Active transport of dimethialium in *Saccharomyces cerevisiae*

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Summary. Dimethialium, a derivative of thiamine which has a methyl group in place of hydroxyethyl group at the 5-position of the thiazole moiety, was found to be accumulated in nonproliferating cells of *Saccharomyces cerevisiae* by the same transport mechanism for thiamine. The results strongly support the supposition that thiamine as well as dimethialium can be transported and accumulated without obligatory phosphorylation in yeast cells, since dimethialium is not phosphorylated by yeast thiamine pyrophosphokinase.

Evidence has been accumulated with shows that the uptake of thiamine by various living cells occurs by active transport[1,2]. Although the exact nature of energy coupling in thiamine transport system is still unknown, the possible role of phosphorylation of thiamine in transport has been argued[3,4].

Thus, phosphorylation appears to be necessary for the effective accumulation of thiamine in the cell, even though free thiamine can be transported and perhaps even accumulated without obligatory phosphorylation.

Thiamine transport in *S. cerevisiae* is characteristic in that there is extensive accumulation of thiamine, primarily in the free form[5]. However, even in this organism, there is a possibility that thiamine transported is trapped within the cell by its phosphorylation and then free thiamine is accumulated after hydrolysis of thiamine pyrophosphate, since yeast has powerful acid phosphatase.

In order to investigate the uptake of thiamine independently of its phosphorylation, the use of some derivatives of thiamine which is not phosphorylated by living cells is preferable. Dimethialium, 3-2′-methyl-4′-aminopyrimidyl-(5′)-methyl-4,5-dimethyl thiazolium chloride hydrochloride, is a thiamine derivative which was synthesized as an effective anticoccidant[6], and has the same structure as thiamine, except that a hydroxyethyl group at the 5-position of the thiazole moiety was substituted by a methyl group.

Table 1. Effect of several metabolic inhibitors on uptake of dimethialium and thiamine

Addition	Dimethialium uptake nmoles/mg dry weight/2 min (%)	Thiamine uptake
Control	13.5 (100)	15.1 (100)
Monoiodoacetate (0.4 mM)	2.0 (14.8)	1.9 (12.6)
KCN (2 mM)	9.7 (71.9)	9.6 (63.6)
2,4-dinitrophenol (0.4 mM)	1.1 (8.1)	1.7 (11.3)

Yeast cell suspensions (30 µg dry weight/ml) were preincubated for 15 min at 37 °C with or without addition as indicated. Dimethialium or thiamine was added to the medium at 1 µM concentration, followed by further incubation at 37 °C. The uptake of thiamine compounds was measured after 2 min of incubation.

Table 2. Effect of thiamine added to the growth medium on uptake of dimethialium and thiamine

Addition	Dimethialium uptake nmoles/mg dry weight/2 min (%)	Thiamine uptake
None	10.6 (100)	14.3 (100)
Thiamine (1 µM)	0.2 (1.9)	0.5 (3.5)

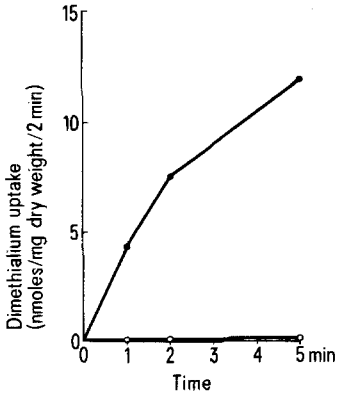
After 18 h of growth in Wickerham's minimal medium with or without 1 µM thiamine, uptake of thiamine compounds was measured.

In this paper we describe that dimethialium can be accumulated in nonproliferating cells of *S. cerevisiae* by the same transport system for thiamine.

Results and discussion. The figure shows a time course of dimethialium uptake by yeast cells. The uptake was rapid at 37 °C, whereas it was insignificant at 0 °C. The intracellular dimethialium concentration after 5 min of incubation was calculated as approximately 8900-fold the external dimethialium concentration from the basis of 2.1 µl of intracellular water per mg of dry yeast[9]. This indicates thast dimethialium was taken up by yeast cells against a large concentration gradient as previously observed with thiamine[5].

Table 1 shows the comparison of the effect of several metabolic inhibitors on the uptake of dimethialium and thiamine. In the absence of the inhibitors, dimethialium uptake was 89.4% of thiamine uptake after 2 min of incubation. Preincubation of yeast cells with monoiodoacetate, potassium cyanide or 2,4-dinitrophenol caused an almost parallel reduction in the amount of dimethialium and thiamine taken up. This result suggests that the process of dimethialium uptake by yeast cells is energy-dependent as that of thiamine.

In a previous paper[10], we reported that thiamine uptake appeared to be subject to regulation by the repression controlled by growth in exogenous thiamine. Therefore, it seemed of interest to examine the effect of thiamine in the growth medium on dimethialium uptake by yeast cells. As shown in table 2, there was a remarkable decrease in the uptake of dimethialium as well as thiamine by yeast cells grown in the presenc of 1 µM thiamine, suggesting that a common transport system for dimethialium and thiamine is



Time course of dimethialium uptake by *S. cerevisiae*. The growth of *S. cerevisiae* and the uptake studies were carried out as previously reported[7]. 5 ml of yeast suspensions (30 µg 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 dimethialium was added to the medium at 1 µM concentration, followed by further incubation at 37 °C (●) and 0 °C (○), respectively. The dimethialium transported into cells was determined fluorometrically as thiochrome by the same procedure with thiamine[8].

repressible by exogenous thiamine. This possibility was finally confirmed since a thiamine transport mutant of *S. cerevisiae,* PT-R2, previously isolated[7], showed no significant uptake of dimethialium.

From the findings obtained above, we conclude that dimethialium, which has the thiamine structure incapable of phosphorylation by thiamine pyrophosphokinase, is accumulated in yeast cells by an energy-dependent process mediated by the same carrier for thiamine. These results strongly suggest that thiamine can be transported and accumulated without obligatory phosphorylation in *S. cerevisiae.*

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Biochemical changes in mango after infection with *Rhizoctonia bataticola*

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Summary. Rhizoctonia bataticola is responsible for the spoilage of mango fruits *(Mangifera india)* during post-harvest preservation and storage. Culture of *R. bataticola* exhibited significant pectinase and cellulase activity. In *Rhizoctonia*-infected fruits an increase of protease and cellulase activity, and a decrease in certain enzymes of carbohydrate metabolism, were observed in comparison to healthy fruits.

Post-harvest preservation, transit and marketing of mango fruits is a challenging problem. Earlier we reported the spoilage of mangoes by *Rhizoctonia bataticola*[1,2]. The present study focuses on biochemical changes after the infection process. An understanding of the physiology of the pathological process may help to develop methods for control.

The preparation of cell-free extracts and the determination of protein were performed as described earlier[3]. Ketoacids were estimated according to the method of Friedemann[4]. Assay methods for aconitase and isocitrate dehydrogenase were as described by Anfinsen[5] and Ochoa[6] respectively. Protease and cellulase activities were assayed by the methods of Ong and Gaucher[7] and Miller[8] respectively, whereas malate dehydrogenase and succinate dehydrogenase were measured according to Ochoa[9] and Slatter and Bonner[10] respectively. Pectinase was determined according to the method of McCready and McComb[11] and aldolase according to Sibley and Lehninger[12]. The mould *R. bataticola* was found to be responsible for the development of blackspot on *Alphonso mango.* An important problem in pathogenesis is the biochemical mechanism of penetration by degradation and solubilization of cell walls of plant tissues in different stages of disease. The encrusting and sheathing substances of plant cell walls such as cutin, suberin, lignin, pectin, protein and cellulose can all be degraded or modified by enzymes of a number of microbial pathogens. It has been shown in apple rot caused by *Penicillium expansum* and *Sclerotinia fructigena*[13] that polygalacturonase and macerating enzymes are important for pathogenesis. Furthermore, several soft rot pathogens like *Pythium* and *Rhizopus* are highly cellulolytic[14,15]. Cellulase activity has also been reported by Bateman[16,17] in bean hypocotyl tissue infected by *Rhizoctonia solani. R. bataticola* from mango tissues also showed considerable pectinase and cellulase activity when grown in the synthetic medium supplemented with carboxy methyl cellulose (table 1). When healthy and affected mango tissues were analysed for cellulase activity, about 4 times more acctivity was observed in black spotted tissues compared with healthy tissues (table 2).

Earlier[1] we reported the accumulation of citrate in mango tissues infected by *R. bataticola.* This accumulation may be due to an increase in the activity of citrate synthase or a decrease in the activity of citrate catabolizing enzymes like aconitase and isocitrate dehydrogenase. It is known that the aconitase reaction is reversible and in equilibrium at pH 7.0, with a citrate:cis aconitate:isocitrate ratio of 90:4:6.

Table 1. Cellulase and pectinase activities from culture filtrates of *R. bataticola*

Enzymes	Enzyme activity (units/100 ml flask)
Cellulase (CMCase)	5.0
Pectinase	46.9

Table 2. Biochemical changes in healthy and infected tissues of ripe mango fruit

Biochemical changes	Mango tissue	
	Healthy	Infected
Aconitase	65.20	6.90
Isocitrate dehydrogenase	14.80	ND
Succinate dehydrogenase	1.90	1.20
Malate dehydrogenase	326.00	4.90
fructose-diphosphate aldolase	197.50	18.90
Protease	9.20	21.50
Carboxymethyl cellulase	6.80	27.80
Monocarboxylic keto acids	7.70	10.30
Dicarboxylic keto acids	8.70	13.50

ND: not detectable. Enzyme activities are expressed as units/mg protein; organic acids are expressed as mg/100 g pulp.