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Effect of thiamin on cordycepin sensitivity in *Saccharomyces cerevisiae*

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The sensitivity of *Saccharomyces cerevisiae* to the antibiotic cordycepin (3'-deoxyadenosine) was found to be decreased by the addition of thiamin to the growth medium. A thiamin transport mutant of *S. cerevisiae* was also found to be resistant to the growth inhibitory effect of cordycepin. Not only the thiamin uptake but also adenosine uptake by this mutant cell was markedly reduced compared to those by the parent yeast cells. This strongly suggested that cordycepin, an analog of adenosine, is virtually taken up by the thiamin transport system of growing yeast cells; thus the drug sensitivity is decreased by the presence of thiamin in the growth medium.

Cordycepin; Thiamin; 3'-Deoxyadenosine; *Saccharomyces cerevisiae*

1. INTRODUCTION

The antibiotic cordycepin (3'-deoxyadenosine) is an analog of adenosine which preferentially inhibits cellular nucleic acid synthesis [1]. It has been reported that some microorganisms are sensitive to inhibition by the drug (e.g. *Bacillus subtilis*), while others are drug-resistant (e.g. yeast) [2]. However, we have recently found that *S. cerevisiae* is sensitive to cordycepin when it is grown in thiamin-free minimal growth medium. In this paper we describe that cordycepin sensitivity of yeast observed in the growth medium free of thiamin is decreased by the addition of low concentrations of thiamin to the growth medium, and a thiamin transport mutant of *S. cerevisiae* is resistant to the growth inhibitory effect of this drug. This suggests that the uptake of the drug by growing yeast cells is affected by the vitamin. Evidence showing that adenosine, and probably cordycepin, share a common transport system with thiamin in growing cells of *S. cerevisiae* is also presented.

2. MATERIALS AND METHODS

2.1. *Organisms and growth conditions*

S. cerevisiae obtained as a clonal isolate of commercial baker's yeast (Oriental's) and a thiamin transport mutant of *S. cerevisiae* (PT-R2) isolated by the procedure previously described [3] were used. Mutants resistant to cordycepin were isolated after treatment with ethylmethane sulphonate [4]. For growth studies the yeasts were incubated at 30°C for 24 h, without shaking, in 5 ml thiamin-free Wickerhams minimal medium [5] in the presence and absence of cordycepin or other drugs. Growth was measured as the optical density at 560 nm.

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2.2. *Assay of [^{14}C]adenosine uptake*

The yeasts were grown at 30°C for 24 h, without shaking, in 5 ml thiamin-free minimal medium in the presence of 10 μM [^{14}C]adenosine (9,774 dpm/nmol). After incubation, the cells in 1.0 ml were quantitatively filtered on a nitrocellulose filter (0.65 μm pore size), followed by one wash with 10 ml of 0.05 M potassium phosphate buffer (pH 5.0). The filters were immediately removed from the suction apparatus, dried, and put into scintillation vials containing 10 ml scintillation fluid, and radioactivity was measured. The incorporation of [^{14}C]adenosine into the acid-insoluble fraction was measured similarly, except that the yeast cells grown with 10 μM [^{14}C]adenosine were collected on the filters and treated with 10 ml cold 5% trichloroacetic acid for 10 min and washed twice with 10 ml cold 5% trichloroacetic acid; the filters were dried and the radioactivity was measured as described above. The [^{14}C]adenosine uptake was expressed as nmol [^{14}C]adenosine (mg dry weight)$^{-1}$.

2.3. *Assay of [^{14}C]thiamin uptake*

The uptake of [^{14}C]thiamin was determined by the method previously described [6].

2.4. *Chemicals*

[^{14}C]adenosine ([8-^{14}C]adenosine, 1.85 GBq/mmol) and [^{14}C]thiamin ([thiazole-2-^{14}C]thiamin hydrochloride, 899 MBq/mmol) were purchased from Amersham International (UK). Cordycepin was the product of Sigma Chemical Co. All other chemicals were purchased from commercial suppliers.

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of cordycepin on the growth of *S. cerevisiae*. The growth of *S. cerevisiae* in the synthetic complete (SC) medium which was used by Anderson et al. [2], was inhibited for 73% by this drug at a concentration of 0.1 mM and the IC_{50} (concentration required for 50% growth inhibition) of cordycepin was 38 μM (Fig. 1). On the other hand, *S. cerevisiae* grown in thiamin-free Wickerham's minimal medium was found to be much more sensitive to cordycepin (IC_{50}: 90 nM) than that grown in SC medium. This suggests that SC medium contains a substance that reduces cordyce-

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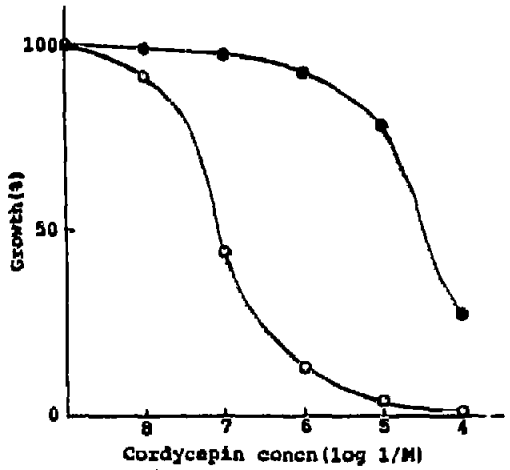

Fig. 1. Inhibition by cordycepin of growth of *S. cerevisiae*. The yeasts were grown at 30°C for 24 h, without shaking, in 5 ml SC medium (●) and thiamin-free Wickerham's minimal medium (○) containing the indicated concentrations of cordycepin. Growth was measured as the optical density at 560 nm and the value in the absence of cordycepin was expressed as 100%. Each point represents the mean of two independent experiments.

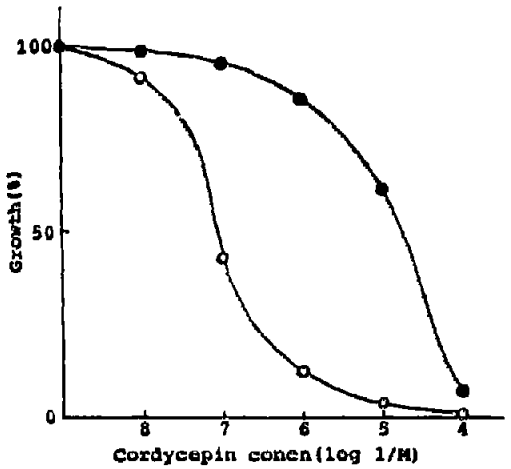

Fig. 2. Effect of cordycepin on growth of a thiamin transport mutant of *S. cerevisiae*. The growth of *S. cerevisiae* (○) and a thiamin transport mutant (PT-R2) (●) in 5 ml thiamin-free Wickerham's minimal medium containing the indicated concentrations of cordycepin was measured as described in the legend for Fig. 1. Each point represents the mean of two independent experiments.

pin sensitivity in *S. cerevisiae*. To identify this substance, we supplemented the minimal medium systematically with compounds that are present in the SC medium but not in the minimal medium. The growth inhibition of *S. cerevisiae* in minimal medium containing 10 μM cordycepin was almost completely restored by the addition of 1 μM thiamin, which is included in the SC medium at approximately 4 μM (Table I). No other compound was found to make the growing yeast sensitive to cordycepin. Neither adenosine nor 2′-deoxyadenosine was effective at 100-fold concentrations higher than cordycepin.

In order to determine the site of thiamin action on the cordycepin sensitivity in *S. cerevisiae* the effect of cordycepin on the growth of a thiamin transport mutant

Table I

Effect of thiamin on the growth inhibition of *S. cerevisiae* by cordycepin

Addition (μM)	Growth (OD_{560})
None	0.700
Cordycepin (10)	0.040
+Thiamin (0.1)	0.170
+Thiamin (1.0)	0.600
+Adenosine (1,000)	0.040
+2′-deoxyadenosine (1,000)	0.040

The yeast was grown at 30°C for 24 h, without shaking, in 5 ml thiamin-free Wickerham's minimal medium in the absence or presence of 10 μM cordycepin. Thiamin, adenosine, or 2′-deoxyadenosine was added to the culture medium at the indicated concentrations. Growth was measured as the optical density at 560 nm. Values are averages of two independent experiments.

of *S. cerevisiae* (PT-R2), which was found to be almost totally defective in thiamin uptake [3], was then investigated. As shown in Fig. 2 the mutant was more resistant to cordycepin than the parent strain. The IC_{50} of cordycepin in this mutant was 21 μM, which was 233-fold greater than that of the parent strain. These findings strongly suggested that cordycepin is taken up by a common transport system with thiamin in the growing yeast cells; thus thiamin can reduce the sensitivity of *S. cerevisiae* to the drug. However, cordycepin at higher concentrations, appeared to enter the yeast cells by another transport mechanism, probably diffusion, since thiamin was no longer effective to restore the growth inhibition induced by 0.1 mM cordycepin (data not shown) and the growth of PT-R2 was inhibited by cordycepin at 1 μM concentrations or above (Fig. 2).

Since the findings described above strongly suggest that the effect of thiamin is related to the cellular availability of cordycepin by growing yeast we examined the uptake of adenosine, an analog of cordycepin, by growing cells of *S. cerevisiae* in the presence of thiamin. The [^{14}C]adenosine uptake was decreased dose-dependently by the addition of thiamin to the culture medium (Table II), and PT-R2 was found to have a markedly reduced adenosine uptake, which indicated that adenosine, probably cordycepin, is virtually taken up by the thiamin transport system of growing yeast cells. The phenomenon that adenosine and thiamin share a common transport system in growing cells of *S. cerevisiae* is interesting, and some enzyme reactions common to nucleotides and thiamin phosphates have been reported in mammalian tissues [7,8]. Among the nucleosides other than adenosine, only cytidine was found to be taken up

Table II

Effect of thiamin on the uptake of [^{14}C]adenosine by growing cells of *S. cerevisiae*

Addition (μM)	[^{14}C]adenosine uptake (nmol/mg dry weight)
None	11.5
+Thiamin (0.03)	5.70
+Thiamin (0.1)	1.63
+Thiamin (1.0)	0.47
None*	1.0

Assay of [^{14}C]adenosine uptake by growing cells of *S. cerevisiae* was carried out as described in section 2. Thiamin was added to the growth medium at the indicated concentrations. Values are averages of two independent experiments.

*A thiamin transport mutant of *S. cerevisiae* (PT-R2) was used.

Table III

Some properties of mutants of *S. cerevisiae* resistant to cordycepin

	Parent strain	CD-R1	CD-R2
IC_{50} of cordycepin	90 nM	7.9 μM	240 μM
[^{14}C]thiamin uptake (%)	100.0	1.8	97.8
[^{14}C]adenosine uptake (%)	100.0	10.9	136.5
Incorporation into acid-insoluble fraction in total uptake (%)	73.2	–	30.2

Assay of [^{14}C]thiamin and [^{14}C]adenosine uptake by yeast cells was carried out as described in section 2. Values are averages of two independent experiments.

by the thiamin transport system of growing yeast cells, but its uptake was only 5.9% of that of adenosine.

Among several nucleoside-type antibiotics tested, other than cordycepin, formycin A and tubercidin, which are adenosine-type antibiotics, were found to have inhibitory activity on yeast growth cultured in Wickerham's minimal medium, but they were less effective than cordycepin. The IC_{50} of formycin A and tubercidin was approximately 0.4 μM and 8 μM, respectively. These values were increased to approximately 70 μM and more than 1 mM, respectively, when PT-R2 was used as a test organism (data not shown). These findings show that both formycin A and tubercidin are also taken up by the thiamin transport system in growing yeast cells and their inhibitory effects on yeast growth can be alleviated by thiamin.

We further isolated two mutants, CD-R1 and CD-R2, which are resistant to the inhibitory action of cordycepin, by methylethane sulphonate treatment from *S. cerevisiae*. Table III shows some properties of these two mutants. CD-R1 and CD-R2 were about 88- and 2670-fold more resistant to cordycepin than their parent strain, respectively. Since CD-R1 had markedly reduced activities of both thiamin and adenosine uptakes, its resistance to cordycepin was thought to have resulted from an impairment in the uptake of cordycepin by this mutant as observed in PT-R2. PT-R2 was selected by the use of pyrithiamin, an antagonist of thiamin [3]. However, the antibiotic cordycepin is also applicable to isolate thiamin transport mutants from *S. cerevisiae*. On the other hand, CD-R2 could take up both thiamin and adenosine just as well as the parent strain, but there was a marked difference in the distribution of [^{14}C]adenosine taken up by growing cells between the parent strain and CD-R2; the incorporation of [^{14}C]adenosine into the acid-insoluble fraction in the parent yeast cells was 73.2% the total uptake, whereas it was only 30.2% in the mutant cells. These findings suggest that the incorporation of adenosine into RNA, namely the conversion of cordycepin into a more active form [1] than the free nucleoside itself, is impaired in CD-R2, resulting in the high resistance of this mutant to cordycepin. However, the precise biochemical lesion in CD-R2 and the mechanism of its resistance to the drug remains to be clarified.

It is finally concluded that the cordycepin sensitivity in *S. cerevisiae* is decreased by thiamin in the growth medium, since the antibiotic and thiamin share a common transport system in the growing yeast cells. Thus, not only the presence of thiamin in the growth medium but also the thiamin transport activity of this organism can affect the cordycepin sensitivity in *S. cerevisiae*.

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