Absorption of Magnesium and Chloride by Excised Corn Roots

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ABSTRACT

Absorption characteristics of Mg⁺ and Cl⁻ were investigated with 5-day-old excised corn (Zea mays) roots. Uptake from both 0.5 and 10 milliequivalents per liter MgCl₂ solutions occurred at steady state rates for the first 6 hours. Inhibition by dinitrophenol and low temperatures established that absorption during this period was metabolically mediated in the absence and presence of Ca²⁺. Absorption isotherms indicated dual mechanisms of Mg⁺ and Cl⁻ absorption from solutions above 1 milliequivalent per liter. The effect of H⁺ on absorption of Mg⁺ and Cl⁻ was typical of that generally reported for other plant roots and other ions. In the physiological pH range, Ca²⁺ greatly suppressed the rate of Mg⁺ absorption but had little effect on Cl⁻. The influence of Ca²⁺ on Mg⁺ appeared to be noncompetitive and independent of its effect on membrane permeability.

Magnesium absorption by excised root tissues has been investigated in the past with plant species that do not absorb significant amounts of Ca²⁺. Results of Moore et al. (14, 15), Leggett and Gilbert (8, 9), and Johnson and Jackson (5) show that excised roots of barley, soybeans, and wheat, respectively, all actively accumulate Mg⁺ but accumulate very little Ca²⁺. Nevertheless, Ca²⁺ may markedly affect the absorption of Mg⁺. Without being absorbed itself, Ca²⁺ greatly inhibits the uptake of Mg⁺ by barley roots (7, 15). However, this effect does not appear to be universal, as Ca²⁺ reduces Mg⁺ uptake only slightly in soybean roots (9). These divergent effects of Ca²⁺ certainly warrant further study. Furthermore, the nature of the interaction between Ca²⁺ and Mg⁺ might be better understood if studied with a species that absorbs both Ca²⁺ and Mg⁺. One species that meets these requirements is corn. Unlike the plant tissues mentioned above, excised corn roots actively absorb Ca²⁺ (3, 10), as well as Mg⁺. This study was undertaken to characterize the general features of Mg⁺ and Cl⁻ uptake by this tissue and to determine the effects of Ca²⁺ under various treatment conditions.

MATERIALS AND METHODS

Experiments were conducted with 6-cm apical segments of primary corn roots (Zea mays, cv. DeKalb 441). The roots were obtained from 5-day-old seedlings that were dark-grown in 0.2 mm CaSO₄, according to the procedure described previously (10). The preparation and treatment of the roots are also indicated therein, except for the following minor modifications: (a) to minimize leaching of the excised roots prior to treatment, the roots were held between several layers of damp cheesecloth rather than in distilled water and (b) both growth and treatment temperatures were maintained at 30 C, except when temperature was a variable. A root to solution ratio of 1 g/liter was used in all treatments except when MgCl₂ concentrations were less than 0.1 meq/liter in which case a ratio of 1 g/4 liters was used. The absorption solutions were aerated continuously and the pH was controlled within ±0.2 unit with acids or bases corresponding to the ions in the solutions.

Treatments were terminated by removing the roots from the absorption solution and rinsing them for 30 sec in 1 liter of distilled-deionized water. Chloride was extracted from the fresh roots with four successive 10-ml aliquots of boiling water and was determined by automatic coulometric-amperometric titration (1). The hot water extracts and titrated solutions were then recombined quantitatively with the root samples, taken to dryness, and digested in nitric and perchloric acids. Cations were determined by atomic absorption spectroscopy. Initial contents of Mg⁺, Ca⁺, Cl⁻, and K⁺ were about 4, 6, 6, and 28 μeq/g fresh weight, respectively. With the exception of the time course experiment, all the absorption data were obtained from two treatment times of 1 and 5 hr and are expressed as absorption rates (μeq/g fresh roots per hr). Several time course experiments established that steady state conditions prevailed within these limits. Negative absorption rates indicate a net loss of the ion during the 4-hr interval. The results presented represent the data of individual experiments, but all of the findings have been substantiated in either identical or similar experiments.

RESULTS AND DISCUSSION

Time Course of Mg⁺ and Cl⁻ Uptake and Role of Metabolism. To determine conditions and rates of steady state absorption for Mg⁺ and Cl⁻, uptake was measured in the low and high concentration ranges as a function of time. As shown in Figure 1, steady state rates prevailed from time zero up to 6 hr and decreased appreciably thereafter. At the pH employed, 5.6, Mg⁺ uptake exceeded that of Cl⁻ at both concentrations. The relative rates of Mg⁺ and Cl⁻ absorption, however, depended upon other factors, as will be seen below.

The characteristics of Mg⁺ and Cl⁻ transport by excised corn roots in the absence of Ca²⁺ are unlike those found for K⁺ (6, 11). Whereas K⁺ uptake in the low concentration range is negligible or even negative for several hours (Fig. 2 in Ref. 6; Fig. 1 in Ref. 11), Mg⁺ and Cl⁻ uptake began immediately (Fig. 1) and continued at rates of 2.8 and 2.2 μeq/g·hr, respectively. Nevertheless, since these data were obtained in the absence of Ca²⁺, the integrity of the root cell membranes should...
logically be questioned. A measure of this parameter was obtained by following the indigenous K+ level in the corn roots throughout the absorption period. During the entire 10 hr in single salt MgCl₂ solutions, K+ losses never exceeded 1 to 3 μeq/g in the low ambient concentration range and 2 to 4 μeq/g in the high range. Since these small losses also occur in CaCl₂ solutions (10), there is no reason to believe that membrane permeability was increased by these conditions. Moreover, CI- was readily absorbed and retained against the concentration gradient. This finding contrasts with that of many plant tissues which become quite "leaky" in the absence of Ca++. Soybeans (8, 9), pinto beans, and mung beans (J. E. Leggett, personal communication) retain very little indigenous K+ or Cl- when placed in MgCl₂ solutions. The difference in permeabilities among these species may be related to the retention of Ca++. Whereas in the present work, the corn roots retained over 70% of indigenous Ca++ when exposed to MgCl₂ solutions, soybean roots, grown in CaSO₄ under similar conditions and having the same initial Ca++ content, rapidly lost up to 90% of their Ca++ (8).

To insure that the above time course experiment was not simply a measure of nonmetabolic sequestering of Mg++ by the root tissue, the role of metabolism was studied. The effects of DNP (adjusted to pH 5.6 with Mg(OH)₂) and temperature on the rates of Mg++ and Cl- absorption between 1 and 5 hr were determined in the absence and presence of Ca++. (Table I). Potassium losses were also measured but are given as the total loss for the entire 5 hr so that the extent of leakage can be fully appreciated.

Studies with barley (15) and soybean (9) indicate that Mg++ transport is metabolically mediated. This finding is also substantiated here by the inhibitory effects of DNP and low temperature. Absorption by corn roots of both Mg++ and Cl- in the low concentration range is clearly dependent upon metabolism. Both 2 x 10^-4 M DNP and low temperature drastically reduced the rate of Mg++ and Cl- absorption in both the absence and presence of Ca++. However, it appears that 10°C was more effective than 0.5°C in inhibiting the metabolic absorption of Mg++. The relative losses of indigenous root K+ to the various treatments are instructive in the explanation of these data. The high losses of K+ at 0.5°C indicate that membrane disruption occurred, presumably exposing additional absorption sites in the cytoplasm having an affinity for the divalent cation. This also seems to be the case for the DNP treatment in the absence of Ca++. With Ca++ present, DNP inhibited uptake without causing undue membrane leakiness.

A further indication of the extent of membrane disruption when K+ losses were high was the concomitant losses of chloride from the tissue. Unlike Mg++, Cl- uptake and retention due to adsorption would be negligible when complete loss of membrane integrity occurs. Undoubtedly, other cellular constituents, including organic acids, were lost, thus maintaining electrostatic neutrality.

The presence of 0.5 meq/liter Ca++ strongly inhibited Mg++ absorption in the control and other treatments. In this respect, corn roots react more like barley roots (15) than soybean roots (9). This interaction between Ca++ and Mg++ is studied in more detail below.

Absorption Isotherms. Absorption rates for Mg++and Cl- as a function of ambient concentration in the absence and presence of 0.5 meq/liter CaSO₄ are shown in Figure 2. Concentrations of MgCl₂ from 0.01 to 10 meq/liter were used and the pH was maintained at 5.6. In both the absence and presence of Ca++, typical logarithmic absorption isotherms were obtained for Mg++ and Cl-, indicating dual absorption mechanisms for these ions. Saturation of the low concentration mechanism for Cl- occurred at about 0.1 or 0.2 meq/liter. Although the Mg++ isotherm did not show the distinct saturation of mechanism 1 that Cl- did, it occurred below 1.0 meq/liter. Above 1.0 meq/liter, the absorption rates of both Mg++ and Cl- were linear functions of the ambient concentration.

The depressive effect of Ca++ on Mg++ absorption, shown in the data of Table I, was found for all Mg++ concentrations above 0.01 meq/liter. Both the low and high concentration mechanisms appeared to be affected by Ca++. Chloride absorption, however, was reduced much less by the presence of CaSO₄ in the treatment solution. This experiment also lends further confidence in the membrane integrity of this tissue when Ca++ is absent. Not only were the Cl- absorption rates comparable in the absence and presence of Ca++, but K+ losses never exceeded 3 μeq/g in either case, even at Mg++ concentrations up to 10 meq/liter.

Influence of pH. Since the effect of Ca++ on ion absorption has been shown to be dependent upon pH for some ions (2, 4)

Table I. Effect of Temperature and DNP on Mg++ and Cl- Absorption and K+ Loss

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absorption Rates</th>
<th>K+ Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg++</td>
<td>Cl-</td>
</tr>
<tr>
<td>Ca++ absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C (control)</td>
<td>3.15</td>
<td>2.82</td>
</tr>
<tr>
<td>20°C</td>
<td>1.20</td>
<td>1.10</td>
</tr>
<tr>
<td>10°C</td>
<td>0.35</td>
<td>0.47</td>
</tr>
<tr>
<td>0.5°C</td>
<td>0.78</td>
<td>-0.32</td>
</tr>
<tr>
<td>DNP (2 x 10^-4 M; 30°C)</td>
<td>1.15</td>
<td>-0.25</td>
</tr>
<tr>
<td>Ca++ present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>1.90</td>
<td>2.48</td>
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<tr>
<td>20°C</td>
<td>0.88</td>
<td>1.52</td>
</tr>
<tr>
<td>10°C</td>
<td>0.15</td>
<td>0.38</td>
</tr>
<tr>
<td>0.5°C</td>
<td>0.25</td>
<td>-0.60</td>
</tr>
<tr>
<td>DNP (2 x 10^-4 M; 30°C)</td>
<td>0.20</td>
<td>0.45</td>
</tr>
</tbody>
</table>

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Graph 2: Absorption isotherms for Mg\(^{2+}\) and Cl\(^{-}\) in the absence and presence of 0.5 meq/liter Ca\(\text{SO}_4\) at pH 5.6. Absorption rates taken between 1 and 5 hr.

Graph 3: Effect of pH on Mg\(^{2+}\) and Cl\(^{-}\) absorption rates from 0.5 meq/liter MgCl\(_2\) in the absence (solid symbols) and presence (open symbols) of 0.5 meq/liter Ca\(\text{SO}_4\). Absorption rates taken between 1 and 5 hr.

Graph 4: Effect of increasing Ca\(^{2+}\) concentration on the rates of Mg\(^{2+}\) and Ca\(^{2+}\) absorption. All solutions contained 0.5 meq/liter MgCl\(_2\) at pH 5.6. Absorption rates taken between 1 and 5 hr.

**Influence of Ca\(^{2+}\).** The data above show that at equivalent concentrations, Ca\(^{2+}\) had a pronounced inhibitory effect on the absorption of Mg\(^{2+}\) by corn roots. To determine the sensitivity of Mg\(^{2+}\) absorption to Ca\(^{2+}\), Ca\(\text{SO}_4\) concentrations from 0.01 to 5.0 meq/liter were tested for their effects. The concentration of MgCl\(_2\) was 0.5 meq/liter and the pH was maintained at 5.6. The inhibitory effect of Ca\(^{2+}\) on the rate of Mg\(^{2+}\) absorption was striking (Fig. 4). Magnesium absorption was markedly reduced by Ca\(^{2+}\) in the low concentration range (0.01 to 0.1 meq/liter). This regulatory effect of Ca\(^{2+}\), however, did not result from competitive absorption of Ca\(^{2+}\). Total net uptake of Ca\(^{2+}\) in this concentration range was less than 1.0 \(\mu\text{eq/g}\) for the entire absorption period of 5 hr. It should be pointed out that, in the absence of Mg\(^{2+}\), corn roots readily absorb Ca\(^{2+}\) in this range, with 0.1 meq/liter saturating mechanism I (10). It appears, therefore, that Ca\(^{2+}\) and Mg\(^{2+}\) are mutually inhibitory in the low concentration range. With progressively higher
Ca$^+$ concentrations above 0.1 meq/liter a gradual but much smaller decrease in the rate of Mg$^{2+}$ absorption occurred, while the rate of Ca$^{2+}$ absorption increased markedly.

Although the inhibition by Ca$^{2+}$ was not as great for corn (Fig. 4) as that reported for barley (15), the mechanism seems to be the same. That is, the effect of Ca$^{2+}$ is not dependent upon its own absorption in either barley or corn. The effect of Ca$^{2+}$ on Mg$^{2+}$ absorption does not appear to be due to permeability changes either. As discussed above, membrane permeability of CaSO$_4$-grown corn roots seems to be little affected by the lack of ambient Ca$^{2+}$ in short term experiments at physiological temperatures and pH conditions. Of course, we do not intend to minimize the role of Ca$^{2+}$ in maintaining membrane integrity under other conditions or with other species as evident in the studies on soybean by Leggett and Gilbert (8, 9), in which roots placed in single salt MgCl$_2$ solutions lost large amounts of indigenous Ca$^{2+}$ and K$^+$ and did not absorb any Cl$^-$. Calcium appears to regulate the rate of Mg$^{2+}$ absorption by corn roots by functioning at some site other than the actual absorption site. A mechanism discussed previously to explain many mutual, noncompetitive interactions between cations observed with barley roots (13) would also explain the Ca$^{2+}$-Mg$^{2+}$ interaction in corn. By binding to or reacting with the carrier, Ca$^{2+}$ may cause a conformational change in the structure of the carrier, thus reducing its transport capacity. Conversely, a regulatory effect of Mg$^{2+}$ also occurs that inhibits the transport of Ca$^{2+}$.

LITERATURE CITED