A yeast with unusual sulphur amino acid metabolism

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A yeast strain highly resistant to propargylglycine (an inhibitor of cystathionine γ -lyase) was isolated from air. It was partially characterized, but it has not been identified with any known yeast species. Its sulphur amino acid metabolism differed from that of other fungi by the lack of the reverse transsulphuration pathway from methionine to cysteine, as no activity of cystathionine β -synthase or cystathionine γ -lyase was found. The functional lack of this pathway was confirmed by growth tests and by experiments with [^{35}S]methionine. In contrast to *Saccharomyces cerevisiae* neither homocysteine synthase nor the sulphate assimilation pathway were repressible by methionine in the new strain; on the contrary, a regulatory effect of cysteine was observed.

Introduction

An outline of sulphur amino acid metabolism is presented in Fig. 1. While the physiological importance of particular enzymic activities and regulatory patterns differs from species to species, in all fungi tested so far catabolism of methionine proceeds via the reverse transsulphuration pathway involving cystathionine β -synthase and cystathionine γ -lyase (steps 5 and 6). The strain described here lacks this pathway. The present report deals with this unusual metabolism and its regulation.

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Methods

Strains. Strain PGR-13 is our isolate from air. Yeast strains used for comparison were: *Saccharomyces cerevisiae* haploid laboratory strain AT-12 (*ade1*) and industrial diploid AS-4 (wild-type), *Pachysolen tannophilus* (wild-type) from our laboratory collection and *Candida utilis* Ct5 (wild-type) from the collection of the Institute of Fermentation Industry, Warsaw. *S. cerevisiae* strain *cys3*, impaired in cystathionine γ -lyase, was kindly supplied by Dr B. Ono.

Media and growth conditions. Preliminary characterization of strain PGR-13 was done by the methods and on the media described by Barnett *et al.* (1983).

For other experiments strains were grown on GO minimal medium as described by Swietlinska *et al.* (1978), supplemented as indicated. For enzyme assays the cultures were grown at 30 °C on a rotary shaker (150 r.p.m.) and harvested in the exponential phase of growth.

Fig. 1. An outline of sulphur amino acid metabolism in fungi. Enzymes: (1) cysteine synthase, (2) cystathionine γ -synthase, (3) β -cystathionase, (4) homocysteine synthase, (5) cystathionine β -synthase, (6) cystathionine γ -lyase. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

Enzyme assays. Yeast cells were suspended in 0.1 mM-potassium phosphate buffer pH 7.5 and disrupted in a Braun homogenizer. Cystathionine γ -lyase (EC 4.4.1.1) and β -cystathionase (EC 4.4.1.8) were assayed according to Paszewski & Grabski (1974). Homocysteine synthase (EC 4.2.99.10) was assayed using the reaction mixture described by Paszewski & Grabski (1973). Cystathionine β -synthase (EC 4.2.1.22) was assayed both as serine sulphydrylase and as cystathionine synthase using DL-[14 C]serine [1.6 μ Ci (59.2 kBq) per reaction mixture] as described by Pieniazek *et al.* (1973). Protein was estimated by the method of Bradford (1976).

Determination of the pool of 35 S-labelled compounds. In experiments with $^{35}\text{SO}_4^{2-}$, cultures were grown in a modified medium in which the sulphate concentration was lowered to 2 mM (substituting the corresponding chlorates for the sulphates omitted). The cells were grown under standard conditions for 6 h, then $\text{Na}_2^{35}\text{SO}_4$ (4.9×10^8 c.p.m. mmol^{-1}) was added and growth was continued. The cells were harvested at the early stationary phase and the labelled compounds isolated as described previously (Paszewski *et al.*, 1984). Methionine was determined by thin-layer chromatography (TLC), and cysteine, glutathione and homocysteine by high-voltage paper electrophoresis as described earlier (Paszewski & Grabski, 1974).

Methionine metabolism was studied in cells grown in GO medium supplemented with 3 mM-L-[35 S]methionine (3.6×10^8 c.p.m. nmol^{-1}). Growth conditions and determination of sulphur-labelled compounds were the same as in experiments with radioactive sulphate. Since S-adenosylmethionine decomposes during water extraction of cells at 100 °C, it was determined as 5-methylthioadenosine by TLC along with methionine. The amounts of radioactive substances were calculated from the specific activities of $^{35}\text{SO}_4^{2-}$ and L-[35 S]methionine, respectively.

Reagents. O-Acetylhomoserine was synthesized by the method of Wiebers & Garner (1967). DL-Propargylglycines sodium selenate and L-amino acids were from Sigma. DL-[14 C]Serine and $\text{Na}_2^{35}\text{SO}_4$ were obtained from the Institute of Nuclear Research, Swierk, Poland. L-[35 S]Methionine (1000 Ci mmol^{-1} ; 37 TBq mmol^{-1}) was from Amersham. On the day it was used, L-[35 S]methionine accounted for 83% of the total radioactivity of the preparation, as determined by TLC.

Results and Discussion

Isolation and preliminary characterization of the strain

During our work on effects of the antibiotic propargylglycine on enzymes of sulphur amino acid metabolism in fungi (Piotrowska & Paszewski, 1986), we isolated from air a yeast strain, designated PGR-13, resistant to the antibiotic at concentrations up to 200 μM . The growth properties of the isolate did not correspond exactly to those of any yeast described by Barnett *et al.* (1983). Morphologically, it strongly resembled *S. cerevisiae*. However, *S. cerevisiae* is unable to utilize lysine and uracil as nitrogen sources, while PGR-13 utilized both.

Sulphur amino acid metabolism of strain PGR-13

The most striking trait of strain PGR-13 concerns its sulphur amino acid metabolism. When enzymes of methionine metabolism were examined, we could find no

Table 1. Activity of cystathionine β -synthase in PGR-13 and *S. cerevisiae*

The results represent the radioactivity of serine and cystathionine present in the reaction mixtures. The radioactivity of about 1000 c.p.m. at the cystathionine position came from a radioactive impurity present in our DL-[14 C]serine preparation. Reaction mixtures contained 600 μg and 550 μg protein for PGR-13 and *S. cerevisiae*, respectively. The results from one representative experiment are presented. Similar ratios of cystathionine and serine were obtained in three experiments with extracts from independent cultures.

Strain	Spot	Radioactivity (c.p.m.)	
		Reaction mixture:	
		+ Homocysteine	– Homocysteine
PGR-13	Serine	16265	14209
	Cystathionine	958	1345
<i>S. cerevisiae</i>	Serine	18241	18524
	Cystathionine	2657	960

activity of two enzymes of the reverse transsulphuration pathway: cystathionine β -synthase and cystathionine γ -lyase (Fig. 1, steps 5 and 6) while both activities were present in the reference yeast strains. In extracts from *S. cerevisiae* the activities of cystathionine β -synthase and cystathionine γ -lyase were 22 and 4 nmol min^{-1} (mg protein^{-1}), respectively.

β -Cystathionase and homocysteine synthase activities in extracts from PGR-13 were higher than those of *S. cerevisiae* (not shown).

Cystathionine β -synthase was first assayed as serine sulphydrylase with serine and sulphide as substrates, i.e. in a non-physiological reaction. We then assayed the reaction with homocysteine and serine, which are the natural substrates, using *S. cerevisiae* cell-free extract as the control (Table 1). It is evident that there was no activity of cystathionine β -synthase in PGR-13.

While it cannot be excluded that there is only one protein and one gene responsible for both of the activities lacking in PGR-13, we think this is highly improbable. A mutant of *Yarrowia lipolytica* has been described which has lost only one of these activities (Morzycka *et al.*, 1976). A mutant of *S. cerevisiae*, *cys3*, with lowered activity of cystathionine γ -lyase (Ono *et al.*, 1984) was tested in our laboratory and found to have a normal level of cystathionine β -synthase.

It was confirmed that the lack of transsulphurating activities observed *in vitro* was a real physiological trait by growth tests with selenate. Selenate is a toxic analogue of sulphate and competes with it for assimilation. When methionine is added together with selenate, it is methionine that serves as sulphur donor for synthesis of cysteine. Thus mutants blocked in the reverse trans-

Table 2. Accumulation of ^{35}S -labelled compounds in cells of PGR-13 and *S. cerevisiae* grown in the presence of L-[^{35}S]methionine

The results from one representative experiment are given. A similar pattern was obtained in three experiments with extracts from independent cultures.

Strain	Amount of ^{35}S or ^{35}S -labelled compound [μmol (g dry wt) $^{-1}$]				
	Total ^{35}S *	Cysteine + glutathione	Homo- cysteine	Methionine	S-Adenosyl- methionine
PGR-13	113	1.3	2.0	53.0	31.0
<i>S. cerevisiae</i>	181	22.0	4.7	66.0	43.0

* Retained on Dowex 50 (H^+).

sulphuration pathway cannot grow on selenate/methionine medium. This feature allowed isolation of mutants devoid of either cystathionine γ -lyase or cystathionine β -synthase in *Aspergillus nidulans* (Pieniazek & Paszewski, 1970) and *Y. lipolytica* (Morzycka *et al.*, 1976). PGR-13 failed to grow on medium with 3 mM-selenate + 1 mM-methionine, whereas *S. cerevisiae* and *C. utilis*, used as controls, grew normally. When 1 mM-cysteine was used instead of methionine, it enabled PGR-13 to grow in the presence of selenate.

The ability of PGR-13 to catabolize methionine was tested directly in experiments with [^{35}S]methionine (Table 2). In *S. cerevisiae*, methionine was easily metabolized to cysteine while in PGR-13 it was metabolized only to a very limited extent. It should be noted that 17% of the ^{35}S added to the growth medium was not in the form of methionine (see Methods). It is possible, therefore, that in the case of PGR-13 the radioactivity found in the cysteine and glutathione spots came from the non-methionine fraction of the radioactivity in the preparation used.

We conclude that PGR-13, presumably a new species, is the first yeast described so far which lacks the reverse transsulphuration pathway.

Regulation of sulphur amino acid metabolism

In all fungi tested, homocysteine synthase is under control of the same regulatory system as the enzymes of the sulphate assimilation pathway. In *S. cerevisiae* these enzymes are repressed, in a coordinate fashion, in the presence of methionine at concentrations less than 1 mM (Breton & Surdin-Kerjan, 1977). In PGR-13, addition of methionine to the growth medium at concentrations up to 5 mM had no effect on homocysteine synthase, while in the presence of 1 mM-cysteine the enzyme specific

Table 3. Accumulation of ^{35}S -labelled compounds in cells of PGR-13 and *S. cerevisiae* grown in the presence of $^{35}\text{SO}_4^{2-}$

The results from one representative experiment are presented. A similar pattern was obtained in three independent experiments. The content of S-adenosylmethionine was not determined precisely; it was lower than that of methionine.

Strain	Medium	Amount of ^{35}S or ^{35}S -labelled compound [μmol (g dry wt) $^{-1}$]			
		Total ^{35}S *	Cysteine + glutathione	Homo- cysteine	Methionine
PGR-13	GO	6.27	4.06	0.58	0.71
	GO + L-Met (3 mM)	5.20	3.07	0.25	0.41
<i>S. cerevisiae</i>	GO	14.30	6.50	2.20	0.51
	GO + L-Met (3 mM)	<0.1	tr.	tr.	ND

ND, Not detected; tr., trace.

* Retained on Dowex 50 (H^+).

activity was lowered threefold [360 ± 17 and 104 ± 24 nmol min^{-1} (mg protein) $^{-1}$ for cells grown without and with cysteine, respectively; means $\pm$ SD].

A marked difference was also observed between PGR-13 and *S. cerevisiae* in the effect of methionine on sulphate assimilation and the synthesis of sulphur amino acids (Table 3). While the *de novo* synthesis of amino acids from sulphate was completely repressed in the *S. cerevisiae* strain grown in the presence of methionine, this amino acid had only a slight effect in PGR-13. Similarly, only a slight effect of cysteine on sulphate assimilation in PGR-13 was observed (not shown). This contrasts with the relatively strong repressing effect of cysteine on homocysteine synthase in this strain.

Neither methionine nor cysteine affected the level in PGR-13 of β -cystathionase, an enzyme of methionine biosynthesis, (Fig. 1, step 3).

Both *S. cerevisiae* and PGR-13 grown in the presence of methionine accumulated high pools of methionine and S-adenosylmethionine (Table 2). It seems clear that the regulation of the sulphate assimilation pathway and homocysteine synthase in PGR-13 is not mediated by these compounds, contrary to what has been suggested for *S. cerevisiae* (Cherest *et al.*, 1975). The absence of methionine repression in PGR-13 is logical, as the presence of such a regulatory mechanism in a strain unable to catabolize this amino acid to cysteine would be lethal.

Our results suggest that cysteine may be a low-molecular-mass effector in the regulation. It is perhaps

the regulatory role of cysteine that is a particular trait of the unusual metabolism in PGR-13. It is possible, however, that cysteine may play a similar role in *S. cerevisiae* since cysteine can be readily synthesized from methionine. In fact, the pool of cysteine is much higher in methionine-grown than in sulphate-grown cells (Tables 2 and 3).

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