Lysine Biosynthesis Pathway and Biochemical Blocks of Lysine Auxotrophs of *Schizosaccharomyces pombe*

ZHI-HAI YE AND J. K. BHATTACHARJEE*

Department of Microbiology, Miami University, Oxford, Ohio 45056

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The α -aminoadipate (AA) pathway for the biosynthesis of lysine was investigated in the wild type and in lysine auxotrophs of the fission yeast *Schizosaccharomyces pombe*. Of the eight enzyme activities of the AA pathway that have been examined so far, six were present in the extract of wild-type *S. pombe* cells. Growth response to AA and accumulation studies indicated that three lysine auxotrophs, the *lys2-97*, *lys4-95*, and *lys8-1* strains, were blocked before the AA step and that four lysine auxotrophs, the *lys1-131*, *lys3-37*, *lys6-3*, and *lys7-2* strains, were blocked after the AA step. Among the mutants investigated, the *lys2-97* mutant exhibited an enzyme lesion at the *cis*-homoaconitate hydratase step, the *lys1-131* and *lys7-2* mutants exhibited lesions at the AA reductase step, and *lys3-37* exhibited a lesion at the saccharopine dehydrogenase step. These results demonstrated the basic similarity of the AA pathway in *S. pombe* and *Saccharomyces cerevisiae*.

Two distinct biosynthetic pathways exist for lysine. The diaminopimelic acid pathway is present in bacteria, green plants, and some phycocyanetes (5, 22). The α -aminoadipate (AA) pathway is present only in blue-green algae and fungi (except certain phycocyanetes) (15, 22). The AA pathway has been investigated in detail in *Saccharomyces cerevisiae* (1, 16, 21) and *Saccharomycopsis lipolytica* (7, 8) and to a lesser extent in *Neurospora crassa* (11), *Rhodotorula glutinis* (13), and *Candida maltosa* (19). Eight enzyme steps (Fig. 1) and more than eight gene functions are required for the AA pathway of *S. cerevisiae* (2, 21). However, the presence of the AA pathway has never been demonstrated in the fission yeast, *Schizosaccharomyces pombe*. Besides cell division by fission, *S. pombe* exhibits a more typical eucaryotic cell cycle, the ability to accurately excise introns of higher eucaryotes, and only three linkage groups (6, 12, 17). Because the AA pathway is present in fungi, this pathway can be used as a selective target for the rapid detection and control of pathogenic yeasts and molds. The lysine mutants of *S. pombe* have been classified into nine complementation groups, and the representatives of several complementation groups have been mapped on linkage group I (10, 14). However, the mutants have never been analyzed biochemically. We report here for the first time the presence of the enzymes of the AA pathway in wild-type *S. pombe* and the biochemical blocks of lysine auxotrophs.

S. pombe strains used in this investigation are shown in Table 1. Strains were maintained on YEPD (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; distilled water to a final volume of 1 liter; agar, 20 g) medium. Minimal medium (MM) used in growth studies and the determination of enzyme activities consisted of Difco yeast nitrogen base without amino acids (6.7 g), glucose (10 g), and distilled water to a final volume of 1 liter. Lysine (30 μ g/ml), DL-AA (60 μ g/ml), or pipercolic acid (60 μ g/ml) was added to MM, when appropriate, for the growth of auxotrophs.

Crude enzyme preparations were obtained by disruption of pre-stationary-phase cells in a Brawn model MSK mechanical cell homogenizer (4, 18). Protein concentrations in cell extracts were determined by the biuret method (9). Lysine biosynthetic enzymes, homocitrate synthase (EC

4.1.3.21), *cis*-homoaconitate hydratase (EC 4.2.1.36), homoisocitrate dehydrogenase (EC 1.1.1.87), α -aminoadipate reductase (AA reductase) (EC 1.2.1.31), saccharopine reductase (EC 1.5.1.10), and saccharopine dehydrogenase (EC 1.5.1.7), were assayed by the procedures described for *S. cerevisiae* (20, 23). Accumulation of α -aminoadipate-semialdehyde (ASA) in lysine auxotrophs was determined by the addition of *p*-dimethylaminobenzaldehyde in 2-methoxyethanol to an equal volume of the culture supernatant (3). The mixture was boiled for 10 min and centrifuged, and the A_{460} of yellow *p*-dimethylaminobenzaldehyde adduct was determined as described previously (18). Accumulation of α -ketoadipate was determined by the previously published procedure (23).

Seven of the nine lysine auxotrophs of *S. pombe* grew in lysine-supplemented MM and not in MM (Table 2). Two of the auxotrophs, the *lys5-391* and *lys9-1* mutants, grew in MM and were not investigated further. Among the seven mutants, the *lys2-97*, *lys4-95*, and *lys8-1* mutants grew in MM supplemented with AA whereas the *lys1-131*, *lys3-37*, *lys6-3*, and *lys7-2* mutants failed to grow in AA-supplemented MM. Also, none grew in MM supplemented with pipercolic acid (1). Growth studies indicated that three of the mutants were blocked in the first half of the pathway, before the AA step, and four were blocked in the second half of the pathway, after the AA step (Fig. 1). Accumulation of ASA and

TABLE 1. *S. pombe* strains used in this study

Trophic level and strain	Genotype	Source
Prototrophs		
972h ⁻	h ⁻ , <i>LYS</i>	G. Lehman
975h ⁺	h ⁺ , <i>LYS</i>	G. Lehman
Auxotrophs		
<i>lys1-131</i>	h ⁻ , <i>lys1</i>	G. Lehman
<i>lys2-97</i>	h ⁻ , <i>lys2</i>	A. Nasim
<i>lys3-37</i>	h ⁻ , <i>lys3</i>	A. Nasim
<i>lys4-95</i>	h ⁻ , <i>lys4</i>	G. Lehman
<i>lys6-3</i>	h ⁻ , <i>lys6</i>	G. Lehman
<i>lys7-2</i>	h ⁻ , <i>lys7</i>	A. Nasim
<i>lys8-1</i>	h ⁻ , <i>lys8</i>	A. Nasim

* Corresponding author.

TABLE 2. Growth responses in three kinds of media and accumulation of ASA in the wild type and in lysine auxotrophs of *S. pombe*^a

Strain	OD ₅₅₀ ^b			Accumulation of ASA ^c (A ₄₆₀)
	MM	MM + AA	MM + lysine	
972h ⁻	1.2	1.1	1.2	0.0
<i>lys2-97</i>	0.04	0.40	1.2	ND
<i>lys4-95</i>	0.05	0.55	1.2	ND
<i>lys8-1</i>	0.20	0.70	1.2	ND
<i>lys1-131</i>	0.03	0.03	1.2	0.1
<i>lys3-37</i>	0.09	0.08	1.2	1.5
<i>lys6-3</i>	0.09	0.09	1.2	2.5
<i>lys7-2</i>	0.06	0.06	1.2	0.0

^a *S. pombe* wild type and lysine auxotrophs were grown for 48 h in MM alone or MM supplemented with AA or lysine, and optical density was determined for up to 72 h.

^b OD₅₅₀, optical density at 550 nm.

^c Lysine auxotrophs blocked after the AA step were grown in MM supplemented with lysine, and accumulated ASA was determined in the culture supernatant. ND, Not determined.

α -ketoadipate was determined to further characterize the biochemical blocks of specific mutants. Among the mutants blocked in the second half, the *lys3-37* and *lys6-3* mutants exhibited significant accumulation of ASA compared with the *lys1-131* and *lys7-2* mutants and wild-type cells (Table 2). None of the mutants blocked in the first half of the pathway exhibited α -ketoadipate accumulation.

Six of the enzymes of the AA pathway, homocitrate synthase, *cis*-homoaconitate hydratase, homoisocitrate dehydrogenase, AA reductase, saccharopine reductase, and saccharopine dehydrogenase were present in the extracts of wild-type 972h⁻ cells (Table 3). Enzymes of the first half of the pathway were assayed in mutants blocked before the AA step, and those of the second half of the pathway were assayed in mutants blocked after the AA step. Among the mutants blocked in the first half of the pathway, the *lys2-97* mutant exhibited significantly lower *cis*-homoaconitate hydratase activity compared with the other two mutants and wild-type cells. Among the mutants blocked in the second half of the pathway, the *lys1-131* and *lys7-2* mutants exhibited little or no AA reductase activity and *lys3-37* exhibited significantly reduced saccharopine dehydrogenase activity (Table 3; Fig. 1). No mutants had a reduced level of saccharopine reductase activity.

On the basis of this investigation, it has been established that the AA pathway is used by *S. pombe* for the biosynthesis of lysine. This conclusion is based on the results of growth responses of *S. pombe* lysine auxotrophs to AA-supplemented MM; amounts of ASA accumulated in specific lysine auxotrophs; presence of six lysine biosynthetic enzyme activities in wild-type cells, including the first (homocitrate synthase) and last (saccharopine dehydrogenase) enzyme activities; and the lack of specific enzyme activity in the lysine auxotrophs studied here. Pipecolic acid is converted to lysine in *R. glutinis* but not in *S. pombe* (1, 13).

FIG. 1. Lysine biosynthetic pathway and the biochemical blocks of lysine auxotrophs of *S. pombe*. Acetyl CoA, Acetyl coenzyme A.

TABLE 3. Lysine biosynthetic enzyme activities of the wild type and of lysine auxotrophs of *S. pombe*

Strain ^a	Enzyme activity					
	Homocitrate synthase ^b	Homoaconitate hydratase ^c	Homoisocitrate dehydrogenase ^d	AA reductase ^e	Saccharopine reductase ^f	Saccharopine dehydrogenase ^g
972h ⁻	0.2	9.3	2.2	6.8	9.6	10.4
<i>lys2-97</i>	0.3	0.5	3.2			
<i>lys4-95</i>	0.3	5.6	2.3			
<i>lys8-1</i>	0.4	9.8	2.2			
<i>lys1-131</i>				0.1	8.8	11.7
<i>lys3-37</i>				6.0	10.5	0.4
<i>lys6-3</i>				5.7	12.2	14.6
<i>lys7-2</i>				0.0	9.2	12.3

^a Wild-type cells were grown in MM, and lysine auxotrophs were grown in MM supplemented with 30 μ g of lysine per ml; extracts were prepared and assayed for specific activities.

^b Homocitrate synthase activity as A₄₁₂ per hour per milligram of protein.

^c Homoaconitate hydratase activity as A₂₄₀ per minute per milligram of protein.

^d Homoisocitrate dehydrogenase activity as A₅₂₀ per hour per milligram of protein.

^e AA reductase activity is expressed as A₄₆₀ per hour per milligram of protein.

^f Saccharopine reductase activity is expressed as A₄₆₀ per hour per milligram of protein.

^g Saccharopine dehydrogenase activity is expressed as 10⁻² A₃₄₀ per minute per milligram of protein.

Among the mutants blocked in the first half of the AA pathway, the lesions of the *lys4-95* and *lys8-1* mutants remain to be determined; among those blocked in the second half of the pathway, the lesion of the *lys6-3* mutant remains to be determined. The latter mutant accumulates large amounts of ASA; therefore, the biochemical block must be after the ASA step. Since the *lys1-131* and *lys7-2* mutants are blocked at the AA reductase step and the *lys3-37* mutant is blocked at the saccharopine dehydrogenase step (Fig. 1), the *lys6-3* mutant could be blocked in the forward reaction of saccharopine reductase and not in the reverse reaction. Because of the lack of availability of substrate for the forward reaction, saccharopine reductase was assayed for the reverse reaction. It is possible that the forward and reverse reactions of saccharopine reductase of *S. pombe* are catalyzed by two different enzymes. However, this is not the case for *S. cerevisiae* (1, 20). The *lys1* and *lys7* mutants of *S. pombe* are blocked at the AA reductase step. It is important to note that two mutants, *lys2* and *lys5*, of *S. cerevisiae* are also blocked in the AA reductase step (3). Results of this investigation demonstrate the presence of the AA pathway in *S. pombe* and biochemically characterize *S. pombe* lysine auxotrophs.

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