TWO PATHWAYS OF CYSTEINE BIOSYNTHESIS IN *SACCHAROMYCOPSIS LIPOLYTICA*

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1. Introduction

In fungi, cysteine can be synthesized from *O*-acetylserine or from homocysteine [1]. The second pathway can be regarded as de novo synthesis of cysteine, if homocysteine is synthesized directly from *O*-acetylhomoserine by homocysteine synthase (fig.1). This activity was described in *Saccharomyces cerevisiae* [2], *Neurospora crassa* [3], *Saccharomycopsis lipolytica* [4] and *Aspergillus nidulans* [5,6], but only in the latter organism was unequivocal evidence obtained that cysteine can be synthesized de novo by both pathways [7]. In *S. cerevisiae* it was shown that the activities of cysteine and homocysteine synthase are attributes of one enzyme protein [8]. The same holds for *S. lipolytica* ([9] and unpublished). The existence of a bifunctional enzyme catalyzing steps 2 and 3 (see fig.1) does not allow one to evaluate the physiological significance of both reactions, as mutations affecting this enzyme abolish both activities [10].

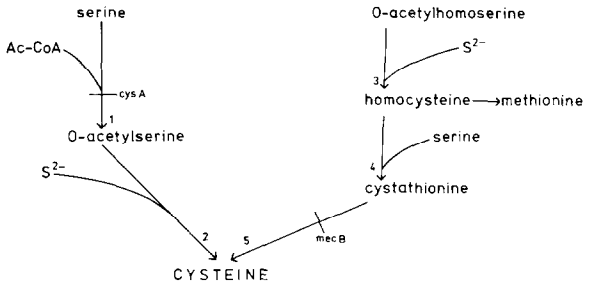

Fig.1. Pathways of cysteine biosynthesis in *Saccharomycopsis lipolytica*. Metabolic blocks in *cysA* and *mecB* mutants are indicated. (1) Serine acetyltransferase (EC 2.3.1.30); (2) cysteine synthase (EC 4.2.99.8); (3) homocysteine synthase (EC 4.2.99.10); (4) cystathionine β-synthase (EC 2.1.22); (5) γ-cystathionase (EC 4.4.1.1).

We have shown [4] that the mutant *mecB1*, impaired in γ-cystathionase (steps 5) is a prototroph, which confirms that cysteine can be synthesized in vivo from *O*-acetylserine. Here we present evidence that the block in *O*-acetylserine formation (step 1) does not lead to auxotrophy when the homocysteine to cysteine pathway is functioning. Therefore it is clear that in *S. lipolytica* there are two pathways for de novo cysteine synthesis, and both have to be simultaneously impaired to cause cysteine auxotrophy.

2. Materials and methods

The wild type S1-1 and *his1,mecB1* strain devoid of γ-cystathionase activity were as in [4]. Minimal G_o medium was that in [11]. In the modified G_o–S medium the amount of sulphate was reduced to 2 mM.

Culture conditions, enzyme preparations, determination of ^{35}S-labelled amino acid pools and extraction procedures were essentially as in [4] with the modifications indicated in the text. Identification of labelled compounds was performed by thin-layer chromatography and high-voltage electrophoresis as in [6].

The activity of cysteine synthase (EC 4.2.99.8) and cystathionine-β-synthase (EC 2.1.22) were assayed as in [12]. Homocysteine synthase (EC 4.2.99.10) and γ-cystathionase (EC 4.4.1.1) were determined by the method in [5,6]. Serine acetyltransferase (EC 2.3.1.30) was estimated according to [13]: *O*-acetyl-L-[^{14}C]-serine was separated from the unreacted L-[^{14}C]-serine by thin-layer chromatography on MN-300 cellulose using butanol:acetic acid:H_2O (120:30:50). Protein content was determined by the Lowry method [14].

3. Results

The cysteine requiring auxotroph *mecB1,cysA1* was obtained by ultraviolet mutagenesis of strain *mecB1*. It grows on cysteine as well as on *O*-acetylserine which suggested a block in serine acetyltransferase (step 1). We have attempted to assay this enzyme but most of our extracts were completely inactive. The activity of serine acetyltransferase in the mutant was lower by a factor of ~10 compared with the wild-type strain.

The analysis of ^{35}S-labelled amino acid pools provides convincing data that both pathways of cysteine synthesis are impaired in *mecB1,cysA1* strain (table 1, expt 2). As expected (fig.1) in the double mutant only trace amounts of radioactive cysteine and glutathione are formed while synthesis of cystathionine and methionine remains unaffected. This strain was crossed with the wild type in order to check if *cysA1* segregants are prototrophs. Unfortunately in all tetrads isolated only one spore germinated so it was impossible to examine the marker segregation. Therefore we mutagenised the *mecB1,cysA1* strain and isolated cysteine prototrophs. They appeared at a frequency of 2×10^{-3} of surviving cells: 3% of them were identified as revertants of the *mecB1* mutation. The rest of the revertants carried the *mecB1* mutation as was demonstrated by a failure to grow on methionine in the presence of selenate [6]. Therefore these strains were revertants of *cysA1* or carried a suppressor of this mutation. In either case they were able to synthesize cysteine independently of homocysteine. It is unlikely that the strains identified as *mecB1* revertants result from simultaneous reversion of both mutations as the expected frequency of such an event is of the order 1×10^{-9} while revertants of *mecB1* appeared with the frequency of 6×10^{-5}. The results of enzymatic examination of *mecB1^{rev1}, cysA1* are presented in table 2. This strain shows ~50% of the wild-type activity of γ-cystathionase and is able to synthesize cysteine and glutathione (table 1).

It is noteworthy that in the strain *mecB1* levels of homocysteine and cystathionine are markedly elevated, whereas the amount of glutathione is slightly reduced, which coincides with a derepression of cysteine and homocysteine synthase (table 2). In the revertant *mecB1^{rev1},cysA1* pools of homocysteine and cysteine are slightly elevated as compared with the wild-type, the pool of glutathione is lower and comparable to that found in *mecB1*. On the other hand the revertant differs both from the wild-type and *mecB1* strains in its growth pattern (fig.2). Particularly striking is the long lag phase. This behaviour is not easily explained by the differences in the pools of sulfur amino acids, and therefore is probably associated with the *cysA1* mutation.

The fact that mutant *mecB1* impaired in γ-cysta-

Table 1
The pool of ^{35}S-labelled amino acids in the wild-type and mutant strains of *Saccharomycopsis lipolytica*

Relevent genotype	Cysteine	Homocysteine	Glutathione	Cystathionine	Methionine	Total
			(nmol/g dry wt)			
Expt 1[a]						
W T	208.8	47.8	2832.3	235.7	235.2	4686.4
mecB1	456.2	614.7	1680.5	3840.0	283.4	16194.7
mecB1^{rev1},cysA1	252.0	82.4	1665.7	382.6	266.0	6086.5
Expt 2[b]						
W T	197.9	92.8	1869.0	216.1	220.2	2968.0
mecB1,cysA1	21.0	101.8	79.9	871.0	107.7	1314.0

[a] Growth on the G_O–S medium containing $Na_2{}^{35}SO_4$ (5×10^8 cpm/mmol) at 30°C for 18 h
[b] Growth on the G_O medium containing L-cysteine (0.1 mM) at 30°C for 18 h. The cells were collected, washed with G_O–S medium, resuspended in the same medium containing $Na_2{}^{35}SO_4$, incubated for a further 6 h and harvested for determination of the ^{35}S-labelled amino acid pool

Table 2
Activities of cysteine and homocysteine biosynthetic enzymes in wild type and mutant strains of *Saccharomycopsis lipolytica*

Relevent genotype	γ-Cysta-thionase	Cystathionine β-synthase	Cysteine synthase	Homocysteine synthase
W T	3.96	15.2	75.6	242.9
mecB1	0.01	15.5	119.2	373.5
mecB1,cysA1	0.01	11.2	41.7	271.0
mecB1^{rev1},cysA1	2.10	12.7	154.5	418.0

The cells were grown in G_0 medium (in the case of *mecB1,cysA1* supplemented with 0.1 mM L-cysteine) at 30°C for 18 h and collected for the assay of enzymes. Activity is expressed in nmol/min/mg protein

thionase accumulates large amounts of cystathionine and homocysteine suggests that at least part of the cysteine is normally synthesized from homocysteine. This accumulation is not correlated with the increase in the cystathionine-β-synthase activity and implies therefore that the pool of cystathionine depends on the cellular content of homocysteine. It is interesting that the pool of methionine is not changed under these conditions indicating that an excess of homocysteine is metabolised to cystathionine.

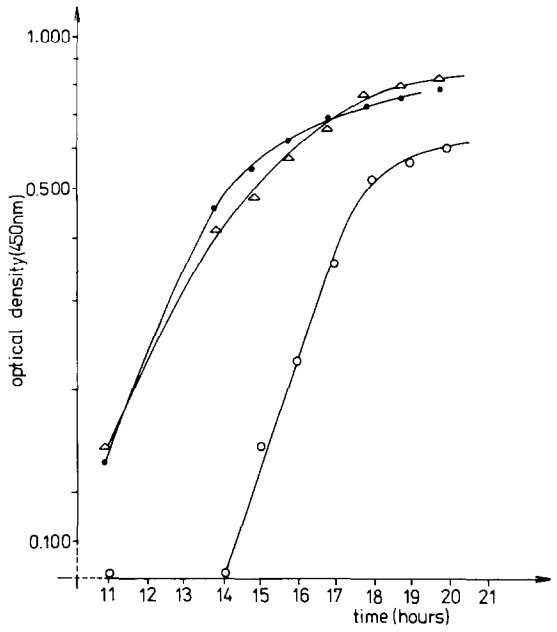

Fig.2. Growth of the wild-type, *mecB1* and *mecB1^{rev1},cysA1* strains in G_0 medium. The cultures were grown on a rotary shaker at 30°C.

In the *mecB1* mutant the total pool of cysteine and glutathione is only slightly reduced. Cysteine and/or glutathione was shown to be involved in the regulation of cysteine and homocysteine synthase [15] but it seems doubtful that the observed reduction of the pool of these amino acids could be responsible for derepression of the enzyme. An alternative hypothesis is that there are two different pools of cysteine and/or glutathione and only one of them, that derived from homocysteine, is involved in the regulation of enzyme synthesis. It may be recalled that two separate pools of cysteine were found in *Chlorella* [16].

Acknowledgements

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