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MULTIPLE SITE UPTAKE OF INDIVIDUAL CATIONS BY ROOTS AS AFFECTED BY HYDROGEN ION[1]

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Many investigators have suggested that metabolic uptake of ions by cells involves a system of carriers. A comprehensive review is given by Epstein (6). The ion combines with the carrier to form an active intermediate. The active intermediate breaks down essentially irreversibly to release the ion inside the cell.

Cations that are similar chemically may compete with each other for the same binding site. However different carriers are involved for different groups of cations. Thus Collander (4), and Epstein and Hagen (7), and Menzel and Heald (19), have indicated that rubidium and cesium and potassium are competitive for the same site. Although calcium utilizes a different site than potassium, Epstein and Leggett (8), and Menzel and Heald (19) have shown that strontium and barium compete with calcium. Neither sodium nor magnesium was found to be competitive for either of these sites.

Over a limited concentration range Epstein and Hagen (7), and Epstein and Leggett (8) noted one carrier site for each cation. Epstein and Hagen noted an anomaly at higher ion concentrations with sodium and Epstein and Leggett an anomaly with strontium. Over a wide concentration range Hagen and Hopkins (12) demonstrated two carrier sites for phosphate uptake.

Various investigators (14, 15, 16, 21) have shown that the hydrogen ion concentration of the bathing medium affects ion uptake. Others (1, 2, 3, 11) have minimized this effect within usual physiological ranges. Jacobsen et al (17), using the model of Ro-

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senberg (22), suggested that the cation carrier is metabolically produced at the external surfaces as the hydrogen complex, HR, and that the anion carriers were metabolically produced at the external surfaces as the hydroxyl complex R′OH. They formalized this in equations a and b,

$$HR + M^{+} \rightleftharpoons MR + H^{+} \qquad (a)$$

$$R'OH + A^{-} \rightleftharpoons R'A + OH^{-} \qquad (b)$$

where HR and R′OH represent the metabolically produced cationic and anionic binding substances. MR and R′A serve as transporting agents, transferring ions from culture medium to the vacuole (17).

On the other hand, Epstein and Hagen (7) and Hagen and Hopkins (12) have described ion uptake by carriers without any detailed assumptions as to the nature of the carrier. Ion absorption is described by these authors in the following equations.

$$R + M_{(outside)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} MR \qquad (1)$$

$$MR \underset{k_4}{\overset{k_3}{\rightleftharpoons}} R' + M_{(inside)} \qquad (2)$$

where R represents a metabolically produced site, M, the ion, MR, the active intermediate and k the rate constant for each reaction.

Hagen and Hopkins (12) have shown by kinetic analysis that hydroxyl ion is a competitive inhibitor of phosphorus uptake and behaves similarly to other competitive inhibitors such as ADP and pyrophosphate in phosphate uptake (13) and selenate in sulfate uptake (18).

The competitive inhibition of phosphate uptake by hydroxyl ion is consistent with the uptake mechanism described by both Hagen and Hopkins (12) and Jacobsen et al (17). However, the present authors prefer the description afforded in equations 1 and 2 since it makes no assumptions as to the location of the carrier nor the specific combination in which the carrier occurs. By analogous interpretation from kinetic analysis as developed for ion absorption (7, 12), the present results demonstrate that hydrogen ion is a competitive inhibitor of cation uptake. The cations used were sodium, potassium, rubidium and strontium. Although different sites are involved for various cations, two sites are shown to be involved in uptake of each of these cations. Competitive inhibition is demonstrated at one of the two sites of ion uptake found for each cation. This is the site that dominates ion uptake at lower ion concentrations. Although hydrogen ion effects are also demonstrated at higher ion concentrations where a second site dominates cation uptake the experiments were not designed to show whether this effect is competitive.

METHODS

THEORY: Where k_3 in equation 2 is rate limiting in the forward direction and k_4 is negligible, the observed absorption of an ion is proportional to MR as shown in equation 3.

$$v = k_3\,[MR]\,t \qquad (3)$$

where v = the amount of ion, M, absorbed in time, t.

From these equations a velocity equation can be derived analogous to case III for enzyme action described by Hofstee (16) and utilized by Hagen, Leggett and Jackson (13) for phosphorus uptake.

$$v = -K_m \frac{v}{[M]} + V_{max} \qquad (4)$$

where K_m equals $\frac{k_2 + k_3}{k_1}$ analogous to the Michaelis constant (20), and V_{max} equals the amount absorbed when all of the carrier is saturated with the ion under study, i.e., $MR = \Sigma R$.

If v is plotted against $\frac{v}{[M]}$ a straight line should result for each site provided [M] remains essentially constant during the steady state uptake. The slope of this straight line is $-K_m$, the apparent dissociation constant of MR, when $k_2 \gg k_3$ (16). The y intercept is V_{max} and $V_{max} = k_3\,[\Sigma R]t$.

If another ion competes for the same site, the V_{max} is the same in the presence of the competing ion (12) but the slope of the line changes. This change in slope reflects a decrease in the amount of ion at a given concentration associated with the site in the presence of a competing ion. Although the presence of one ion may affect the rate of uptake of another ion without being competitive, such an effect always results in a changed V_{max} and may also result in a change in slope.

It is more convenient to determine the total ion sorbed (absorbed plus adsorbed) instead of the total absorbed. Since adsorption not involved in uptake is negligible for monovalent cations as shown by Fried, Noggle and Hagen (10) and for phosphate as shown by Hagen and Hopkins (12) this would change equation 3 to 3a below:

$$v = [MR]k_3t + [MR] \qquad (3a)$$

However it would not affect equation 4 except that

$$V_{max} = [\Sigma R]k_3t + [\Sigma R] \text{ instead of}$$

$$V_{max} = [\Sigma R]k_3t \qquad (3b)$$

Thus, whether amount of ion absorbed or total sorbed is measured a plot of v vs $\frac{v}{[M]}$ will give a slope, $-k_m$, the apparent dissociation constant.

TECHNIQUE: The methods employed are similar to those described by Epstein and Hagen (7). Seeds of *Hordeum vulgare*, var. Atlas 46, were germinated in demineralized water and grown for 6 days in continuously aerated 2×10^{-4} M $CaSO_4$. Twelve hours before an experiment was to begin, the roots were rinsed and demineralized water was substituted for the $CaSO_4$ solution. The roots were excised, rinsed and suspended in two liters of continuously aerated, demineralized water just prior to making the sorption studies.

Chloride salts of Rb, Na, K and Sr were labeled with Rb^{86}, Na^{24}, Na^{22}, K^{42}, and Sr^{89} respectively. The pH of Rb and K solutions were adjusted to the desired value with 0.1 *N* NaOH or 0.1 *N* HCl. The pH of Na solution was adjusted to desired value with 0.1 *N* KOH or 0.1 *N* HCl.

The excised roots were blotted on washed, dry cheesecloth. One half gram portions of roots were weighed for Rb, K, or Na studies and 1.5 g portions for Sr studies and placed in beakers of continuously aerated water. The water was decanted and replaced by the appropriate radioactive solution to begin the sorption period. The cation concentration was maintained essentially constant by proper selection of root : volume ratios. At the end of the sorption period for Rb, Na or K, the solution was decanted and the roots were rinsed four times with demineralized water to remove adhering solutions and "outer space" (5) ions. The ion still associated with the root was considered total ion sorbed. At the end of the sorption period for Sr the solution was decanted, roots were rinsed with demineralized water, and adhering ions desorbed for a period of 30 minutes in continuously aerated solution of nonradioactive 10^{-2} M $CaCl_2$. The Sr still associated with the root was considered total Sr absorbed. After rinsing, the roots were placed into circular, ¼-ounce seamless tin boxes and dried under an infrared lamp prior to measurement of activity. Aliquots of the radioactive solutions were pipetted into tin boxes, dried, measured and used as a standard for computing ion absorption by roots.

Experimental Results

The uptake of cation in a given period of time was plotted against the uptake divided by the concentration of cation in solution. The times chosen were in the steady state region as shown by Fried, Noggle and Hagen (10). This was 20 minutes for rubidium and potassium, 30 minutes for sodium and one hour for strontium.

The uptake of rubidium at various concentrations from 10^{-6} to 10^{-2} M was measured in the presence and absence of 10^{-5} and 10^{-4} M potassium. The results are presented in figure 1.

One site, assuming the carrier concept of ion uptake, should give a straight line relationship. However a curve was obtained. Since the curved line can be resolved into at least two linear components a and b, by the use of polar coordinates more than one site must be involved in rubidium uptake. Because the relationship appeared to approach linearity at both extremes of the curve, the linear components can also be approximated by extrapolation to the intercepts at both coordinates (fig 1). This approximation is feasible because the apparent K_m for each component differs widely (12). Therefore, a likely explanation for the experimentally observed phenomenon was that two different sites were operative for rubidium absorption, one dominant at lower concentrations of the ion and the other at higher concentrations.

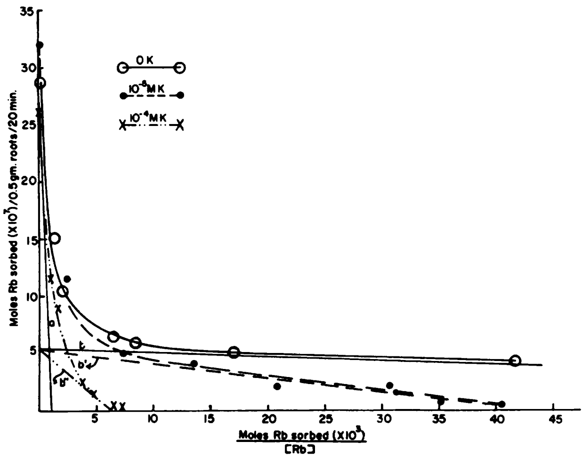

FIG. 1. The effect of potassium concentration on the uptake of rubidium by 0.5 g (fresh wt) of excised barley roots in 20 minutes. The uptake from solutions of various RbCl concentrations is plotted as a function of uptake divided by Rb concentration. The b-component of the reactions is separated at all potassium concentrations and the a-component at the zero-k level.

The uptake of rubidium in the presence of different concentrations of potassium also gives curved lines when plotted in the same manner. Again these curves can be separated into two linear components. The linear component at lower concentration of rubidium are illustrated by lines b′ and b″. These linear components have the same V_{max} as the linear component, b, in the absence of potassium. This indicates that the decrease in the uptake of rubidium represents direct competition by potassium. Apparently potassium combines with the same carrier as rubidium.

The uptake of sodium, potassium, rubidium and strontium at various concentrations was measured in presence of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M hydrogen ion. Hydrogen ion concentration was maintained constant during the sorption period. The uptake of ion was then plotted against the uptake divided by concentration. For the sake of simplicity only the results at pH 4 and 6 are shown. At these pH levels no adjustment of pH was necessary during the experiment. The results at pH 5 were intermediate and those at pH 7 did not differ appreciably from pH 6 except for sodium which continued to show a hydrogen ion effect at the higher pH levels. For rubidium, potassium and sodium the total ion sorbed was measured. For strontium only the total ion absorbed was measured. Results are presented in figures 2 a, b, c, and d.

Again curved lines were obtained at all pH levels. The two linear components were again approximated by extrapolation to the intercept of both coordinates.

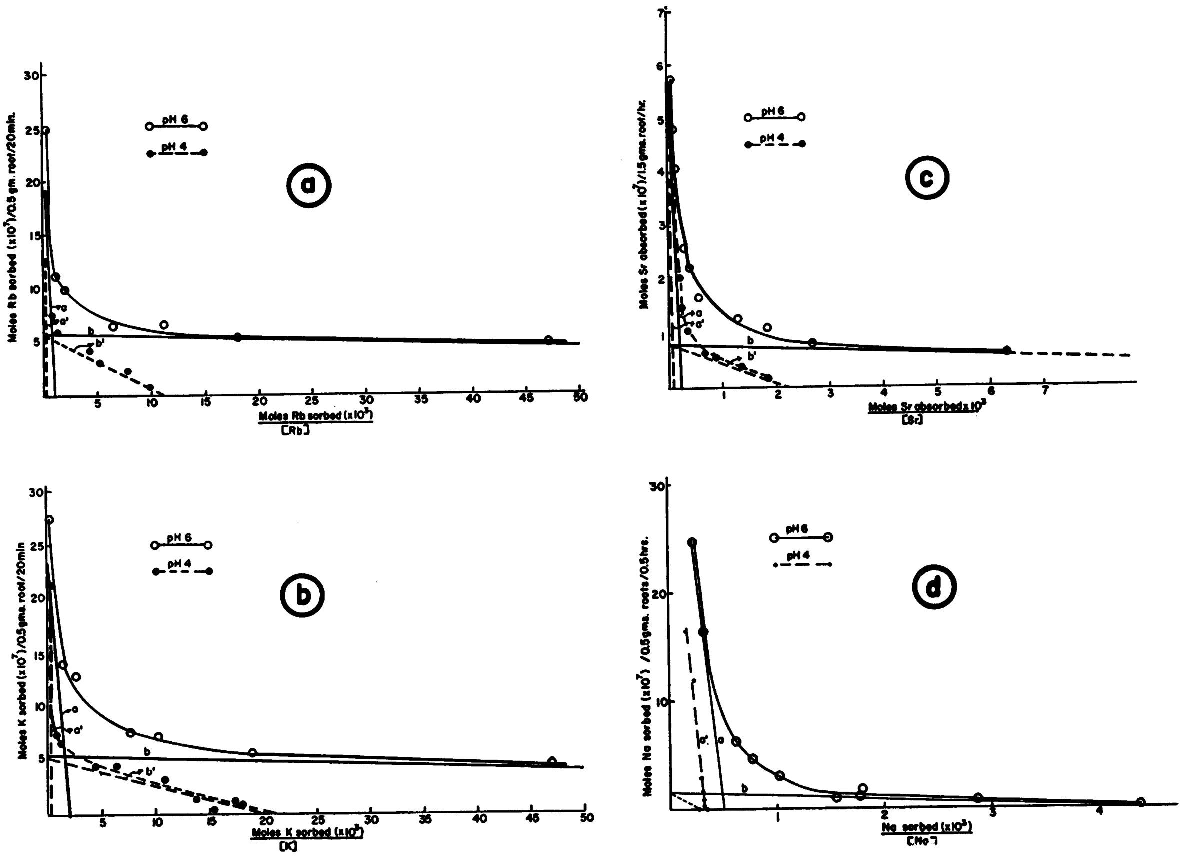

FIG. 2 a–d. The effect of pH on the uptake of cations by excised barley roots. The uptake from various concentrations of the cation is plotted as a function of uptake divided by concentration. The component reactions are separated into lines a and b for pH 6 and a′ and b′ for pH 4. Fig. a. Moles of Rb sorbed (x 10^7) by 0.5 g (fresh wt) of roots in 20 minutes. Fig. b. Moles of K sorbed (x 10^7) by 0.5 g (fresh wt) of roots in 20 minutes. Fig. c. Moles of Sr absorbed (x 10^7) by 1.5 g (fresh wt) of roots in 60 minutes. Fig. d. Moles of Na sorbed (x 10^7) by 0.5 g (fresh wt) of roots in 30 minutes.

Apparently the uptake of the four cations studied takes place from at least two distinct sites for each of the cations.

The results recorded in figure 2 also show that hydrogen ion is strictly competitive with each of the cations for b, the site that dominates ion uptake at low concentrations of the cation and probably competitive for the other site, a, that dominates ion uptake at the higher cation concentrations. The strict competition of hydrogen ion is evident because the V_{max} (y intercept) is the same at both pH levels and the slope of the component lines differs at the two pH levels.

DISCUSSION

The uptake of each cation, apparently involves at least two distinct sites. This is somewhat analogous to the finding that two sites are involved in phosphorus uptake by excised barley roots as described by Hagen and Hopkins (12). However, the two sites for phosphorus were shown to be related to two separate ion species, $HPO_4^=$ and $H_2PO_4^-$. No such explanation is apparent for cations, particularly monovalent cations.

At least two sites of ion uptake were demonstrated for strontium, rubidium, potassium and sodium. Other cations may also have multiple sites. The potassium sites may be identical to the rubidium sites as suggested by figure 1 and the work of Epstein and Hagen (7). The calcium sites may also be identical to the strontium sites as indicated by both Epstein and Leggett (8) and Menzel and Heald (19) at relatively high ion concentrations.

The slope of line a and b equal the respective K_m values, the apparent dissociation constant of MR (the active intermediate as discussed under "METHODS"). These K_m values were calculated for Rb, K and Sr. The results recorded in table I under the heading K_m are a mean of several experiments. The results indicate that potassium, rubidium, and strontium carrier complexes have approximately the same apparent dissociation constants.

To calculate the dissociation constant, K_i, of the complex formed with the competing ion, requires a

TABLE I

APPARENT DISSOCIATION CONSTANTS OF Na, K, Rb, AND Sr AND THE APPARENT DISSOCIATION CONSTANTS OF COMPETING IONS

CATION SORBED	SITE DESIGNATION	APPARENT DISSOCIATION CONSTANTS		
		K_m	K_i (HYDROGEN ION)	K_i (POTASSIUM)
Na	a	4×10^{-3}		
Na	b	1×10^{-5}	2×10^{-6}	
K	a	1×10^{-3}		
K	b	3×10^{-6}	1×10^{-5}	
Rb	a	2×10^{-3}		
Rb	b	4×10^{-6}	1×10^{-5}	4×10^{-6}
Sr	a	3×10^{-3}		
Sr	b	3×10^{-6}	1×10^{-5}	

generalized relationship for uptake in the presence and absence of the competing ion.

The equation for a competing ion used by Hagen and Hopkins (12) and Epstein and Hagen (7) when operating in the same system for the ion under test is formally analogous to equation 1 and is shown below as equation 5

$$I + R \rightleftharpoons IR \qquad (5)$$

where R in equation 1 and 5 is the same carrier, I is the competing ion and IR a complex of the competing ion and the carrier. By considering the equations for the equilibrium constants of the two reactions and the conservation equation

$$[\Sigma R] = [R] + [IR] + [MR] \qquad (6)$$

a mass action equation can be derived relating the concentration of the ions and the apparent dissociation constants to the amount of active intermediates.

$$[MR] = [\Sigma R] - \left(K_m + \frac{[K_m][I]}{K_i}\right)\frac{[MR]}{[M]} \qquad (7)$$

By substituting for MR and ΣR the respective relationships obtained from kinetic equations of steady state, 3a and 3b, equation 8 is obtained:

$$v = V_{max} - \left(K_m + \frac{[K_m][I]}{K_i}\right)\frac{v}{[M]} \qquad (8)$$

where a plot of v vs v/[M] has a slope =

$$-\left(K_m + \frac{[K_m][I]}{K_i}\right).$$

Thus the apparent dissociation constant for potassium at the site that dominates at lower rubidium concentrations can be calculated from slope of line b (K_m), and either b′ or b″, $-\left(K_m + \frac{[K_m][I]}{K_i}\right)$ in figure 1. The apparent dissociation constants for hydrogen ion at the sites dominant at lower ion concentrations of rubidium; potassium and strontium sites can also be calculated from the appropriate lines b, pH 6 and b′, pH 4 in figure 2. Each run gave a slightly different value. The averages of several determinations are presented in table I.

The apparent dissociation constant, K_m, of potassium as determined by the uptake of potassium was approximately the same as the apparent dissociation constant of potassium, K_i, from the rubidium site. Again it appears likely that the rubidium site b and potassium site b are one and the same site.

The apparent dissociation constant of the hydrogen ion complex, K_i, at site b is approximately the same for the site complexes of the three cations K, Rb, and Sr. This is higher than the dissociation constants for the cations themselves but only by a factor of approximately three. The magnitudes of the dissociation constants indicate that both hydrogen ion and the cations are relatively tightly held.

The K_i (hydrogen ion) for sodium could not be calculated from data in figure 2 d. A 10^{-4} molar solution of hydrogen ion almost eliminated the appearance of site b, figure 2 d, in the sodium uptake system, indicating that hydrogen ion was more tightly held than sodium at this site. The same hydrogen ion concentration also had a marked effect on site a. Since hydrogen ion had a greater affinity for the sodium site than did sodium, pH 6 was not adequate to eliminate the effect of hydrogen ion at lower concentrations of sodium.

The K_m for a cation must be calculated in a system in which the concentration of competing ion is negligible. The K_m for sodium was therefore calculated from uptake data at pH 7. The K_i (hydrogen ion) was calculated from comparative uptake data at pH 5.0. The results are recorded in table I.

The K_m for sodium at the site that dominates at lower concentrations appears to be somewhat greater than that for the other cations tested. This indicates a slightly lower affinity of sodium for the sodium site than of the other cations for their respective sites. The K_m of site a is similar to that found for the other cations. The K_i (hydrogen ion) is smaller for sodium than the K_m of sodium indicating again that hydrogen is more tightly held than sodium. Hydrogen ion also appears to be more tightly held to the sodium sites than to the other cation sites.

The hydrogen ion competes with rubidium, potassium, sodium and strontium, and possibly most other cations. However, the dissociation constants for hydrogen ion on the b site are of same order of magnitude as the dissociation constants for the cation. Thus, when cation concentration is appreciably higher than hydrogen ion concentration there is little, if any, pH effect due to competition. However, if the concentrations are similar, marked pH effects may be noted.

SUMMARY

The uptake of rubidium, potassium, sodium and strontium by excised barley roots under steady state conditions indicates at least two distinct carrier sites of absorption for each of the cations. One site domi-

nates at higher ion concentrations while the second dominates at lower ion concentrations.

The apparent dissociation constants were determined at each of the two sites for each of the four cations studied. They were similar in magnitude for potassium, rubidium and strontium and slightly higher for sodium.

Hydrogen ion was shown to compete directly for each of the sites that dominates at lower concentrations of all cations tested. The apparent dissociation constant for hydrogen ion at the strontium, rubidium and potassium site that dominates at lower concentrations was 1×10^{-5}. The comparable dissociation constant, K_1, at the sodium site was 2×10^{-6}. The apparent dissociation constants for hydrogen were similar in magnitude to the apparent dissociation constants of the ions themselves. Rubidium and potassium were shown to compete for the same site.

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GROWTH SUBSTANCES IN CORN POLLEN[1,2,3]

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Growth substances have been detected in the pollen of apple (3), orchid (7), tobacco (5), hazelnut shrub and pine (14), and corn (6, 10, 13). Studies of the growth substances in corn pollen have been concerned solely with crude ether soluble extracts. This communication describes the growth substances found in the acid and non-acidic or " neutral " fractions in ethyl ether extracts of corn pollen, *Zea mays*, var. Golden Cross.

Freshly harvested pollen (166 grams) was extracted with cold, peroxide-free ethyl ether at 2° C for four hours. The supernatant was decanted and extracted with 5 % aqueous sodium bicarbonate. The

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