Biosynthesis of Lysine in *Saccharomyces cerevisiae*: Regulation of Homocitrate Synthase in Analogue-Resistant Mutants

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INTRODUCTION

Homocitrate synthase (EC. 4.1.3.21), the first enzyme of the 2-aminoadipic acid pathway for biosynthesis of lysine, is feedback inhibited and repressed by L-lysine in *Neurospora crassa* (Hogg & Broquist, 1968). There are two homocitrate synthases in *Saccharomyces cerevisiae*; both of them are feedback inhibited by L-lysine, but only one is repressed by L-lysine (Tucci & Ceci, 1972). 5-Hydroxy-DL-lysine (DL-hydroxylysine) inhibits the growth of wild-type *S. cerevisiae*, and the enzymes of lysine biosynthesis are non-coordinately repressed by L-lysine or DL-hydroxylysine (Sinha, Kurtz & Bhattacharjee, 1971). This paper compares the regulation of homocitrate synthase and the excretion of lysine from the wild-type strain and from mutant strains of *S. cerevisiae* resistant to the analogues of lysine, DL-hydroxylysine and to S-(3-aminoethyl)-L-cysteine (L-thialysine).

METHODS

Organisms and growth conditions. Thialysine-resistant strain AEC-R1, hydroxylysine-resistant strain OHL-1A, lysine auxotroph 46952 (*lys13*), and wild-type *S. cerevisiae* (x2180) were used. Stock cultures were maintained on a complete medium containing: glucose, 20 g; MgSO₄·7H₂O, 1 g; KH₂PO₄, 2 g; peptone, 6 g; yeast extract, 4 g; agar, 25 g; distilled water, 1 l. The pH value was adjusted to 5.5 and the medium was sterilized by autoclaving for 15 min at 121 °C. Cells for growth studies and enzyme preparations were grown in shaken cultures at 30 °C in minimal medium containing: Difco yeast nitrogen base without amino acids, 6.7 g; glucose, 10 g; distilled water, 1 l. This medium was supplemented with L-lysine or an analogue of lysine as required.

Enzyme preparation. Cells from the late-exponential growth phase were disrupted with a Bronwill MSK homogenizer (Bronwill Scientific Co., San Mateo, California, U.S.A.). Homogenates were centrifuged at 14000 g at 1 °C for 20 min and the supernatant solutions were dialysed against 100 vols 0.02 M-Tris/HCl buffer pH 8.0 at 8 °C for 16 h with three changes of buffer. Dialysed extracts were used as the source of enzyme (Sinha & Bhattacharjee, 1970). Protein content was determined by the biuret method using crystalline bovine serum albumin as a standard (Gornall, Bardawill & David, 1949).

Measurement of homocitrate synthase. The method was based on that described by Wieland, Weiss & Eger-Neufeldt (1964) for citrate synthase except that oxaloacetate was substituted by 2-oxoglutarate. The reaction mixture contained (in 1 ml): 2-oxoglutarate, 100 µM; acetyl-CoA, 35 µM; 2,6-dichlorophenolindophenol, 35 µM; Tris/HCl buffer pH 8.0, 100 mM; and dialysed extract equivalent to 1 mg protein. These concentrations of substrates gave maximum activity. The reaction was initiated by adding the enzyme. Where applicable, potential inhibitors were added to the reaction mixture before adding the enzyme. The 2-oxoglutarate-dependent cleavage of acetyl-CoA and the subsequent reduction

of 2,6-dichlorophenolindophenol was measured spectrophotometrically at 600 nm using a recording spectrophotometer. Activity was expressed as nmol product formed min^{-1} (mg protein^{-1}).

Isolation of analogue-resistant mutants. Wild-type strain x2180 was inoculated into minimal medium supplemented with 10^{-3} M L-thialysine or DL-hydroxylysine. Growth occurred after 5 to 6 days of shaking at 30 °C. The cells were then diluted and plated on to minimal agar medium and subsequently replica-plated on to minimal medium supplemented with 10^{-3} M L-thialysine or DL-hydroxylysine. Representative colonies capable of growth after 24 h in medium supplemented with the appropriate analogue were selected as thialysine-resistant (AEC-RI) or hydroxylysine-resistant (OHL-IA) mutants.

Lysine excretion. Three Erlenmeyer flasks, each containing 48 ml minimal medium supplemented with 5 μCi of DL-2-amino[1- ^{14}C]adipic acid (20 mCi mmol^{-1}), were inoculated with 2 ml starter culture of strains x2180, AEC-RI and OHL-IA, respectively. Radioactivity from 2-amino[1- ^{14}C]adipic acid is selectively incorporated into lysine (Bhattacharjee & Tucci, 1969). Flasks were shaken at 30 °C for 48 h. Cells were harvested by centrifuging and washed by resuspension in 5 ml cold distilled water followed by centrifuging. The washings were combined with the respective supernatant solutions and lyophilized; this was the 'extracellular fraction'. Washing with cold distilled water did not impair the intracellular pool of lysine. The washed cells were resuspended in 10 ml distilled water, heated in a boiling water bath for 30 min, and centrifuged; the supernatant solution was the 'intracellular fraction'. Intracellular and extracellular fractions were analysed chromatographically to determine radioactive lysine and 2-aminoadipic acid (Kurtz & Bhattacharjee, 1975).

Chemicals. DL-2-Amino[1- ^{14}C]adipic acid was obtained from The Radiochemical Centre, Amersham. All other fine chemicals, including L-thialysine and DL-hydroxylysine, were obtained from Sigma.

RESULTS AND DISCUSSION

L-Thialysine and DL-hydroxylysine (10^{-3} M) inhibited growth of wild-type strain x2180 for 3 to 4 days. The inhibition was reversed by 10^{-3} M L-lysine. Feedback sensitivity of homocitrate synthase activity in extracts of x2180 was examined (Table 1). L-Lysine and L-thialysine, and to a lesser extent DL-hydroxylysine, decreased the synthase activity. The enzyme was also inhibited by 10^{-2} M L-arginine, L-norleucine and L-norvaline (Table 1), but not by 10^{-2} M 2-aminoadipic acid (an intermediate of the lysine biosynthetic pathway) or by 10^{-2} M L-glutamic acid, L-asparagine, D-lysine or L-glutamine. Norleucine and norvaline also inhibit saccharopine dehydrogenase which catalyses the last reaction of lysine biosynthesis (Fujioka & Nakatani, 1972).

Homocitrate synthase activity in extracts of analogue-resistant strains AEC-RI and OHL-IA was less sensitive than the wild-type enzyme to feedback inhibition by L-lysine, L-thialysine, and slightly less resistant to inhibition by DL-hydroxylysine (Table 1). The activity in strain AEC-RI was also resistant to inhibition by 10^{-2} M L-norleucine and L-norvaline; 10^{-2} M L-arginine gave only 25 % inhibition of the enzyme from this strain.

The possibility of increased lysine production by AEC-RI and OHL-IA compared with x2180 was investigated in experiments using DL-2-amino[1- ^{14}C]adipic acid as substrate (Table 2). Strain AEC-RI accumulated almost four times as much extracellular lysine as did x2180, but only a slight increase of extracellular lysine was seen with OHL-IA. The intracellular concentrations of lysine and 2-aminoadipic acid were greatly reduced in strain AEC-RI compared with x2180 and OHL-IA. Total non-protein lysine (intracellular plus extracellular) from AEC-RI was more than 250 % that of x2180. The concentration of extracellular 2-amino-

Table 1. *Feedback inhibition of homocitrate synthase activity from wild-type (x2180), thialysine-resistant (AEC-R1) and hydroxylysine-resistant (OHL-1A) strains of S. cerevisiae*

Extracts were prepared and the homocitrate synthase activity was determined as described in Methods.

Inhibitor	Concn (mM)	Homocitrate synthase activity [nmol product formed min ⁻¹ (mg protein) ⁻¹]		
		x2180	AEC-R1	OHL-1A
None		0.280	0.280	0.280
L-Lysine	5	0.070	0.056	0.056
	1	0.070	0.210	0.070
	0.1	0.091	0.280	0.280
L-Thialysine	5	0.00	0.280	0.245
	1	0.105	0.245	0.245
	0.1	0.210	0.280	0.280
DL-Hydroxylysine	10	0.105	0.140	0.140
	3	0.175	0.280	0.280
	1	0.245	ND	ND
L-Arginine	10	0.070	0.210	ND
	5	0.210	ND	ND
L-Norleucine	10	0.210	0.280	ND
	5	0.245	ND	ND
L-Norvaline	10	0.245	0.280	ND

ND, not determined.

Table 2. *Production and excretion of non-protein lysine by wild-type (x2180), thialysine-resistant (AEC-R1) and hydroxylysine-resistant (OHL-1A) strains of S. cerevisiae*

Cells were grown in medium supplemented with DL-2-amino[1-¹⁴C]adipic acid. 'Intracellular' and 'extracellular' concentrations of [¹⁴C]lysine and 2-amino[1-¹⁴C]adipic acid were determined after growth for 48 h.

Strain	2-Amino[1- ¹⁴ C]adipate remaining after growth (counts/10 ⁷ cells)		[¹⁴ C]Lysine (counts/10 ⁷ cells)	
	Extracellular	Intracellular	Extracellular	Intracellular
x2180	5824	2041	511	181
AEC-R1	5745	107	1911	18
OHL-1A	5301	2251	655	198

adipic acid from all three cultures was about the same. Excretion of excess lysine by the thialysine-resistant strain was confirmed by the ability of the concentrated culture supernatant from AEC-R1 to support growth of a lysine auxotroph (*lys13*) of *S. cerevisiae*.

Activities of homocitrate synthase were compared in strains x2180, AEC-R1, and in a lysine auxotroph lacking saccharopine reductase, *lys13* (Bhattacharjee & Sinha, 1972) after growth with or without various additions to the minimal medium. The synthase activity of auxotroph *lys13* grown in minimal medium supplemented with L-lysine (20 µg ml⁻¹) was considered as 100 %. L-Lysine (10⁻⁴ M) or L-thialysine (10⁻⁵ M) repressed the biosynthesis of homocitrate synthase in strain x2180 by 80 % and in AEC-R1 by 50 %.

L-Lysine and DL-hydroxylysine inhibit both homocitrate synthases and exert non-coordinate repression of the enzymes of lysine biosynthesis including one of the two homocitrate synthases of *S. cerevisiae* (Sinha *et al.*, 1971; Tucci & Ceci, 1972). We propose a

similar mechanism for the action of L-thialysine, and this would explain the inhibition of growth of wild-type *S. cerevisiae* by L-thialysine. Mutation in strain AEC-RI for resistance to thialysine appears to have a pleiotropic effect; causing excretion of lysine, impairment of feedback inhibition and repression of homocitrate synthase. The results in this paper do not show whether one or both homocitrate synthases of AEC-RI are insensitive to feedback inhibition and resistant to repression by L-lysine and L-thialysine. Mutant OHL-1A does not excrete any lysine and is somewhat less resistant than mutant AEC-RI to inhibition. The precise nature of the mutations involved in strains AEC-RI and OHL-1A and whether they affect structural or regulatory genes for homocitrate synthase are not known.

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