1. Effect of sulfhydryl inhibitors on the absorption of Rb by excised mung bean roots in the absence of Ca.

The presence of 10^{-3} M Ca in the absorption medium drastically changed the direction of the effects of the strong -SH inhibitors on Rb uptake (fig 2). Instead of increasing Rb absorption, Cu and PMN at very low concentrations (less than 1 ppm) greatly reduced Rb uptake in the presence of Ca. In

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[2] Abbreviations used: -SH, sulfhydryl; PMN, phenylmercuric nitrate; IDA, iodoacetic acid; AHP, 3-amino-4-hydroxy-phenyldichloroarsine hydrochloride; RNA, ribonucleic acid; RNase, ribonuclease.

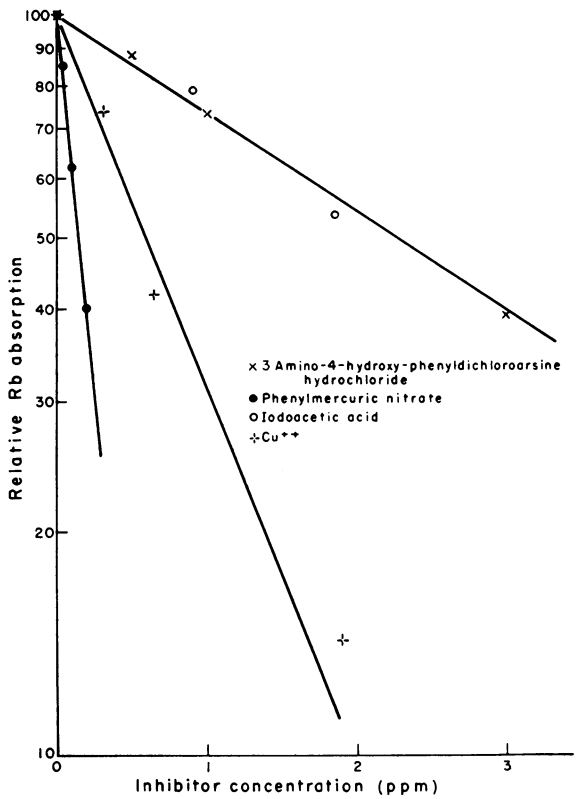

FIG. 2. Interaction of sulfhydryl inhibitors and Ca on the absorption of Rb by excised mung bean roots. The absorption medium contained 10^{-4} M RbCl, 10^{-3} M $Ca(NO_3)_2$, and various concentrations of inhibitors except in the case of the Cu treatment. The Cu-treated roots were pretreated with various concentrations of $CuSO_4$ for one min and washed before placement in the absorption medium containing RbCl and $Ca(NO_3)_2$.

the case of Cu this depressing effect was observed only when the roots were first pretreated with $CuSO_4$ for one min before placement in the RbCl solution containing $Ca(NO_3)_2$. When Ca and Cu were added together in the absorption medium, the Ca prevented any effect of the Cu. On the other hand, no such antagonism was observed between Ca and PMN when they were added together. The weaker -SH agents showed little interaction with Ca and were effective only at fairly high concentrations. When a monovalent cation such as Na was used in the form of $NaNO_3$ in place of Ca, no interaction was observed between the Na and the strong -SH inhibitors as between Ca and the strong -SH inhibitors. Cysteine and glutathione were able to reverse completely the Cu effect and partially the PMN effect.

The interaction between the effects brought about by strong -SH poisons and by Ca on Rb absorption appears to take place at low concentrations of Ca. Table I shows the effect of PMN and increasing Ca concentration on Rb absorption by excised mung bean roots. It can be seen from the data that even low concentrations of Ca have a marked depressing effect on the "enhanced" Rb uptake brought about by PMN. The data also indicate that the Ca reduction of the PMN effect increases with increasing Ca concentration.

DISCUSSION

From the results reported in this paper, it is obvious that certain -SH groups play an important part in the normal absorption of ions by mung bean roots. What that role is can not be unequivocally answered in these in vivo experiments. However, some of the inhibitory effects of the weak -SH agents could be due to their poisoning of some critical metabolic reactions which are necessary for normal salt absorption. But, such an explanation does not appear to explain adequately the unusual effects of the strong -SH inhibitors, which can greatly increase or decrease Rb absorption depending upon the presence of Ca in the absorption medium. One probable explanation of the striking effects of PMN and Cu on increasing Rb uptake is that these poisons caused changes in the permeability of the membrane which limits the active uptake of ions. This point was studied by treating excised roots, which had previously absorbed Rb^{86}, with $CuSO_4$ for short periods. After washing, the Cu-treated and control roots were then placed in 10^{-4} M inactive RbCl. The loss of Rb^{86} from the roots was then measured after suitable intervals. Both control and Cu-treated roots showed little or no loss of Rb^{86} after 15 and 30 min in the inactive RbCl. Obviously, if permeability changes occurred in the cells following the Cu treatment, the loss of Rb^{86} from the treated roots would be very much higher than untreated roots unless the unlikely possibility occurred that only permeability into the cells was changed. In addition, a few experiments with P^{32} showed that PMN had only a moderate inhibiting effect on phosphate absorption. This inhibition increased with increasing PMN concentration with Ca having little or no effect on the course of the inhibition. Lewin (7) likewise has found that -SH poisons inhibited silicon uptake by diatoms. A somewhat comparable phenomenon has been observed on the effect of traces of Cu on the absorption of glycerol by human erythrocytes. The literature on this subject has been reviewed by Le Fevre (6) and Jacobs (4).

TABLE I

EFFECT OF PHENYLMERCURIC NITRATE AND INCREASING CALCIUM CONCENTRATION ON RUBIDIUM ABSORPTION BY EXCISED MUNG BEAN ROOTS

COMPOSITION OF ABSORPTION MEDIUM		Rb ABSORPTION (CPS)
PMN (PPM)	Ca^{++} (M)	
0	0	10.3
2.0	0	19.8
2.0	10^{-5}	9.9
2.0	5×10^{-5}	5.1
2.0	10^{-4}	4.2
2.0	10^{-3}	3.2

These workers and coworkers have found that trace amounts of Cu inhibited uptake of glycerol by erythrocytes without affecting the uptake of some other compounds. They believe the Cu effect to be on -SH groups at cell surfaces. Unless permeability changes in the cells can be restricted only to cations, the strong -SH inhibitors probably affect cation absorption through some mechanism other than permeability changes. In addition, the drastic effects of Ca on PMN- and Cu-treated roots are also difficult to ascribe to permeability changes. Ca, as found by Viets (16) and others (11, 14), stimulates salt uptake in untreated roots.

If the results reported here can not be attributed to permeability changes, perhaps an explanation can be found in one of the current hypotheses on ion absorption. The more popular hypotheses do not offer much hope that an adequate explanation can be found in any one of them. They have been recently reviewed by Overstreet and Jacobson (10), Lundegårdh (8), and by Steward and Millar (13). However, a suitable explanation can be found in the less well-known postulate made recently by Lansing and Rosenthal (5) in which RNA[2] plays the role of an ion-binding carrier compound in salt absorption. Incidentally, Steward and Millar (13) have suggested that RNA takes part in salt absorption through its role in protein synthesis. Lansing and Rosenthal have found that treating Elodea cells with RNase[2] brought about a reduction in Ca uptake. From these results they suggested the role of RNA as an ion-binding carrier compound in salt absorption. They have cited several biochemical and cytological data which make their proposal plausible. Tanada (15), using excised mung bean roots, has recently confirmed their findings that RNase has a marked effect on salt uptake. In using Lansing and Rosenthal's postulate to explain ultraviolet and Ca effects on ion uptake by excised mung bean roots, Tanada (14) has slightly modified their suggestion by identifying the carrier as a ribonucleoprotein with the negatively charged nucleic acid binding cations and the positively-charged protein binding anions.

Now, if the ribonucleoprotein hypothesis is used to explain the results reported here, one can visualize the nucleic acid and the protein moieties being held together by certain critical H bonds formed between -SH groups of the protein and other H-bonding groups of the nucleic acid. Henry and Stacey (2) have found that -SH groups in the cytoskeleton have to be reduced before RNA becomes attached to it. Immobilization of the -SH groups by strong -SH agents would break the H bonds. Without these critical H bonds with the -SH groups of the protein, the nucleic acid would be held weakly or become separated from the protein. Under this condition changes in the spatial configuration of the RNA could result and make available more sites for cation binding. This could explain the increased uptake of Rb by the action of the strong -SH agents, Cu and PMN, in the absence of Ca. It is likely, however, that the protein determines to a large extent the properties of the RNA, and the RNA is physiologically stable only when combined with the protein (3). If "free" RNA were unstable, small amounts of Ca could have marked deleterious effects on the physico-chemical properties of the "free" RNA and result in the RNA losing much of its cation-binding potential. Ambrose and Butler (1) and Shack et al (12) have found that small concentrations of polyvalent cations have large effects on certain physico-chemical properties of nucleic acids and nucleoproteins.

At present, until more information is obtained on salt absorption, it would be premature to speculate on the actual mechanics of ion absorption involving a ribonucleoprotein as an ion-binding carrier compound. However, if the above explanations present the true picture on the action of -SH agents, the effect of ultraviolet irradiation on Rb absorption reported recently by Tanada (14) would be suitably explained. Tanada found that ultraviolet irradiation has an effect on Rb absorption by mung bean roots similar to that brought about by the strong -SH inhibitors. Ultraviolet irradiation increased Rb uptake in the absence of Ca; but in the presence of Ca, it decreased Rb absorption. These results indicate that one of the major results of ultraviolet irradiation on salt absorption could be the oxidation of -SH groups of a ribonucleoprotein.

Summary

The absorption of Rb by excised mung bean roots has been found to be markedly affected by strong -SH inhibitors. Cu and phenylmercuric nitrate at low concentrations have been found to increase markedly Rb uptake in the absence of Ca. Rb absorption in the presence of Ca, however, was greatly reduced by these inhibitors. Weaker -SH poisons showed little or none of these effects. The suggestion was made that Rb absorption is mediated by a ribonucleic acid and protein complex which is held together by H bonds formed by certain critical -SH groups on the protein moiety, and that the strong -SH agents bring about their effects by their action on these -SH groups.

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