Chloride Accumulation in Plant Cells as a Homeostatic System: Energy Supply as a Dependent Variable

W.J. Cram
School of Biological Sciences (A12), University of Sydney, New South Wales 2006, Australia

Summary. During the accumulation of KCl by excised tissue of the carrot storage root, Cl− influx falls by 90% or more. The possibility that this reduction is caused by depletion of respiratory substrate and consequent limitation of Cl− influx by energy supply is disproved. If energy were limiting, then after accumulating KCl (i) the rate of O2 uptake would be less; (ii) the ATP content might be less; (iii) external glucose and/or sucrose would stimulate O2 uptake and Cl− influx; and (iv) respiratory uncouplers would stimulate O2 uptake less. In each case the opposite was found. The ratio of O2 uptake in KCl-loaded tissue to that in nonloaded tissue was 1.25 ± 0.21 (7); the ATP concentration was the same after KCl loading; glucose did not stimulate O2 uptake (except indirectly in the same way as a nonmetabolized glucose analogue) nor did glucose or sucrose stimulate Cl− influx; and the maximal O2 uptake in the presence of the uncoupler CCCP was the same after KCl loading.

The decrease in Cl− influx must therefore be due to a negative feedback signal from internal Cl−, as previously suggested. It is pointed out that normal energy supply must be regarded not as an independent, controlling variable; but rather as a dependent variable of the KCl accumulation system.

Conditions where energy supply is or becomes limiting are discussed. The distinction is drawn between pumps operating near equilibrium when energy supply may normally be a controlling factor (e.g., many H+−ATPases); and other pumps (possibly most of those for the uptake of nutrients and osmotica) which operate far from equilibrium and for which energy supply is a dependent variable.

Key Words chloride flux/accumulation · plant root cells · homeostasis · energy supply · sugar levels · respiration · dependent/independent variables

Introduction

This paper examines the relationship of energy supply to the active transport of ions in higher plant cells. The main conclusion is that energy supply does not limit transport, particularly in pumps far from equilibrium. A picture of a generalized transport system in plants is presented, which includes both signals to transport and its energy supply.

The system investigated experimentally is KCl accumulation by excised tissue of the carrot storage root. During the accumulation of KCl to a final steady level of around 160 μmol (g fresh wt)−1 (independent of external KCl concentration - manuscript in preparation), Cl− influx falls by 90% or more. The fall is correlated specifically with the (Cl− + NO3−) concentration in the vacuole (Cram, 1973), but the possibility remains that this correlation is not due to a negative feedback signal from the internal (Cl− + NO3−) concentration to Cl− influx, but rather is an indirect relationship.

The alternative proposal (which it is the purpose of this paper to disprove) is that the decrease in Cl− influx during accumulation is due to limitation by energy supply, due in turn to depletion of respirable sugars (see, e.g., discussions in Lüttge & Pitman, 1976; Pitman & Cram, 1977; Cram, 1980a). The same hypothesis can be invoked to explain reduced uptake of K+, H2PO4−, and NO3− by roots under conditions in which their supply of carbohydrate from the shoot is reduced.

The “energy limitation” hypothesis suffers from the disadvantage of being inconsistent with our knowledge of respiratory control in mitochondria. Respiration is not an overall limitation on the energy-dependent activites of a cell; rather it is a self-regulated activity that automatically matches supply of ATP to demand for its use (Wiskitch, 1980). The cell could only operate with its multitude of energy-dependent, self-regulating and interacting biochemical pathways if their common energy supply were maintained more or less constant and unlimited, though some major, overall regulation of cellular activities, in the direction of synthesis, for example, may have the potential energy supply as an important coordinator (Atkinson, 1972; see Discussion).

Nevertheless, it is clear that energy supply may
come to limit transport when it (energy supply) naturally, or more frequently artificially, falls towards zero. This has been amply demonstrated in the work designed to characterize energy supply routes by selective inhibition of components of the cell's energy supply system (e.g., MacRobbie, 1970; Raven, 1976). But it does not follow that normal variations in transport rates (of the sort occurring during Cl− accumulation by plant cells) are also caused by variation in their energy supply.

The first, experimental, part of this paper demonstrates that the change in Cl− influx during KCl accumulation in carrot cells is not controlled by energy supply.

The second part of the paper discusses the general question of what controls normal variations in ion fluxes in plant cells, and, in particular, how energy supply can be included in this picture. The solution offered relies on the distinction between dependent and independent variables. Energy input to the Cl− transport process is pictured as being a variable that depends on the rate of Cl− transport, rather than an independent variable that determines it.

Tests of the Hypothesis that Energy Supply Limits Cl− Transport in KCl-Loaded Carrot Tissue

If a reduced rate of energy supply limits the Cl− influx in KCl loaded tissue, the following would be predicted:

1) The rate of oxygen uptake would be less in the KCl-loaded tissue.

If the stoichiometry between O2 uptake and Cl− influx were tight, then the component of O2 uptake linked to Cl− influx would fall by 90% during KCl accumulation. This component will be taken as being the 25% stimulation of respiration in water-washed tissue when it is placed in KCl solution (e.g., Robertson & Wilkins, 1948, and similar values observed in the present experiments). The predicted decrease in O2 uptake rate would then be 0.9 × 0.2 = 0.18 (18%) of the rate in water-washed tissue in 1 mM KCl.

2) The ATP level would be less in KCl-loaded tissue, if Cl− influx depends directly or indirectly on energy supplied via ATP.

If the pump rate were proportional to the ATP concentration, then in KCl-loaded tissue the ATP concentration would be 10% of its value in non-loaded tissue. In Neurospora the electrogenic pump has a rectangular hyperbolic relationship to ATP concentration in vivo, with a K1 of about 2 mM, which is comparable to other ATP-dependent pumps (Slayman, Long & Lu, 1973). If the same were true of carrot then the ATP level would have to fall to less than 10% of the control level to cause a 90% fall in Cl− influx in the KCl-loaded tissue.

3) Supplying external glucose and/or sucrose would stimulate both the rate of oxygen uptake and Cl− influx in KCl-loaded tissue if the supply of respiratory substrate were limiting.

For glucose in the cytoplasm to limit respiration, it must be present at concentrations comparable to the Kj for hexokinase (around 0.1 mM – Turner & Turner, 1975; Turner & Copeland, 1981). In the absence of external glucose this must be supplied by hydrolysis of sucrose or influx to the cytoplasm from the vacuole. From the cytoplasmic volume (about 2% of the tissue volume, or 0.02 ml g fresh wt−1) the cytoplasmic glucose content must be around 0.1 μmol ml−1 × 0.02 ml g fresh wt−1 or 0.002 μmol g fresh wt−1. From the rate of O2 uptake the rate of utilization of glucose in respiration can be calculated to be 3/6 or 0.5 μmol g fresh wt−1 hr−1. For glucose turnover in the cytoplasm the rate constant must therefore be 0.5/0.002 = 250 hr−1, giving a half time of 0.2 min. If glucose supply limits respiration then it follows from the calculations above that an additional glucose influx to the cytoplasm of 3.5 μmol g fresh wt−1 hr−1 from 10 mM externally supplied glucose (Grant & Beevers, 1964) would increase the rate of O2 uptake by 100% (hexokinase activity would be saturated) within minutes and would increase Cl− influx similarly if it is limited by energy supply from respiration.

Externally supplied sucrose, which is hydrolyzed to UDPG and fructose or glucose and fructose in vivo, would have an effect similar to externally supplied glucose.

4) Oxygen uptake in KCl-loaded tissue would be stimulated less by respiratory uncouplers, and the maximally uncoupled O2 uptake rate would be significantly less in the KCl-loaded tissue.

Materials and Methods

Phloem or xylem tissue of the storage root of carrot (Daucus carota L) and tissue of red beet (Beta vulgaris L) was cut into rectangles approximately 5 × 5 × 1 mm and washed in aerated distilled water for several days. After 4 or 5 days part of a batch of tissue was allowed to accumulate ions from 10 mM KCl + 0.1 mM CaSO4 for a further 4 to 5 days. At this stage the cellular KCl level would be about 120 μmol g−1, which is 80% of the final steady 160 μmol g−1 that would be accumulated after 10 days (Cram, 1980b).

Chloride influx was estimated using 36Cl. The tissue was placed in 36Cl-labeled solution for 30–60 min and then extracel-