NET Rb⁺ TRANSPORT BY Rb⁺ LOADED YEAST CELLS AND THE ELECTROKINETICAL FORMULISM

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SUMMARY

It has been shown that Rb⁺ efflux from Rb⁺ loaded yeast cells is almost unaffected by the concentration of Rb⁺ in the medium, whereas the influx rate of Rb⁺ into the yeast cells increases largely on increasing the external Rb⁺ concentration.

It is possible to describe the kinetics of Rb⁺ net transport by the electrokinetical formulism provided that a non linear diode like model is chosen in preference to a varistant one.

1. INTRODUCTION

It has been pointed out by THELLIER (1971) that the kinetics of biological processes may be described by "the electrokinetical formulism". In this formulism the speed of a biological reaction is formally equivalent with an electrical intensity (I), and the structure catalyzing the reaction is equivalent with a conductor, whereas the force driving the reaction can be considered to be equivalent with an electrical potential difference ($\triangle E$).

The equation used in order to describe the dependence of the reaction rate upon the value of the driving force contains a term linear to $\triangle E$ (ohmic behaviour) and a term proportional with $(\triangle E)^m$ (non-ohmic behaviour):

$$I = \triangle E/r \; + \; (\lambda \triangle E)^m \tag{1}$$

r, λ and m are constants. The corresponding rate equation for a biological process is found by replacing $\triangle E$ by 2.3 A log Bsp^{-1}:

$$v = 2.3(A/r) \log Bsp^{-1} + (2.3\lambda \, A\log Bsp^{-1})^m \tag{2}$$

r, λ and m are characteristic constants of the structure catalyzing the process involved, B is a constant characteristic of the thermodynamic state of the cell, p and s are the concentrations of the product of reaction and the substrate respectively, and A is a constant proportional to the absolute temperature. As long as one is not too far from the equilibrium state the second term of equation (2) will be negligibly small and v will be proportional to log s, whereas

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on increasing the concentration of s higher powers of log s will determine also the rate of the process involved. As shown by THELLIER (1970) the electrokinetical formulism can be applied to describe the concentration dependence of ion transport by plant cells, see also AYADI & THELLIER (1970). At low substrate concentrations a linear relationship between v and log s is found as expected whereas at higher substrate concentrations an apparent "non-ohmic behaviour" is found.

We have now examined whether the electrokinetical formulism can be applied also to Rb^+ transport by yeast cells (BORST-PAUWELS et al. 1971). When equation (2) holds one should expect that the net rate of transport at infinitely low substrate concentrations becomes negative and infinitely large. This means that the rate of efflux would increase largely on decreasing the external concentration. We have carried out our studies with yeast cells in which the greater part of cell K^+ is replaced by Rb^+ in order to be capable to study both the influx and the efflux of Rb^+ as a function of the external Rb^+ concentration.

2. METHODS

Preparation of yeast in which the greater part of cellular K^+ is replaced by Rb^+ is carried out as follows. 2% (w/v) yeast, *Saccharomyces cerevisiae*, Delft 2, is incubated for one day in 100 mM Na citrate buffer of pH 4.5 provided with 50 mM RbCl and 5% w/v glucose under anaerobic conditions at about 20 °C. The medium is renewed 5 times during the incubation period. Finally the yeast is washed with ice cold 20 mM $MgCl_2$ solution, and subsequently with distilled water of 0 °C.

Yeast is labelled with radioactive ^{86}Rb by incubating the "Rb^+ cells" for one night at room temperature in 50 mM sodium citrate buffer of pH 4.5 provided with both 0.5% glucose and carrier-free radioactive $^{86}RbCl$. These cells are used for efflux experiments. The cells used for influx experiments are treated in the same way except that no radioactive Rb^+ is added.

Efflux experiments: 5 ml of yeast cells labelled with radioactive Rb^+ are preincubated for one hour with 5% glucose at a yeast concentration of 10% w/v in 50 mM Tris citrate buffer of pH 4.5 at 25 °C. In order to prevent exhaustion of glucose from the medium an extra amount of glucose is added at 30 minutes (0.15 g). At 60 minutes the 5 ml yeast sample is added to 45 ml of 50 mM Tris citrate buffer provided with various amounts of cold RbCl and 3% glucose. 1 ml samples are taken at appropriate times, and these samples are centrifuged at 18,000 r.p.m. The radioactivity of 0.5 ml samples of the supernatants is determined by means of an end window Geiger-Müller tube. Initial rates of efflux are obtained from the efflux curves.

Influx of radioactive Rb^+ is determined with yeast cells pretreated in the same way as the yeast used for the efflux experiments except that no radioactive Rb^+ is added during the over night preincubation period. The uptake is started by adding 2 ml of yeast cells preincubated for one hour at 25 °C, and

pH 4.5 in the presence of glucose as in the efflux experiments to 18 ml of Tris citrate buffer containing various amounts of cold RbCl and a fixed amount of ^{86}RbCl. The rate of uptake of radioactive Rb$^+$ is determined according to BORST-PAUWELS et al. (1973).

Concentrations of K$^+$ and Rb$^+$ in the medium and amounts of K$^+$ or Rb$^+$ in the cells have been determined as follows: 5 ml of 2% yeast cell suspension is filtrated by suction and is washed with 2 ml of ice cold water. The cells are boiled in 0.5 ml of water for 30 seconds, whereafter the cells are extracted with an appropriate amount of water. The K$^+$ and Rb$^+$ content of these extracts have been determined by flame photometry.

3. RESULTS

It appeared that a small amount of Rb$^+$ leaked out the cells during the one hour preincubation of the 10% yeast. The concentration of Rb$^+$ in the medium after this preincubation was 0.065 mM corresponding with a concentration of 0.0065 mM in the medium of the yeast after diluting the yeast ten times. The concentration of K$^+$ in the diluted yeast suspension amounted to 0.007 mM arising mainly from contamination of the buffer with small amounts of K$^+$. The K$^+$ and Rb$^+$ concentrations in the cell water were 38 mM and 175 mM respectively.

Fig. 1 shows the dependence of both the efflux rate and the net transport rate of Rb$^+$ observed with Rb$^+$ cells upon the external Rb$^+$ concentration. We have taken for this Rb$^+$ concentration the sum of the concentrations of added Rb$^+$ and of Rb$^+$ already present in the medium. It is seen that the efflux rate is almost independent of the external Rb$^+$ concentration. The mean efflux rate amounts to 0.27 mmoles min^{-1}kg^{-1}. The net transport rate increases largely on increasing the external Rb$^+$ concentration. Zero net uptake rate occurs at about 0.08 mM Rb$^+$. The rates of influx are not shown in this figure. These rates do not differ much from the net rates of transport and are on the average 0.27 mmoles min^{-1}kg^{-1} greater than the net transport rates.

Fig. 2 shows a plot of the influx rate of Rb$^+$ (v_i) against the quotient of this influx rate and the external Rb$^+$ concentration. A linear relationship between v_i and v_i/s should be expected when the concentration dependence of the influx rate is described by the Michaelis-Menten equation:

$$v_i = \frac{V_m s}{K_m + s} = V_m - K_m v_i/s \tag{3}$$

Apparently this is true only at the higher Rb$^+$ concentrations, whereas the influx rate observed is smaller than the influx rate expected according to equation (3) at relatively low concentrations of Rb$^+$ in the medium. In fact this is equivalent with a sigmoidal relation between v and s, and can be ascribed to an inactivation of the Rb$^+$ transport mechanism at the lower Rb$^+$ concentrations. It is possible to obtain values of the maximal rate of influx (V_m) and

the K_m from the linear part of the line drawn in *fig. 2*. These values are 8.4 mmoles $min^{-1}kg^{-1}$ (dry weight of yeast) and 1.5 mM respectively. We have now calculated how the relation between v and s would be when the rate of influx is described by a Michaelis - Menten equation over the whole range of Rb^+ concentrations by taking V_m = 8.4 mmoles $min^{-1}kg^{-1}$ and K_m = 1.5 mM. By adding the mean efflux rate (-0.27 mmoles $min^{-1}kg^{-1}$) to the influx rates calculated according to equation (3) one obtains the net rate of transport expected when the influx rate is activated also at the lower Rb^+ concentrations. It is seen from the course of the dotted line in *fig. 1* that the course of the dotted line does not differ much from the course of the line drawn through the experimental points.
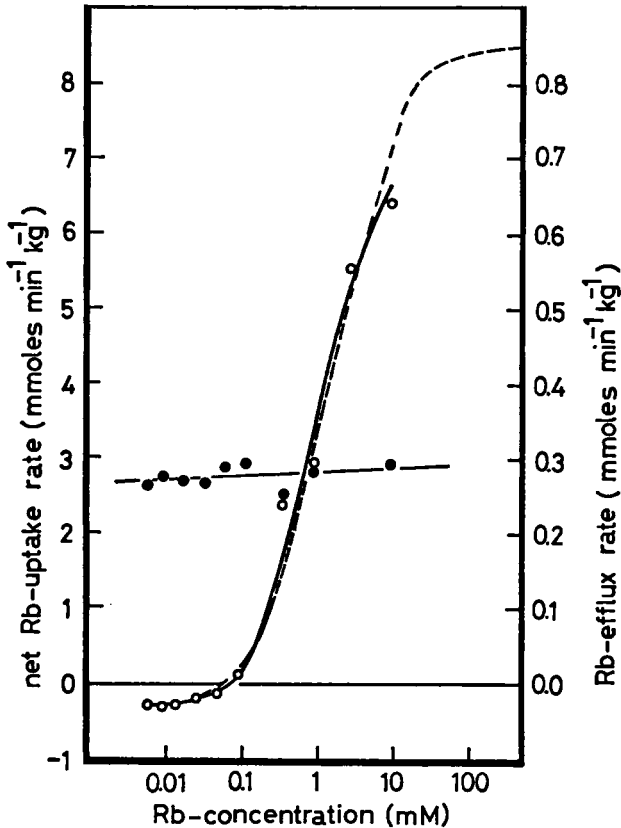

Fig. 1. Plot of the net transport rate and the efflux rate against the logarithm of the Rb^+ concentration in the medium.

O : net transport rate

● : efflux rate

The dotted line is the line calculated for a net transport rate given by $v = 8.4 s(1.5 + s)^{-1} - 0.27$ mmoles $min^{-1}kg^{-1}$ representing the net transport rate for Rb^+ transport in case that the Rb^+ transport mechanism is activated already at low Rb^+ concentrations.
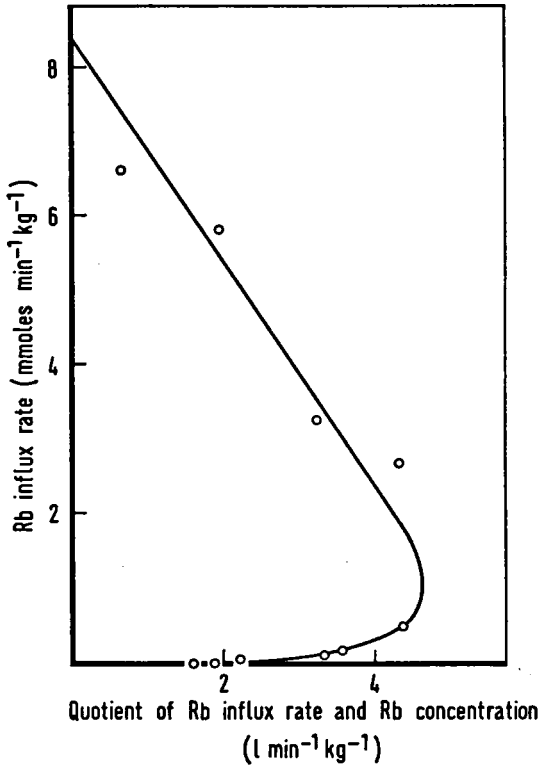
Fig. 2. Hofstee plot of Rb$^+$ influx
A plot is made of the influx rate (v)
against the quotient of the rate and
the concentration of Rb$^+$ in the
medium (v/s) according to equation
(3).

4. DISCUSSION

The kinetics of Rb$^+$ influx into Rb$^+$ loaded yeast cells is similar to the kinetics
of influx of Rb$^+$ into normal yeast cells, and can be described very well by a
transport via a two-site transport mechanism (BORST-PAUWELS 1973, BORST-
PAUWELS et al. 1971, 1973). This shows that replacing the greater part of the
cell K$^+$ by Rb$^+$ does not lead to a dramatic change in the type of transport
process.

Our finding that the Rb$^+$ efflux rate does not depend much upon the external
Rb$^+$ concentration is in apparent contradiction with the observation of
ROTHSTEIN & BRUCE (1958) that the efflux rate of K$^+$ from normal yeast cells
decreases greatly on increasing the external K$^+$ concentration. This difference in
results is probably not due to the fact that we are using Rb$^+$ loaded cells, whereas
ROTHSTEIN & BRUCE are using "normal cells". The K$^+$ concentration in the
medium of "normal cells" is at flux equilibrium even lower than that found
for Rb$^+$ with Rb$^+$ loaded cells namely approximately 0.02 mM K$^+$ as compared
with 0.065 mM Rb$^+$ indicating that the K$^+$ efflux rate from normal cells is very
low even at low K$^+$ concentrations in the medium. However, ROTHSTEIN &
BRUCE applied a different technique for measurement of the K$^+$ efflux namely

a column technique. They let the medium flow through a column of yeast cells, and they measured the difference in K^+ concentration before and after passing the yeast column. It may be possible that some essential compounds are removed from the yeast cells by this technique, and that these substances regulate the permeability of the yeast cell membrane together with the concentration of K^+ ions. This is now under examination in our laboratory.

We will now discuss our results in view of the electrokinetical formulism of THELLIER. According to this formulism a linear relationship between v and log s should be expected near equilibrium, thus in the range of concentrations at which v is almost zero, see also equation (2). It is seen in *fig. 1* that no linear relationship is found near the equilibrium concentration (0.08 mM) or that linearity applies only for a very small range of concentrations near 0.08 mM, whereas as already stated in the introduction normally linearity occurs over a rather large range of concentrations near the equilibrium concentration. We have examined whether this "deviation" from what is found normally may be due to the fact that we are dealing with an anomalous type of transport kinetics by calculating the net transport rates for the case that the Rb^+ influx rate depends upon the external Rb^+ concentration according to a Michaelis-Menten equation over the whole range of Rb^+ concentrations applied. It is seen from *fig. 1* (dotted line) that still no linear relation is found near the equilibrium Rb^+ concentration.

According to equation (2) one should expect that the net rate of transport tends to decrease to infinitely high negative values on decreasing the Rb^+ concentration in the medium. This is not found experimentally. A horizontal asymptot is observed on the left in *fig. 1*, which should be ascribed to the fact that the efflux rate hardly depends upon the external Rb^+ concentration.

We will now show that the electrokinetical formulism can be modified in order to account for the observed dependence of the net Rb^+ uptake rate upon the external Rb^+ concentration. It is supposed that the net flux rate is equivalent with the electrical current via two diodes in series and of opposite sign, see also THELLIER (1973). The current via the first diode will be equal to the current via the second diode. The two currents are given by the equations for the current voltage characteristics for a current flowing via two diodes of opposite sign. The currents via each diode are given by I' and I'' respectively:

$$I' = I'_o \, (1 - e^{qE'}) \tag{4}$$

$$I'' = I''_o \, (1 - e^{-qE''}) \tag{5}$$

E' and E'' are the potentials over the first and the second diode respectively. The currents via the two diodes will be equal to each other, and can be represented by I. The sum of E' and E'' is given by E. Writing E' and E'' in an explicit way and adding these two values leads to:

$$E = E' + E'' = q^{-1} \ln \frac{(I'_o - I) \, I''_o}{(I''_o - I) \, I'_o} \tag{6}$$

I can be written explicitly:

$$I = \frac{I_o' \, I_o'' \, (e^{qE} - 1)}{I_o' \, e^{qE} - I_o''} \tag{7}$$

When E is infinitely high and positive then I will approximate to I_o''. Infinitely high values of E correspond with infinitely high values of s, when $_qE$ is equivalent with $\ln Bsp^{-1} = \ln cs$ (c is equal to Bp^{-1}). Then I'' is equivalent with the maximal rate of net transport or with the sum of the maximal rate of influx (V_m) and the efflux rate (v_e). When E is infinitely high but of negative sign, which corresponds with s approximating to zero values then I approximates to I_o' which is equivalent with v_e. We shall now replace the electrical parameters by the equivalent transport parameters. This leads to the following equation for the net transport rate:

$$v = \frac{v_e \, (V_m + v_e) \, (cs - 1)}{v_e cs - V_m - v_e} \tag{8}$$

The rate equation for influx is given by:

$$v_i = v - v_e = \frac{V_m s}{- \dfrac{V_m + v_e}{v_e c} + s} = \frac{V_m s}{K_m + s} \tag{9}$$

Because v_e is negative, the value of K_m will be positive. The value of c is found by determining the concentration of the substrate at which the net transport rate equals zero. Then $\ln cs = 0$ or $cs = 1$.

This analysis shows clearly that when the net transport rate is described by the sum of a Michaelis-Menten equation and a constant term, the efflux term, the net transport is equivalent with the electrical current via two diodes.

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