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# THIAMINE TRANSPORT MUTANTS OF *SACCHAROMYCES CEREVISIAE*

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## SUMMARY

Two mutants, PT-R1 and PT-R2, which are resistant to the inhibitory action of pyrithiamine, were isolated by nitrosoguanidine treatment from *Saccharomyces cerevisiae*. They were found to be, respectively, partially and almost totally defective in the thiamine-specific transport system. The mechanism of resistance of the mutants to pyrithiamine is discussed.

## INTRODUCTION

It has been reported that a specific transport system for thiamine is present in baker's yeast (*Saccharomyces cerevisiae*) [1–3]. The transport process is energy and temperature dependent and it displays structural specificity and saturation kinetics.

Pyrithiamine is a potent antimetabolite of thiamine which inhibits not only the growth of *S. cerevisiae*, but also the uptake of thiamine by yeast cells [2]. The existence of such analogs which competitively inhibit transport has been proved useful in the selection of mutants with altered transport properties [4, 5].

In this paper we demonstrate that two mutants of *S. cerevisiae* resistant to pyrithiamine, PT-R1 and PT-R2, were found to be partially and almost totally defective, respectively, in both thiamine and pyrithiamine uptake, suggesting that a common carrier stereospecific for thiamine and the analog is involved in the transport system for thiamine in *S. cerevisiae*.

## MATERIALS AND METHODS

The microorganism used was *S. cerevisiae* obtained as a clonal isolate of commercial baker's yeast (Oriental's). Mutants of *S. cerevisiae* resistant to pyrithiamine (pyrithiamine hydrobromide, Sigma) were isolated by the procedure for mutation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [6].

Growth studies were carried out in 5 ml of thiamine-omitted Wickerham's synthetic medium [7] in the presence and absence of pyrithiamine at 30 °C for 16 h without shaking. Growth was measured turbidimetrically at 560 nm.

The thiamine in yeast cells was extracted by heating at 85 °C for 15 min the cells from 25 ml culture after adjusting the pH to 4.5, and the amount of total thiamine

was estimated by the thiochrome method after Takadiastase hydrolysis [8]. The pyrithiamine transported into the cells was determined as pyrichrome by the same procedure with thiamine as described above. Pyrichrome was determined fluorometrically using a Shimadzu spectrophotofluorometer at 460 nm, with an excitation wavelength of 430 nm [9].

Thiamine pyrophosphokinase was extracted by sonication (10 kcycles, 20 min) of the cells from 300 ml culture in 3 ml of 0.05 M Tris · HCl, pH 7.5, containing 2 mM 2-mercaptoethanol and 1 mM EDTA. The supernatant fluid obtained by centrifugation at 26 000 $\times g$ for 15 min was used as crude extracts. The enzyme activity was measured by the procedure described by Kaziro [10].

The uptake of [$^{14}$C]thiamine was determined by the method previously described [2].

## RESULTS AND DISCUSSION

The effect of several concentrations of pyrithiamine on the growth of the parent and mutant strains of *S. cerevisiae* is shown in Fig. 1. The growth of the parent strain was completely inhibited by pyrithiamine at a concentration of $2 \cdot 10^{-6}$ M, which was completely abolished by addition of thiamine ($1 \cdot 10^{-6}$ M) at the same time as pyrithiamine (data not shown). On the other hand, the growth of *S. cerevisiae* PT-R1 was inhibited about 50 % in the presence of the analog of the same concentration, but the growth of PT-R2 was not inhibited at all by pyrithiamine up to a concentration of $1 \cdot 10^{-5}$ M.

The development of resistance to antimetabolite has sometimes been attributed to overproduction of the metabolite which was caused by derepression of controlled biosynthetic systems or to the loss of regulatory mechanisms. *Escherichia coli* resistant

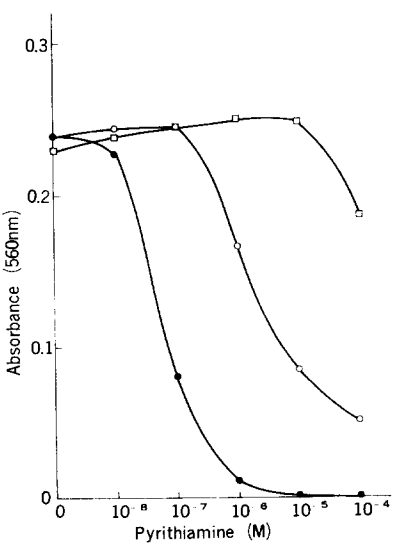


Fig. 1. Effect of pyrithiamine on the growth of parent and mutant strains of *S. cerevisiae*. The cultivation of the yeast and the growth measurement were carried out as described in the text. ●, *S. cerevisiae*; ○, PT-R1; □, PT-R2.

TABLE I

CELLULAR THIAMINE CONTENT

All strains were grown on 25 ml of thiamine-omitted Wickerham's synthetic medium, and the cells were washed once with distilled water, suspended in 0.05 M acetate buffer (pH 4.5). Aliquots of the suspensions were used for the assay of cellular thiamine content as described in the text.

| Strain | Thiamine content (nmol/mg dry weight) |
|---|---|
| *S. cerevisiae* | 0.12 |
| PT-R1 | 0.11 |
| PT-R2 | 0.11 |

TABLE II

THIAMINE PYROPHOSPHOKINASE AND EFFECT OF PYRITHIAMINE

The reaction mixture, in a final volume of 1.5 ml, contained: 1.5 μmol thiamine, 1.5 μmol ATP, 3 μmol $MnSO_4$, 30 μmol Tris · HCl, pH 8.6, and the crude extract (0.6–0.7 mg protein). The values in parentheses are the enzyme activities in the presence of 15 μmol pyrithiamine.

| Strain | Thiamine pyrophosphate formed (n mol/mg protein per h) | |
|---|---|---|
| *S. cerevisiae* | 0.81 | (0.38) |
| PT-R1 | 0.92 | (0.28) |
| PT-R2 | 0.87 | (0.36) |

to pyrithiamine isolated by Kawasaki and Nose [11] has a three times higher content of intracellular thiamine than wild strain, *E. coli* K12. As shown in Table I, however, no evidence could be obtained for the overproduction of thiamine by PT-R1 and PT-R2 as a mechanism of their resistance to pyrithiamine.

It is well-known that pyrithiamine inhibits microbial growth by interfering with thiamine metabolism in the cell. The principal enzyme target of pyrithiamine is thiamine pyrophosphokinase, the $K_i$ of baker's yeast kinase being $8.5 \cdot 10^{-5}$ M [10]. As shown in Table II, the activities of thiamine pyrophosphokinase in the crude extracts from the parent and mutant strains were almost the same, and they were effectively inhibited by pyrithiamine. These results would rule out the possibility that the mechanism of resistance to pyrithiamine may be caused by some altered metabolism of thiamine in mutant cells.

Based on these findings, the effect of mutation on the cell membrane permeability for pyrithiamine was then estimated by comparing initial rates of pyrithiamine uptake by yeast cells. The results are shown in Fig. 2. The parent strain can accumulate pyrithiamine intracellularly, whereas the uptake of pyrithiamine was markedly affected in two mutant strains. The rate of uptake of pyrithiamine in PT-R1 and PT-R2 is 7.0 and 1.3 %, respectively, of that of the parent strain.

Fig. 3 shows the time course of [$^{14}$C]thiamine uptake by *S. cerevisiae*. It can be seen that pyrithiamine-resistant mutants are also deficient in the uptake of thiamine. The rate of thiamine uptake in PT-R1 and PT-R2 is 1.9 and 0.1 %, respectively, of that of the parent strain.

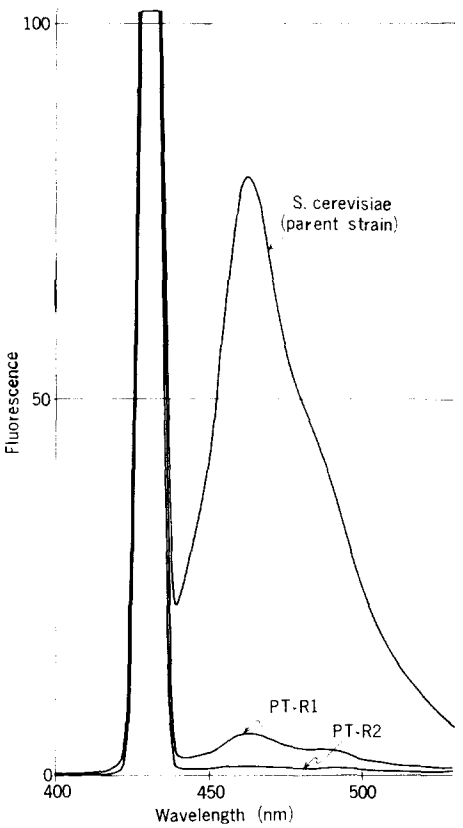


Fig. 2. Uptake of pyrithiamine. 100 ml of yeast cell suspensions (73 $\mu$g dry weight/ml) in 0.05 M potassium phosphate buffer (PH 5.0) containing 0.1 M glucose, were preincubated for 15 min at 37 °C, and then pyrithiamine was added to the medium at 1 $\mu$M concentration, followed by further incubation at 37 °C for 2 min. Pyrithiamine transported into the cells was determined as described in the text.

In bacteria, a mutant of *E. coli* which is defective in thiamine uptake and has higher activity of thiamine pyrophosphate synthesis from thiamine than that of the parent strain has been isolated [12]. On the other hand, a pyrithiamine-resistant mutant of *Staphylococcus aureus* isolated by Sinha and Chatterjee [13] was found to be devoid of thiamine pyrophosphokinase in the cell-free extract. However, the transport of thiamine and pyrithiamine by whole cells was not investigated.

The findings derived from the present studies with *S. cerevisiae* specifically relate mutations to an impairment of transport capacity for pyrithiamine. The rate of uptake of both pyrithiamine and thiamine determined by each mutation revealed a definite correlation with the corresponding resistance properties. It was therefore concluded that pyrithiamine-resistant mutants isolated from *S. cerevisiae* in this experiment are thiamine transport-negative mutants which are defective in a specific transport system both for thiamine and for pyrithiamine. It is supposed that PT-R1 is genetically partially defective in a common component for the transport (probably carrier protein for thiamine transport), which is almost totally lost in PT-R2.

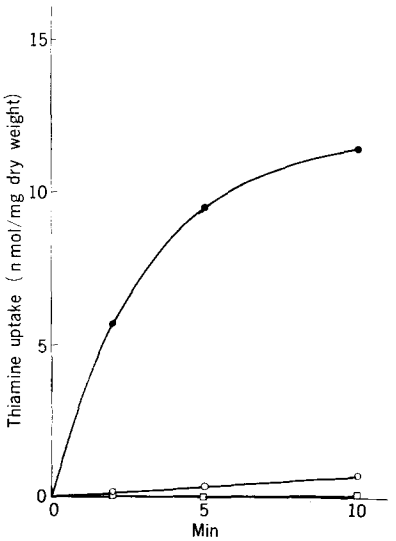


Fig. 3. Uptake of [$^{14}$C]thiamine. 5 ml of yeast cell suspensions (73 $\mu$g dry weight/ml) in 0.05 M potassium phosphate buffer (pH 5.0) containing 0.1 M glucose, were preincubated for 15 min at 37°C, and then [$^{14}$C]thiamine (18.9 Ci/mol) was added to the medium at 1 $\mu$M concentration, followed by further incubation at 37 °C. The uptake of [$^{14}$C]thiamine was measured using a membrane-filter technique [2]. ●, *S. cerevisiae*; ○, PT-R1; □, PT-R2.

Recently we have found a protein in *S. cerevisiae* which specifically binds to thiamine. The intracellular localization and physiological role of this protein concerning thiamine transport are now under investigation.

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