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Effect of tunicamycin on thiamine transport in *Saccharomyces cerevisiae*

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The activity of thiamine transport in *Saccharomyces cerevisiae* was decreased by the treatment with tunicamycin without affecting the growth of yeast cells. Although the total activity of a soluble thiamine-binding protein in yeast periplasm, which is known to be a glycoprotein, was decreased by tunicamycin treatment, the activity of thiamine uptake by yeast protoplasts was inhibited as much as by whole cells. Furthermore, tunicamycin decreased the activity of the membrane-bound thiamine-binding protein in a dose dependent way and in parallel with the thiamine transport activity. These findings suggested that the membrane-bound thiamine-binding protein is a glycoprotein which plays a functional role in thiamine transport in *S. cerevisiae*.

Previous studies have shown that *Saccharomyces cerevisiae* accumulates a large amount of thiamine in the cells by an active transport system [1,2]. It was also found that there exist soluble and membrane-bound thiamine-binding proteins in yeast cells and they appeared to be repressed by exogenous thiamine with a concomitant decrease in the thiamine transport in *S. cerevisiae* [3–5]. The soluble thiamine-binding protein has been purified and characterized to be a glycoprotein present in yeast periplasm [4,5], but the role of this protein in the thiamine transport has been still unknown.

In this paper, the effects of tunicamycin, an antibiotic that blocks the glycosylation of glycoproteins [6], on the activities of thiamine-binding proteins and thiamine transport in *S. cerevisiae*, were investigated to clarify the functions of these proteins in the thiamine transport in yeast.

[^{14}C]Thiamine ([*thiazole*-2-^{14}C]thiamine hydrochloride, 24.3 Ci/mol) was purchased from Amersham International (U.K.). Tunicamycin was the product of Sigma Chemical Co. All other chemicals were purchased from commercial suppliers. The microorganism used was *Saccharomyces cerevisiae* obtained as a clonal isolate of commercial baker's yeast Company (Orientals).

TABLE I

EFFECT OF TUNICAMYCIN OR CYCLOHEXIMIDE ADDED TO THE MEDIUM ON THE YEAST GROWTH AND THIAMINE TRANSPORT

S. cerevisiae was grown at 30°C for 18 h in 500 ml of thiamine-omitted Wickerham's synthetic medium [8]. The yeast cells were washed and suspended in the same medium freshly prepared (500 ml) with an absorbance at 560 nm of 0.20. After shaking at 30°C for 2 h with or without either of antibiotics, [^{14}C]thiamine uptake and cell density were measured as previously described [1].

Addition	Growth (A_{560nm})	Thiamine transport	
		nmol/min per mg dry wt.	%
None (before incubation)	0.20	4.75	100
None (after incubation)	0.30	6.28	132.2
Tunicamycin (5 μg/ml)	0.29	2.91	61.3
Cycloheximide (0.2 μg/ml)	0.22	4.74	99.8

Yeast cells harvested in the early log phase of the growth were suspended in the freshly prepared medium and incubated with shaking at 30°C for 2 h. As shown in Table I, the incubation caused a 1.3-fold increase in the specific activity of the thiamine transport in *S. cerevisiae*. Under this condition, the addition of tunicamycin to the medium (5 μg/ml) not only induced a complete inhibition of the increase in the transport activity, but also reduced the basal transport activity of the cells by 38.7%. However, the growth of yeast cells incubated with tunicamycin was almost the same as that of the untreated control cells. On the other hand, cycloheximide (0.2 μg/ml) caused an almost complete inhibition of the growth and of the increase of the thiamine transport activity, but the basal transport activity was fully retained even after the incubation for 2 h. These results suggested that some protein components involved in the thiamine transport was newly synthesized during the incubation and tunicamycin inhibited their post-translational modification, probably glycosylation, resulting in the decrease of the thiamine transport in *S. cerevisiae*. Furthermore, the fact that the basal thiamine transport activity was not affected by cycloheximide but markedly decreased by tunicamycin during the incubation may suggest that the glycosylation reaction is involved in the formation and recycling of functional thiamine transport proteins in *S. cerevisiae*.

It has been reported that secretion of glycoproteins such as acid phosphatase and invertase present in yeast periplasm is completely inhibited by tunicamycin [7]. Since the soluble thiamine-binding protein is a periplasmic glycoprotein, the effects of tunicamycin on the thiamine-binding activity of this protein and the thiamine transport activities of whole cells and protoplasts of *S. cerevisiae* were examined. As shown in Tables II and III, tunicamycin (5 μg/ml) inhibited both total thiamine-binding activity of the soluble thiamine-binding protein secreted into the peri-

TABLE II

EFFECT OF TUNICAMYCIN ON SOLUBLE THIAMINE-BINDING PROTEIN

Yeast cells were treated with or without tunicamycin as described in Table I. Then 10 ml of the shock fluid prepared from the same number of cells ($1.55 \cdot 10^{10}$ cells) by an osmotic shock treatment as previously reported [4] was used for the assay of thiamine-binding activity. Thiamine-binding activity was measured by an equilibrium dialysis as previously described [3].

Addition	Total binding activity (pmol thiamine bound)	Total protein (mg)
None	439.2	0.37
Tunicamycin (5 μg/ml)	263.1	0.25

TABLE III

EFFECT OF TUNICAMYCIN ON THIAMINE TRANSPORT IN YEAST PROTOPLASTS AND WHOLE CELLS

Yeast cells were treated with or without tunicamycin as described in Table I. Then protoplasts prepared using Zymolyase-5000 and cellulase-Onozuka R-10 were tested for [^{14}C]thiamine uptake. The preparation of protoplasts and the assay of thiamine transport in yeast whole cells and protoplasts were carried out as previously reported [9].

Addition	% Uptake	
	protoplasts	whole cells
None	100	100
Tunicamycin (5 μg/ml)	54.1	46.4

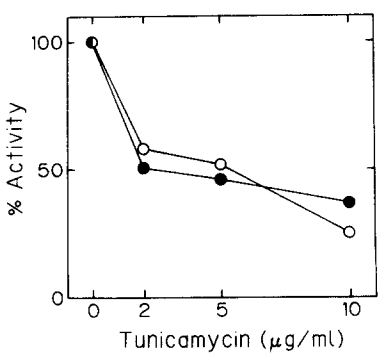

Fig. 1. Effects of various concentrations of tunicamycin on thiamine transport and binding activity of the membrane fraction of *S. cerevisiae*. After the yeast cells were treated with or without tunicamycin as described in Table I, thiamine transport (●) and binding activity of the membrane fraction (○) were measured. The membrane fraction was prepared by the method previously described [5]. The specific thiamine-binding activity of the membrane fraction was 32.2 pmol/mg protein at pH 5.0. Each value is the mean of three separate determinations.

plasm and thiamine transport activity of whole cells to about the same extent. However, the antibiotic also inhibited the thiamine transport in protoplasts which are free from periplasmic proteins. These results indicated that tunicamycin treatment might affect membrane component(s) of the thiamine transport system in *S. cerevisiae*. Therefore, the effect of tunicamycin on the membrane-bound thiamine-binding protein was examined. As shown in Fig. 1, the activities of the thiamine transport and membrane-bound thiamine-binding protein were inhibited in parallel by tunicamycin. Previous report from this laboratory [5] showed that several properties of the thiamine transport system in *S. cerevisiae* are similar to those of the membrane-bound thiamine-binding protein suggesting that the protein is involved in the thiamine transport system. From the results obtained in this study with tunicamycin, it was presumed that the membrane-bound thiamine-binding protein is a glycoprotein sensitive to tunicamycin as well as the soluble thiamine-binding protein and it directly participates in thiamine transport in *S. cerevisiae*.

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