A POSSIBLE MECHANISM OF ENERGY COUPLING IN PURINE TRANSPORT OF *SACCHAROMYCES CEREVISIAE*

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1. Introduction

In a previous report of this laboratory [1], experimental evidence for the existence of a common transport system for adenine, guanine, hypoxanthine, and cytosine in *Saccharomyces cerevisiae* was given. Hypoxanthine and guanine were found considerably accumulated within the cells against a concentration gradient. Attempts to demonstrate the participation of purine pyrophosphorylases (EC 2.4.2.7, 8) in the accumulation process, similar to the situation in bacteria [2], were without success [1,3].

An interesting point of our studies were strong differences in the kinetic parameters for the uptake of the distinct bases when compared with the results of other authors [4,5]. In order to check on the reason for these differences we performed kinetic measurements at different pH with different buffer systems and found that purine uptake is activated by H^+ and inhibited by monovalent cations (Na^+, K^+) especially giving rise to changing affinities of the transport system for its substrates depending on the ratio of protons and monovalent cations present. Considering that *Saccharomyces* maintains as well a K^+ as in the reverse sense a H^+ gradient between the cytoplasm and the medium whereby in the presence of metabolic energy the uptake of K^+ is connected with the transfer of protons from the cell [6] we suggest by virtue of our kinetic results that the cells are using this gradient energy to accumulate purine bases. In the terminology of Mitchell [7], we suppose that the purine transport system in *Saccharomyces* is acting as a proton symporter and a potassium antiporter.

2. Materials and methods

Saccharomyces cerevisiae strain R XII (a kind gift of Dr A. Kotyk, Prague) was cultivated to the early stationary phase (about 10^8 cells/ml) in GYNP (2% glucose, 1% Difco yeast nitrogen base, 0.5% Merck peptone) at 30°C with agitation. Cells of a 50 ml culture were washed and incubated in 250 ml of glucose–citrate buffer (0.05 M sodium citrate, pH 5.4, 2% glucose) at 30°C under aeration for 60 min. After this pretreatment the cells were resuspended in 100 ml of 2% glucose in water. 200 µl of the cell suspension were mixed with 750 µl of the buffer mentioned below and 50 µl of different concentrations of [^{14}C]hypoxanthine (58 Ci/mol) and incubated for 1 min at 25°C. The incubation time was extended to 3 min at pH values above 6.5 because of the low uptake activity in this range. After stopping the reaction by the addition of 10 ml of 1 mM hypoxanthine solution the suspension was immediately filtered on glassfiber filters (pretreated with 1 mM hypoxanthine) using the Millipore 3025 sampling manifold. The filters were washed with the stop solution, completely dried and assayed for radioactivity by liquid scintillation counting in a toluene cocktail.

The buffer system was of the McIlvaine type [8]. 50 mM citric acid was mixed with 100 mM Na_2HPO_4 or K_2HPO_4 in different ratios. The concentrations of Na^+ or K^+ were increased by the addition of the corresponding hydroxide to a double-concentrated aliquot of the buffer. After readjusting the pH with HCl or H_2SO_4 the solution was diluted to the final volume.

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3. Results and discussion

Fig.1a shows the combined action of pH and the concentrations of K^+ and Na^+ on the apparent K_m value (K_m') for the uptake of hypoxanthine. One may suspect from these results that protons enhance the affinity of the transport system to the purine base whereas Na^+ and K^+ decrease it. That it is the cations that are acting and not the corresponding anions may be concluded from fig.2 where the anions Cl^- and SO_4^{2-} exert exactly the same effect at diverse K^+ concentrations. This figure also demonstrates that the influence of K^+ on the affinity of the transport system may be described by competitive inhibition kinetics indicating that the maximum uptake rate V_{max} is obviously not affected by the presence of monovalent cations. This statement holds also true for another set of measurements performed at pH 3.1 (not shown). The dependency of V_{max} on the pH is given in fig.1b. In fig.3, the K_m' values obtained at pH 3.1 and 6.1 are replotted against the K^+ concentration according to the equation

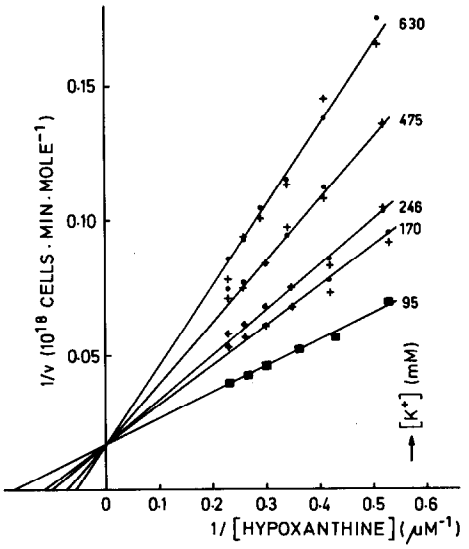

Fig.2. Lineweaver-Burk plot of hypoxanthine uptake in the presence of increasing K^+ concentrations at pH 6.1. The values are plotted for a citric acid -K_2PO_4 mixture (■) supplemented with different amounts of KOH and readjusted to pH 6.1 by means of HCl (●) and H_2SO_4 (×).

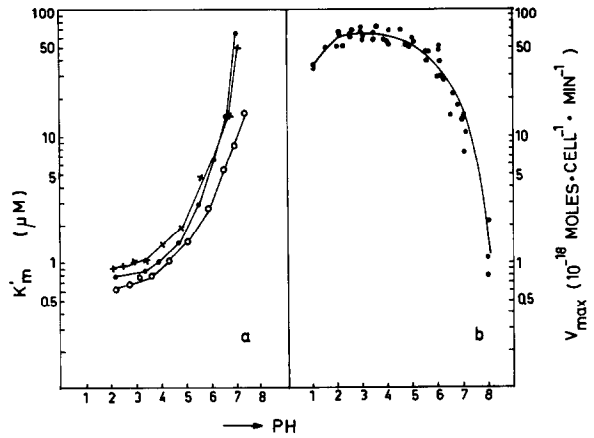

Fig.1. The apparent K_m value K_m' a) and the maximum velocity V_{max} b) of hypoxanthine uptake relative to the pH of the uptake buffer. K_m' values are plotted for citric acid -Na_2HPO_4 mixtures (○) with final concentrations of Na^+ increasing from 0 mM (pH 2.2) in steps of 15 mM to 135 mM (pH 7.35). The other two curves represent the K_m' values of the same buffer mixtures supplemented with 375 mM NaCl (●) and 375 mM KCl (×). The V_{max} values are obtained from 6 different sets of experiments under diverse conditions.

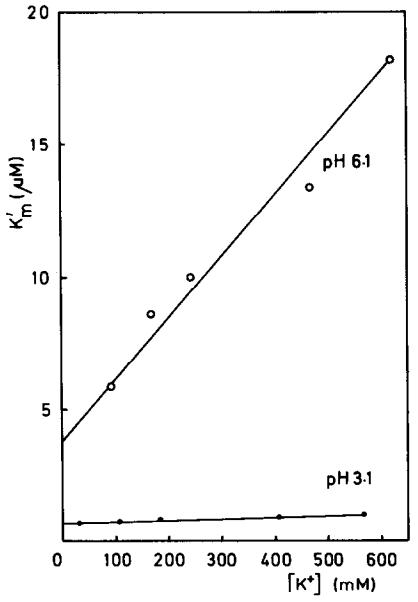

Fig.3. Secondary plots of K_m' against the concentration of potassium ions at pH 3.1 and 6.1.

$$K_m' = K_m + (K_m/K_i) \cdot i \quad [9]$$

with i the K^+ concentration and K_i the inhibitor constant of K^+. The K_m values in the absence of K^+ and the K_i values for K^+ may be read off from the intercepts with the ordinate and the negative abscissa after extrapolation. They are K_m = 0.69 μM, K_i = 1.3 M at pH 3.1 and K_m = 3.7 μM, K_i = 0.16 M at pH 6.1. These data support the assumption that both, protons and monovalent cations, react with the transport system changing in a reverse sense its affinity to hypoxanthine. With regard to the action of protons, however, it cannot be distinguished from our results between the two possibilities i) that the transport system only recognizes the protonated substrate which competes with monovalent cations for a common site or ii) that the transport system binds proton and uncharged substrate at different sites. In fig.4, a tentative scheme is given connecting the present findings with a possibility to explain the previously found accumulation of purines against a concentration gradient [1]. This working model makes use of the known fact that in metabolically active yeast protons and potassium ions are unsymmetrically distributed between cytoplasm (compartment 2) and medium (compartment 1) due to the action of a K^+–H^+ exchanging pump [6]. Outside of the cell, substrate molecules together with a relatively high amount of high-affine protons compete with monovalent cations of low affinity for the active site(s) of the transport system. Inside of the cell, a relatively high amount of potassium ions compete with substrate molecules and a relatively low amount of protons.

In the net-balance, an inflow of substrate molecules and protons and an outflow of potassium ions should be the consequence. In a second step, the K^+–H^+ exchanging pump recreates the original distribution between H^+ and K^+ by the use of a metabolic energy source.

Further experiments mainly have to prove that a transfer of potassium ions from the cells in fact accompanies the entry of substrate molecules.

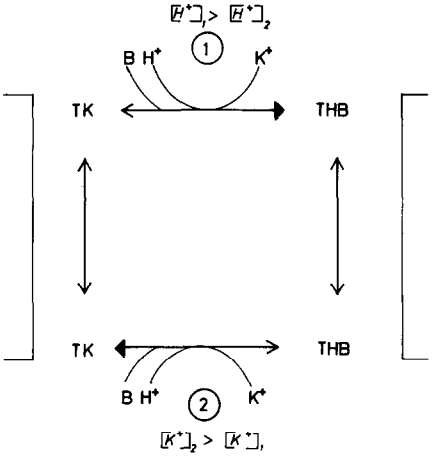

Fig.4. Tentative model explaining the accumulation of purine bases (B) by a H^+ symporting and K^+ antiporting transport system (T).

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