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PROPERTIES OF REVERTANTS OF lys2 AND lys5 MUTANTS AS WELL AS α-AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE FROM SACCHAROMYCES CEREVISIAE

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α -Aminoadipate-semialdehyde dehydrogenase catalyzes the conversion of α-aminoadipate to α-aminoadipate-semialdehyde in the biosynthetic pathway of lysine in yeasts and molds. Mutants belonging to lys2 and lys5 loci of Saccharomyces cerevisiae lacked the α-aminoadipate-semialdehyde dehydrogenase activity. Complementation in vitro was demonstrated by combining the extracts from different lys2 and lys5 mutants. Some of the revertants of lys2 and lys5 mutants exhibited lower specific activity and higher thermolability of α-aminoadipate-semialdehyde dehydrogenase than the enzyme from wild-type cells. The enzyme was partially purified from wild-type cells and the molecular weight of the enzyme was estimated on a Sephacryl S-300 column at 180,000. Results from the revertant analysis and in vitro complementation indicated LYS2 and LYS5 as structural genes, each encoding a subunit of this large enzyme.

α-Aminoadipate-semialdehyde dehydrogenase (EC 1.2.1.31; commonly known as α-aminoadipate reductase) catalyzes, in the presence of ATP, Mg^{2+}, and NADPH, the conversion of α-aminoadipate to α-aminoadipate-semialdehyde in the biosynthetic pathway of lysine in yeasts and other higher fungi (1,2). A three-step scheme for the overall reaction was proposed which involved: (a) an ATP and Mg^{2+} dependent adenylation of α-aminoadipate, (b) an NADPH and Mg^{2+} dependent reduction of δ-adenyl- α-aminoadipate, and (c) hydrolysis of the adenyl group to yield α-aminoadipate-semialdehyde (3,4) (Figure 1).
The formation of α-aminoadipate-adenylate, a novel intermediate, has been demonstrated in vitro and in vivo (3-5). An adenylate formation prior to the reduction of an intermediate is unique among the biosynthetic pathways (1,6). The molecular properties of the enzyme which catalyzes this unique and complex reaction remains to be elucidated.

Mutations in the LYS2 gene mapped on chromosome II or in the LYS5 gene mapped on chromosome VII of Saccharomyces cerevisiae (7) result in a total or partial loss of α-aminoadipate-semialdehyde dehydrogenase activity (8,9). Two genes are also required for this step in Yarrowia lipolytica and Schizosaccharomyces pombe (10,11), whereas only one has been identified to date in Neurospora crassa (12). Mutations at the LYS2 gene of S. cerevisiae are obtained with a much higher frequency than at the LYS5 (13,14). The LYS2 gene has been implicated as a structural gene (14,15). However, nothing is known regarding the role of the LYS5 gene. The cloned LYS2 gene is localized in a 4.2 Kb DNA insert with a transcript size of 3.9 Kb (16,17) and the LYS5 gene has been cloned on a 7.5 Kb DNA insert of the recombinant plasmid pSC5 (18). Complementation of mutant extracts in vitro and the properties of enzyme from revertants are very important methods to determine the structural genes of a

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α-Aminoadipate $\xrightarrow{ATP,\ Mg^{2+}}$ δ-Adenyl-α-aminoadipate

δ-Adenyl-α-aminoadipate $\xrightarrow{NADPH}$ δ-Adenyl-α-aminoadipate-semialdehyde

δ-Adenyl-α-aminoadipate-semialdehyde $\xrightarrow{H_2O}$ α-aminoadipic-semialdehyde

Fig. 1 Proposed steps for the conversion of α-aminoadipate to α-aminoadipate-semialdehyde by α-aminoadipate-semaldehyde dehydrogenase.

heteropolymeric enzyme (19,20). We report here the results of such investigations of lys2 and lys5 mutants as well as the molecular properties of the partially purified α-aminoadipate-semialdehyde dehydrogenase.

Methods

Wild-type S. cerevisiae x2180 and lysine auxotrophs P49 lys2 and AB9 lys5 were obtained from the Yeast Genetics Stock Center. Additional lys2 and lys5 strains were derived from wild-type cells following UV mutagenesis (13) and slow-growing revertants of lys2 and lys5 mutants were selected on minimal agar medium. Cell extracts were prepared and assayed for α-aminoadipate-semialdehyde dehydrogenase activity according to published procedure (21,22). Protein was estimated by the biuret method (23). In vivo temperature sensitivity was determined by growing wild-type and revertant cells at permissive (30°C) and nonpermissive (37°C) temperatures. In vitro temperature sensitivity of α-aminoadipate-semialdehyde dehydrogenase was determined by preincubating the cell extracts for 0 to 60 min at 40°C and samples were placed on ice prior to being assayed for residual α-aminoadipate-semialdehyde dehydrogenase activity. Extract maintained at 4°C served as the control. The enzyme from wild-type S. cerevisiae was partially purified through several steps using published procedures (22) and the molecular weight of the enzyme was estimated by gel filtration chromatography (24) on a Sephacryl S-300 (Pharmacia) column calibrated with proteins of known molecular weight (thyroglobulin, 669,000; ferritin, 440,000; catalase, 232,000; and aldolase, 158,000).

Results and Discussion

To determine whether both LYS2 and LYS5 gene-products contribute to the enzyme activity, in vitro complementation and revertant analysis of the mutants were performed. Although crude extract from P49 lys2 or AB9 lys5 failed to exhibit α-aminoadipate-semialdehyde dehydrogenase activity individually, mixtures of the two extracts at appropriate concentrations yielded a small but reproducible amount of enzyme activity (Table 1). In vitro complementation was also demonstrated between the extracts of SR36 lys2 and AB9 lys5 as well SR36 lys2 and X4004-3A lys5. Control experiments, in which the extracts were not mixed or the substrate (α-aminoadipate) was excluded from the assay mixture, failed to demonstrate any activity.

Two lys2+ revertants, 8211-7bR5 and 8211-7bR9, derived from 8211-7b lys2 mutant, exhibited temperature sensitivity by their inability to grow at 37°C in the absence of lysine supplementation (Table 2). These revertants also demonstrated no detectable α-aminoadipate-semialdehyde dehydrogenase activity in vitro, possibly due to enzyme instability. The revertants 3-45R1b, derived from 3-45 lys2, and 3-211R9, derived from 3-211 lys5 mutant, demonstrated significantly lower enzyme activity than wild-type cells. Strain 3-45R1b also had a temperature sensitive mutation unrelated to its lysine requirement. Both strains demonstrated increased α-aminoadipate-semialdehyde dehydrogenase thermolability in vitro (Fig. 2). The enzyme obtained from 3-211R9 lys5+ was completely inactivated by preincubation at 40°C for 60 min, and that

Table 1. In vitro complementation between crude extracts of lys2 and lys5 auxotrophs

Extract, ul[1]		Aminoadipate	
P49, lys2	AB9, lys5	(substrate)	Sp. Act.[2]
0 +	0		
135	15	+	0.64 +/- 0.32
120	30	+	0.87 +/- 0.03
100	50	+	0.89 +/- 0.04
75	75	+	0.68 +/- 0.09
0	150	+	0
75	75	-	0

[1]Protein adjusted to 10 mg/ml
[2]Aminoadipate-semialdehyde dehydrogenase activity (umol /min/mg x 10^{-2})
+/- standard deviation of three independent determinations

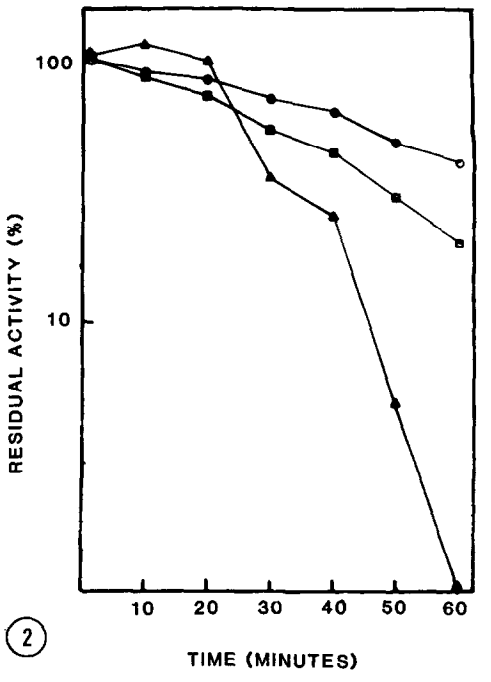
from 3-45R1b lys2+ demonstrated less than 50% of the activity observed in wild-type extract following exposure to 40°C for 60 min. The enzyme from wild-type cells was also thermolabile to a lesser extent. The reduced specific activity and increased thermosensitivity of the enzyme suggested that these revertants produced an enzyme with properties distinct from wild-type enzyme. Results of in vitro complementation among different mutants and revertant analyses are consistent with the hypothesis that both LYS2 and LYS5 encode different subunits of α-aminoadipate-semialdehyde dehydrogenase.

α-Aminoadipate-semialdehyde dehydrogenase from wild-type S. cerevisiae cells was purified using DEAE-cellulose, gel-filtration, hydroxylapatite, and Reactive Red-120 agarose column chromatographic procedures (22) to a single activity peak. However, the preparation was not homogenous based on analysis by nondenaturing PAGE (25). Poor yield and instability of the enzyme following storage at 30°C, 4°C, -20°C, and -70C, as well as in low-ionic strength environments precluded further purification. Addition of 10 mM 2-mercaptoethanol and protease inhibitors such as 5 mM EDTA and 1 mM phenylmethlsulfonyl fluoride to the crude extract improved the stability of the enzyme somewhat but altered the elution profile of the enzyme from the columns employed for purification. Purified enzyme activity eluted with a Kav

Table 2. Lysine dependent temperature sensitivity and the α-aminoadipate-semialdehyde dehydrogenase activity of the lys2 and lys5 revertant strains

		Growth in				
		MM[1]		MML[2]		
Strain	Genotype	30°C	37°C	30°C	37°C	Sp. Act.[3]
X-2180	wildtype	+	+	+	+	0.28
8211-7b	lys2	-	-	+	+	0
8211-7bR5	lys2+	+	-	+	+	0
8211-7bR9	lys2+	+	-	+	+	0
3-45	lys2	-	-	+	+	0
3-45R1b	lys2+	+	-	+	-	0.16
3-211	lys5	-	-	+	+	0.01
3-211R9	lys5+	+	+	+	+	0.05

[1]Minimal medium.
[2]Minimal medium supplemented with 20 ug lysine/ml
[3]Aminoadipate-semialdehyde dehydrogenase activity (umol /min/mg)



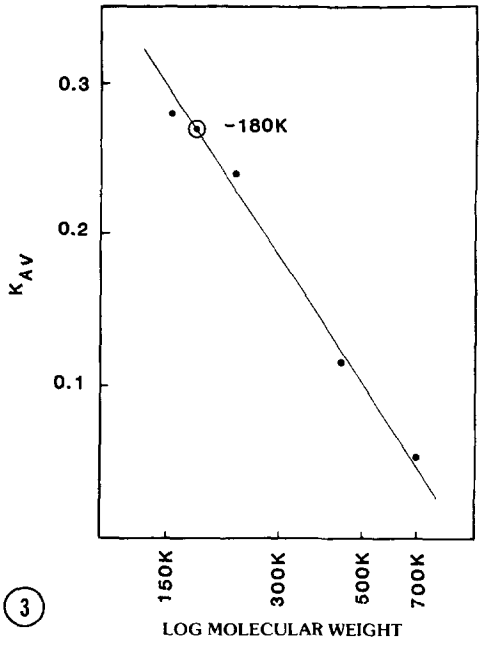


Fig. 2 Thermosensitivity of α-aminoadipate-semialdehyde dehydrogenase from lys2$^+$ and lys5$^+$ revertants. Crude extracts from wildtype X-2180 (●-●), 3-45R1b lys2$^+$(◇-◇) and 3-211R9 lys5$^+$ (△-△) were pre-incubated at 40°C for the time indicated. The extracts were then assayed for residual α-aminoadipate-semialdehyde dehydrogenase activity at 30°C.

Fig. 3 Determination of the native molecular weight of α-aminoadipate-semialdehyde dehydrogenase. The K_{av} for several marker proteins (●) was determined and plotted against their known molecular weights. The molecular weight of α-aminoadipate-semialdehyde dehydrogenase (○) was then extrapolated from the standard curve.

of 2.7 corresponding to a molecular weight of 180,000 upon chromatography on a calibrated Sephacryl S-300 column (Fig. 3). The enzyme activity eluted in the void volume when chromatographed on Sephadex G-200 Superfine (Pharmacia) column, reinforcing the large molecular weight of the enzyme estimated on the basis of Sephacryl, S-300 column chromatography.

Large molecular weight of the enzyme may account for the need of two different (LYS2 and LYS5) structural genes. Since the cloned LYS2 gene has been localized in a 4.2 Kb DNA insert, which elicits a 3.9 Kb transcript (17), it could encode a large polypeptide of 150,000 molecular weight and LYS5 gene could encode a much smaller polypeptide of a heteropolymeric enzyme. A large molecular weight (160,000) of this enzyme has also been reported from Candida maltosa (26). An increased frequency of mutation in the LYS2 gene would be consistent with its relatively large size and transcript. Results presented here demonstrate for the first time the molecular weight of the novel enzyme, α-aminoadipate-semialdehyde dehydrogenase, encoded by two different genes (LYS2 and LYS5) of S. cerevisiae. This unique enzyme and the α-aminoadipate pathway could serve as a valuable marker for phylogenetic studies and the rapid detection and possible control of opportunistic fungal pathogens in immunocompromised patients.

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