Alkylguanidines as Inhibitors of K+ Transport in Isolated Barley Roots

Beatriz Gómez Lepe and Epifanio Jiménez Avila
Instituto de Biología, Universidad Nacional Autónoma de México, México, D. F., México

ABSTRACT

It has been shown that plants can accumulate K+ through an energy-dependent process. The effect of alkylguanidines, in particular octylguanidine on the uptake of 86Rb+ by excised barley roots (Hordeum vulgare var. Apizaco LV-72), has been studied. 86Rb+ was used as tracer of K+. The uptake of 86Rb+ which is linear with time and shows saturation kinetics is inhibited by octylguanidine. Half-maximal inhibition of 86Rb+ uptake is attained at 50 µM octylguanidine. Octylguanidine induces a decrease in the V_max of the process and increases the Km of the system for Rb+. When the effects of various alkylguanidines were studied, the following order of effectiveness was encountered: octylguanidine > butylguanidine > ethylguanidine > guanidine. This suggests that guanidines inhibit Rb+ uptake by interacting through its positively charged guanidinium group with a Rb+ carrier while the alkyl chain interacts with the hydrophobic milieu of the membrane.

It has been established that plants can accumulate K+ through a process that apparently depends on metabolic energy (5, 6, 14, 15). Little is known with respect to the molecular events that occur during K+ translocation. It has been suggested that a membrane-bound ATPase participates in the process (8, 9, 18, 20, 22, 25) and that the uptake of K+ involves the combination of a hypothetical carrier with the ion in such a way that the intermediate ion-carrier complex that is formed on the outer part of the membrane breaks down irreversibly on the internal side of the membrane releasing the ion inside the cell (3, 6, 10, 11). In this work, a possible action of various alkylguanidines on the absorption of K+ by excised barley roots has been explored. The effect of alkylguanidines on K+ uptake in plants was studied because these lipophilic cations (their pKa is around 12) have been shown to inhibit numerous metal ion-dependent systems (1, 2, 12, 13, 16, 23, 24, 27–29) and thus, it was thought that alkylguanidines could prevent K+ movements by interacting with the K+ carrier system of the membrane of plants. The results indicate that alkylguanidines are very effective inhibitors of the uptake of K+ that occurs in excised barley roots.

MATERIALS AND METHODS

Seeds of barley (Hordeum vulgare var. Apizaco MV-72)1 were rinsed several times with distilled H2O, soaked for 24 hr in continuously aerated distilled H2O, and rinsed again 3 times with distilled H2O. Seedlings were grown essentially as described by Epstein (4). The seeds were placed on a plastic screen covered with cheesecloth which was supported by a 7-liter plastic tank containing 5 liters of 0.5 mM CaSO4; the corners of the cheesecloth were dipped into the solution. The tank was placed in the dark and covered with a plastic cover. The temperature was 24°C ± 3 and vigorous aeration was provided.

Roots from 6-day-old seedlings were used for the absorption experiments. The roots were cut in 2-cm segments (the 3-cm basal portions and 3-cm apical portions of the roots were discarded) and washed 3 times with ice-cold distilled H2O. The tissue prepared in this form is a highly homogeneous material. Batches of 16 segments were maintained in distilled H2O until the experiment was initiated. The uptake experiments were carried out by incubating barley roots in 4 ml of a mixture that contained 20 mM maleic buffer (pH 6), 0.05 mM CaSO4, and different concentrations of the indicated alkylguanidines and RbCl according to the experiment. Experiments on the ion absorption by excised tissues of higher plants have shown that potassium and rubidium behave virtually as isotopes of the same element (21). Therefore, 86Rb+ was used as a radioactive analog of K+. The incubation medium was maintained at 30°C and continuously shaken in a Dubnoff incubator. The solution was equilibrated for a 10-min period prior to the addition of the roots. At the times indicated in the respective figures (see under "Results"), the solution was decanted, and the roots were rinsed for 30 sec with a mixture of 30 ml of ice-cold 0.5 mM CaSO4 and 5 mM RbCl to eliminate adsorbed 86Rb+. Subsequently the roots were washed for 30 min in 15 ml of an identical ice-cold solution and finally they were washed for 15 min more with ice-cold distilled H2O. The roots were blotted with paper towels, weighed, and ashed in aluminum planchets for 2 hr at 500°C. The ash was moistened with 0.2 ml of distilled H2O, dried with an infrared lamp, and counted for radioactivity. This procedure has been used by several authors (7, 9).

RESULTS

The characteristics of 86Rb+ uptake by excised barley roots with respect to the time of incubation are shown in Figure 1. The uptake of 86Rb+ is directly proportional to the time of incubation. Octylguanidine inhibits the uptake regardless of whether it is added from the beginning of the experiment (Fig. 1A) or after a substantial uptake of 86Rb+ has taken place (Fig. 1B). It should be noted that when octylguanidine is added at the beginning of the experiment it does not affect 86Rb+ uptake immediately, but after 3 min of incubation. The same phenomenon is observed if octylguanidine is added after some accumulation of 86Rb+ has occurred, its inhibiting effect is detected after a lag period of 3 min.

A competition between the action of monovalent cations and...
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Fig. 1. Absorption of rubidium by isolated barley roots as a function of time in absence and presence of 441 μM octylguanidine. External concentration of RbCl was 1 mM. A: Octylguanidine was added when no 86Rb⁺ accumulation occurred; B: octylguanidine added after 3 min of 86Rb⁺ absorption.

Fig. 2. Double reciprocal plots to show the effect of 228 μM and 441 μM octylguanidine on the absorption of different external concentrations of 86Rb⁺ in isolated barley roots. Incubation period was 10 minutes; r: correlation coefficient.

Fig. 3. Effect of different concentrations of octylguanidine on transport of 86Rb⁺ by isolated barley roots. External concentration of RbCl was 1 mM and incubation period was 10 min.

Octylguanidine has been reported (12, 13, 24, 27). A Lineweaver-Burk plot of 86Rb⁺ uptake at various concentration of 86Rb⁺ is shown in Figure 2. The uptake of 86Rb⁺ shows saturation kinetics and octylguanidine induces an increase in the Km for Rb⁺ and a decrease in the maximum velocity of 86Rb⁺ uptake (Fig. 2).

The effect of various concentrations of octylguanidine on 86Rb⁺ in a 10-min incubation period with 1 mM RbCl in the reaction mixture is shown in Figure 3. Although octylguanidine is a very effective inhibitor of 86Rb⁺ uptake (half-maximal inhibition being attained at 50 μM), octylguanidine does not inhibit completely the uptake of 86Rb⁺; in our experimental conditions, the uptake of approximately 0.35 μmole of 86Rb⁺ is octylguanidine-insensitive.

Octylguanidine may be considered a lipophilic cation, it possesses a positive charge in its guanidine moiety and an 8 carbon chain that confers to the molecule a significant degree of lipophilicity. In order to assay the contribution of these two components of octylguanidine in the inhibition of 86Rb⁺ uptake, the effect of guanidines with a variable degree of lipophilicity was studied. Figure 4 shows that the inhibiting action of alkylguani-
Fig. 5. Effect of atom carbon number of the hydrophobic chain of guanidine on different concentrations on the absorption of $^{86}$Rb$^+$ by isolated barley roots. External concentration of RbCl was 1 mm and absorption period 10 min.

dines on $^{86}$Rb$^+$ uptake is highly dependent on the length of the alkyl chain. The more polar of the guanidines assayed, butylguanidine and ethylguanidine, inhibited the uptake by approximately 50% and 25%, respectively, while hexylguanidine and octylguanidine induced an inhibition of about 80%. Free guanidine at low concentration induces a slight enhancement of $^{86}$Rb$^+$ uptake, while at higher concentrations it inhibited the process. A plot of the number of carbons in guanidine with respect to the inhibiting effect on $^{86}$Rb$^+$ uptake shows that there is a very close relationship between these two parameters (Fig. 5). Clearly the alkyl chain plays a role in the presently reported action of alkylguanidines.

**DISCUSSION**

The objective of the present work is to describe the inhibiting action of octylguanidine on the uptake of $^{86}$Rb$^+$ by barley roots. It may be argued that alkylguanidines inhibit $^{86}$Rb$^+$ uptake by perturbing the energy transducing systems of the cell. Indeed, the time delay for onset of octylguanidine action may suggest that diffusion to sites other than the plasma membrane may operate. The reports that show oligomycin inhibits K$^+$ uptake in plant cells (17, 19) argue in favor of this possibility.

Although with the presently described experiments the latter alternative cannot be fully discarded, the fact that octylguanidine shows a partially competitive effect with external Rb$^+$ is highly suggestive that octylguanidine may act on the transport systems of the cell. It should be recalled that most of the reported effects of alkylguanidines are on metal ion-dependent systems (1, 24, 27, 29) and therefore it is very probable that in the presently described experiments alkylguanidines act on a cation-dependent process and logically this could correspond to the Rb$^+$ transport machinery.

The fact that the action of alkylguanidine on the $^{86}$Rb$^+$ uptake is related directly to the length of their alkyl chain is strongly indicative that hydrophobic interactions occur between the hydrophobic regions of the membrane and the alkyl chain of the guanidines. Two alternatives could account for the inhibitory action of alkylguanidines on the Rb$^+$ transport. First, there may be an alteration of the surface charges of the membrane which results from the localization of the alkyl chain of guanidine in the hydrophobic region of the membrane, with the guanidinium group oriented toward the surface of the membrane. This type of arrangement of alkylguanidines has been shown to occur in biological and model membranes (29). It is conceivable that an increase in the positive charges at the surfaces of the membrane results in inhibition of the uptake of monovalent cations. It should be recalled that inhibition of K$^+$ uptake by local anesthetics in certain biological membranes occurs through this mechanism (26). A second alternative would be that alkylguanidines interact (either through H-bonding or electrostatic interactions) with a Rb$^+$ carrier through its guanidinium group. This octylguanidine-carrier complex would be further stabilized if a hydrophobic region is in close contact with the K$^+$ carrier. In other words, the simultaneous interaction of alkylguanidines with the carrier and the hydrophobic milieu of the membrane would result in inhibition of Rb$^+$ through immobilization of the hypothetical K$^+$ carrier.

Whichever of these two alternatives is responsible for the inhibition of Rb$^+$ uptake, we would like to propose that alkylguanidines are effective inhibitors of Rb$^+$ transport by acting at the level of the membrane structures of the plant cell that are related to the transport of Rb$^+$. To our knowledge, this is the first report of an inhibitor of metal ion transport in plant cells that possesses the aforementioned property.

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**LITERATURE CITED**

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