Occurrence of a novel yeast enzyme, L-Lysine ϵ -dehydrogenase, which catalyses the first step of lysine catabolism in *Candida albicans*

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The yeast *Candida albicans* is able to utilize L-lysine as the sole nitrogen and carbon source accompanied by intracellular accumulation of α -aminoadipate- δ -semialdehyde. A novel yeast amino acid dehydrogenase catalysing the oxidative deamination of the ϵ -group of L-lysine was found in this yeast. The enzyme, L-lysine ϵ -dehydrogenase, is strongly induced in cells grown on L-lysine as the sole nitrogen source. The enzyme is specific for both L-lysine and NADP⁺. The K_m values were determined to be 0.87 mM for L-lysine and 0.071 mM for NADP⁺. An apparent M_r of 87 000 was estimated by gel filtration. The enzyme has maximum activity at pH 9.5 and a temperature optimum of 32 °C under our assay conditions.

Introduction

Both the biosynthesis and the catabolism of L-lysine show considerable variation from one life form to another. This amino acid is biosynthesized via two mutually exclusive pathways and no fewer than eight distinct catabolic routes have been proposed for animals, fungi and bacteria. In yeasts, two enzymic steps to initiate lysine degradation have been described so far. In *Rhodotorula glutinis* (Kinzel *et al.*, 1983) and *Pichia guilliermondii* (Schmidt *et al.*, 1988a) the first step of lysine catabolism is the conversion of this amino acid to α -aminoadipate- δ -semialdehyde by L-lysine ϵ -amino-transferase. In *Hansenula saturnus* (Rothstein, 1965), *Yarrowia lipolytica* (Gaillardin *et al.*, 1976) and *Candida maltosa* (Schmidt *et al.*, 1988b), lysine is catabolized via acetylated intermediates.

We present here evidence for the occurrence of a third initial step of lysine catabolism in *Candida albicans* caused by a novel yeast amino acid dehydrogenase that catalyses the oxidative deamination of the ϵ -amino group of L-lysine.

Methods

Strain and growth conditions. *C. albicans* SBUG 182 is a strain from our laboratory and was grown in minimal salt medium (Tanaka *et al.*, 1967) supplemented with biotin (1 mg l⁻¹) and thiamin (1 mg l⁻¹), at 30 °C on a rotary shaker (100 ml medium in 500 ml flasks, 100 r.p.m.).

Enzyme preparation. Cells were harvested and subsequently washed with distilled water. The washed cells were resuspended in 100 mM-

Tris/HCl buffer (pH 9.5) and disrupted by passing them twice through an X-pressure cell (110 MPa). Crude extract was obtained after removal of cell debris by centrifugation at 20 000 g for 20 min. To purify the enzyme, the broken yeast cells were resuspended in 100 mM-Tris/HCl buffer (pH 9.5) containing 1 mM-dithioerythritol, 10 mM-L-lysine and 2.25 M-ammonium sulphate, and the homogenate was centrifuged. The supernatant was chromatographed on a Sephadex G-200 column (1.6 × 50 cm), equilibrated and eluted with the same buffer. Fractions of 0.7 ml were collected at a flow rate of 15 ml h⁻¹. The pooled enzyme fractions were used to determine some properties of the enzyme. The L-lysine ϵ -dehydrogenase was purified sevenfold from the original extract with a recovery of 80%. Under standard conditions the enzyme expressed a specific activity of 1.4 nkat (mg protein)⁻¹.

Enzyme assay. The dehydrogenase activity was determined at 30 °C by measuring the rate of increase in the absorbance at 340 nm. The standard reaction mixture consisted of 10 mM-L-lysine, 1 mM-NADP⁺, 100 mM-Tris/HCl buffer (pH 9.5) and enzyme solution in a final volume of 1.0 ml.

L-lysine aminotransferase and L-lysine acetyltransferase activity were determined according to Schmidt *et al.* (1988a, b).

Protein concentration in enzyme extracts was determined by the Lowry method using bovine serum albumin as standard.

Lysine and α -aminoadipate- δ -semialdehyde determination. Lysine concentration in the medium was determined according to a previously described method (Vogel & Shimura, 1971), while α -aminoadipate- δ -semialdehyde was determined according to Sagisaka & Shimura (1962).

Results

C. albicans SBUG 182 was tested for its ability to utilize L-lysine as a sole source of carbon or nitrogen. Results of growth experiments demonstrated that the yeast can use L-lysine in both ways. At the same molar nitrogen

concentration of L-lysine and ammonium (5 mM) no significant difference between the growth rates was observed ($\mu = 0.32 \text{ h}^{-1}$) but at the same carbon concentration (1%) the growth rate of the yeast with glucose was five times higher than with L-lysine as the sole carbon source. Similar to *R. glutinis* (Kinzel *et al.*, 1983) and *P. guilliermondii* (Schmidt *et al.*, 1988a), growth of *C. albicans* with lysine as the nitrogen source was accompanied by intracellular accumulation of α -aminoadipate- δ -semialdehyde [$0.4 \text{ mmol (g dry wt)}^{-1}$]. In the presence of lysine as the sole carbon source no semialdehyde accumulation was found.

On the basis of these findings we tested the activities of lysine aminotransferase and lysine acetyltransferase, two enzymes known to be involved in the first step of lysine catabolism in other yeasts, but neither of these could be detected. Only when L-lysine was incubated with the crude extract of *C. albicans* in the presence of NADP^+ or NAD^+ , was the synthesis of α -aminoadipate- δ -semialdehyde observed. The reaction proceeded linearly as a function of the amount of enzyme and incubation time (Fig. 1). Both reaction products, NADPH and α -aminoadipate- δ -semialdehyde, were determined at the same concentration in the assay, indicating an ϵ -dehydrogenase reaction.

The dehydrogenase activity was normally present in only small amounts in *C. albicans* cells grown in minimal medium, but activity was much higher in extracts from cells grown in the presence of L-lysine (Table 1). In cells grown in medium containing L-lysine as the sole nitrogen source, the enzyme activity was increased more than 21-fold in comparison with that reached in cells grown in minimal medium. In the presence of cycloheximide no noticeable increase of the dehydrogenase activity was observed.

We followed the time course of both enzyme activity and lysine utilization of *C. albicans* cells grown with lysine as the sole nitrogen source. As shown in Fig. 2, the dehydrogenase activity strongly increased during growth and the maximum enzyme level was achieved between 12 and 20 h of growth; thereafter the enzyme activity decreased rapidly during further incubation.

To characterize the enzyme activity, cells were harvested after 16 h cultivation in medium containing L-lysine as the sole nitrogen source and the crude extract was chromatographed on Sephadex G-200. The dehydrogenase activity was very sensitive to preparation procedures and could only be stabilized in the presence of ammonium sulphate, L-lysine and dithioerythritol. The enzyme eluted as one peak from the column and an M_r of 87000 ± 5000 was determined. Catalase, alcohol dehydrogenase, bovine serum albumin and ovalbumin served as calibration proteins.

The enzyme had its maximum activity around pH 9.5;

Fig. 1. Effect of incubation time on the activity of lysine ϵ -dehydrogenase in crude extract of cells grown in medium with ammonium as the sole nitrogen source. Both α -aminoadipate- δ -semialdehyde (filled symbols) and NADPH concentration (open symbols) were measured in the presence of 2.8 mg protein ($\square$, $\blacksquare$) and 5.6 mg protein ($\circ$, $\bullet$) in the assay.

Fig. 2. Time course of lysine ϵ -dehydrogenase activity ($\circ$) from *C. albicans* and lysine concentration in the medium ($\bullet$). Cells were first grown for 16 h in minimal medium with 40 mM-ammonium as the sole nitrogen source, then transferred to medium with 5 mM-L-lysine as the sole nitrogen source and incubated at 30 °C.

50% of the maximum activity was achieved at pH 7.0 and 11.5.

The dehydrogenase reaction was carried out at various temperatures in the standard reaction system. The reaction velocity increased linearly with temperature from 15 to 28 °C. Maximum activity was found at 32 °C and the activation energy calculated from Arrhenius plots was 22.1 kJ mol^{-1} .

The amino acid specificity of the enzyme was studied using the standard assay system, in which the com-

Table 1. Level of lysine ϵ -dehydrogenase in *C. albicans* incubated for 9 h in minimal medium of different composition

Carbon source (1%, w/v)	Nitrogen source	Supplement	Enzyme activity* (pkat mg ⁻¹)
Glucose	40 mM-Ammonium	—	10
Glucose	40 mM-Ammonium	1 mM-L-Lysine	18
Glucose	40 mM-Ammonium	5 mM-L-Lysine	48
Glucose	40 mM-Ammonium	10 mM-L-Lysine	65
Glucose	5 mM-L-Lysine	—	215
Glucose	5 mM-L-Lysine	0.1 mM-Cycloheximide	14
L-Lysine	40 mM-Ammonium	—	79
Glucose	5 mM-L-Leucine	—	12
Glucose	5 mM-L-Ornithine	—	14

* Crude extract was used.

Table 2. Substrate specificity of lysine ϵ -dehydrogenase

Substrate* (10 mM)	Relative activity† (%)			
	Uninduced cells		Induced cells	
	Crude extract	Gel filtration eluate	Crude extract	Gel filtration eluate
L-Lysine	100	100	100	100
D-Lysine	0	0	0	0
S-(β -Aminoethyl)-L-cysteine	66	48	65	42
δ -Hydroxylysine	38	25	40	21
L-Leucine	20	20	20	21
L-Isoleucine	5	4	10	5
L-Ornithine	9	10	12	14

* None of the following amino acids could be used as substrate: L-histidine, L- α -aminoadipate, β -alanine, L-alanine, L-arginine, L-valine, L-phenylalanine, L-tryptophan, L-tyrosine, L-glutamate, L-aspartate, L-proline, L-serine, L-threonine, L-methionine. Only the gel filtration eluate was used.

† The specific activities were: 10 pkat mg⁻¹ (uninduced cells, crude extract), 55 pkat mg⁻¹ (uninduced cells, gel filtration eluate), 205 pkat mg⁻¹ (induced cells, crude extract), 1400 pkat mg⁻¹ (induced cells, gel filtration eluate).

pounds in question were substituted for L-lysine, usually at the same concentration (10 mM). Table 2 shows the specificity of the enzyme obtained from induced and uninduced cells, both before and after gel filtration. The highest activity was obtained with L-lysine. The enzyme was also able to convert lysine derivatives [*S*-(β -aminoethyl)-L-cysteine, δ -hydroxylysine] and L-leucine or L-ornithine, but to a lesser extent.

The enzyme required NADP⁺ as a natural coenzyme for the oxidative deamination of L-lysine. NAD⁺ could also serve as coenzyme, but the enzyme activity in the presence of 1 mM-NAD⁺ was only a third of the activity with 1 mM-NADP⁺.

Apparent K_m values for L-lysine and NADP⁺ were determined either by varying concentrations of L-lysine at a fixed concentration of NADP⁺ or by varying concentrations of NADP⁺ at a fixed concentration of L-lysine. The double reciprocal plots were linear, indicating normal Michaelis-Menten kinetics, and the K_m values for L-lysine and NADP⁺ were determined to be 0.87 mM and 0.071 mM, respectively (not shown).

The effect of different potential effectors on the enzyme activity was tested. As shown in Table 3, the enzyme was inhibited completely by *p*-chloromercuribenzoate and HgCl₂. Some divalent metal ions also decreased the activity, but to a lesser extent.

Table 3. Influence of potential effectors on activity of lysine ϵ -dehydrogenase

Effector (1 mM)	Relative activity* (%)
None	100
CaCl ₂	89
p-Chloromercuribenzoate	0
CoCl ₂	33
CuCl ₂	24
EDTA	93
FeCl ₂	100
HgCl ₂	0
KCN	95
MgCl ₂	100
MnCl ₂	29
NaN ₃	78
SnCl ₂	100

* Gel filtration eluate was used.

Discussion

The results reported in this study show that *C. albicans* is capable of catabolizing L-lysine as the sole nitrogen and carbon source by a novel dehydrogenase that converts L-lysine to α -amino adipate- δ -semialdehyde. This compound is probably converted to α -amino adipate, 2-oxoadipate and glutarate via a pathway similar to that of *Pseudomonas aeruginosa* (Fothergill & Guest, 1977). The high derepression of the L-lysine ϵ -dehydrogenase activity from yeast cells grown in lysine-containing medium indicates that the enzyme plays an important role in the catabolism of lysine in *C. albicans*. The levels of enzyme derepression in cells grown with lysine as the sole nitrogen source and in cells grown with lysine as the sole carbon source were different and corresponded with the yeast growth rate in the media. In addition, the absence of any other enzyme activities of lysine degradation suggests that this dehydrogenase reaction represents the first step in degradation of L-lysine in this yeast. A significant derepression by lysine of lysine aminotransferase and lysine acetyltransferase, the first enzymes of lysine degradation in *R. glutinis*, *P. guilliermondii* and *C. maltosa*, respectively, was also observed (Kinzel *et al.*, 1983; Schmidt *et al.*, 1988a, b).

L-Lysine ϵ -dehydrogenase has not been demonstrated in any eukaryote, but the enzyme activity was found in some bacteria (Misono & Nagasaki, 1983). The highest activity was obtained in *Agrobacterium tumefaciens*. The enzyme from this organism was purified and characterized in detail (Misono & Nagasaki, 1982; Misono *et al.*, 1989). In contrast to the enzyme from *C. albicans*, the *A. tumefaciens* enzyme was specific for NAD⁺ and did not

react with NADP⁺. In addition to L-lysine, only S-(β -aminoethyl)-L-cysteine showed reactivity (2.9%) with the *A. tumefaciens* enzyme. The dehydrogenase from *C. albicans* is able to deaminate lysine derivatives as well as ornithine and leucine. Since the enzyme was not purified to homogeneity, other dehydrogenase activities can not be excluded. The latter two amino acids, however, were unable to derepress the lysine ϵ -dehydrogenase activity (Table 1). Therefore, a physiological role of deamination of both ornithine and leucine by lysine ϵ -dehydrogenase is probably excluded.

The basic kinetic properties of the dehydrogenase obtained here (L-lysine K_m 0.87 mM; NADP⁺ K_m 0.071 mM) are comparable with those reported for the enzyme from *A. tumefaciens* (L-lysine K_m 1.5 mM; NAD⁺ K_m 0.06 mM). The pH optimum of the enzyme reaction and the behaviour of the enzyme from both sources with mercury-containing compounds are also similar. The M_r of the native enzyme from *C. albicans* was determined to be 87000, which is somewhat different from that found for the *Agrobacterium* dehydrogenase (78000).

It is interesting to note that among 28 yeast species (17 genera), which were studied with respect to their enzyme recruitment, besides *C. albicans* only *Kluyveromyces marxianus* possessed lysine ϵ -dehydrogenase activity (T. Hammer, unpublished). This suggests that L-lysine ϵ -dehydrogenase is not widely distributed among yeasts.

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