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Regulation of Potassium Absorption in Barley Roots

AN ALLOSTERIC MODEL

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ABSTRACT

Plasmalemma influx isotherms for K^+ were measured in the system I concentration range (0.01-0.32 mM), for barley (*Hordeum vulgare* L.) roots of varying internal K^+ concentration, and *Km* values for influx calculated. In plants grown for several days in $CaSO_4$ or in $CaSO_4$ plus KCl solutions, as well as in plants grown in $CaSO_4$ for several days and then rapidly loaded with KCl during a pretreatment period, Michaelis constant values were positively correlated with internal K^+ concentrations. Influx of K^+ is shown to be sigmoidally related to internal K^+ concentration and Hill plots of influx data give linear transformations with $n = 4$. This information is taken as support for an allosteric model for the regulation of K^+ influx in which the "carrier" is envisaged as possessing a single external binding site for K^+ as well as four internal sites for allosteric control of influx.

The absorption of inorganic ions by tissues of higher plants has been shown to be negatively correlated with the internal (vacuolar + cytoplasmic) concentrations of these ions. Thus, plasmalemma influx of K^+ into barley roots is negatively correlated with internal K^+ concentration (5); in carrot tissue, Cl^- influx is negatively correlated with the log of $Cl^- + NO_3^-$ concentration (2); and in cultured tobacco cells, SO_2^{2-} transport is negatively correlated with internal SO_4^{2-} concentration (13). As a consequence of such observations, and by analogy with what is known of the regulation of biosynthetic pathways, it has been suggested that ion absorption may be sensitive to negative feedback inhibition by the absorbed ion (5, 8, 9). It has been proposed that this type of control might be mediated via repression of carrier synthesis or by a form of allosteric inhibition of influx in which the absorbed ion serves as the allosteric effector (5). While conceptually attractive, and in line with current dogma, there exists to date little direct evidence for any of the above models of regulation. The experiments described below were designed to examine the hypotheses of regulation of influx by repression of carrier synthesis or by direct allosteric inhibition. The fact that the Michaelis constant (*Km*), which has frequently been employed as a measure of the affinity of uptake systems for their substrates, is not constant for K^+ influx by barley roots, but rather, increases (indicating reduced affinity) as the K^+ concentration of the tissue ($[Ki^+]$) increases, together with the demonstrated sigmoidal relationship between influx and $[Ki^+]$ is taken as strong support for an allosteric model of regulation.

MATERIALS AND METHODS

Barley plants (*Hordeum vulgare* L. cv. Zephyr) were grown at 26 C in darkness, according to methods described previously (5). In order to obtain plants of differing K^+ status, two different methods were employed. In the initial experiments, plants were grown for 6 days in solutions containing 0.5 mM $CaSO_4$ plus various concentrations of KCl. In later experiments, plants were grown in 0.5 mM $CaSO_4$ solutions for 6 days and transferred at the beginning of an experiment to 50 mM KCl plus 0.5 mM $CaSO_4$. By sampling root tissue at various intervals after immersion in KCl solutions, roots of differing K^+ status were obtained.

Influx determinations were made using 0.5-g samples of excised roots which were allowed to absorb ^{86}Rb-labeled K^+ from solutions containing 0.5 mM $CaSO_4$ or $CaCl_2$ plus concentrations of KCl ranging from 0.01 to 0.32 mM. The influx period was 10 min at 30 C followed by a 5-min wash in ice-cold isotope-free influx medium. In all experiments involving pretreatment in KCl solutions, care was taken to remove any free space KCl by rinsing root samples for two 5-min periods in 0.5 mM $CaSO_4$ solution prior to influx determination. Data presented in this paper express influx measurements as $\mu mol\ g^{-1}\ hr^{-1}$ and are based upon fresh weight determinations. Values depicted in graphs are most usually the average of four replicates, rarely three. Standard errors so commonly lay within the symbols that for purposes of clarity, they are not shown. *Km* values throughout this paper were determined by means of Lineweaver-Burk plots of the reciprocal of influx *versus* the reciprocal of external K^+ concentration. Regression lines were obtained by the statistical method of least squares, and *Km* values calculated, in each case, from the corresponding regression equations. Potassium concentrations of tissue samples $[Ki^+]$ were determined by extracting the tissue repeatedly in boiling distilled H_2O. These pooled aqueous extracts were then analyzed by flame photometry.

RESULTS AND DISCUSSION

Figure 1 shows Lineweaver-Burk plots of $1/v$ against $1/[C_K]$, where v represents K^+ influx ($\mu mol\ g^{-1}\ hr^{-1}$) and $[C_K]$ represents external K^+ concentration of the influx medium. Closed circles represent data taken from an experiment using roots which had been grown for 6 days in solutions containing 5 mM KCl plus 0.5 mM $CaSO_4$. Triangles represent data for plants grown in 1 mM KCl plus $CaSO_4$, and open circles show data for plants grown in 0.5 mM $CaSO_4$. *Km* and V_{max} values calculated from linear regression equations were 0.09 mM and 2.25 $\mu mol\ g^{-1}\ hr^{-1}$ for the 5 mM KCl-grown roots, 0.036 mM and 2.9 $\mu mol\ g^{-1}\ hr^{-1}$ for 1 mM KCl-grown roots, and 0.03 mM and 7.6 $\mu mol\ g^{-1}\ hr^{-1}$ for the $CaSO_4$-grown roots. Figure 2 shows a Lineweaver-Burk plot for K^+ influx by roots grown in 10 mM KCl plus 0.5 mM $CaSO_4$ for 6 days. *Km* and V_{max} values were calculated to be 0.25 mM and 0.4 $\mu mol\ g^{-1}\ hr^{-1}$, respectively.

Similar studies in *Lemna* grown in media ranging in K^+ concentration from 0.015 mM to 1 mM demonstrate that V_{max} values declined from 74.1 mol $cm^{-2}\ sec^{-1}$ to 9.8 mol $cm^{-2}\ sec^{-1}$ with increasing K^+ concentration of the media (14). However, *Km* values remained constant at 0.027 mM. In the present study, no

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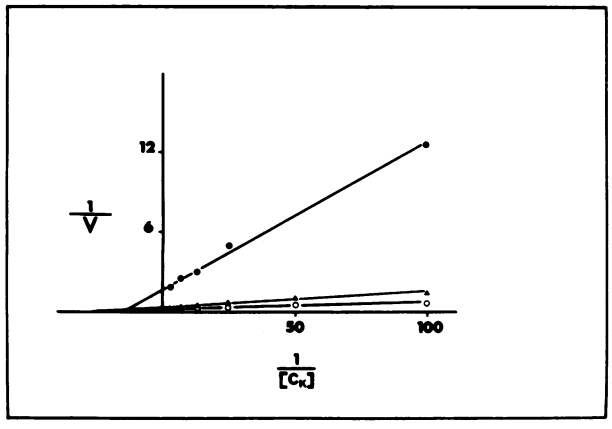

FIG. 1. Lineweaver-Burk plots of $1/v$ against $1/[C_K]$, in which v = plasmalemma influx in μmol g^{-1} hr^{-1} and $[C_K]$ = external potassium concentration (mM), for roots grown in $CaSO_4$ (○), 1 mM KCl plus $CaSO_4$ (▲), and 5 mM KCl plus $CaSO_4$ (●). *Km* values were 0.03 mM, 0.036 mM, and 0.09 mM, respectively.

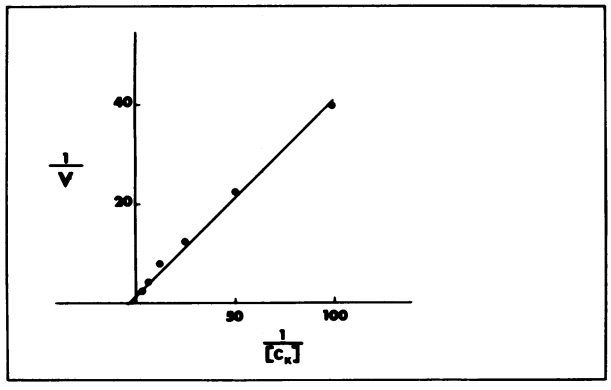

FIG. 2. Lineweaver-Burk plot (symbols as for Fig. 1) for 10 mM KCl-grown roots. *Km* value was 0.25 mM.

significant changes in *Km* values were obtained for roots grown in K^+ concentrations up to 1 mM. Above 1 mM KCl, *Km* values increased significantly.

How do the above observations relate to the question of the mechanism of regulation of influx? The following hypotheses may be considered.

In response to increasing internal K^+ concentration: *(a)* carrier systems of lowered affinity for K^+ are synthesized; *(b)* the absolute numbers of carriers are reduced by repression of carrier synthesis; *(c)* a direct "allosteric" inhibition of influx by K^+, or some allosteric effector synthesized in response to increased $[Ki^+]$ occurs; *(d)* influx of K^+ is regulated by some combination of *a* through *c*. Alternative *a* seems, *a priori*, the least likely since to account for increasing *Km* values associated with increasing $[Ki^+]$, several discrete "structural" genes, each coding for carrier systems of different K^+ affinity would be required. Alternative *b* adequately accounts for the reduced V_{max} values but clearly is unable to explain the increased values of *Km* observed. Nevertheless, this does not eliminate the possibility that increased internal K^+ concentration may result in repression of carrier synthesis in addition to other effects.

Ion influx over the concentration range up to 1 mM (system I) is very different in character from influx in the high concentration range 1 to 50 mM (system II) (3, 4) for example system I "carriers," in low K^+ roots, demonstrate a high affinity for K^+ (*Km* approximately 0.02 mM) and an indifference to the presence of Na^+ and the anion accompanying K^+. By contrast, uptake in the high concentration range is characterized by a low affinity for K^+ and extreme sensitivity to the presence of Na^+ and the nature of the associated anion.

Considering the observed low affinity for K^+ displayed by the K^+-loaded roots in the experiments described above, it was decided to examine their sensitivity to Na^+ and to replacement of Cl^- as the accompanying anion, by SO_4^{2-}. If high potassium levels resulted in selective inhibition of system I activity, then influx isotherms might reflect the operation of a low affinity "system II" mode of ion uptake. When K^+ influx to 10 mM KCl-grown roots was measured in the presence of 0.5 mM Na^+, or from KCl compared with K_2SO_4 solutions (Fig. 3) ranging in concentration from 0.01 to 0.32 mM, there was no inhibitory effect of Na^+ or SO_4^{2-}. These observations confirm recent studies by Johansen (7) showing that the absorption of K^+ by KCl-pretreated roots from 0.5 mM KCl or K_2SO_4 solutions does not differ significantly. Thus, it would appear fairly certain that the low affinity for K^+ exhibited by KCl-grown roots is, in fact, due to the operation of system I carriers. These observations and arguments do not altogether invalidate the second alternative and this question will be reconsidered below.

In order to evaluate the third alternative, it was decided to examine the influence of fairly rapid changes of $[Ki^+]$ upon *Km* values, as described under "Materials and Methods," the rationale for this approach being that changes in the affinity of the carrier system resulting from genomic transcriptional events would be relatively slow, whereas direct allosteric effects should be effective without delay. Unfortunately, the worker on transport systems is limited by his inability to change the environment of the postulated allosteric system instantaneously. Neverthe-

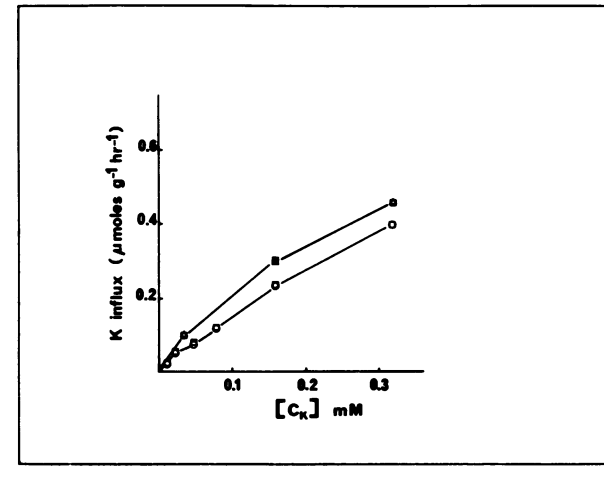

FIG. 3. Influx isotherms for K^+ absorption by roots grown in 10 mM KCl (symbols as for Fig. 1), from KCl (□), or K_2SO_4 (○) solutions, and from KCl solutions (□) or from KCl plus 0.5 mM NaCl (△), obtained from two separate experiments.

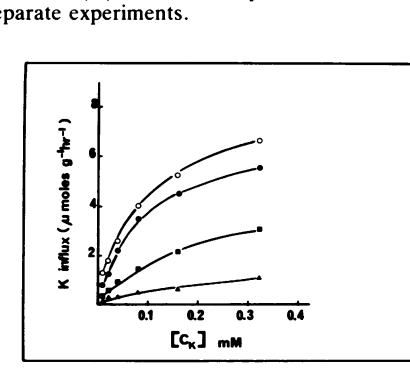

FIG. 4. Influence of duration of pretreatment in 50 mM KCl upon influx isotherms for $CaSO_4$-grown roots (symbols as for Fig. 1) 0 hr (○), 3 hr (●), 6 hr (■), and 12 hr (▲).

less, following 3-, 6-, and 12-hr immersions of excised $CaSO_4$-grown roots in 50 mM KCl, [Ki^+] was increased from 26.3 μmol g^{-1} to 64.6, 93.9, and 130.5 μmol g^{-1}, respectively. Figure 4 shows influx isotherms from such an experiment, in which by 3 hr, influx rates had declined significantly. Figure 5 displays the same data in the form of Lineweaver-Burk plots. Km values were calculated to 0.036 mM (time 0 hr, ○), 0.054 mM (3-hr pretreatment in KCl, ●), 0.066 mM (6-hr pretreatment, ■), and 0.133 mM (12-hr pretreatment, ▲), respectively. It is extremely difficult to evaluate how long genomic transcriptional events involved in carrier synthesis might be involved. Observations on the influence of inhibitors of protein synthesis upon ion accumulation by barley roots (11) suggest that carriers for K^+ are relatively long lived. It might be argued that changes of affinity induced within 3 hr are unlikely to be the result of gene-mediated activity. Furthermore, it has recently been shown (6) that the reduction of plasmalemma influx associated with rapid loading of barley roots with KCl can occur in the absence of DNA, RNA, and protein synthesis. When the data for influx (Fig. 4) were replotted against [Ki^+], it became apparent (Fig. 6) that the relationship between influx and [Ki^+] was clearly sigmoidal. Such a relationship between effector and enzyme is highly characteristic for enzymes subject to allosteric control (1). Because of the limited number of observations obtained in this experiment, and out of concern for the possibility of artifact created by the use of excised roots over long pretreatment periods, this experiment was repeated employing intact tissue. $CaSO_4$-grown plants were transferred intact at the beginning of the KCl pretreatment period and sampled at intervals of 2 hr. Figure 7 shows the results of this experiment and confirms the clear sigmoidal relationship between influx and [Ki^+].

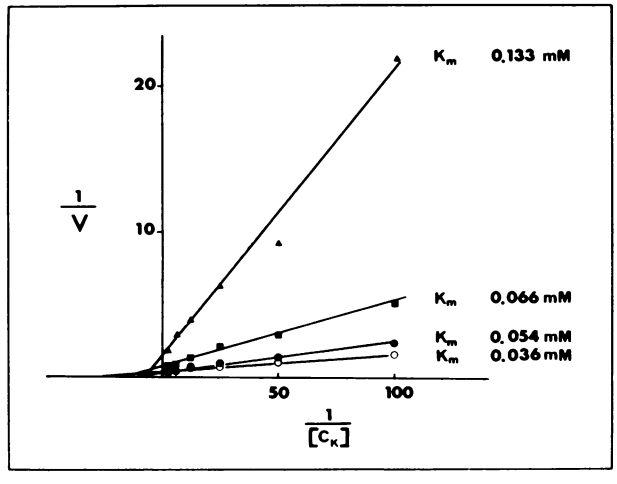

FIG. 5. Data of Fig. 4 redrawn in the form of Lineweaver-Burk plots (symbols as for Fig. 4).

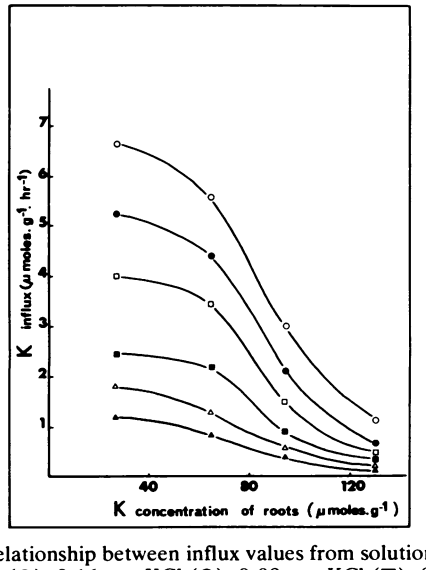

FIG. 6. Relationship between influx values from solutions containing 0.32 mM KCl (○), 0.16 mM KCl (●), 0.08 mM KCl (□), 0.04 mM KCl (■), 0.02 mM KCl (△), and 0.01 mM KCl (▲) and internal K^+ concentration of roots.

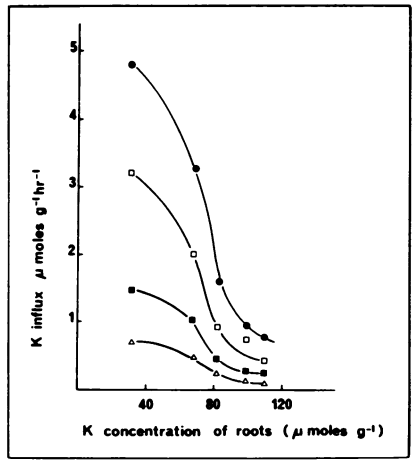

FIG. 7. Relationships between influx values from solutions containing 0.16 mM KCl (●), 0.08 mM KCl (□), 0.04 mM KCl (■), and 0.02 mM KCl (△) (employing intact barley plants during the pretreatment period in 50 mM KCl), and internal K^+ concentration of roots.

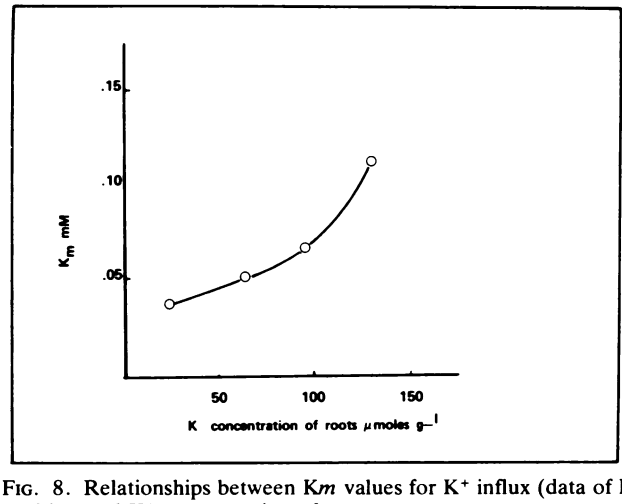

FIG. 8. Relationships between Km values for K^+ influx (data of Fig. 5) and internal K^+ concentration of roots.

Figure 8 shows the Km values obtained from Figure 5 plotted against [Ki^+]. Km values were highly correlated with [Ki^+] (correlation coefficient 0.93) and exhibited an exponential relationship. This observation reiterates the apparent cooperativity of internal K^+ in reducing affinity for influx, an important diagnostic feature of allosteric systems.

Influx isotherms for K^+ into barley roots have been shown to take the form of rectangular hyperbolas at K^+ concentrations up to 1 mM (3). These isotherms have been interpreted in terms of Michaelis Menten enzyme kinetics:

$$\frac{v = V_{max} \cdot [S]}{Km + [S]}$$

in which v represents influx, S represents the external concentration of K^+, and V_{max} and Km have their usual meanings. Sig-

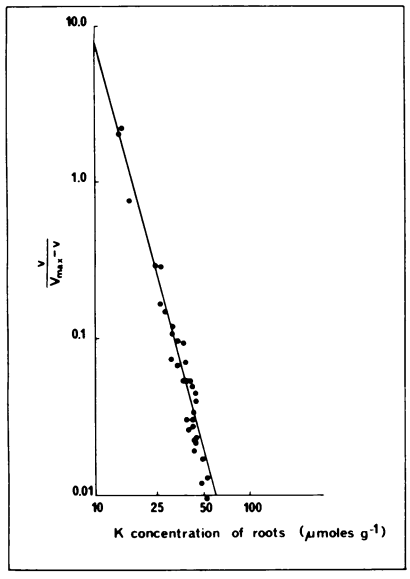

FIG. 9. Hill plot ($v/V_{max} - v$ against internal K^+ concentration, plotted on log-log scales) of previously published influx data (5) obtained from several separate experiments; n (slope of regression line) = 3.7 ± 0.2, and the correlation coefficient = −0.96.

moidal enzyme kinetics, by contrast, may be interpreted in terms of the Hill equation which was derived empirically for the sigmoidal haemoglobin-oxygen dissociation curve. The linear transformation of this equation:

$$\log K' = \log \frac{V_{max} - v}{v} + n \log [S]$$

is commonly employed by enzymologists to evaluate the number of allosteric modifier sites *(n)* (12). Adapting this concept to apply to carrier-mediated transport, the Hill plot might be used to investigate the number of substrate or inhibitor interaction sites upon the carrier. Now, as previously stated, influx data plotted against external K^+ concentration give hyperbolic plots with no indication of sigmoidicity. Hill plots of such data naturally give $n = 1$, implying the existence of a single binding site for external K^+. However, influx data plotted against $[Ki^+]$ gave sigmoidal plots which transform to linear Hill plots in which n values vary from 3.7 to 4. The data from a large number of separate experiments are shown in Figure 9 in which $n = 3.7 \pm 0.2$ and the correlation coefficient for the regression line = −0.96. Data from a single experiment gave $n = 3.97 \pm 0.15$. It is attractive to consider, therefore, that the K^+ carrier might possess a single binding site for external K^+, as well as four allosteric binding sites for internal K^+. Saturation of these binding sites at high internal concentrations of K^+ might inhibit further influx of K^+ by causing conformational changes in the carrier which reduce the affinity of the external binding site for K^+. This model is shown diagramatically in Figure 10.

It is perhaps dangerous to extrapolate from the kinetics of simple enzyme reactions to the kinetics of whole root K^+ absorption. However, bearing in mind these hazards, the following arguments may be advanced in favor of the above model:

A. The vast majority of regulatory enzymes, including hemoglobin, which have been studied appear to consist of four subunits (10). The observed n values of 4 are, therefore, highly suggestive.

B. The relationship between velocity and substrate concentration in systems (such as oligomeric allosteric enzymes) which display sigmoidal kinetics is such that substrate saturation occurs within a narrow range of allosteric effector concentration. In terms of the above model, such a system is ideally adapted to maintain internal levels of nutrients within a limited physiological range.

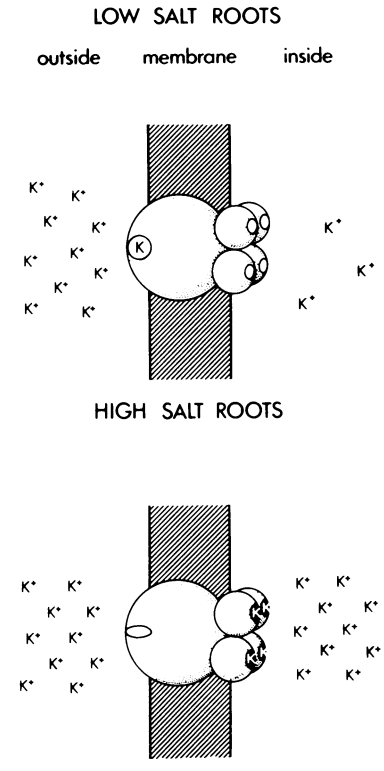

FIG. 10. Model of allosteric control system for K^+ influx into barley root cells. The large centrally located structure represents the K^+ carrier. On the outer surface, a single K^+-binding site is represented by the small circle. On the inner surface, four allosteric binding sites located on four subunits are represented by four circles. In low salt roots, the allosteric sites are vacant, and K^+ can be bound to the external site giving high initial rates of influx. When the internal concentration of K^+ is high (high salt roots), the allosteric sites are saturated, and the conformation of the external binding site is modified resulting in lowered affinity for K^+.

C. Biological systems are characteristically conservative at the biochemical level. There is no reason *a priori*, why the subunit structure which so effectively serves the regulatory requirements of allosteric enzymes should not also serve the regulatory requirements of carrier-mediated transport.

It seems most logical to assume that this allosteric action is mediated by cytoplasmic K^+, although clearly, the measurements of internal K^+ reported here represent the sums of cytoplasmic and vacuolar K^+ concentrations. Although the changes of Km induced by high internal K^+ can not be accounted for by repression of carrier synthesis, this does not eliminate the possibility that repression does, in fact, occur. Thus, it may be that in the long term, reduction of K^+ influx is mediated through repression of carrier synthesis in addition to the postulated allosteric effects.

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