Inhibition by Bicarbonate of Divalent Cation Activated Adenosine Triphosphatase from the Microsomal Fraction of Wheat Roots *

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ATPase activity in the plasmalemma-rich, microsomal fraction from plant roots is of interest, because of its possible role in the regulation of ion transport into plant cells. Influence of cations, both monovalent [1-3] and divalent,[4,5] on microsomal ATPases have been extensively studied. Effects of anions are not as well documented, although an HCO_3^--dependent ATPase has been associated with secretion of bicarbonate from pancreas.[6] To investigate regulation of microsomal ATPase activity further, the interaction between some anions and divalent cations were studied. After preliminary work by Henning Kaufholz in Copenhagen, the effects of bicarbonate were regarded as particularly interesting.

Materials and methods. Seeds of *Triticum vulgare* Vill., *cv.* Svenno Spring Wheat were soaked for 24 h, then grown for 7 days in the dark at 18 °C over aerated Cl^--free medium containing 1 mM $Ca(NO_3)_2$, 0.15 mM $NaHPO_4$, 0.3 mM KH_2PO_4, 0.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.5 μM $MnSO_4$, 1.6 μM H_3BO_3, 20 μM Titriplex III (EDTA) and 30 μM $NH_4Fe(SO_4)_2$. Roots were rinsed with distilled water and ground in preparation buffer (0.25 M sucrose, 1 mM EDTA and 10 mM Tris-HCl, pH 7.5 [1]). Following differential centrifugation of the homogenate, the 12 000 (20 min) to 30 000 (1 h) $\times g$ fraction was collected and resuspended in preparation buffer to a protein concentration of 75 μg/ml. The preparation was stored at −80 °C until use.

The assay was carried out in a reaction volume of 1 ml, the final concentration of ATP (H^+ form) and cations (as acetate) being 0.5 mM in a 12.5 mM Tris-HEPES buffer, pH 7.8. Although the pH optimum of this enzyme is 6.8, the high assay pH was required to prevent the protonation of the active species of particular interest – the bicarbonate ion. It was also difficult to maintain a constant pH while varying only the bicarbonate concentration at a lower pH. Anions were present in the concentrations noted (0 – 50 mM), all as K^+ salts. The reaction proceeded for 30 min at 30 °C and was stopped by addition of 0.1 ml of 33 % trichloroacetic acid and 4 ml of distilled water. Phosphate was assayed by a modified Fiske-Subbarow method [7] and protein according to Potty.[8] Non-enzymatic ATP hydrolysis was subtracted in all cases. All experiments were performed at least twice with independent preparations and there were three replicates within each experiment. The standard errors were in the order of 5 – 10 % of the averages.

Results. Ca-dependent ATPase was found to be inhibited by bicarbonate and by chloride (Fig. 1). Ionic strength and potassium concentration had only minimal effect (KAc line). The inhibitory effect of chloride and bicarbonate were apparently additive (Fig. 2). As for Mg- and Mn-dependent ATPase, the bicarbonate effect was small and of the same order of magnitude as the ionic strength effect. The chloride effect was small but more variable.

Discussion. These data, along with the classical physiological concept [9] that the first step of anion uptake in roots is exchange of, for instance, chloride from the outside against bicarbonate (or OH^-) from the inside, lead us to propose a model for active transport of anions in wheat roots. It consists of an anion carrier coupled by calcium or a calcium-dependent factor to an ATPase.

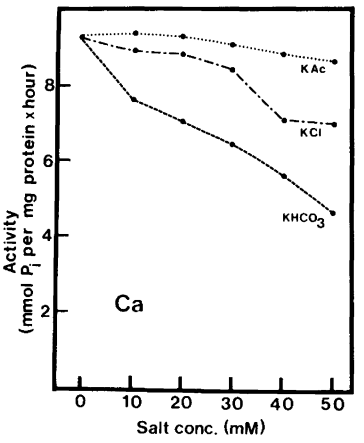

Fig. 1. The effect of different anions on Ca-dependent ATPase.

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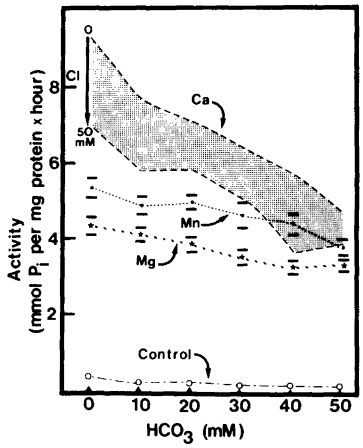

Fig. 2. Bicarbonate and chloride effects on Ca-, Mn- and Mg-dependent ATPases. Activity in the absence of a divalent cation was very low (control). The inhibiting effect of chloride on the Ca-dependent ATPase is shown by the shaded area, the top border of which represents the ATPase activity in the bicarbonate concentration as given on the abscissa. For the Mn- and Mg-dependent ATPases, all the effect of chloride is between the horizontal bars, and the effect of bicarbonate is represented by the respective curves.

Bicarbonate, a waste product of respiration, is transported out and other anions are transported in. Mg- and Mn-dependent ATPases are uneffected by these anions and are apparently not involved in anion transport. The Ca-dependent ATPase (anion carrier) system may be inhibited by high external bicarbonate through a simple equilibrium effect, while high chloride when entering would allosterically slow the system to prevent excessive accumulation of chloride (*cf.* Ref. 10). An influence of calcium on physiological transport of anions has been demonstrated, for instance, by Cuppoletti and Segel[11] in *Penicillium* and by Skjelbreid and Nissen[12] in barley roots. The K,Mg-ATPase related to cation transport in corn roots is inhibited by calcium,[2] which would appear logical if calcium contributes to the coupling between energy supply and transport of anions.

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1. Fisher, J. and Hodges, T. K. *Plant Physiol. 44* (1969) 385.
2. Leonard, R. T. and Hotchkiss, C. W. *Plant Physiol. 58* (1976) 331.
3. Lindberg, S. *Physiol. Plant. 48* (1980) 65.
4. Kawasaki, T., Kähr, M. and Kylin, A. *Physiol. Plant. 45* (1979) 45.
5. Caldwell, C. R. and Haug, A. *Physiol. Plant. 50* (1980) 183.
6. Simon, B., Kinne, R. and Sacks, G. *Biochim. Biophys. Acta 282* (1972) 293.
7. Lindeman, W. *Proc. 2nd Int. Conf. of the UN on the Peaceful Uses of Atomic Energy 24* (1958) 8.
8. Potty, V. H. *Anal. Biochem. 29* (1969) 535.
9. Lundegårdh, H. *Plant Physiology*, Oliver & Boyd, Edinburgh and London 1966.
10. Pettersson, S. and Jensén, P. *Physiol. Plant 45* (1979) 83.
11. Cuppoletti, J. and Segel, I. H. *Biochemistry 14* (1975) 4712.
12. Skjelbreid, E. and Nissen, P. *Physiol. Plant. 49* (1980) 383.

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