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UPTAKE AND ACCUMULATION OF PURINE BASES BY STATIONARY YEAST CELLS PRETREATED WITH GLUCOSE

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SUMMARY

1 Dependent on the growth phase, the ability of *Saccharomyces* cells to take up the purine bases adenine, guanine, and hypoxanthine shows a maximum in the first half of exponential growth (Fig 1). Stationary cells, however, can also be prepared for maximum uptake by preincubation in glucose solution. The stimulating effect of this treatment is repressed in the presence of actidione (Fig 2)

2 Since only low differences in the specific activities of those enzymes involved in the metabolization of hypoxanthine were observed after and without glucose preincubation (Table I), the stimulating effect rather may be explained by the de novo synthesis of the purine transport system itself or a component of it

3 Kinetic studies with glucose pretreated stationary cells reveal a strong competitive inhibition pattern between adenine, guanine, hypoxanthine, and cytosine, indicating that all the four bases are accepted by this special transport system (Table II) Maximum uptake rates are in the range of 1–4 10^{-17} moles/cells per minute at 25 °C The temperature optimum is between 35 °C and 40 °C The activation energy has been calculated to 15 ± 3 kcal/mole

4 From the three purine bases, only hypoxanthine and guanine are considerably accumulated by the cells against the external concentration (Fig 4)

5 Attempts to demonstrate a participation of the IMP pyrophosphorylase in the accumulation process were without success

INTRODUCTION

In contrast to sugar and amino acid transport, studies on the mechanism of purine uptake are rather scarce The most instructive investigations in this field were performed by Stadtman and co-workers [1–3] referring to the purine pyrophosphorylases (E C 2 4 2 7 8) an essential role in the uptake of purine bases by bacteria

In yeasts, experimental data are still incomplete and their interpretation at least sometimes confusing In *Schizosaccharomyces pombe*, adenine and hypoxanthine

Abbreviation 5-*P*-Rib-1-*P*-*P*, 5-phosphoribosyl-1-pyrophosphate

compete for guanine uptake sites [4]. A mutant of the IMP pyrophosphorylase is strongly affected in the uptake of these three bases (strain *pur-1*), quite in contrast to a mutant of the AMP pyrophosphorylase (strain *dap-1*) (Pourquié, J. personal communication). In *Saccharomyces cerevisiae*, more recent studies speak of at least two purine "permeases" existing in the exponential growth phase, an "adenine permease" and a "guanine permease", either recognizing hypoxanthine [5] The "adenine permease" also accepts the pyrimidine base cytosine [6] No evidence for the participation of purine pyrophosphorylases in the uptake process has been found [5].

In the following we want to demonstrate some properties of a common uptake system for all the four bases mentioned. This uptake system or a part of it is newly synthesized by stationary *Saccharomyces* cells in the presence of glucose.

MATERIALS AND METHODS

Strain and growth conditions

The tetraploid wild type strain 2200 of *Saccharomyces* [7] 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

Glucose pretreatment

Cells of a 50 ml culture were washed and incubated in 200 ml of glucose–citrate buffer (0.05 M sodium citrate, pH 5 4, 2 % glucose) at 25 °C under aeration for 90 min (standard conditions).

Uptake rates

1 ml of the preincubation suspension was each time agitated for 30 or 60 s with different concentrations of ^{14}C-labelled substrates in the absence or presence of 10 μM of an unlabelled competitive base. After adding 10 ml of ice-cold stop solution (5 mM adenine, 5 mM hypoxanthine, glucose–citrate buffer) the cells were separated from the liquid on glassfiber filters, washed with the stop solution, and assayed for radioactivity by liquid scintillation counting

Intracellular accumulation and conversion of purine bases

10^{10} glucose-pretreated cells were suspended in 10 ml glucose–citrate buffer containing 100–200 μM labelled or unlabelled base At intervals, cells were separated from the medium by filtration (cellulose nitrate, pore size 1 2 μ) Intracellular pools were extracted by the lyophilization procedure of Nazar et al. [8] with slight modifications. The total of labelled and unlabelled hypoxanthine, inosine, and guanine, respectively, was measured in the medium and pool extracts with the enzymatic procedure of Kalckar [9] The enzymes necessary for this assay were obtained from Boehringer Mannheim Adenine was estimated by ion exchange chromatography on Beckman M-71 resin according to the technical data sheet M-TI 4003 (Unichrom amino acid analyzer) The labelled bases and conversion products were separated by two-dimensional thin-layer chromatography using precoated thin-layer chromatography plates Cellulose F (Merck No 5718) and 0 01 % EDTA as the first, 2-methyl-2-butanol, concentrated formic acid, water (90/60/30, v/v/v) as the second solvent

[10]. Adenine, guanine, hypoxanthine, xanthine, adenosine, guanosine, inosine, and xanthosine appear as distinct spots The nucleotides flock together The different ultraviolet active spots (intensified by unlabelled reference compounds) were scratched out and assayed for radioactivity by liquid scintillation counting

Enzymes assays

Crude extracts were prepared in the cold by grinding 1 g of wet cells with 1 5 g kieselguhr (Merck No. 8119) and 1 ml of buffer (0 1 M potassium phosphate, pH 7 6, 5 % (v/v) glycerol, 1 mM dithioerythritol) for approximately 15 min After addition of 1 ml of the same buffer, the homogenate was centrifuged at 30 000 × *g* for 15 min and the supernatant dialyzed overnight against 200 ml of 0 01 M potassium phosphate, pH 7 6, containing 10 % (v/v) glycerol and 1 mM dithioerythritol The activity of 5-*P*-Rib-1-*P*-*P* synthetase (EC 2 7 6 1) was measured with a modification of the two step procedure of Kornberg et al [11]. The first step included an ATP regenerating system, the second was carried out with the 5-*P*-Rib-1-*P*-*P* assay of P-L-Biochemicals Inc , Milwaukee, Wis 53205, U S.A IMP pyrophosphorylase (EC 2 4 2 8) was assayed radiochemically [12], [^{14}C]hypoxanthine and [^{14}C]IMP were separated by thin layer chromatography using cellulose sheets and 0 01 % EDTA as solvent

RESULTS AND DISCUSSION

Relations between growth phase and purine uptake

Dependent on the growth phase, the initial rate of purine uptake attains a maximum in the first half of exponential growth Thereafter it decreases to a level of about 10 % of the maximum This finding, demonstrated for adenine in Fig 1, also holds true for hypoxanthine and guanine

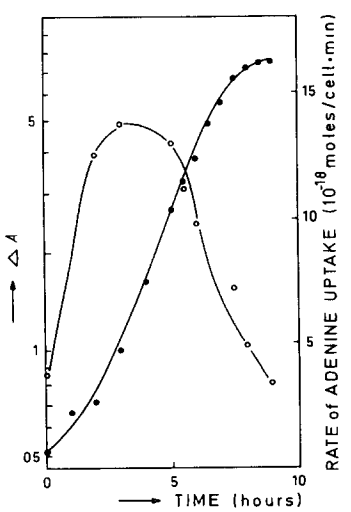
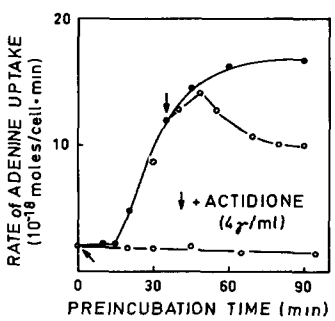


Fig 1. Initial rate of adenine uptake (○) relative to the growth phase (●)

The influence of glucose pretreatment on the uptake in stationary cells

One possibility to explain the decrease in uptake during proceeding growth may be an exhaustion of the external energy supply. To prove this, stationary cells were incubated for different times in glucose solution without any nitrogen source. As may be seen from Fig. 2, this treatment indeed activates purine uptake. In addition to the acceleration of the initial rate, an increase in the capacity of the cells to remove purines from the medium is observed (Fig 3)



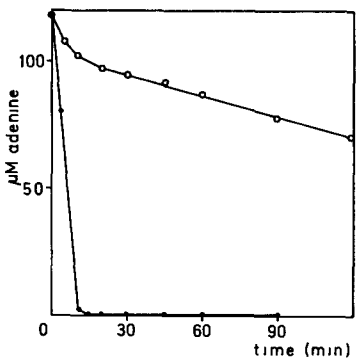


Fig 2. Initial rate of adenine uptake by stationary cells relative to the preincubation in glucose–citrate buffer (●) The arrows indicate the addition of actidione to the preincubation mixture at two different times (○)

Fig. 3. Removal of [^{14}C]adenine from citrate buffer without glucose by glucose pretreated (●) and untreated (○) stationary cells (4 5 10^8 cells/ml)

The stimulating effect of glucose may be due (i) to a rapid increase in the pool of energy-rich compounds necessary for an active transport mechanism, or (ii) to the de novo synthesis of one or more proteins involved in the transport process or in the metabolization of purine bases, or (iii) to both. To check the second possibility, actidione, a potent inhibitor of protein biosynthesis in yeasts [13], was added at different times to the preincubation mixture The results (Fig 2) attribute at least one part of the effect to de novo protein synthesis

The question which now remains is: What kind of protein is newly synthesized, a transport or a metabolizing protein? Since the effects observed so far hold for all the three purine bases under investigation (but not for xanthine) and since in the case of hypoxanthine only the IMP pyrophosphorylase is known to metabolize this

TABLE I

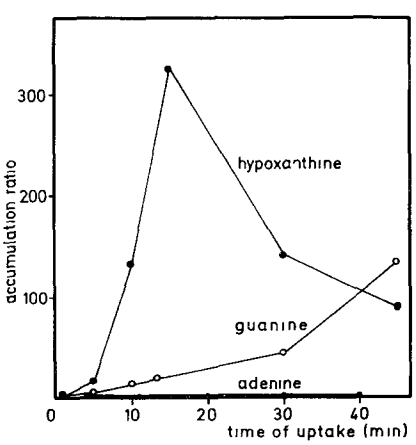
EFFECT OF GLUCOSE PRETREATMENT ON THE SPECIFIC ACTIVITIES OF IMP PYROPHOSPHORYLASE AND 5-*P*-Rib-1-*P*-*P* SYNTHETASE

Preincubation	Specific activity (munits/mg protein)	
	IMP pyrophosphorylase	5-*P*-Rib-1-*P*-*P* synthetase
Without	7 2	1 5
With	12 8	1 3

base in *Saccharomyces*, we have measured its specific activity in glucose pre- and untreated cells As 5-*P*-Rib-1-*P*-*P* is the second substrate in the conversion of hypoxanthine to IMP we also included the 5-*P*-Rib-1-*P*-*P* synthetase in this investigation. The results given in Table I do not reveal such striking differences to explain the 10-fold stimulation in the uptake rate by the de novo synthesis of one of these enzymes For this reason it seems most likely that during the glucose pretreatment the transport system itself, or a component of it, is newly synthesized

Intracellular accumulation of purine bases

As it was shown in Fig 3, the glucose pretreatment not only influences the uptake rate but also the uptake capacity of the cells Therefore, it is likely that glucose, in addition to its inducing effect, enables the cells to accumulate purine bases To prove this we estimated the ratio between internal and external base concentrations during the uptake of the three bases The results demonstrate that guanine and hypoxanthine are considerably accumulated within the cell (Fig 4) The highest accumulation ratio we ever measured was about 350-fold for hypoxanthine and 1500-fold for guanine In contrast to this, adenine is not accumulated but is mainly stored up in the form of hypoxanthine and nucleotides (Fig 5)



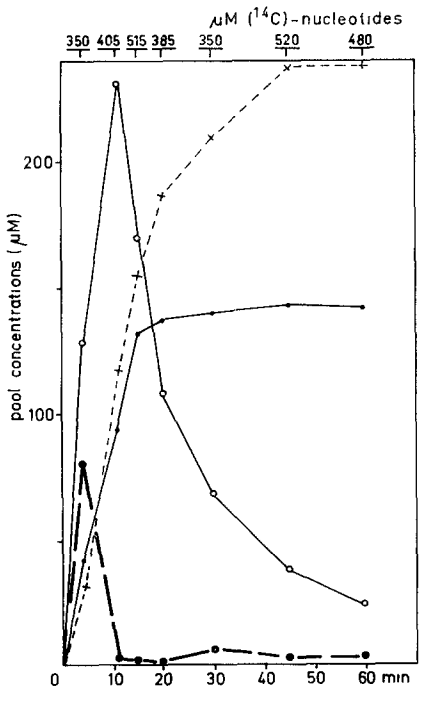


Fig 4 Ratio of the internal to external base concentration during the uptake of the indicated base More than 90 % of the external base supply (initial concentrations are about 200 μM) are found within the cells after 10 min (adenine, hypoxanthine) and 45 min (guanine), respectively

Fig 5 [^{14}C]labelled pool during the uptake of [^{14}C]adenine by glucose pretreated cells The external adenine concentrations are given in Fig 3 (●) ●--●, adenine, ○–○, hypoxanthine, ●–●, inosine, ×–×, insoluble compounds

Parameters of the transport system

After it was shown that the glucose pretreatment does not change the substrate affinity (K_m values) of the transport system under investigation (Fig. 6), the

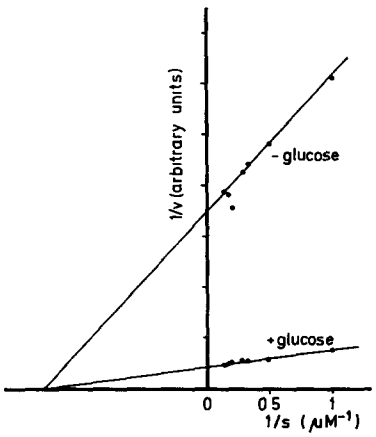


Fig. 6. Lineweaver-Burk plot of adenine uptake by glucose pre- and untreated stationary cells.

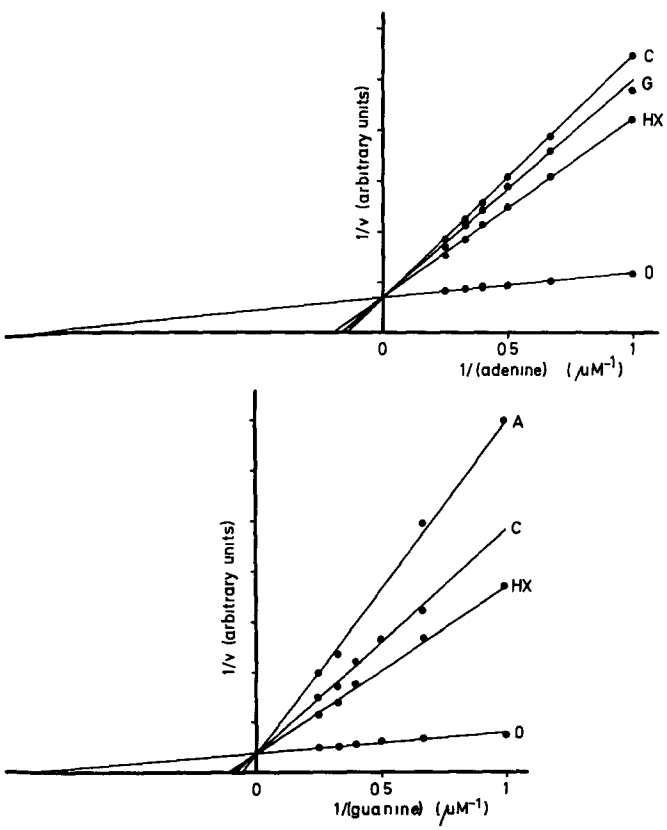


Fig 7 Lineweaver-Burk plot of adenine (a) and guanine (b) uptake in the absence (0) or presence of 10 μM adenine (A), guanine (G), cytosine (C), and hypoxanthine (HX), respectively

TABLE II

K_m AND K_i VALUES AT 25 °C

K_m values (μM) are in brackets the other values are the K_i values (μM) of the different inhibitors given in the head of the table for the different substrates noted at the left side For the K_m values, standard deviations are noted

Substrate	Inhibitor			
	Adenine	Hypoxanthine	Guanine	Cytosine
Adenine	(0 7±0 15)	1 9	1 35	1 1
Hypoxanthine	0 9	(1 9±0 2)	1 4	1 2
Guanine	0 65	1 7	(1 3±0 2)	1 0

following kinetic experiments were performed with glucose preincubated stationary cells.

The initial rate of uptake strongly depends on the temperature It attains a maximum in the range of 35–40 °C. The activation energy has been calculated to 15±3 kcal/mole. At 25 °C, maximum velocities were found in the range of $1–4 \cdot 10^{-17}$ moles/cell per min for the three bases The pH dependency of the uptake rate shows a broad optimum in the range of pH 5 to 6 5.

In contrast to the maximum velocities, K_m values are rather reproducible The three purine bases inhibit the uptake of each other in a competitive manner Furthermore, the pyrimidine base cytosine is a potent competitive inhibitor (Fig 7a, b) Table II summarizes the mean K_m and K_i values obtained from several experiments The mutual and competitive inhibition pattern and the obvious similarity between corresponding K_m and K_i values strongly suggest the existence of only one common uptake system under our conditions

On the mechanism of purine accumulation

An interesting point of the preceding investigations is that adenine does not appear to be accumulated within the cell This does not mean that *Saccharomyces* principally lacks the capacity to do this A fast metabolic interconversion of adenine to AMP and hypoxanthine may keep the pool of free adenine low On the other hand, it might be possible that the IMP pyrophosphorylase recognizing hypoxanthine and guanine, but not adenine, is an obligatory constituent of the accumulation process. It might be possible that this enzyme is capable of converting its carrier-bound substrates to free nucleotides which would then, in part, be degraded to the corresponding bases in a second step Some evidence in this direction is given by the finding that the hypoxanthine-excreting IMP pyrophosphorylase-less mutant *pur-1* of *S pombe* lacks the capacity to accumulate hypoxanthine, quite in contrast to the wild type and to other hypoxanthine excreting mutants (Reichert, U , Nagy, M and Heslot, H , unpublished)

The results of Table I revealing only slight differences in the specific activities of IMP pyrophosphorylase between glucose pre- and untreated cells does not instantly disprove the hypothesis of a participation of the IMP pyrophosphorylase in the accumulation process, because a specific protein coupling this enzyme to the loaded carrier might be synthesized during glucose preincubation Also the observation that adenine uptake depends on glucose, too, is no argument against the hypothesis.

since we found that a large part of the external adenine is accumulated in form of hypoxanthine (Fig. 5).

At the moment therefore, at least two possibilities to explain purine accumulation do exist, (i) via the "carrier" coupled to a suitable energy source, and (ii) via the IMP pyrophosphorylase with 5-*P*-Rib-1-*P*-*P* as the source of energy.

To decide initially between these and with the scheme of purine interconversion in view (Fig 8), we have measured the labelling of the hypoxanthine and inosine pool in dependence of [^{14}C]hypoxanthine uptake. The choice of these purine derivatives has been favoured by the observation that *Saccharomyces* always possesses a well-measurable pool of unlabelled hypoxanthine and inosine, whereas the content of IMP, guanine, and guanosine is very low. The results (Fig 9) indicate that the hypoxanthine pool is labelled faster than the inosine pool. This finding clearly disagrees with the assumption of a participation of the IMP pyrophosphorylase in the accumulation process.

However, the localization of hypoxanthine and inosine in distinct compartments of the cell which are extracted altogether by the lyophilization procedure or a

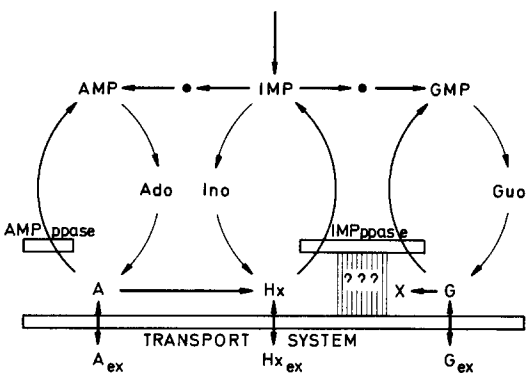


Fig 8 Scheme of purine interconversion The possible interaction of the IMP pyrophosphorylase with the transport system is indicated by question-marks. Purine bases are abbreviated as in Fig 7 Further unusual abbreviations are X (xanthine), Ado (adenosine), Ino (inosine), Guo (guanosine), and ppase (pyrophosphorylase)

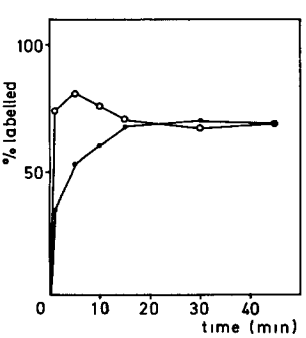


Fig 9 Labelling of hypoxanthine (○) and inosine (●) pool during [^{14}C]hypoxanthine uptake.

degradation of IMP to hypoxanthine by enzymes other than the recycling enzymes 5-nucleotidase and nucleoside phosphorylase possibly might falsify our results Therefore, further investigations including genetic work with mutants affected in the transport and accumulation of purines are necessary for a definitive decision

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