ENERGY COUPLING IN HYPOXANTHINE TRANSPORT OF YEAST

Potentiometric evidence for proton symport and potassium antiport

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1. Introduction

In *Saccharomyces cerevisiae*, the uptake of the purine bases hypoxanthine, adenine, guanine and the pyrimidine base cytosine is catalyzed by a common energy-dependent transport system [1]. In a previous report we proposed a model of energy coupling with the purine–cytosine transport system being active as a proton symporter and a potassium antiporter [2]. The model was substantiated essentially by the finding that a decrease in the extra-cellular pH enhances over a wide pH range the apparent affinity of the transport system to its substrates, while cations like K^+ and Na^+ act as competitive inhibitors the inhibitory effect of which increases with the pH. Meanwhile, the possibility of a proton co-transport has found further support by the experimental result reported that proton-conducting uncouplers of oxidative phosphorylation inhibit the influx and stimulate the efflux of the substrates not only under aerobic but also under anaerobic conditions as well as in respiratory deficient strains [3,4]. Furthermore, pH variation studies indicate that the association of one proton is necessary to bind one hypoxanthine molecule to the transport system [5].

In the present paper we demonstrate by potentiometric measurements that the transport of hypoxanthine into yeast cells is indeed accompanied by the absorption of protons as well as by the ejection of potassium ions with a 1 : 1 : 1 stoichiometry.

2. Materials and methods

2.1. *Organism*

Commercial baker's yeast (Hamelner Hefe, Nord-West-Deutsche Hefe- und Spritwerke AG, Hameln) was employed in all experiments.

2.2. *Chemicals*

Hypoxanthine was obtained from Pharma-Waldhof, Düsseldorf. Antimycin A, 2-deoxy-D-glucose and cycloheximide were purchased from Serva, Heidelberg. Xanthine oxidase (from cow milk; EC 1.2.3.2; spec. act. about 4 units/ml) was supplied by Boehringer Mannheim. The general inorganic and organic chemicals were of analytical grade.

2.3. *Pretreatment of the cells*

Fresh yeast, 10 g, was preincubated at room temperature for 5–7 h in 2 liter glucose–citrate buffer (0.05 M sodium citrate, pH 5.4, 2% (w/v) glucose) with agitation, but without aeration.

The glucose pretreatment is necessary to stimulate the cells for a well measurable uptake of purines [1].

2.4. *Assay of hypoxanthine uptake, proton absorption and potassium ejection*

The cells were collected by centrifugation and resuspended in 60 mM NaCl to give final vol. 100 ml. The suspension was brought into a stirred 150 ml-

vessel of an experimental device that was similar to that described by Eddy and Nowacki [6].

To record changes in the concentrations of protons and potassium ions the following electrodes were immersed into the suspension: the Ingold pH electrode, type 405-88, and the Orion ion specific electrode pair, types 93-19 (potassium) and 90-01 (reference filled with 60 mM NaCl). The electrodes were connected with the Knick pH-mV-meter, model 510, and the Philips digital ion activity meter, model PW 9414, respectively. Servogor S recorders were used.

The system was allowed to equilibrate for about 5 min at room temperature before the uptake experiment was started by the addition of 1–2 ml saturated hypoxanthine solution. In intervals of about 15 s aliquots of 1 ml were withdrawn and filtered over glassfiber discs. The hypoxanthine concentration in the filtrate was determined spectrophotometrically by measuring the conversion of hypoxanthine into uric acid by means of xanthine oxidase [7].

At the end of the uptake experiment 0.1 ml 100 mM KCl and 1 ml 10 mM HCl were added successively to the suspension in order to calibrate the electrode system.

3. Results and discussion

Figure 1 reports the characteristics of an experiment carried out according to the standard procedure given in Materials and methods.

The resuspension of glucose-pretreated cells in 60 mM NaCl is followed by a continuous increase in the extracellular potassium concentration and by a continuous decrease in the external proton concentration during the equilibration period, only the last minute of which is considered in the figure. Since the effect is not found with yeast inactivated by heat, it must not be attributed to electrode artefacts or instrumental drift, but rather to the cells absorbing protons from their surroundings and ejecting potassium ions from their interior. The total potassium concentration of the cells at the beginning of the experiment has been determined to 1.75 mequiv./10 g wet yeast. After an equilibration time of about 5 min extracellular proton and potassium concentrations of 4 μM (pH 5.4) and 700 μM (pK 3.15) respectively, have been found. The addition of hypoxanthine at this point gives rise to a sudden jump in the pK and pH traces which is attributed to dilution effects. After

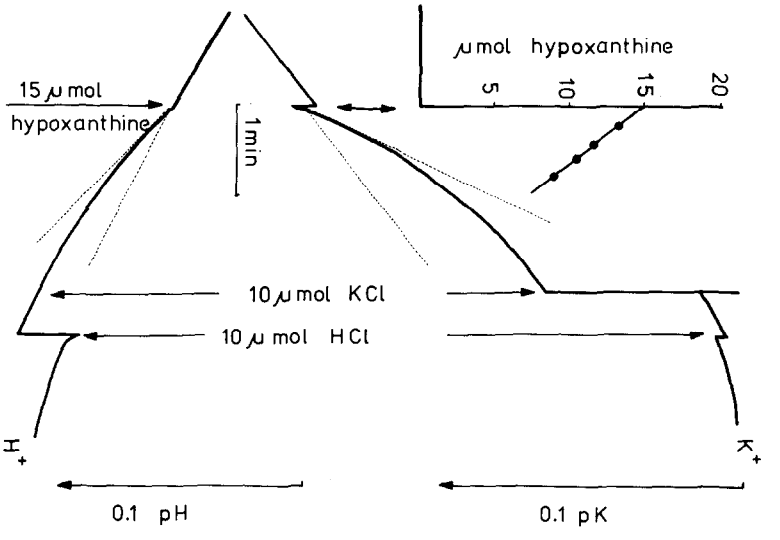

Fig.1. Absorption of protons and ejection of potassium ions caused by addition of 15 μmol hypoxanthine to 100 ml yeast (10 g wet wt) at pH 5.4 and pK 3.15. The disappearance of hypoxanthine from the medium is indicated. The experiment was performed with cells that were glucose-pretreated as described in Materials and methods.

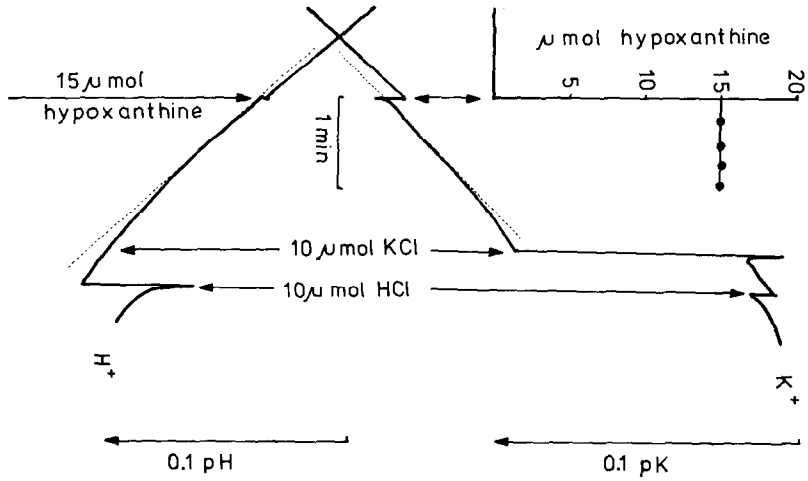

Fig.2. The same experiment as in fig.1, but carried out with cells that were glucose-pretreated in the presence of 10 mg cycloheximide/litre.

that, the traces continue with an evidently steeper slope than prior to the addition of the purine base. The enhanced movement of protons and potassium ions is obviously connected with the uptake of hypoxanthine by the cells.

To check up on this assumption the same experiment was performed with cells that were glucose-pretreated in the presence of cycloheximide which is known to suppress the stimulatory effect of the glucose-pretreatment on the uptake of purines [1]. Essentially the same picture as in the first experiment is obtained (fig.2) but with the difference that now neither the purine base disappears in measurable amounts from the medium nor significant changes occur in the slopes of the pH and pK traces after the addition of hypoxanthine.

In order to evaluate the stoichiometry between hypoxanthine uptake, proton absorption and potassium ejection we estimated the initial velocities for the hypoxanthine-induced proton and potassium movements from the differences in the slopes of the pH and pK traces before and after the addition of hypoxanthine. The jumps in pK and pH accompanying the addition of 0.1 ml 100 mM KCl and 1 ml 10 mM HCl, respectively, were used to calibrate the system. The results of 13 independent experiments are summarized in fig.3. Nine of them were performed with the standard procedure, one was the experiment with cycloheximide and three experiments were carried out with cells to which antimycin A

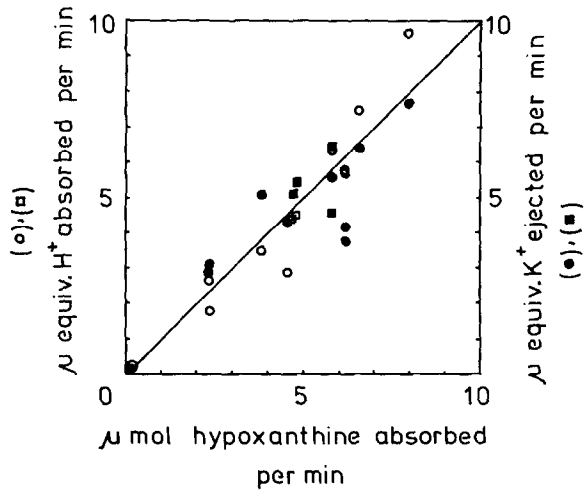

Fig.3. The hypoxanthine induced initial movements of protons and potassium ions relative to the initial velocity of hypoxanthine uptake. The data of 13 independent experiments are plotted. The squares indicate experiments in which the energy metabolism of the cells was blocked by the addition of 1 mg antimycin A and 400 µmol 2-deoxy-D-glucose at the beginning of the equilibration period [8].

and 2-deoxy-D-glucose were added at the beginning of the equilibration period. Antimycin and deoxyglucose block cooperatively the energy metabolism and cause a depletion of the cellular ATP content [8]. As may be seen from the figure the data of all these experiments are distributed around the straight line that indicates a 1 : 1 : 1 stoichiometry. This means that under our experimental conditions the uptake of one mole hypoxanthine is accompanied by the absorption of one equivalent of protons and by the extrusion of one equivalent of potassium ions.

Acknowledgement

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