ION TRANSPORT KINETICS IN PLANT TISSUE:
COMPLEXITY OF THE CHLORIDE ABSORPTION ISOTHERM

O. E. Elzam, D. W. Rains, and Emanuel Epstein
Department of Soils and Plant Nutrition
University of California, Davis, California

Received February 17, 1964

We have examined the kinetics of chloride absorption by barley root tissue in experiments similar to those in which we demonstrated the duality of potassium absorption mechanisms in this tissue (Epstein, Rains, and Elzam, 1963). In those experiments, rates of potassium absorption were found to be a function of the external concentration of KCl in accord with Michaelis-Menten kinetics over a low concentration range, approaching the theoretical maximal velocity at an external concentration of 0.2 mM KCl. At higher concentrations (0.5 to 50 mM), another mechanism of potassium absorption was shown to come into play. In the present experiments, we measured the rate of chloride absorption from solutions of KCl ranging in concentration from 0.005 mM to 50 mM.

METHODS

Seedlings of barley, Hordeum vulgare, var. Arivat, were grown in 2×10^{-4} M $CaSO_4$ solution (Epstein, 1961) and the absorption experiments were done as described earlier (Epstein, Schmid, and Rains, 1963), with slight modifications. The excised root samples were 0.50 g. fresh weight. Prior to the absorption period proper, the samples were kept in an aerated solution of 0.50 mM $CaSO_4$, at 30° C. They were rinsed with fresh identical $CaSO_4$ solution and then transferred to the vigorously aerated experimental solutions for a 20-minute absorption period at 30° C. The experimental solutions contained KCl labeled with Cl^{36}, and 0.50 mM $CaSO_4$. The pH (unbuffered) was 5.6 $\pm$0.2.

The absorption period was discontinued by rinsing the tissue with a cold (5° C) solution of 5 mM KCl, 0.50 mM $CaSO_4$, followed by transfer of the samples to an aerated identical solution for 30 minutes. Exposure of the samples to this solution serves to free the tissue of diffusible or readily exchangeable Cl^{36} in the "outer space" of the tissue, leaving for final assay only that fraction that has been transported into the "inner" (trans-membrane) space or spaces where it is not readily available for isotopic exchange with external chloride. The radiochloride was quantitatively extracted from the tissue samples with boiling water, and aliquots of the extracts pipetted into counting cups, dried, and counted with a thin-window gas flow counter. Absorption was found to be a strictly linear function of time for at least one hour.

RESULTS

Figure 1 shows the results of an experiment in which the rate of chloride absorption was determined as a function of the external chloride concentration over a 10,000 fold range of external concentrations. At low concentrations (0.005 to 0.2 mM), a mechanism with high affinity for chloride operates (see the insert of fig. 1). The Michaelis constant, K_m, for this mechanism is 0.014 mM. This high-affinity mechanism 1 reaches virtual saturation at 0.1 mM chloride, doubling the concentration causing no further increase in the rate of absorption.

However, at higher concentrations (main part of fig. 1) the curve depicting the absorption rate as a function of the concentration rises, with two further inflections at about 10 and 28 mM chloride, eventually leveling off at about 50 mM. The mechanism operating over the 0.5 to about 10 mM range of chloride concentrations has a Michaelis constant of about 5 mM, higher by a factor of 350 than that of the high-affinity mechanism 1. On the other hand, all three mechanisms coming into play at the high concentrations have Michaelis constants differing from each other by much smaller factors.

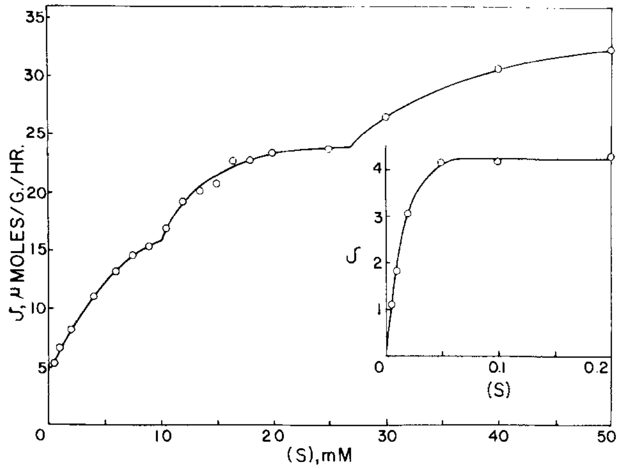

Figure 1. Rate of chloride absorption by excised barley roots as a function of the concentration of KCl. Results for the 0.005 to 0.2 mM range of concentrations shown in the insert.

On the supposition that the abrupt changes in chloride absorption rates observed at certain concentrations might be functions of the particular ratios of KCl to $CaSO_4$ prevailing in the solutions we performed an experiment identical with the one shown, except that the $CaSO_4$ concentration was 10 mM instead of 0.5 mM. Up to a concentration of about 10 mM KCl, results were virtually identical with those shown in fig. 1. At the higher concentrations, actual rates were somewhat lower than those depicted in fig. 1, but the shape of the isotherm was similar, with the points of inflection within 2 mM of those shown for the experiment done at 0.5 mM $CaSO_4$.

DISCUSSION

Conformance of ion transport kinetics in plant tissue with enzyme kinetics, together with other findings, has been interpreted as evidence for the operation of carriers which effect the transport of the ions across cellular membranes (Epstein and Hagen, 1952; Epstein, Rains, and Elzam, 1963). From the beginning, the kinetic evidence has been to the effect that a single ionic species (the substrate ion) may be bound and transported by more than one type of active carrier site, the sites having different affinities for the substrate ions and other (interfering) ions. Two transport mechanisms differing widely in their affinities for potassium ions, and in other features, have been implicated in potassium absorption by barley roots (Epstein, Rains, and Elzam, 1963).

The present experiments on absorption of chloride show a striking parallelism with those on potassium absorption. At low concentrations, a high affinity mechanism 1 operates which is highly selective, being indifferent to the presence of sulfate at concentrations higher than the chloride concentration by several orders of magnitude. The high-affinity potassium mechanism is also very selective, showing little affinity for sodium. As with potassium absorption, a second mechanism of chloride transport comes into play at high concentrations (0.5 to 50 mM). It is not homogeneous. We interpret the inflections of the absorption isotherm in this range of concentrations as evidence for the existence of carrier sites differing perceptibly, though not greatly, in their affinities for chloride ions.

The present experiments show that a given rate of absorption of chloride, like potassium absorption rates, may represent the sum of rates contributed by different transport mechanisms. Theories of "anion respiration" (Lundegårdh, 1955) or "salt respiration" (Robertson, 1960) will have to be re-examined in view of the heterogeneity of anion transport revealed here.

Acknowledgements. This research was supported by grants to one of us (E. E.) from the National Science Foundation and the Office of Saline Water, U. S. Department of the Interior. Chlorine-36 was obtained from Oak Ridge National Laboratory, Oak Ridge, Tennessee.

REFERENCES

Epstein, E., Plant Physiol. 36, 437 (1961).
Epstein, E., and Hagen, C. E., Plant Physiol. 27, 457 (1952).
Epstein, E., Rains, D. W., and Elzam, O. E., Proc. Nat. Acad. Sci. (U.S.) 49, 684 (1963).
Epstein, E., Schmid, W. E., and Rains, D. W., Plant and Cell Physiol. 4, 79 (1963).
Lundegårdh, H., Ann. Rev. Plant Physiol. 6, 1 (1955).
Robertson, R. N., Biol. Rev. 35, 231 (1960).