Discrimination between Alkali Metal Cations by Yeast

I. *Effect of pH on uptake*

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ABSTRACT Extracellular pH markedly influences the ability of yeast cells to discriminate between K^+ and Na^+, with K^+ favored to a greater degree at low pH. Studies of the kinetics of uptake of individual alkali metal cations by fermenting yeast indicate three zones relative to pH. Between pH 6 and 8, H^+ has no effect. Below pH 4, H^+ competitively inhibits the transport of each cation. Between pH 4 and 6, H^+ acts kinetically as a predominantly non-competitive inhibitor. Both effects can be reversed by increasing the concentrations of cations. However, the concentrations required to reverse the competitive effect are considerably lower than those required to reverse the apparently non-competitive effect. It is suggested that H^+ and the alkali metal cations can combine with two sites, a transport or carrier site, and a second, non-transporting site that influences the maximal rate of transport. Because the non-competitive inhibitory effect of H^+ is considerably greater on the other cations than on K^+, the discrimination in favor of K^+ is increased severalfold at low pH, beyond that predicted on the basis of the relative affinities for the transport site.

The yeast cell possesses a transport system by means of which monovalent cations can be absorbed in large amounts. This transport system requires the presence of an utilizable substrate and, in well starved cells, the cation absorbed is balanced by the excretion of an equivalent amount of hydrogen ion whose source is the metabolism of the cell (3, 9). Kinetically, cation uptake appears to follow a saturation curve that can be adequately described by the Michaelis-Menten equation (5, 11). If pairs of ions of the alkali metal series are present, each interferes in a competitive manner with the uptake of the other (5).

The uptake of the alkali metal cations is markedly influenced by pH. As the pH is reduced the net rate of potassium uptake not only decreases but also may even become negative at pH levels below 2. Similar effects of pH on the net uptake of other cations have been reported (4). The mechanism by which

extracellular pH influences cation uptake has not, however, been systematically investigated. Rothstein and Bruce (12) have studied the efflux of potassium from yeast cells as a function of pH and have shown that below pH 6 potassium efflux increases in a regular fashion with increasing external hydrogen ion concentration. No comparable studies, however, have been made to determine the effect of extracellular pH on the rate of influx of potassium into the yeast cell.

A few observations indicate that the influence of extracellular pH on K^+ uptake is not the same as that on Na^+ uptake. For example, at low pH the efflux of potassium is not increased by the addition of extracellular sodium, but above pH 4.5 potassium efflux is markedly increased by sodium ions which are taken up in equivalent quantity to the potassium lost (12). At pH values near neutrality, cells suspended in sodium chloride take up large amounts of sodium but at low pH sodium uptake is minimal (5, 7). It appears, therefore, that a change occurs in the properties of the transport system with respect to Na^+ as compared to K^+, as the external pH is increased.

In the present paper the effects of extracellular pH on the kinetics of uptake of the alkali metal cations by yeast are described in some detail. Special attention is paid to the factors contributing to the ability of the cells to discriminate between them. A preliminary account of part of this work has already been given elsewhere (1).

METHODS

Fresh baker's yeast (Standard Brands, Inc.) was used in these experiments. The fresh cells were washed twice with deionized water, resuspended, and aerated continuously for several hours. They were then centrifuged and rewashed twice. The final suspension contained 20 mg wet weight of yeast/ml. The uptake by these suspensions of lithium, sodium, potassium, rubidium, and cesium, added as their chlorides, was studied over the pH range 3 to 8. The suspensions were fermented at room temperature (25°C) for 3 minutes,[1] timed from the moment at which glucose was added to give a final concentration of 0.1 M. The pH was maintained at the desired level by means of a pH stat using dilute HCl at pH 3 and 3.5 and triethylamine at higher pH values. The latter compound has minimal effects on ion transport and on metabolism (10). During fermentation, the cells were kept in suspension by continuous bubbling with air. At the end of the run the cells were centrifuged, quickly washed with cold water,[2] and again resuspended in deionized water. The uptake of sodium, potassium, rubidium, and cesium by the cells was determined isotopically using Na^{24}, K^{42}, Rb^{86}, and Cs^{137} as tracers. Following fermentation and washing, suitable aliquots of the suspension were dried on planchets and their radioactivity assayed using a G-M

[1] Three minutes were chosen because the uptake of K^+ is linear during the first 5 minutes of fermentation. Also, during this time the rate of uptake of K^{42} is not significantly different from the net rate of uptake of K^+ (11).

[2] Because yeast cells are relatively impermeable to cations, washing (especially in the cold) results in virtually no loss of cellular cations (11).

counter and an automatic sample changer. Aliquots of the labeled salt solutions were dried and counted as controls.

Lithium uptake was estimated as follows. Aliquots of the washed cell suspensions following fermentation were digested with 1 ml of concentrated nitric acid and evaporated to dryness when oxidation was complete. The residue was dissolved in a measured volume of deionized water and lithium was determined by flame photometry at 671 mμ using a Beckman DU flame photometer.

RESULTS

The effect of pH on the uptake of K^+ and of Na^+ from a mixture containing both ions is shown in Fig. 1. The ratio of their concentrations is equal to the

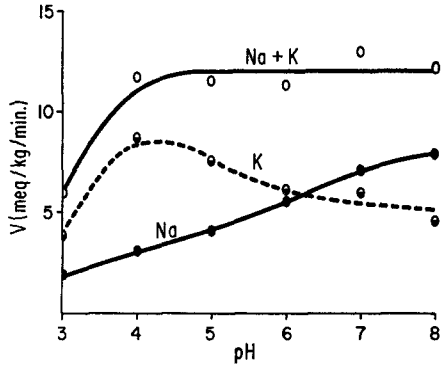

FIGURE 1. Effect of pH on the rate of uptake of Na^{24} and K^{42} from solutions containing 125 mM NaCl and 5 mM KCl/liter.

reported ratio of their Michaelis constants (5), and their absolute concentrations (K^+ = 5 mM; Na^+ = 125 mM) were sufficient to "saturate" the transport system. If only Na^+ and K^+ compete for a carrier site, then the ratio of Na^+ to K^+ uptake should be independent of pH. If H^+ also competes, then at low pH the total of Na^+ and K^+ uptake should decrease. The data of Fig. 1 suggest that H^+ does compete below pH 4, but they also demonstrate that a large change in the relative uptakes of Na^+ and K^+ occurs above pH 4.0, apparently unrelated to the competitive effect. The effect relative to pH is more apparent if the ratio of K^+/Na^+ uptake is plotted against pH (Fig. 2). The discrimination in favor of K^+ is increased almost fourfold with most of the change occurring between pH 6 and 4. A similar pattern was found in experiments with mixtures of K^+ and Cs^+, K^+ and Rb^+, and K^+ and Li^+.

Because the experiments of Fig. 1 indicated a complex relationship of pH to Na^+ and K^+ uptake, a kinetic analysis of cation uptake in cells exposed to individual alkali metals was undertaken. The relationship of the rate of cation uptake to cation concentration was determined with the pH maintained at various levels between 3.0 and 8.0. A representative set of data for K^+ is given in Fig. 3. At all pH levels studied, the rate of uptake tends to reach a limiting value as the external potassium concentration is increased. However, the shape

of the uptake curve and the maximal rates achieved are markedly influenced by pH. The curves for pH 6, 7, and 8 were superimposable (to avoid confusion only one set of data is given in Fig. 3). At pH 4.5 and 3.5, the asymptote is lower with little displacement of the curves to the right. At pH 3.0, however, the asymptote approaches that of pH 3.5, but the curve is displaced to the right.

The effects of pH on the kinetics of K^+ uptake are most conveniently de-

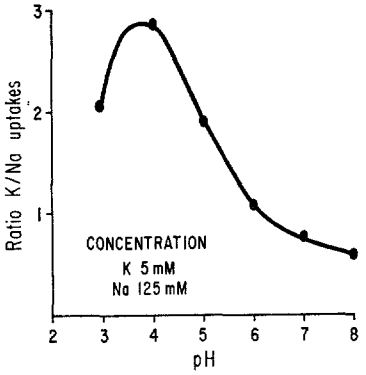

FIGURE 2. Ratio of K/Na uptake as a function of pH. Data taken from Fig. 1.

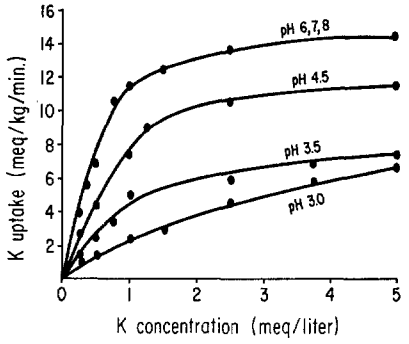

FIGURE 3. The effect of K^+ concentration on the rate of uptake of K^{42} at different pH levels.

scribed in terms of the Lineweaver and Burk form of the Michaelis-Menten equation (8) in which the reciprocal of the rate of uptake, $1/V$, is plotted against the reciprocal of the potassium concentration (Fig. 4). Two distinct effects of H^+ on potassium uptake are apparent. Below pH 3.5, H^+ behaves as though it were a competitive inhibitor of K uptake; that is, the slope of the reciprocal plot is increased but the intercept is unaltered. Between pH 3.5 and pH 5.0, H^+ behaves kinetically as if it were a predominantly non-competitive inhibitor of potassium uptake, both the slope and the intercept of the double reciprocal plot increasing with increasing hydrogen ion concentration. Between pH 5 and pH 8, H^+ has no apparent effect on the rate of potassium uptake.

The effects of pH can be quantitatively described in terms of the parameters K_M and V_{max} of the Michaelis-Menten equation. In Fig. 5 the calculated values of K_M and V_{max} for potassium uptake, taken from the data of Fig. 4 and additional experiments, are plotted as a function of pH. Three zones of behavior

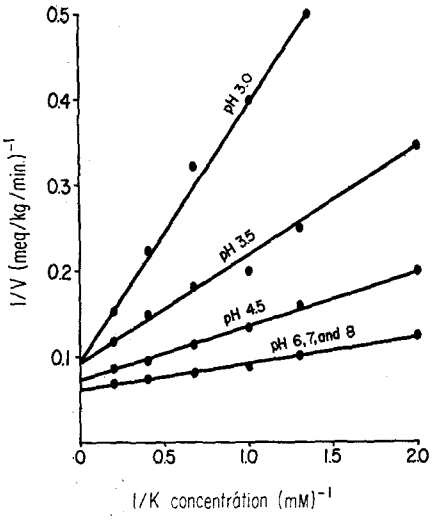

FIGURE 4. Double reciprocal plots of the data shown in Fig. 3.

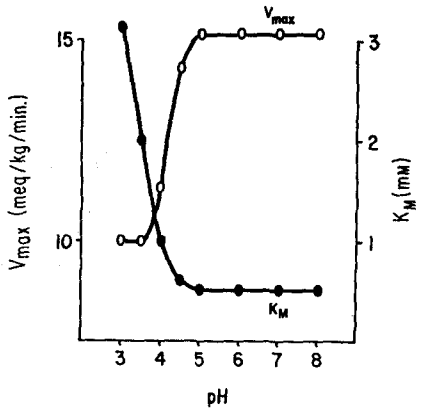

FIGURE 5. Effect of pH on K_M and V_{max} for potassium uptake. Computed from the data of Fig. 4 plus additional experiments.

are evident. Between pH 3 and pH 4, V_{max} is relatively little changed while K_M decreases threefold (predominantly competitive inhibition). Between pH 4 and pH 5, V_{max} increases markedly with relatively smaller changes in K_M predominantly non-competitive inhibition). Between pH 5 and pH 8, both V_{max} and K_M are independent of extracellular pH (no inhibition).

The effect of pH on the transport of other ions in the alkali metal series is summarized in Figs. 6 and 7 in terms of V_{max} and K_M. In each case the pattern observed was similar to that found with potassium. At low pH the apparent K_M for each ion increased with little change in V_{max}, indicating a predomi-

nantly competitive effect of hydrogen ion on uptake. In the pH range 4 to 6, V_{max} increased markedly with relatively little change in K_M, indicating a largely non-competitive effect of hydrogen ion. Above pH 6 relatively little change in V_{max} or K_M was observed. The competitive and non-competitive[3] effects of H^+ on cation transport seem to involve two distinct proton-binding sites, slightly overlapping in terms of required H^+ concentrations. Furthermore, the calculated affinity constants differ by a factor of 6. The non-competitive effect spans a range of approximately 2 pH units, suggesting a single proton dissociation with a pK of about 4.5 or a dissociation constant of 0.03 mM. The apparent dissociation constant (K_I) for the competitive effect calculated from the Lineweaver-Burk equation (from the data of Fig. 4) is 0.18 mM (pK = 3.85).

The concept of two sites to explain the effects of H^+ is also supported by data relating to the action of high concentrations of cations. The competitive effect of H^+ on K^+ demonstrated in Figs. 3 and 4 (pH 3.5 compared to pH 3.0) is virtually reversed at a K^+ concentration of 5 mM but the non-competitive effect (pH 3.5 compared to pH 5.0) is not. At very high K^+ concentrations (50 mM or more), however, all effects of H^+ are virtually overcome. The reversal of the non-competitive effect can be demonstrated by applying the Hunter and Downs (6) form of the Michaelis-Menten equation. Competitive inhibition is represented by

$$(H^+)\frac{\alpha}{1-\alpha} = K_I + \frac{K_I}{K_K}(K^+)$$

where α is the fractional activity, equal to $\frac{V_I}{V_0}$, where V_I is the inhibited rate and V_0 the uninhibited rate at a particular concentration of K^+, K_K is the Michaelis constant for K^+ and K_I is the dissociation constant for the inhibitor. For non-competitive inhibition, the equation reduces to

$$(H^+)\frac{\alpha}{1-\alpha} = K_I$$

which is a horizontal line with an intercept equal to K_I. The data plotted in Fig. 8 are taken from an experiment in which the uninhibited rate was obtained at pH 8.0 and the inhibited rate at pH 4.5. At concentrations of K^+ up to 5 mM, the system behaves as a non-competitive inhibition by H^+,

[3] The term "non-competitive" is used here in a formal kinetic sense only. At low external cation concentrations the kinetics of the inhibition by H^+ of cation uptake in the pH range 4 to 6 are similar to those of a non-competitive inhibitor of enzyme action (8). However, the reversal of this inhibition by high cation concentrations indicates that it is not a simple non-competitive effect.

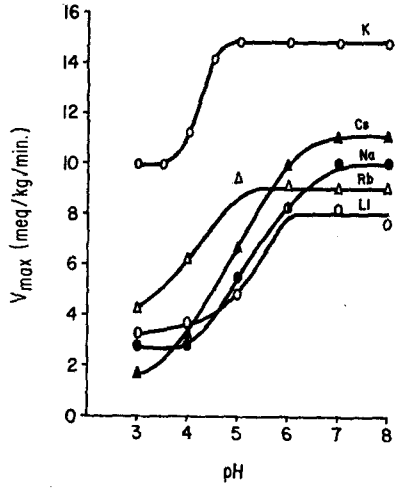

FIGURE 6. Effect of pH on V_{max} for the uptake of alkali metal cations. The point marked ◐ indicates a common value for Li and Na.

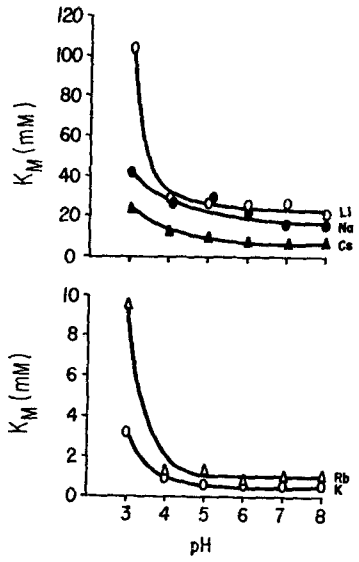

FIGURE 7. Effect of pH on K_M for the uptake of alkali metal cations.

with a K_I of 0.05 mM or a pK_H of 4.3 (in agreement with the half-value for the curve for V_{max} in Fig. 5).[4] Above 5 mM K^+, however, the inhibiting effect is reversed as indicated by the positive slope. The concentrations of K^+ associated with this reversal are considerably higher than those required to reverse the competitive effect, suggesting that two sites are involved with different affinities for K^+ as well as for H^+. The K_M for K^+ transport is 0.5 mM (from

[4] The K_I value for H^+ calculated from Fig. 8 is necessarily an approximate one since the non-competitive effect of H^+ does not result in complete inhibition of cation uptake. The kinetic consequences of this incomplete inhibition will be discussed more fully elsewhere (2).

Fig. 5), whereas the K_K (for reversal of the non-competitive effect), calculated from the slope in Fig. 8, is 2.8 mM, five times as high.

The calculated affinity constants of the two sites for K^+ and H^+ are in a reciprocal relationship. The affinity of the non-competitive site for H^+ is

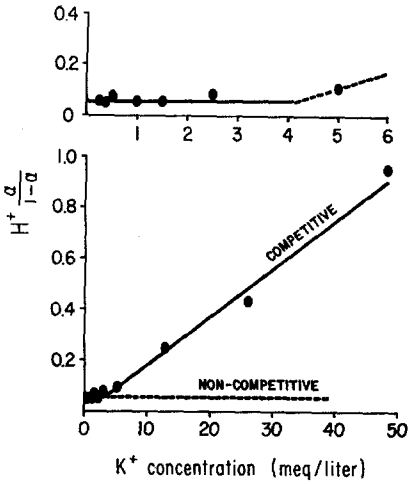

FIGURE 8. Hunter and Downs plots of the uptake of potassium at pH 4.5. Fractional activity, $\alpha(= V_I/V_0)$, computed using the rate of uptake at pH 8 as a control.

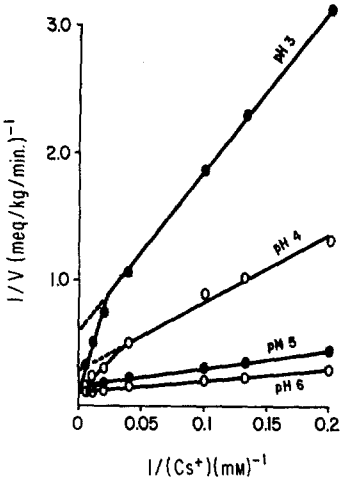

FIGURE 9. Double reciprocal plots of the effect of pH on the rate of uptake of cesium.

greater than that of the competitive site, but for K^+ the reverse is true. For this reason, relatively high concentrations of H^+ are required to produce the competitive effect, but relatively low concentrations of K^+ result in reversal. On the other, relatively low concentrations of H^+ are required for the non-competitive effect, but relatively high concentrations of K^+ are required for reversal.

That the non-competitive and competitive effects involve two distinct ca-

tion-binding sites is further demonstrated in experiments with Cs^+. The data are plotted as reciprocals (Fig. 9) according to the Lineweaver-Burk equations, for pH levels ranging from 3 to 6. At pH 6, Cs^+ uptake over the entire range of concentrations studied can be adequately described in terms of simple Michaelis-Menten kinetics. The data obtained in the pH range 3 to 5, however, are more complex. On the right-hand side of the graph, representing lower Cs^+ concentrations, the data can be fitted by a series of lines corresponding to a predominantly non-competitive inhibition of Cs^+ uptake by H^+. At higher concentrations of Cs^+ the lines show a distinct break and extrapolate to a common point on the vertical axis. In other words, at low concentration of Cs^+ the data are typical of non-competitive inhibition, but at high concentrations they are typical of a competitive inhibition. Similar data were obtained with Li^+ and Na^+.

DISCUSSION

The results reported herein indicate that in the pH range 6 to 8 the uptake of any single ion in the alkali metal series follows simple saturation kinetics which can be described in terms of the Michaelis-Menten equation. The specificity array, in order of decreasing affinity, is K > Rb > Cs > Na > Li (Table I). The relative affinities are similar to those reported by Conway and Duggan (5). As the external pH is reduced below 6, two effects of H^+ ion on the kinetics of cation transport are observed, one behaving as a non-competitive inhibition and the other as a competitive inhibition. The competitive effect can be interpreted as an interaction of H^+ ions with a carrier site directly involved in transporting K^+ and other alkali metal cations into the cell. The K_M of this site for H^+ ions calculated from the data of Fig. 4 (pH 3.5 and 3.0) is 0.18 mM. Thus, the carrier has a greater affinity for H^+ than for K^+ by a factor of about three (Table I). Hydrogen ion bound to the carrier is transported into the cell in a one-for-one exchange for cellular K^+, resulting in a markedly enhanced K^+ efflux (12).

The non-competitive effect of pH on cation transport can be interpreted as arising from the combination of H^+ with a second site, and the reversal of this effect by high concentrations of cations is probably due to competitive displacement of H^+ from the site. The existence of the second site is suggested not only by the two kinds of kinetic effects of H^+ on transport, but also by the large differences in the apparent binding affinities. It has already been indicated that the second site (non-competitive site) has a sixfold greater affinity for H^+ than the first, whereas the affinity ratio for K is 1 to 5 in the other direction. A more detailed study (2) indicates that the specificity array for the second site is quite different in many respects, particularly in its high affinity for bivalent cations.

The exact role of the second site is not clear. When the site is filled with H^+,

the maximal rate of K^+ transport is reduced to 65 per cent, but it is not completely inhibited. When the site is filled with K^+, the maximal rate is returned to 100 per cent. Yet it does not behave like a second transport site. In the virtual absence of H^+ (pH 8), the kinetics of K transport are simple kinetics with a single Michaelis constant, rather than those of a two-component system Furthermore, in another paper (2) it will be demonstrated that other cations such as Cs^+ and Ca^{++} can mimic the non-competitive effects of H^+ without themselves being transported into the cell. An incomplete non-competitive inhibition of a complex system such as ion transport could result from a number of actions of H^+. In some way, unspecified, when the second site is filled with H^+, the maximal turnover rate of the transport system is reduced or modified with no change in the affinity of the carrier for the cation.

TABLE I

K_M VALUES AND RELATIVE AFFINITIES (K = 100) OF THE CATION CARRIER IN YEAST FOR ALKALI METAL IONS AND FOR H^+

Ion	K_M	Relative affinity
	mM	
Li^+	27	2
Na^+	16	3
K^+	0.5	100
Rb^+	1.0	50
Cs^+	7.0	8
H^+	0.18	275

Although the mechanism of the H^+ effect on the second site cannot be specified, the consequences for the cell in terms of discrimination between cations are substantial. At high pH the maximal rate of K transport is 50 to 100 per cent higher than that of any of the other ions tested (Fig. 6). The affinity of the transport site for potassium, as indicated by the K_M value, is also considerably higher than its affinity for any of the other cations (Table I). At low pH, provided the cation content of the external medium is not too high, the maximal rates of transport of all the alkali metal cations are reduced, but the reduction in the case of potassium is considerably less than that for any other cations. Consequently at low pH, the V_{max} for potassium is from two to five times that for the other cations so that, even though the relative affinities of the transport site for the various alkali metal cations are unaffected by pH, the discrimination in terms of total transport is markedly increased in favor of potassium, as demonstrated in Fig. 1, for K^+ *vs.* Na^+. These findings offer an explanation for the observation of Conway and Duggan (5) that whereas so-

dium and other alkali metal cations can compete effectively with potassium for entrance into the yeast cell at pH 7, they do not appear to do so at low pH.

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