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EFFECT OF SURFACE POTENTIAL ON Rb^+ UPTAKE IN YEAST

THE EFFECT OF pH

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The apparent K_m of Rb^+ uptake and the zeta potential of yeast cells are appreciably affected by changes in the pH, variation of the concentration of the buffer cation $Tris^+$ and addition of Ca^{2+} to the suspending medium. Irrespective of the way in which the zeta potential is affected, a direct relationship between the apparent K_m of the Rb^+ uptake and the zeta potential is observed. A reduction of 8 mV in the zeta potential is accompanied by a 20-fold increase in the apparent K_m, which illustrates that electrostatic effects in ion uptake cannot be ignored. Measured zeta potentials are, to a good approximation, linearly related to surface potentials evaluated from a kinetic analysis of the Rb^+ uptake. This shows the practical use of the zeta potential as a measure of the surface potential in studies of electrostatic effects in ion uptake by yeast. It is concluded that $Tris^+$ and the alkaline earth cations inhibit the Rb^+ uptake in yeast exclusively via a reduction in the surface potential. Protons, in addition, exert a competitive inhibition.

Introduction

In previous articles we have presented evidence for the influence of the surface potential on ion uptake in yeast (see Ref. 1 and references therein). The inhibition of the Rb^+ uptake by polyvalent cations, for example, could be ascribed to a decrease in the yeast surface potential and not to competitive inhibition [2,3]. Typically, the apparent K_m of the Rb^+ uptake was not linearly related to the inhibitor concentration, as would be expected in case of a competitive inhibition. Linear relationships were obtained, however, on plotting the apparent K_m against the square-root of a divalent cation concentration, or in the case of a trivalent cation (e.g., La^{3+}) against the cube-root of the inhibitor concentration [2,3]. As a matter of fact, these so-called z_pth root relations (z_p being the valency of the inhibitor) were predicted by application of classical double-layer theory [4,5]. In agreement with this theory, the effectiveness of the polyvalent cations in inhibiting the Rb^+ uptake was related mainly to their valency in the series of salts tested, being divalent < trivalent [2,3]. Moreover, the same differential effectiveness was found for their effect on the zeta potential of the yeast cell [6]. These results indicated an electrical effect of the polyvalent cations on the Rb^+ uptake in yeast.

Not only polyvalent cations effectively reduced the zeta potential of yeast cells. Changes in the zeta potential of yeast cells with pH have also been reported [7,8]. It might be hypothesized, therefore, that the reduction in the rate of the Rb^+ uptake with a decrease in pH [9,10] is also due to a reduction in the surface potential. There are indications, however, that protons are taken up by yeast via the monovalent cation transport system of the cell [10]. Therefore competitive inhibition by protons might also contribute to the observed re-

duction in the Rb^+ uptake.

In the present article we have examined the effect of the medium pH on the kinetics of the Rb^+ uptake by yeast. As changes in pH result in concomitant changes in cell pH, also 'indirect effects' of the pH will come to the fore, as we have described in an earlier paper [11]. After appropriate correction for effects of the cell pH, the dependence of kinetic coefficients of the Rb^+ uptake on the extracellular concentration of protons can be evaluated.

It will be argued that protons reduce the rate of the Rb^+ uptake at low Rb^+ concentrations both via a reduction in the surface potential and via competitive inhibition.

Materials and Methods

Rb^+ uptake by fermenting yeast, *Saccharomyces cerevisiae* Delft II, was studied by means of $^{86}Rb^+$ as a tracer according to Ref. 2 at 25°C. The concentrations of K^+ and Na^+ in the suspending medium were determined by flame photometry 30 s after the addition of Rb^+. The pH of the medium was also measured. The effect of the pH was studied in 45 mM Tris adjusted with succinate (pH < 7.2) or HCl (pH ⩾ 7.2) to the desired pH. The effect of $Tris^+$ was studied with cells which had been preincubated anaerobically for 1 h in 45 mM Tris buffer of either pH 4.5 or pH 7.2, in the presence of 3% (w/v) glucose at a cell density of 4% (w/v). At zero time of the Rb^+ uptake, the cell suspension was diluted 1 : 1 with Tris-HCl of the desired concentration and of the relevant pH. The concentration of protonated Tris (henceforth denoted $Tris^+$) was calculated from its thermodynamic dissociation constant of $8.39 \cdot 10^{-9}$ at 25°C [12]. The effect of Ca^{2+} on the Rb^+ uptake was studied in 45 mM Tris-HCl (pH 7.2) as described before [2].

Cell electrophoretic mobilities were measured at 25°C using a rectangular cuvette and apparatus as described by Fuhrmann et al. [13]. From the electrophoretic mobility, the zeta potential was calculated by using the Helmholtz-Smoluchowski equation [14].

^{86}Rb was purchased from Amersham International, U.K. All other reagents were A.R. grade and were obtained from commercial sources.

Theory

The concentration-dependence of the Rb^+ uptake by yeast can be accounted for by a two-stie immobile carrier transport model [15]. The two sites are called the activation site and the substrate site, respectively [16]. The relation between the rate of Rb^+ uptake (v_i) and the concentration of Rb^+ in the suspending medium (s_i) has the mathematical form:

$$v_i = \frac{A_i s_i + B_i s_i^2}{C_i + D_i s_i + s_i^2} \tag{1}$$

The kinetic coefficients A_i, C_i and D_i are independent of s_i, but depend on the concentration of other ions also present in the suspending medium [16]. B_i is not only independent of s_i, but also of concentrations of other ions. We have shown that the coefficients C_i and D_i and the quotient of A_i and B_i are independent of the cell pH [11]. On the other hand, both A_i and B_i depend upon the cell pH. C_i and D_i also depend on the surface potential [2,3]. D_i is the sum, and C_i is the product of the dissociation constants of the complexes of Rb^+ with the activation site and with the substrate site. Since the affinity of Rb^+ for the activation site is much larger than for the substrate site [1], D_i approaches the apparent dissociation constant referring to the substrate site and represents the apparent K_m for the Rb^+ uptake under conditions that the activation site is saturated with Rb^+. The dependence of A_i/B_i, C_i and D_i on the concentration of other monovalent cations s_j and s_k also present in the suspending medium are given by Eqns. 2–4 [2,3]:

$$A_i/B_i = \sum_{j \neq i} a_{i,j} s_j \tag{2}$$

$$C_i = \frac{c_{i,00}}{y^2} + \sum_{j \neq i} c_{i,j} \frac{s_j}{y} + \sum_{j \neq i} \sum_{k \neq i} c_{i,jk} s_j s_k \tag{3}$$

$$D_i = \frac{d_{i,0}}{y} + \sum_{j \neq i} d_{i,j} s_j \tag{4}$$

with

$$y = \exp(-F\psi_0/RT) \tag{5}$$

where ψ_0 is the surface potential and F, R and T have their usual meaning.

For convenience we define corrected values of A_i and D_i by subtracting the contribution due to the small amounts of the competitive inhibitors Na^+ and K^+ [10], which are always present in the suspension (namely 0.05 mM Na^+ and 0.010–0.115 mM K^+ depending on the pH applied [6]). These corrected values are marked by a prime.

According to the Gouy-Chapman equation (see, for example, Ref. 2) the factor y is a function of the concentrations of both cations and anions present in the medium, and of the surface charge density of the membrane. For negatively charged membranes, y ($y > 1$) reduces when the negative surface potential is reduced. Obviously, ions which reduce the surface potential may also reduce the rate of Rb^+ uptake, even when the ions have no affinity for the transport sites (Eqns. 3–4). As protons reduce the surface potential appreciably by protonation of the negative surface groups on the yeast cell membrane [16], they may reduce the rate of the Rb^+ uptake via a reduction in the surface potential, besides via competitive inhibition. To what extent the reduction in the rate of the Rb^+ uptake by protons may be attributed to a reduction in the surface potential is the subject of the present investigation. For quantitative treatment of the problem, knowledge of the way in which the Rb^+ uptake is affected by other monovalent cations (K^+, Na^+ and $Tris^+$) also present in the medium is obligatory. The $a_{i,j}$ and $d_{i,j}$ values for Na^+ and K^+ have been determined by Derks et al. [17]. However, the values of these coefficients for $Tris^+$, the buffering cation, are unknown. We therefore also examined the effect of $Tris^+$ on the kinetic coefficients of the Rb^+ uptake.

Results

The effect of $Tris^+$ on the concentration dependence of the Rb^+ uptake rates is shown in Fig. 1. The concentration dependence of the uptake rates is represented graphically according to Hofstee [18]. In accordance with earlier observations [11], the maximum rate of the Rb^+ uptake (coefficient B_i) decreased if the pH is raised. With a curve-fitting program and the use of a digital computer the coefficients A_i–D_i of Eqn. 1, describing the isotherms of the Rb^+ uptake at the various $Tris^+$ concentrations and at the two pH values have been computed. At both pH values, the Rb^+ uptake is inhibited in an apparently competitive way; the maximum rate of uptake is namely not affected. Also the quotient A_i/B_i (see Eqn. 2) is not affected by $Tris^+$, which means that coefficient $a_{i,j}$ for $Tris^+$ in Eqn. 2 is zero. This is typical for inhibition caused by a decrease in the surface potential and has also been found with divalent cations [2,3]. The pH-dependence of the effectivity of $Tris^+$ in inhibiting the Rb^+ uptake is also in conflict with the concept of a simple competitive inhibition. According to a competitive inhibition,

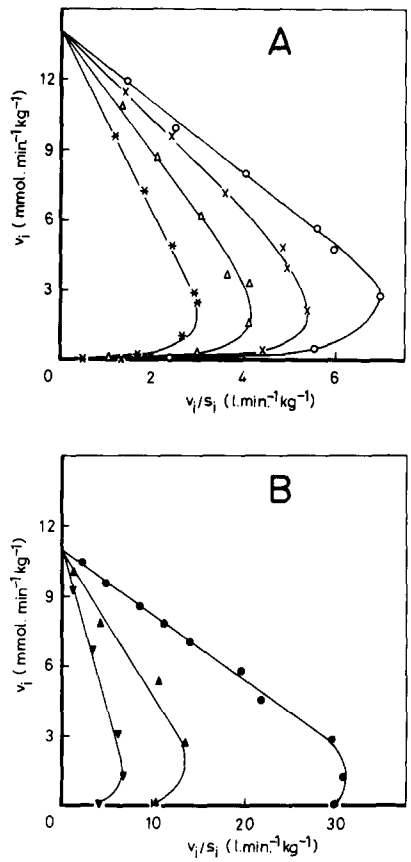

Fig. 1. Effect of $Tris^+$ on the concentration dependence of the Rb^+ uptake rates. The initial rate of Rb^+ uptake (v_i) is plotted against the quotient of this rate and the Rb^+ concentration (s_i). (A) At pH 4.5 and at $Tris^+$ concentrations of (○) 22.5 mM, (×) 45 mM, (△) 90 mM, (*) 180 mM. (B) At pH 7.2 and at $Tris^+$ concentrations of (●) 40 mM, (▲) 80 mM and (▼) 160 mM. Each point represents the mean of triplicates.

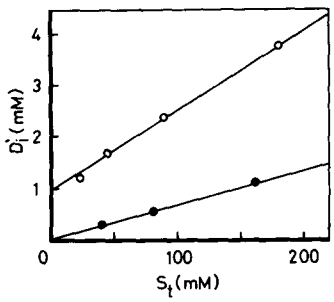

Fig. 2. Dependence of D_i' upon the $Tris^+$ concentration in the suspending medium s_t. ○, at pH 4.5; ●, at pH 7.2.

the effectivity should be independent of pH [15]. Fig. 2 shows that coefficient D_i' is linearly increased with the $Tris^+$ concentration, at pH 4.5 and at pH 7.2 as well, but the slope of the line is greater at pH 4.5 than at pH 7.2.

As, at pH 7.2, the relation between D_i' and the $Tris^+$ concentration is a straight line through the origin, it may be concluded that the value of $d_{i,0}/y$ at this pH is close to zero in the absence of added $Tris^+$. If this also applies to the data obtained at pH 4.5, the intercept of the line with the ordinate would reflect the contribution of protons to the D_i value via competitive inhibition. From the value of this intercept, a $d_{i,j}$ coefficient for protons of 31.3 is calculated.

Subsequently, we have determined the effect of the pH on the Rb^+ Hofstee plots (data not shown). At all the pH values (3.5–7.2) applied, the concentration dependence of the Rb^+ uptake rates can be described by Eqn. 1 and the coefficients A_i'/B_i and D_i' have been calculated as described above. On plotting A_i'/B_i against the proton concentration, to a good approximation, a linear relationship is obtained (Fig. 3), which means that, for protons, coefficient $a_{i,j}$ (Eqn. 1) is non-zero.

Fig. 4. shows that a linear relationship also exists between D_i' and the proton concentration, which is to be expected if protons compete with Rb^+ for the same binding sites. However, the slope of the line is larger than the estimated value of $d_{i,j}$ for protons in the 'Tris experiment' (see Fig. 2), indicating that, apart from competitive inhibition, a reduction in the surface potential is also involved. By making use of the $d_{i,j}$ value for protons as estimated from the Tris experiment, the D_i' values can be corrected for the competitive inhibition by protons. The resulting values should then equal the $d_{i,0}/y$ term in Eqn. 4. Similarly, the D_i' values calculated from the Tris experiment can be corrected for competitive inhibition by protons and $d_{i,0}/y$ values can be obtained at the various $Tris^+$ concentrations at the two pH values, as well. If the variation of the $d_{i,0}/y$ value with pH and $Tris^+$ concentration is due to changes in the surface potential, a direct relationship between $d_{i,0}/y$ and the surface potential should exist, irrespective of whether the surface potential is affected by changing the pH or the $Tris^+$ concentration. As the

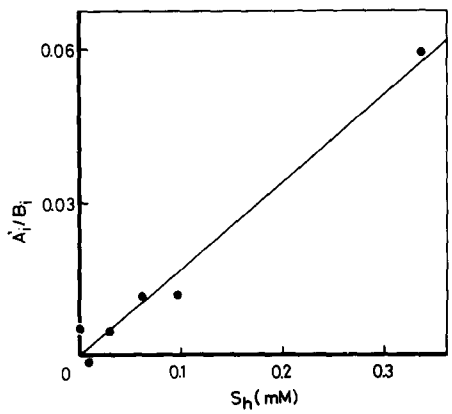

Fig. 3. Dependence of A_i'/B_i upon the proton concentration in the suspending medium s_h.

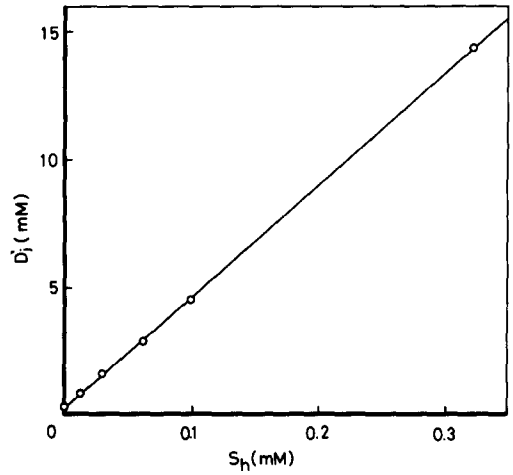

Fig. 4. Dependence of D_i' upon the proton concentration in the suspending medium s_h.

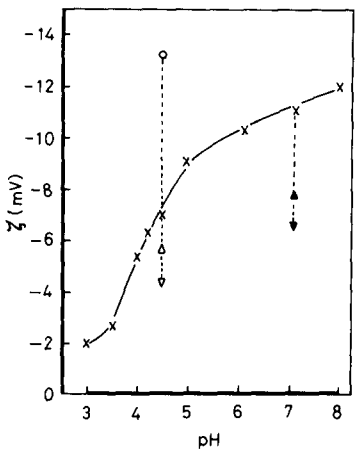

Fig. 5. Effect of the pH and of $Tris^+$ at pH 4.5 and pH 7.2 on the zeta potential of yeast cells. (×) variation of pH in 45 mM Tris-succinate buffer; variation of $Tris^+$ at pH 4.5: (○) 22.5 mM, (△) 90 mM, (▽) 180 mM; variation of the $Tris^+$ at 7.2: (▲) 80 mM and (▼) 160 mM.

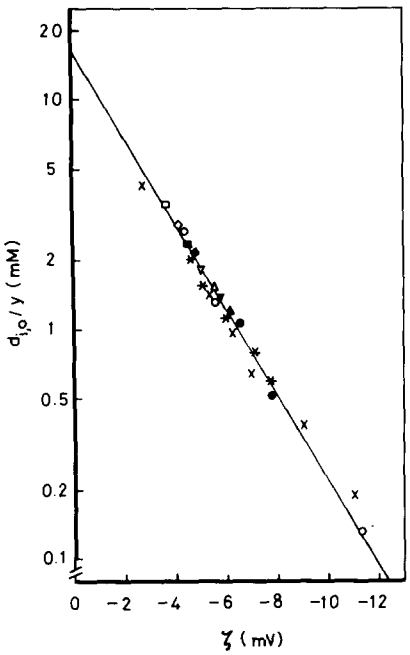

Fig. 6. Relationship between calculated values of $d_{i,0}/y$ and the zeta potential (ζ). The values of $d_{i,0}/y$ are calculated by correcting D_i values for competitive inhibition by Na^+, K^+ and H^+ (see text). The experimental conditions are varied by changing the pH (×), $Tris^+$ at pH 4.5 (○), $Tris^+$ at pH 7.2 (●) and Ca^{2+} at pH 7.2 (*). Data of Figs. 2, 4 and 5 are used. Data on the effect of 10 mM (closed symbols) and 25 mM (open symbols) of the alkaline earth cations on coefficient D_i at pH 4.5 which have been reported previously [2] are included. Corresponding zeta potentials have been determined in the present study. △, Mg^{2+}, ▽, Sr^{2+}, ◇, Ca^{2+} and □, Ba^{2+}.

surface potential cannot be measured directly, we have taken the zeta potential as a measure of the surface potential. Fig. 5 shows the pH dependence of the zeta potential and the effect of $Tris^+$ at pH 4.5 and at pH 7.2 upon this zeta potential. It is clear that the zeta potential is appreciably affected by changes in both the pH and the $Tris^+$ concentration.

In Fig. 6 the calculated values of $d_{i,0}/y$ are plotted as a function of corresponding zeta potentials. Irrespective of the way in which the zeta potential (ζ) is changed, to a good approximation, a single relationship between the two parameters is found, which is described by Eqn. 6:

$$\ln d_{i,0}/y = I + \gamma\zeta \qquad (6)$$

where I is the intercept of the line with the ordinate and γ represents the slope of the line.

We have also determined the effect of Ca^{2+} on the D_i value of the Rb^+ uptake at pH 7.2. Just as was found at pH 4.5 [2], the Rb^+ uptake is inhibited apparently competitively (data not shown) and the D_i value is linearly increased with the square-root of the Ca^{2+} concentration instead of with its concentration direct (data not shown). However, the effectivity of Ca^{2+} in increasing D_i was less than at pH 4.5, just as was found for $Tris^+$. The slope of the line at pH 7.2 is 0.19, whereas at pH 4.5 a value of 0.46 for this slope was obtained [2]. The D_i values determined here and also those reported earlier in the presence of the alkaline earth cations at pH 4.5 [2] have now been corrected for competitive inhibition by Na^+, K^+ and for H^+ as well, as described above. The resulting values of $d_{i,0}/y$ then obtained are also plotted in Fig. 6 against corresponding zeta potentials measured in the present study. The data points are fitted by the same curve and, therefore, support our earlier conclusion that the alkaline earth cations inhibit the Rb^+ uptake mainly via a reduction in the surface potential [2,3].

Discussion

The results presented in this article stress the importance of accounting for effects of changes in the surface potential in the determination of the apparent affinity of an ion for its transport system.

The effect of the buffer cation $Tris^+$ on the apparent K_m (which approximates the D value dealt with in this article) of the Rb^+ uptake may be exclusively attributed to a reduction in the surface potential of the yeast. Previously we suggested that $Tris^+$ may have some affinity for the monovalent cation carrier [19]. The present results, however, clearly show that this is not the case. In line with this, there are no indications for a cotransport of $Tris^+$ with Rb^+, as would be possible when $Tris^+$ also had an affinity for the two-site carrier involved in Rb^+ transport. Similarly, cotransport of divalent cations with Rb^+ does not occur [2,3]. On the other hand, cotransport of protons with Rb^+ is shown to be possible. Under conditions whereby only one site of the carrier is occupied by Rb^+ and the other one is occupied by protons, both ions may be translocated simultaneously across the yeast cell membrane. This supports the suggestion of Rothstein [10], that protons and monovalent cations share the same transport system. Accordingly, part of the increase in K_m observed on decreasing the pH can be ascribed to a competitive inhibition by protons. A significant part of the increase in the K_m, however, is due to the decrease in the surface potential caused by decreasing the pH. This is evident from the data presented in Fig. 6. Irrespective of the way in which the zeta potential is affected, either by varying the pH, the $Tris^+$ concentration or upon adding polyvalent cations, a direct relationship is found between the corrected apparent K_m for the Rb^+ uptake and the zeta potential.

On extrapolating the corrected D_i value to zero zeta potential, a value of 16 mM is obtained. This value represents the sum of the dissociation constants for the complexes of Rb^+ with the activation site and substrate site according to the transport model in which the carrier has two independent sites [15]. Since the dissociation constant of the complex of Rb^+ with the substrate site is much larger than that with the activation site, the extrapolated D_i value approximates the 'true' value of the dissociation constant of the substrate site under conditions of zero surface potenial. This value is much larger than the value found experimentally at high pH at which pH the effect of competitive inhibitors is minimal. Obviously, the experimentally determined K_m of ion transport across the yeast membrane depends not only upon intrinsic physical and chemical properties of the transporter, but also upon the electrostatic properties of the cell membrane. In fact, affinities for cations determined under non-zero conditions of the surface potential may be greatly overestimated.

Changes in the surface potential of the cell may greatly affect the apparent K_m of the Rb^+ uptake in yeast. The ignorance of this may lead to misinterpretation of experimental data. For example, the difference in apparent K_m of the Rb^+ uptake observed between mutant strains of yeast [20] might not be the result of mutagenic alterations in the transport system, but may be due to changes in charge density in the various strains obtained. Besides this, differences in apparent K_m for monovalent cation transport reported in the literature [1] might be due to differences in the ionic strength of the medium instead of to differences in affinity of the transport sites.

Upon reducing the zeta potential from -12 to -4 mV, the factor y decreases 20-fold, as deduced from the increase in the corrected value of D_i. According to Eqn. 5 a reduction of about 75 mV in the surface potential is needed for the 20-fold decrease in y. The zeta potential is thus much lower than the surface potential. This is possible if the negative charges are not uniformly distributed over the surface of the membrane and the transport sites are located in close proximity of these negatively charged groups. A relatively high discrete charge potential is then experienced by the transport sites. The zeta potential, which depends upon the average charge density, will consequently be lower (see, for example, Ref. 21). Recent work of Schmidt et al. [22] showed that cationic silica microbeads are clustered in spots and are not evenly distributed over the membrane surface of the yeast cell. Their observation would support the notion of a non-uniform distribution of negative charges over the plasma-membrane surface.

We have finally made an attempt to evaluate the magnitude of the electrostatic potential (ψ_0) experienced by the Rb^+ transport system. The relation between the corrected apparent K_m ($d_{i,0}/y$) and the zeta potential is given by Eqn. 6. Since $y = \exp(-F\psi_0/RT)$, apparently $\zeta = \alpha\,\psi_0$ with $\alpha = 0.093$. With this value of α, measured zeta potentials can be converted into corresponding

electrostatic potentials in the vicinity of the transport system. In the 45 mM Tris-succinate buffer, for example, at pH 7.2, $\psi_0 = -115$ mV and at pH 4.5 $\psi_0 = -75$ mV with corresponding tentative y values of 99.5 and 20.1, respectively, can be calculated. Electrostatic potentials of the same order of magnitude have been reported for artificial membranes (e.g. Refs. 23, 24) and also for a variety of other biological membranes (e.g. thylakoids [25,26] and red blood cells [27], chosen here just as an example of a plant membrane and an animal membrane system, respectively), of course depending on the pH and the ionic composition of the bathing medium.

Most biological membranes bear a net negative charge [28]. The results of this study are, therefore, of importance for the interpretation of data on ion transport in living cells in general, and not restricted to the yeast cell. As a matter of fact, numerous studies have been published showing that physiological phenomena associated with ion movements across the biological membrane may be affected greatly by changes in the surface potential (e.g., proton pumping by mitochondria [29], gating of ion channels [30], nerve excitation [31] and photosynthesis [32]). We have presented evidence that the effect of the surface potential on ion transport in yeast may not be neglected and described an experimental approach of distinguishing competitive from electrostatic effects of ions on the kinetics of the Rb^+ uptake. We have shown that for the unravelling of these effects, measurements of the zeta potential are of great importance in the diagnostic procedure followed.

Even under conditions where the ionic strength is kept constant, effects of the surface potential on ion transport cannot be ignored. Apparent dissociation constants of complexes of positively charged substrates and carrier may be greatly underestimated, whereas those of anionic substrates will then be overestimated. On comparing the affinity of isolated binding and transport proteins of the membrane with the affinity evaluated from transport studied, one should keep in mind that the electrostatic potential experienced by these proteins may have drastically changed upon their extraction from the membrane (e.g., by a delipidation of the proteins), thereby affecting the apparent affinity for their substrates. Appropriate corrections for differences in this potential (if possible) should then be made. One should also realize that on observing an increase in the K_m a competitive inhibition is not necessarily involved, unless it can be ascertained that the electrostatic potential is not changed.

Finally, as pointed out above, differences in K_m of ion uptake found between various species might be traced to differences in the surface potential instead of to differences in the properties of the transport system.

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References

1 Borst-Pauwels, G.W.F.H. (1981) Biochim. Biophys. Acta 650, 88–127
2 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Biochim. Biophys. Acta 426, 745-756
3 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Bioelectrochem. Bioenerg. 3, 230-240
4 Gouy, M. (1910) J. Phys. Radium 9, 457-468
5 Chapman, D.L. (1913) Philos. Mag. 25, 475-481
6 Theuvenet, A.P.R. (1978) Thesis, Nijmegen.
7 Fuhrmann, G.F., Boehm. C. and Theuvenet, A.P.R. (1976) Biochim. Biophys. Acta 433, 583-596
8 Eddy, A.A. and Rudin, A.D. (1958) Proc. R. Soc. B 148, 419-432
9 Armstrong, W.McD. and Rothstein, A. (1964) J. Gen. Physiol. 48, 61-71
10 Derks, W.J.G. and Borst-Pauwels, G.W.F.H. (1980) Biochim. Biophys. Acta 596, 381-392
11 Theuvenet, A.P.R., Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 469, 272-280
12 Robinson, R.A. and Stokes, R.H. (1955) Electrolyte Solution, Butterworths, London.
13 Fuhrmann, G.F., Granzer, E., Bey, E. and Ruhenstroth-Bauer, G. (1964) Z. Naturforsch. 196, 613–621
14 Smoluchowski, M. (1903) Bull. Acad. Sci. Cracovie, p. 182
15 Borst-Pauwels, G.W.F.H. (1973) J. Theor. Biol. 40, 19–31
16 Borst-Pauwels, G.W.F.H., Wolters, G.H.J. and Henricks, J.J.G. (1971) Biochim. Biophys. Acta 225, 269-276
17 Derks, W.J.G. (1978) Thesis, Nijmegen.
18 Hofstee, B.J. (1952) Science 116, 329-331
19 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1980) in Plant Membrane Transport: Current Conceptual Issues

(Spanswick, R.M., Lucas, W.J. and Dainty, J., eds.), pp. 429–430, Elsevier/North-Holland, Amsterdam.
20 Brunner, A., Carrasco, N. and Peña, A. (1982) Arch. Biochem. Biophys. 217, 30-36
21 Sauvé, R. and Ohki, S. (1979) J. Theor. Biol. 81, 157-179
22 Schmidt, R., Ackermann, R., Kratky, Z., Wasserman, B. and Jacobson, B. (1983) Biochim. Biophys. Acta 732, 421-427
23 McLaughlin, S.G.A., Szabo, G. and Eisenman, G. (1971) J. Gen. Physiol. 58, 667-687
24 Loosley-Millman, M.E., Rand, R.P. and Parsegian, V.A. (1982) Biophys. J. 40, 221-232
25 Chow, W.S. and Barber, J. (1980) J. Biochem. Biophys. Methods 3, 173-185
26 Schapendonk, A.H.C.M., Hemrika-Wagner, A.M., Theuvenet, A.P.R., Wong Fong Sang, H.W., Vredenberg, W.J. and Kraayenhof, R. (1980) Biochemistry 19, 1922-1927
27 Yen, W.S., Mercer, R.W., Ware, B.R. and Dunham, P.B. (1982) Biochim. Biophys. Acta 689, 290-298
28 McLaughlin, S. (1977) in Current Topics in Membranes and Transport (Bonner, F. and Kleinzeller, A., eds.), Vol. 9, pp. 71-144, Academic Press, New York.
29 Schäfer, G. and Rowohl-Quisthoudt, G. (1976) J. Bioenerg. 8, 73-81
30 Lindemann, B. (1982) Biophys. J. 39, 15-22
31 Gilbert, D.L. and Ehrenstein, G. (1969) Biophys. J. 9, 447-463
32 Barber, J. (1982) Annu. Rev. Plant Physiol. 33, 261-295