intracellular concentrations of roquefortine and 3,12-dihydroroquefortine make up 300 µg/g and 30 µg/g of dry biomass, respectively.

The dynamics of changes in the concentration of roquefortine and of 3,12-dihydroroquefortine in *P. farinosum* differ from the dynamics of accumulation of these compounds in the culture of *P. roqueforti;* in the latter the maximal quantity of the metabolites under study was observed on the 5th–7th day of growth[2]. Evidently, the difference seen in the time-course of the accumulation of roquefortine and 3,12-dihydroroquefortine is conditioned by distinctive features of the regulation of the biosynthesis and metabolism of these compounds. Specifically, *P. roqueforti* synthesizes an appreciable amount of clavine alkaloids, which appear in the mycelium and culture liquid at the very early stages of cultivation, while in *P. farinosum* they were not found.

The fact that *P. farinosum* does not produce clavine alkaloids makes this culture a convenient object for studying the biosynthesis of roquefortine and related compounds. *P. roqueforti* is an attractive tool for investigations into the simultaneous synthesis of 2 different groups of alkaloids as well as into the possibility of regulation of the directed synthesis of these or other metabolites.

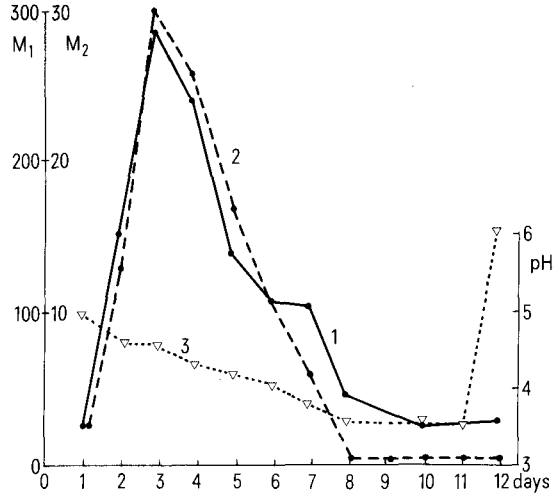

Fig. 2. Changes in contents of roquefortine and 3,12-dihydroroquefortine in *P. farinosum* mycelium and changes in pH during fermentation. *1* M_1, roquefortine (µg/g dry mycelium); *2* M_2, 3,12-dihydroroquefortine (µg/g dry mycelium); *3* changes in pH.

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Interaction of basic dyes with the thiamine transport system in *Saccharomyces cerevisiae*[1]

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Summary. Basic dyes such as methylene blue and triphenyltetrazolium chloride were found to inhibit thiamine transport in *Saccharomyces cerevisiae.* Conversely, the reduction of methylene blue and triphenyltetrazolium chloride by yeast cells was inhibited by thiamine. A thiamine transport mutant of *Saccharomyces cerevisiae* showed decreased utilization of these dyes. From the results, the possibility that the uptake of basic dyes may proceed via a membrane-bound thiamine-binding protein in the thiamine transport system of the yeast is discussed.

It has been demonstrated that the transport of thiamine is *Saccharomyces cerevisiae* occurs by a carrier-mediated active process which is specific for thiamine[2–5]. Experimental inhibition of thiamine transport by various analogs of thiamine suggested that at least the intact pyrimidine moiety of the thiamine molecule is necessary to bind to some components of the transport system[6,7]. Recently, however, evidence suggesting that uptake of dibenzyldimethylammonium (DDA) proceeds via the thiamine transport system of the yeast has been shown[8]. During the course of the study on structural specificity of the yeast thiamine transport system we found that methylene blue inhibits thiamine transport competitively and the dye is effective in abolishing the growth inhibition of *Saccharomyces cerevisiae* by pyrithiamine, which is known to be taken up by a common transport system for thiamine in yeast cells[9].

In this paper we show that basic dyes such as methylene blue and triphenyltetrazolium chloride (TTC) inhibit thiamine transport in *Saccharomyces cerevisiae,* and conversely their utilization by yeast cells is inhibited by thiamine and it is also decreased considerably in a thiamine transport mutant of *Saccharomyces cerevisiae.* Figure 1 shows inhibitory effects of methylene blue, TTC, rhodamine 6GO and safranin O, which are common to be basic dyes, on thiamine transport in *Saccharomyces cerevisiae.* The inhibition was strongest with safranin O among these dyes tested, whereas TTC was less inhibitory.

Among these, both methylene blue and TTC are known to be reduced in living yeast cells. Therefore the reduction of methylene blue during the growth of *Saccharomyces cerevisiae* was examined. During growth without shaking at 30 °C for 20 h, approximately 81% of the methylene blue added (10 µM) was reduced, whereas the presence of 10 µM thiamine in the growth medium was remarkably effective in preventing the reduction of the dye (table 1). Since dimethialium, an analog of thiamine which is also taken up by yeast cells but not converted to the coenzyme form in the cells, showed the same effect, the site of their action appeared to be on the surface of the cell membrane. On the other hand no preventive effect on the reduction of methylene blue was observed with oxythiamine, which is ineffective in inhibiting thiamine transport[3]. DDA at the concentration of 0.1 mM could partly inhibit the utilization of methylene blue by growing yeast.

These results strongly suggest that thiamine and the inhibitors of yeast thiamine transport have a common inhibitory

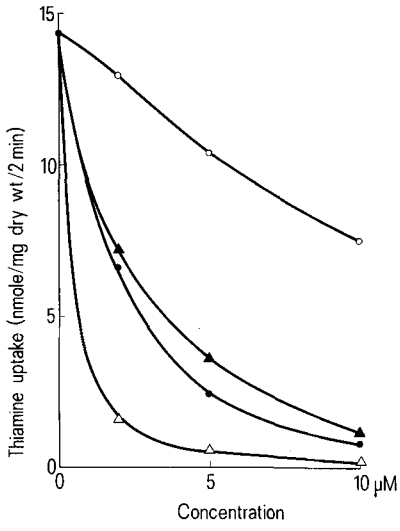

Fig. 1. Inhibitory effect of several basic dyes on thiamine uptake by resting cells of *Saccharomyces cerevisiae*. The growth of *S. cerevisiae* and the uptake studies were carried out as previously reported[5]. 5 ml of yeast suspensions (30 μg dry weight/ml) in 0.05 M potassium phosphate (pH 5.0) containing 0.1 M glucose, were preincubated for 15 min at 37 °C, and then each dye was added to the medium at indicated concentration simultaneously with 1 μM [^{14}C] thiamine, followed by further incubation at 37 °C. ●, plus methylene blue; ○, plus triphenyltetrazolium chloride; ▲, plus rhodamine 6GO; △, plus safranin O.

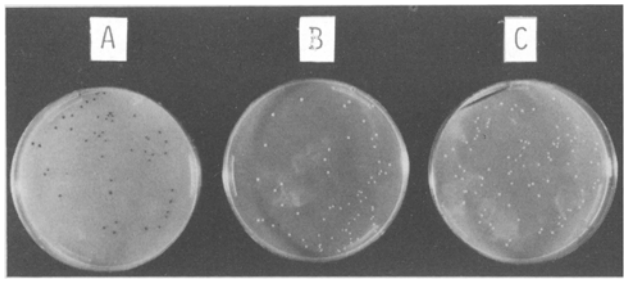

Fig. 2. Reduction of triphenyltetrazolium chloride by yeast cells. Yeast cells were grown on 2% agar plate containing Wickerham's synthetic medium (20 ml) with or without thiamine at 30 °C for 4 days. After then 10 ml of TTC solution dissolved 1.0% agar, 0.1% glucose and 0.005% TTC (1.5×10^{-4} M) was added to the plate, followed by incubation at 30 °C for 30 min. A, *S. cerevisiae;* B, *S. cerevisiae* with 0.1 mM thiamine; C, Thiamine transport mutant (PT-R_2).

effect on the utilization of methylene blue, probably at the step of the uptake of the dye. This speculation seems to be supported by the fact that a thiamine transport mutant of *Saccharomyces cerevisiae* (PT-R_2) is much less active in the reduction of methylene blue. Table 1 also shows that the degree of cell growth does not significantly affect the reduction of the dye by yeast cells.

Figure 2 shows the reduction of TTC by *Saccharomyces cerevisiae* and PT-R_2. Colonies of the yeast grown on an agar plate containing Wickerham's synthetic medium without thiamine colored to red within 30 min after TTC was added to the plate (A). On the other hand the cells grown in the presence of 10 μM thiamine (B) and PT-R_2 (C) remained unchanged after the addition of TTC. These results indicate that TTC is available to *Saccharomyces cerevisiae*, but neither to the cells in the presence of thiamine nor to PT-R_2 cells. Furthermore, this suggests that detection with TTC is applicable not only to the selection of respiratory-deficient mutants[10] but also to the isolation of thiamine transport mutants of yeast.

Table 1. Effect of thiamine and thiamine analogs on reduction of methylene blue by growing yeast cells

Addition	Cell growth (OD at 560 nm)	Methylene blue (OD at 660 nm)
None*	–	0.650
None	0.840	0.125
Thiamine (10 μM)	0.800	0.630
Dimethialium (10 μM)	0.810	0.630
Oxythiamine (10 μM)	0.336	0.098
DDA (0.1 mM)	0.520	0.315
None**	0.675	0.470

Growth studies were carried out using Wickerham's synthetic medium with thiamine omitted as previously described[5]. Growth was measured turbidimetrically at 560 nm[9] and the concentration of methylene blue in the medium was measured spectrophotometrically at 660 nm after the cells were removed by centrifugation. * Inoculation was omitted. ** A thiamine transport mutant (PT-R_2) was inoculated.

Table 2. Effect of methylene blue, triphenyltetrazolium chloride and dibenzyldimethylammonium on membrane-bound thiamine-binding activity in *Saccharomyces cerevisiae*

Addition	Thiamine bound (pmole/mg)	Inhibition (%)
None	34.9	
Methylene blue (10 μM)	18.1	48.2
Methylene blue (0.1 mM)	1.91	94.5
TTC (0.1 mM)	17.5	49.9
DDA (0.1 mM)	11.2	68.0

Membrane-bound thiamine-binding activity was assayed as previously described[7].

It was strongly presumed from the results obtained above that the utilization, probably the uptake, of methylene blue and TTC occur via a common component in the transport system for thiamine. Table 2 shows the effect of the dyes on the binding of thiamine to a yeast membrane fraction in which some protein components of the thiamine transport system may be located. Both methylene blue and TTC, and also DDA, showed inhibitory effects on the thiamine-binding activity in yeast membrane.

In conclusion basic dyes such as methylene blue and TTC were found to inhibit thiamine transport in *Saccharomyces cerevisiae* and may be utilized by yeast cells via the thiamine transport system. Although the mechanism of the interaction of these dyes with the thiamine transport system is unknown, lipophilic and cationic properties of the dyes may be involved in the binding to membrane-bound thiamine-binding protein in the thiamine transport system in *Saccharomyces cerevisiae*.

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