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SHORT REPORTS

EFFECT OF AMINES AND GUANIDINES ON Rb^+ ABSORPTION BY EXCISED CORN ROOTS

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Key Word Index—*Zea mays*; Gramineae; maize; Rb^+ absorption by excised roots.

Abstract—The effect of alkyl-amines and -guanidines on the absorption of rubidium by the excised roots of the corn plant was tested. Inhibition of Rb^+ absorption was observed with both amines and guanidines, where guanidines were more effective. The effect of alkylamines on Rb^+ transport depends on their molecular structure.

INTRODUCTION

Alkylguanidines, particularly octylguanidine (OG), inhibit K^+ transport in excised barley and oat roots [1, 2]. The basis for this inhibition in plants was further studied by investigating the effect of OG on energy transfer reactions in mitochondria and in the plasma membrane of oat roots. It was concluded that this inhibitor acts at the cell surface by interfering with the plasma membrane ATPase complex [2]. Results from plasmolysis experiments with onion epidermis monolayers confirmed the previous conclusion that OG acts primarily on the protoplast surface [3]. The present work was undertaken to explore the contributions of both the lipophobic moiety and the lipophilic carbon chain of the OG to the inhibition of the Rb^+ absorption. Accordingly, various amines (mono amines with different chain lengths, secondary and tertiary amines and hydroxylamine, HYA) and guanidines (OG and decamethylenediguanidine, DDG) were tested on the absorption of Rb^+ by excised roots of the corn plant.

RESULTS AND DISCUSSION

Effects of amines

The effects of primary amines (225 μM) with different chain length (1 to 16 carbon atoms) were tested on Rb^+ absorption and results are shown in Fig. 1. Methylamine (MA) produces a slight stimulation (5%) while ethylamine (EA), butylamine (BA), and pentylamine (PA) produce a slight inhibition (less than 20%). With longer chain lengths, inhibition increased to 50% in hexadecylamine (HDA). Amine concentration curves with various degrees of lipophilicity (2, 4, 6 and 8 carbon chains, Fig. 2), show that at 45 μM only the less polar amines, hexylamine (HA) and octylamine (OA), inhibit the Rb^+ uptake by *ca* 20%, while EA and BA slightly stimulates the absorption by *ca* 5%.

The present results agree with the findings of Tuena de Gómez-Puyou *et al.* [8], in animal aystems where the effect of alkyl ammonium salts was studied on ATPase activity of submitochondrial particles. These authors concluded that the presence of 6 carbon atoms on the alkyl chain were necessary for effective inhibition of the K^+ transport. Although the inhibition is highly dependent on the length of the alkyl chain, inhibitions are smaller by the alkylamines in corn roots in the present study than in the previous similar experiments conducted with alkylguanidines in barley and oat roots [1, 2]. Results presented here are strongly indicative of the role of the polar moiety of the amphiphilic molecules in the absorption of Rb^+.

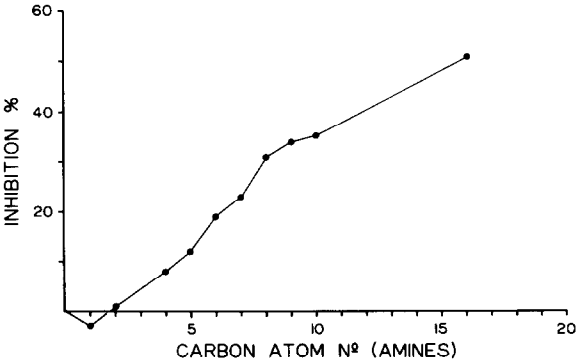

Fig. 1. Effect of length of the alkyl chain of amines at 225 μM on $^{86}Rb^+$ absorption by excised corn roots. External RbCl concentration was 1 mM and absorption was for 8 min.

Effect of guanidines

Figure 3 shows the effect of different concentrations of OG and DDG on the Rb^+ absorption. Inhibition of Rb^+ uptake by these molecules was found at all concentrations studied, with half maximal inhibition values occurring at about 50 and 81 μM respectively. Inhibition of the Rb^+ uptake was not complete with DDG and OG which showed maximal inhibition of *ca* 68 and 50% at concentrations of 225 and 36 μM respectively. This effect is consistent with earlier reports on the effect of OG on the K^+ absorption by barley and oat roots [1, 2]. However, maximal inhibition (*ca* 80%) was found for these plants at similar concentrations as for corn roots. A comparison of the data in Figs. 2 and 3 shows that OA is less effective than OG in the inhibition of K^+ transport.

Additive effects

Experiments were performed to investigate the specificity of the OG effect on the Rb^+ uptake. OA was tested at different concentrations and to each amine concentration, OG was added to a concentration of 56 and 225 μM in separate experiments. Figure 4 shows the results of these additive effects and the expected additive curves. At 56 μM OG, greater increments in the percent inhibition of Rb^+ absorption was produced. At higher concentrations, this increment decreases. At 225 μM OG, a higher and constant additive effect was observed at all OA concentration studied. These findings indicate that guanidines interfere with Rb^+ transport by acting at a common site.

Comparative effects of amines and guanidines

The contribution of both the hydrophobic and the hydrophilic parts of cationic compounds on the Rb^+ absorption, was further investigated as illustrated in Fig. 5. The order of effectiveness for the hydrophobic part of the cations on the inhibition of Rb^+ absorption was: 2 guanidines > guanidine > 1 amine = hydroxyl > > > methyl and for the hydrophilic chain was 10 carbons > 8 carbons > 6 carbons > > > 1, 2 or 3 carbons.

In this work the effect of various amines and guanidines on Rb^+ absorption in corn seeds was explored. The data show that the effect of alkyl-amines on Rb^+ absorption depends on their molecular structure. From the exper-

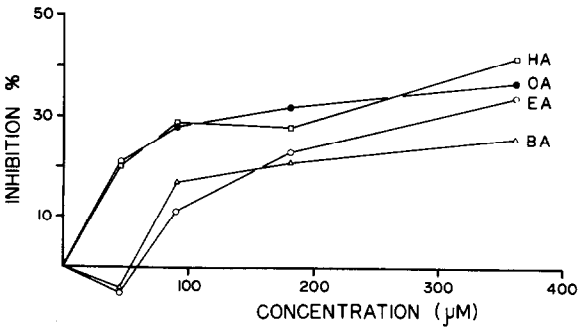

Fig. 2. Effect of length of alkyl chain of amines (ethylamine, EA; butylamine, BA; hexylamine, HA; octylamine, OA) at different concentrations on the absorption of $^{86}Rb^+$ by excised corn roots. External concentrations of RbCl was 1 mM and absorption was for 8 min.

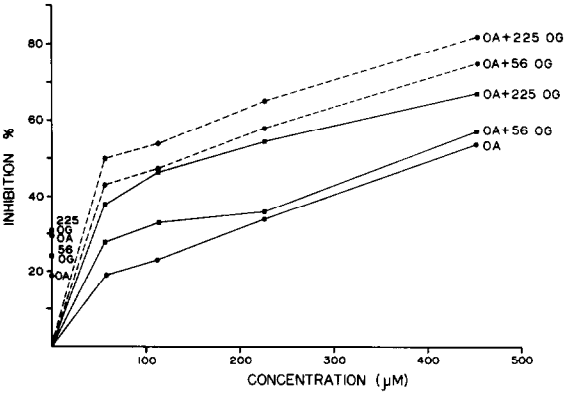

Fig. 4. Additive effect of octylamine (OA) and octylguanidine (OG) on the absorption of $^{86}Rb^+$ by excised corn roots. External RbCl was 1 mM and incubation was for 8 min. The broken lines represent the theoretical expected additive results.

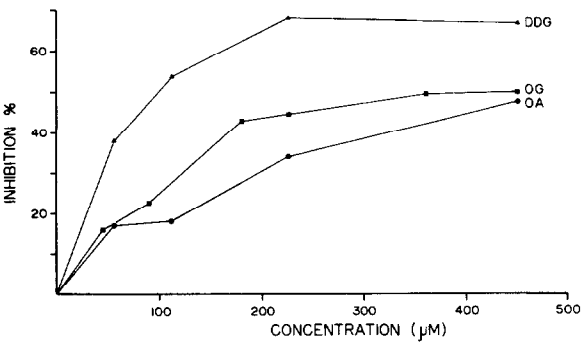

Fig. 3. Effect of different concentrations of decamethylene diguanidine, (DDG) octylguanidine (OG), and octylamine (OA) on uptake of $^{86}Rb^+$ by excised corn roots. External concentration of RbCl was 1 mM and absorption was for 8 min.

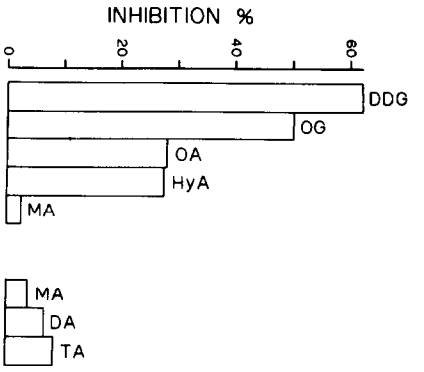

Fig. 5. Effect of amines and guanidines at 225 μM on the $^{86}Rb^+$ absorption by excised corn roots. External RbCl was 1 mM and incubation was for 8 min.

iments described in this work, several conclusions may be drawn. DDG showed a greater effect than OG on Rb^+ uptake. This result could be explained by the possible interaction of DDG at the cell surface as reported for OG in barley and oat roots [1, 2] and an interference of the diguanidine with ATP sources due to its greater permeability compared to OG. In fact, Peña [9] has shown that OG and DDG are effective inhibitors of K^+ transport in yeast and that both molecules can inhibit respiration, but that in addition DDG has an uncoupling effect. Moreover, plasmolysis experiments with onion epidermis cells [3] indicated that OG acts at the cell surface, since cytomorphological alterations were detected only after 30 min in OG treated epidermal cells and that protoplast streaming decreased within 15 min after transfer of cells to a plasmolysing solution containing OG. In addition, it was observed that the substitution of the methyl by a hydroxyl group as in hydroxylamine (HYA) shows an enhanced inhibition of the Rb^+ uptake, due probably to the higher permeability of this molecule. Unfortunately, the effects of this compound on respiration and/or ATP synthesis have not been described, and its greater inhibiting effect could be due to the interference with the energy transducing system of plant cells.

Inhibition of Rb^+ absorption was observed with both amines and guanidines, but guanidines are more effective, which suggest that they act in a specific molecular component involved in Rb^+ transport. Moreover, due to the sensitivity of the system to increasing carbon chain length, the molecular entity would appear to be in the hydrophobic phase of the membrane. The exact locus of action of alkyl guanidines on Rb^+ absorption cannot be ascertained at present. However, it is interesting that the uptake of short chain alkylamines by a marine diatom is inhibited by K^+ [10], which suggests that they may share a common mechanism for transport. Thus, the inhibition by long chain alkylamines and guanidines could be due to their interaction with this particular site. However, further studies are required to establish this alternative.

Finally it is interesting to point out that full inhibition of Rb^+ absorption has not been observed. This suggests that in the preparation employed there may be two separate active systems involved in the absorption of Rb^+.

EXPERIMENTAL

Seeds of corn (*Zea mays* var. Tuxpeño) were rinsed several times with H_2O and placed in rows between layers in cheesecloth held by a stainless steel screen which was supported by a 3 l. beaker containing 2.5 l. of 1 μM $CaSO_4$. Seedlings were essentially grown as described earlier [4]. Five-day-old seedlings were removed from the cheesecloth screen and rinsed several times with H_2O and their roots cut into 1 cm segments (the 3.5 cm apical portions and 5 cm basal portions of the roots were discarded). The uptake expts were carried out by incubating 25 root segments during 8 min in 10 ml of the absorption solns. Experiments on the ion absorption by excised tissues of higher plants have shown that K^+ and Rb^+ behave virtually as isotopes of the same element [5]. Therefore, ^{86}Rb was used as a radioactive analog of K^+. The pH of the absorption soln (6.5) was adjusted with KOH or HCl. The experiments were conducted at ambient temp (23°) and continuous aeration was provided. The absorption period was finished by collecting the roots on filter paper in a Büchner funnel. They were then transferred to 30 ml of washing soln at 0° for a 7 min period. The composition of the absorption solns are given in the figure legends. Roots were collected again in the Büchner funnel, dried between paper towels, placed in tared aluminium planchets, weighed, and ashed at 500°. The ash was moistened with 0.1 ml of H_2O, dried in a hot plate and counted in a Geiger counter. This procedure has been used by several authors [6, 7]. All expts were conducted in triplicate and repeated at least two times. The standard error of the mean was less than 5% of the mean of each datum.

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