CHANGES OF MEMBRANE ASSOCIATED ATPASE ACTIVITY OF CORN ROOT DUE TO SALT STRESS

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It is well established that salt stress reduces plant growth through osmotic effects on water availability as well as the specific toxic effects of the salt ions. There is growing evidence that salt stress inhibits the uptake and transport of mineral nutrients (Lynch and Lauchli 1984, Cramer et al. 1987). It is widely accepted that cation-stimulated membrane-associated ATPase is involved in the transport of mineral nutrients. The membrane-associated ATPase is regulated by the nature of lipids, and some of them are affected by the physical and chemical environment of the roots (Lynch et al. 1987, Yapa et al. 1986). Accordingly, less functional association between lipids and enzymes participating in the activation by divalent cation such as Mg^{2+} and Ca^{2+} might be induced by the stress with NaCl. In this work, we investigated how membrane-associated ATPase of corn roots responds to various cations due to the treatment with NaCl in vivo.

MATERIALS AND METHODS

Plant materials —— Corn (*Zea mays* cv. Nagano No. 1) was used. Plants were cultured in a greenhouse with a basic culture solution containing 4 mM KNO_3, 1 mM NH_4Cl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM KH_2PO_4 and micronutrients. Micronutrients were 1ppm Fe, 0.5ppm B, 0.5ppm Mn, 0.05ppm Zn, 0.02ppm Cu and 0.001ppm Mo. After 7 or 10 weeks of culture, plants were treated with 110 mM NaCl for 4 or 6 days.

Preparation of membrane-associated ATPase —— The harvested roots were washed with distilled water and weighed. Then the roots were homogenized with three times their volume of 0.25 M sucrose in 0.1 M Tris-HCI buffer (pH 7.5) containing 10 mM 2-mercaptoethanol. The homogenate was passed through four layers of cheesecloth and centrifuged at 10,000xg for 20min. The supernatant was centrifuged in a Titan 50 rotor (Beckmann ultracentrifuge Model L5-75) at 100,000xg for 60min. The precipitate was suspended in 0.25 M sucrose in 0.1 M Tris-HCI buffer (pH 7.5) using a Potter homogenizer and used as a microsomal membrane-associated ATPase.

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ATPase assay —— The standard assay system of ATPase contained 50μl of 0.5 M Tris-HCI buffer (pH 7.5), 50μl of 50 mM Tris-ATP (pH 6.0), 50μl of 30mM of $MgCl_2$ and 50μl of enzyme, and the volume was made 0.5ml with distilled water. The reaction was started by the addition of ATP and allowed to progress at 30°C for 30min. The reaction was stopped by the addition of 0.5ml of cold 5% TCA. The liberated Pi was determined by the modification of the Lowry-Lopetz method (Hirasawa et al. 1979). The specific activity was expressed as μmoles Pi released by 1mg protein for 1min. The basal activity means the activity measured in the absence of added metal ions. Activity measured in the presence of Mg^{2+} minus basal activity is called Mg^{2+}-activated ATPase activity. Protein was determined by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

Determination of optimal pH of ATPase —— Fig. 1 shows the pH curve of Mg^{2+}−activated ATPase activity of the control and NaCl-

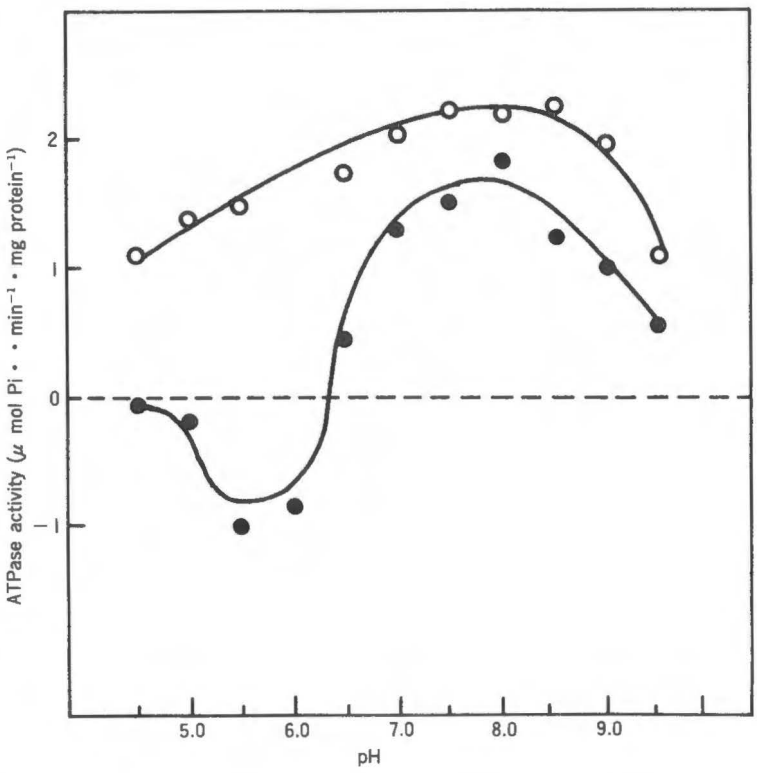

FIG. 1 pH curve of ATPase prepared from the control roots and NaCl-treated roots for 6 days. Activity was measured in the presence of 3.0 mM $MgCl_2$. The dotted line shows the basal activity assayed in the absence of $MgCl_2$. (○) : ATPase prepared from the control roots, (●) : ATPase prepared from NaCl-treated roots.

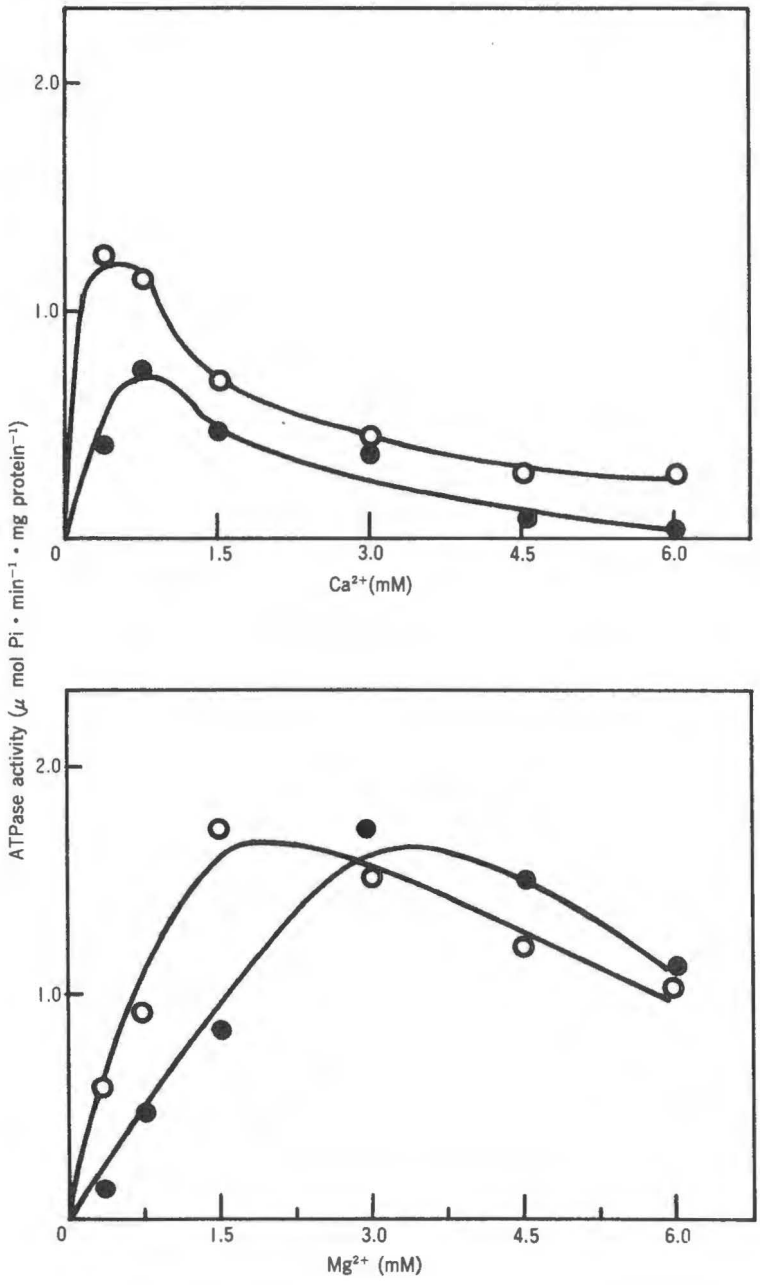

FIG. 2 Effect of different concentrations of Ca^{2+} and Mg^{2+} on ATPase prepared from the control roots and NaCl-treated roots for 4 days. (○) : ATPase prepared from the control roots, (●) : ATPase prepared from NaCl-treated roots.

treated roots for 6 days. Both enzymes showed the maximal activation of the basal ATPase activity by Mg^{2+} at pH 8.0. Basal ATPase prepared from NaCl-treated roots was inhibited by Mg^{2+} at the acidic pHs although ATPase from the control roots was activated by Mg^{2+} at all pHs from 4.5 to 9.5. The ATPase prepared from NaCl-treated roots was slightly activated by Mg^{2+} for an unknown reason. A similar weak response of membrane-associated ATPase to Mg^{2+} was also induced by Ca^{2+} deficiency in cucumber roots and the ATPase from roots heavily injured by Ca^{2+} deficiency showed even lower activity in the presence of Mg^{2+} than the activity in the absence of Mg^{2+} (Matsumoto and Kawasaki 1981).

Effect of different concentrations of Ca^{2+} and Mg^{2+} on ATPase activity prepared from control and NaCl-treated roots —— As shown in Fig. 2, the maximal activation of ATPase by Ca^{2+} was observed at 0.375 mM with ATPase prepared from the control roots, while 0.75 mM of Ca^{2+} was required for the maximal activation of ATPase prepared from the roots treated with NaCl for 4 days. Furthermore, the rate of activation of ATPase by Ca^{2+} at any concentration tested was higher in ATPase prepared from the control roots than NaCl-treated roots. The maximal activation by Ca^{2+} of ATPase prepared from roots treated with NaCl for 4 days was approximately half as much as the ATPase from the control roots.

The maintenence of the cell membrane in a functional state is important for the absorption and transport of ions, and it is believed that Ca^{2+} is clearly related to the function of membranes. Ohnishi and Ito (1974) directly demonstrated that Ca^{2+} induces phase separation of the phosphatidylserine (PS)-phosphatidylcholine (PC) bilayer membrane into a solid phase of PS aggregates bridged by Ca^{2+} chelation and a fluid phase of PC molecules. In the mean time, Lynch et al. (1987) reported that mechanical injury of the plant membrane by salinity was due to the replacement of functional Ca^{2+} on the membrane by Na^{+}. These facts may relate to the reduced response of Ca^{2+} to the activation of basal ATPase activity prepared from the NaCl-treated roots.

Contrary to the case of Ca^{2+}, the maximal rate of activation of ATPase by Mg^{2+} was not affected by the treatment of the roots with NaCl. However, the Mg^{2+} concentration required for the maximal activation of ATPase differed between the control and NaCl-treated roots. In the control roots, 1.5 mM Mg^{2+} gave a maximal activation, while 3.0 mM of Mg^{2+} was required for the maximal activation of ATPase prepared from the NaCl-treated roots.

Effect of various concentration of Mg^{2+} and Ca^{2+} on ATPase activity —— In the following experiment, the behavior of ATPase prepared from

the control and NaCl-treated roots for 4 days under various ratios of Ca^{2+} and Mg^{2+} added to maintain 3 mM in total was investigated (Fig. 3). ATPase activity prepared from the control roots showed the rather stable activity under the differential ratio of Mg^{2+} and Ca^{2+} and the alteration of the activities from 3 mM Ca^{2+} to 3 mM Mg^{2+} was less than 200%. On the other hand, the enzyme prepared from NaCl-treated roots

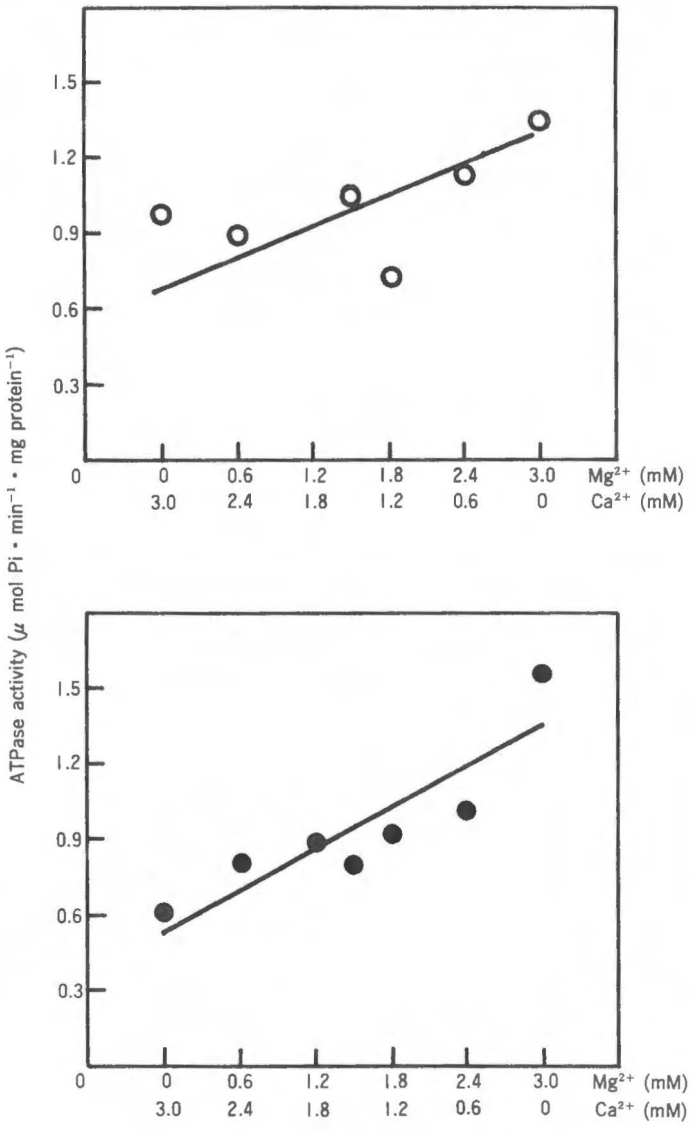

FIG. 3 Effect of various concentrations of Mg^{2+} and Ca^{2+} on ATPase prepared from the control roots and NaCl-treated roots for 4 days. Changing ratio of Ca^{2+} and Mg^{2+} was maintained at 3 mM in total. ATPase prepared from the control roots (upper) and NaCl-treated roots (lower).

increased clearly according to the increasing ratio of Mg^{2+} to Ca^{2+} with the difference in the activities being more than 300%. The result showed that Mg^{2+} was the preferred activator for ATPase prepared from corn roots and this property became more conspicuous with ATPase prepared from the roots treated by NaCl when the total concentration of Mg^{2+} plus Ca^{2+} was kept constant at 3 mM.

Effect of various divalent cations on the ATPase activity —— An activation of ATPase prepared from the control and NaCl-treated roots for 4 days was investigated in the presence or absence of various cations at 3 mM (Fig. 4). ATPase activity prepared from the control roots was

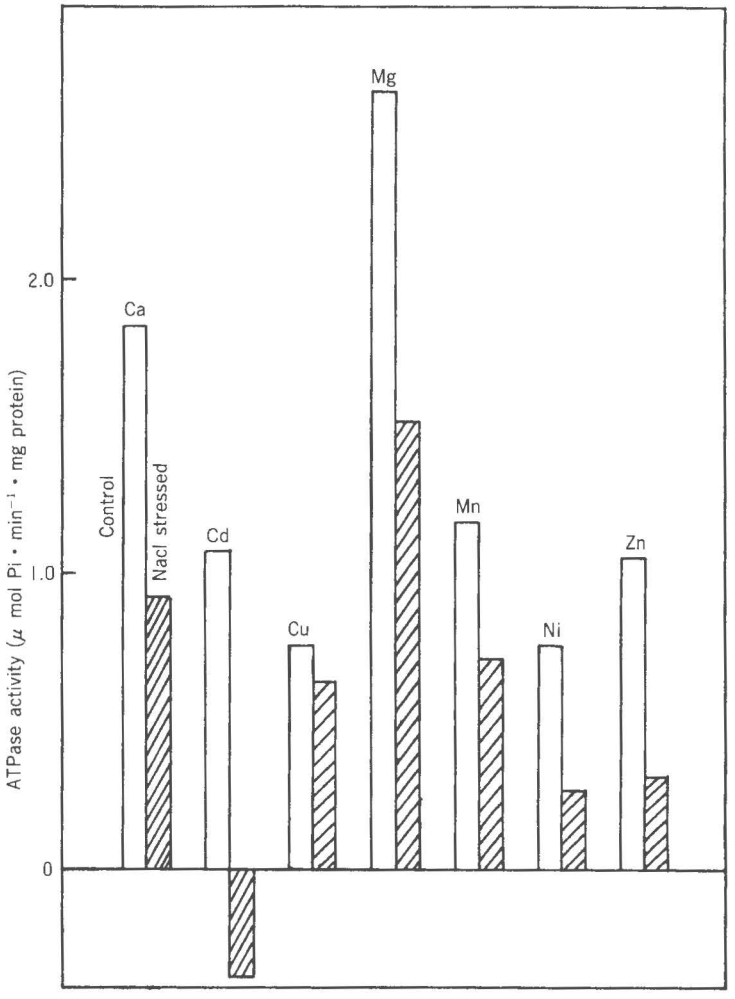

FIG. 4 Effect of various cations on the activation of basal ATPase activity prepared from the control roots and NaCl-treated roots for 4 days. Cation consentration was 3mM. Basal activity assayed without cation is shown as zero.

activated more or less by any kind of metal cation tested. The rate of cation-stimulation of ATPase prepared from the control roots varied as $Mg^{2+} > Ca^{2+} > Mn^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+} = Ni^{2+}$. On the other hand, vanadate, an inhibitor of plasma membrane ATPase, and molybdate, an inhibitor of phosphatase, are inhibitory (data not presented). Unlike the ATPase prepared from the control roots, ATPase from the roots treated with NaCl for 4 days was much less activated by ions, especially Cd^{2+} inhibited basal ATPase. Furthermore, ATPase prepared from the roots treated with NaCl for 20 days was not activated by any cations except

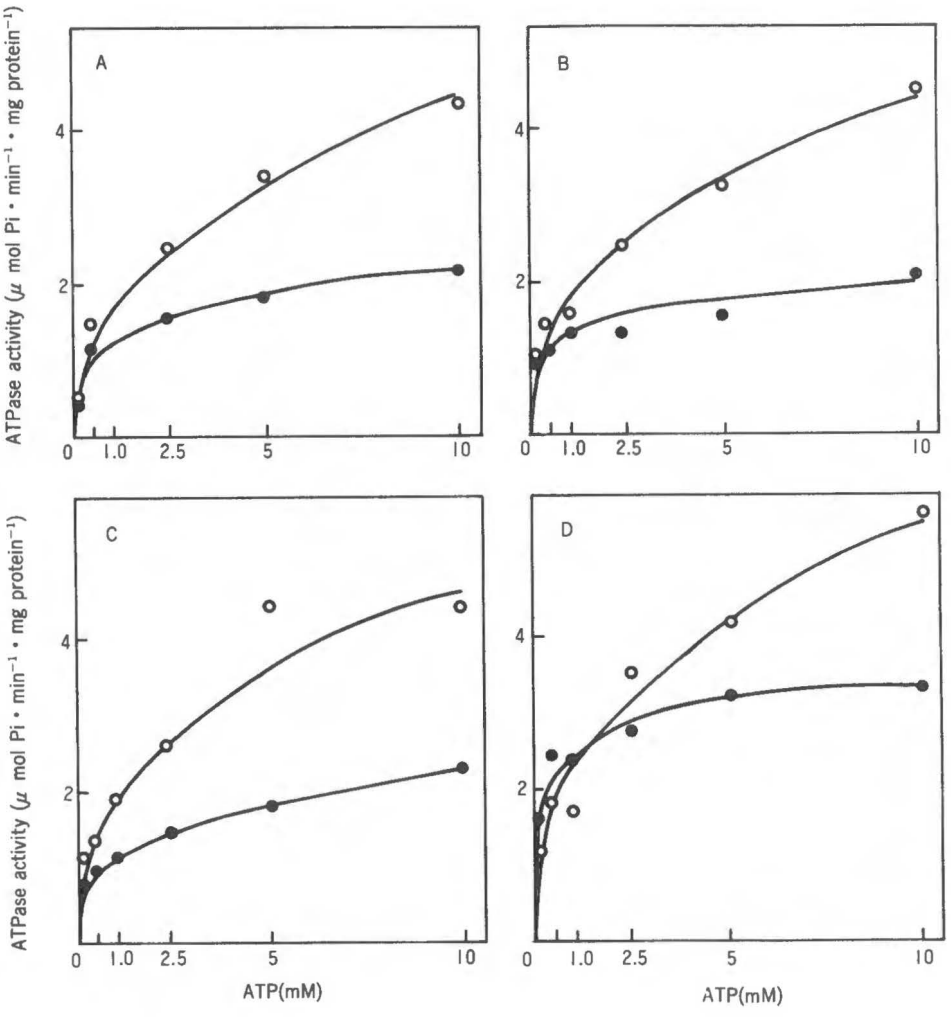

FIG. 5 Effect of different ATP concentrations on basal and Mg^{2+}-ATPase activity prepared from the control and NaCl-treated roots. A : Control roots, B : NaCl-treated roots for 4 days, C : NaCl-treated roots for 6 days, D : NaCl-treated roots for 20 days. (○) : Mg^{2+}-ATPase, (●) : basal ATPase activity.

Ca^{2+} and Mg^{2+} (data not presented).

Effect of ATP concentration on the basal and Mg^{2+}-ATPase of the control and NaCl-treated roots —— Basal and Mg^{2+}-ATPase activity prepared from the control roots and roots treated with NaCl for 0, 4, 6 and 20 days were assayed in the presence of different concentrations of ATP (Fig. 5). Both basal and Mg^{2+}-ATPase activities of the roots treated with NaCl for 0, 4 and 6 days did not change significantly with ATP concentrations up to 10 mM. However, basal ATPase increased distinctly after the treatment of the roots with NaCl for 20 days. Furthermore, basal ATPase was not activated by Mg^{2+} in the presence of ATP concentration less than 1.5 mM. This suggests that non-specific phosphatase activity increases and the affinity of ATPase to Mg-ATP decreases with the NaCl stress for 20 days.

SUMMARY

The properties of microsomal membrane-associated ATPase of the control and NaCl-stressed roots of corn were investigated. The maximal activation of the enzyme prepared from both control and NaCl-stressed roots by Mg^{2+} occurred at pH 8.0, but ATPase prepared from the NaCl-stressed roots was not activated by Mg^{2+} at acidic pH. The activation of ATPase by various divalent cations was lower for the enzyme prepared from the NaCl-stressed roots than the enzyme prepared from the control roots. When ATPase was assayed under the changing ratio of Ca^{2+} and Mg^{2+} maintaining 3 mM in total, the alteration of activity was lower in ATPase prepared from the control roots than from NaCl-stressed roots. The basal and Mg^{2+}-ATPase in the presence of different concentrations of ATP did not change up to 6 days of NaCl treatment, but basal activity increased distinctly after the treatment of NaCl for 20 days and basal ATPase activity was not activated by Mg^{2+} at an ATP cocentration less than 1.5 mM.

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