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LACK OF CORRELATION BETWEEN GLUTATHIONE TURNOVER AND AMINO ACID ABSORPTION BY THE YEAST *SACCHAROMYCES CEREVISIAE*

RICHARD J. ROBINS* and DAVID D. DAVIES

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

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Key Word Index—*Saccharomyces cerevisiae*; yeast; glutathione turnover; amino acid uptake; γ-glutamyl cycle.

Abstract—The yeast *Saccharomyces cerevisiae* was grown in the presence of 1.0 mM L-methionine and the half-life of degradation of glutathione determined for the strains Σ1278b (444 min) and the amino-acid-uptake deficient mutant 2512c (368 min). There is no significant difference in these values, yet the rate of uptake of L-methionine is 5–7 times lower in the mutant. In neither strain is the turnover of glutathione sufficient to account for amino acid uptake. We conclude that there is no correlation between the γ-glutamyl cycle and amino acid uptake by this yeast.

INTRODUCTION

The hypothesis that the γ-glutamyl cycle [1] is involved in amino acid uptake has gained considerable support from work with mammalian tissues [2] and, despite continuing controversy, is now appearing in text books as an acceptable mechanism [3]. Workers with plants and bacteria have tended not to accept the cycle, for which there has been little evidence in these organisms.

Recently, however, Mooz [4] has reported that the *gap* mutant of *Saccharomyces cerevisiae* (2512c) has a low glutathione content and diminished glutathione synthetase (EC 6.3.2.3) activity as compared with the wild-type (Σ1278b). In *Candida utilis*, Osuji claims that the turnover of glutathione can be dependent on the external amino acid concentration [5] and is sufficient to account for amino acid uptake [6, 7].

Other workers, however, fail to confirm these reports. Penninckx *et al.* [8] find no strain-dependent differences in glutathione synthetase activity in *S. cerevisiae* and no difference in the glutathione content (M. Penninckx, personal communication). We have already questioned Osuji's results [9] and, in a detailed analysis of the turnover of glutathione in *C. utilis*, find that the rate of amino acid uptake greatly exceeds the rate of glutathione turnover [10].

The strains Σ1278b and 2512c of *S. cerevisiae* present an ideal physiological case in which to test the hypothesis. These organisms are isogenic, yet the mutant is deficient in the uptake of a number of amino acids [11]. These include L-methionine, the best acceptor of the γ-glutamyl group for γ-glutamyltransferase (EC 2.3.2.2) in *S. cerevisiae* [8]. Hence we have compared the turnover of glutathione in the wild-type and the mutant growing in the presence of L-methionine. We find that there is no correlation between the rate of uptake of L-methionine and glutathione degradation in this organism.

*Present address: Food Research Institute, Colney Lane, Norwich NR4 7UA, U.K.

RESULTS

Growth of yeast

With urea as the nitrogen source, it was found that the wild-type (Σ1278b) grew appreciably more slowly (mean doubling time = 6.2 hr) than the mutant (2512c) (mean doubling time = 4.0 hr). This had no effect on the morphology of the cells or on the relationship between cell density and the absorption at 640 nm (A_{640}).

Glutathione content of the yeast

Samples of yeast were taken at various cell densities, harvested and killed in 80% ethanol. Debris was removed by centrifugation and washed several times with 80% ethanol. The pooled supernatants were evaporated to dryness and the glutathione content of the residue was determined [12]. In both strains the glutathione content remained constant per unit cell number with growth and no difference was found between the two. Σ1278b contained 199.8 ± 19.4 (9) and 2512c contained 193.0 ± 23.5 (12) pmol/(10^6 cells), giving a mean of 196.0 ± 22.0.

Release of ^3H *from the* C-2 *of glutamate*

Yeast (2512c) was grown on full medium plus ^3H_2O at 37 MBq/ml for 4 hr. Immediately prior to harvesting a 40 ml sample was taken. The culture was harvested, washed and resuspended in untritiated medium. Samples (40 ml) were taken during the next 60 min, harvested and extracted with 80% ethanol. L-Glutamate (1.8 mg) was added as carrier. After evaporating the supernatants to dryness, the residues were taken into 2.0 ml 50 mM acetic acid and glutamate purified on Dowex resins [13]. The ^3H at C-2 was determined [14] and recovery measured with glutamic dehydrogenase [15].

The loss of ^3H from the C-2 of glutamate is found to be biphasic. There is an initial rapid loss with a half-life of 5.1 min followed by a more slowly transaminated pool of half-life 258 min. The rapidly transaminated pool

represents about 75% of the total and turns over substantially more rapidly than glutathione.

Degradation of glutathione

In Table 1 the half-lives obtained by measuring the loss of ^3H from total-^3H and the ^3H at the C-2 of the γ-glutamyl moiety of glutathione are given for both strains. The decay in all cases is satisfactorily fitted by a single exponential with a correlation better than 2%. There is no significant difference (*t*-test) between the rates of degradation of glutathione in the two strains. The total-^3H gives a value significantly greater ($P < 0.05$: *t*-test) than that determined by measuring the ^3H at the C-2 of the γ-glutamyl moiety.

Uptake of L-*methionine*

The uptake of L-methionine was confirmed to be dependent on two functions [16], the rate of uptake in Σ1278b being 5–7 times greater than in 2512c (Table 2).

DISCUSSION

When yeasts are grown in the presence of ^3H_2O the amino acid pool becomes labelled. Excess primary label is removed from the cells during the brief wash and ^3H at the C-2 is lost from the rapidly transaminated pool of glutamate during the subsequent 'cold' chase at a rate much greater than from the ^3H of the C-2 of the γ-glutamyl moiety of glutathione. The slowly transaminated pool may be vacuolar as is that in *Lemna minor* [17] or, as in *Candida utilis*, represent cytoplasmic amino acids that do not undergo transamination, notably L-arginine (R. J. Robins, unpublished results).

In ref. [10] evidence is presented that the method of racemization with acetic anhydride effectively releases all the ^3H from the C-2 of the γ-glutamyl group of glutathione. By resolving this ^3H alone, the turnover of that part of the molecule pertinent to amino acid uptake is obtained, as this is the ^3H exchanged during transamination of free glutamate. Theoretically this could differ from the turnover of the ^3H of the C-2 of the glycine group and we show it to be more rapid than the turnover of the total-^3H of the molecule (Table 1).

In healthy aerobically growing yeast, the half-life of glutathione is similar for the two strains, showing no correlation with the observed decrease in the rate of L-methionine uptake in 2512c. We have confirmed that the uptake of L-methionine by both strains follows the kinetic pattern previously established [16] and that in 2512c it is diminished by 5–7 times. Using urea as the source of nitrogen, it was not found possible totally to de-repress the amino acid uptake system, probably due to traces of ammonia. Despite this, the rate of uptake in both strains is 50–600 times greater than the turnover of the total pool of glutathione on a 1:1 molar ratio, as required by the hypothesis [1].

We also find no difference in the glutathione content of the two strains, in contrast to Mooz [4]. In this we are supported by other workers, who used identical growth conditions to Mooz and whose levels of glutathione are comparable to those reported here. (M. Penninckx, personal communication). The same group has shown that the growth conditions affect the levels of the enzymes of glutathione metabolism in a number of mutants [8] and that urea, as used here, is a good nitrogenous source to promote high activity of γ-glutamyltransferase. The present study was therefore conducted under conditions expected not in themselves to limit glutathione turnover.

From direct measurements, as made here, and as reported in ref. [10], it is clear that the γ-glutamyl cycle [1] cannot function in yeast to effect the uptake of amino acids.

Table 1. The half-life of glutathione in *S. cerevisiae* wild-type Σ1278b and *gap* mutant 2512c

^3H determined	Half-life (min) Σ1278b	2512c
Total-^3H	598	641
^3H at the C-2 of the γ-glutamyl moiety	444	368

Yeasts were grown in the presence of 1.0 mM L-methionine and ^3H_2O at 37 MBq/ml for 5–7 generations, harvested, washed and resuspended in ^3H-free medium. Samples were taken at various times, glutathione was extracted and the ^3H at the C-2 of the γ-glutamyl moiety resolved as described in the text. No significant difference (*t*-test) is found between the half-lives for the two strains. In both strains the half-life measured by the total-^3H counts is significantly greater ($P < 0.05$) than for the ^3H at the C-2 of the γ-glutamyl moiety.

Table 2. The uptake of L-methionine by *S. cerevisiae* wild-type Σ1278b and *gap* mutant 2512c

Concentration of L-methionine (mM)	Rate of uptake (pmol/min/10^6 cells) Σ1278b	2512c
0.01	119.2	17.7
0.1	135.7	25.1
0.5	202.9	37.6

Yeast were grown on medium lacking L-methionine for 5–7 generations and the rate of uptake determined on aliquots by the method of Grenson *et al.* [18]. The rate of uptake was linear over the first 5 min.

EXPERIMENTAL

Materials. ^3H_2O (185 GBq/ml) and L-[1-^{14}C]methionine (2.25 GBq/mmol) were purchased from the Radiochemical Centre, Amersham. All other reagents were of AR standard.

Culture of yeast. Saccharomyces cerevisiae Σ1278b and 2512c were kindly supplied by Dr. M. Grenson (Laboratoire de Microbiologie, Faculté des Sciences, Université Libre de Bruxelles, 1070 Bruxelles, Belgium). Cultures were grown using Medium 149 [18] to which vitamins as in ref. [19] were added prior to sterilisation. Immediately before inoculation D-glucose (28 mM), urea (5 mM) and L-methionine (1 mM) were added. Yeasts were grown at 30° with vigorous aeration (4 l./min) and constant stirring. All experiments were conducted during exponential growth.

Determination of the degradation constant of glutathione. Yeast were cultured for 5–6 generations in 400 ml medium containing ^3H_2O at 37 MBq/ml. At about 1.5×10^7 cells/ml a sample was taken, the remainder harvested, washed with 100 ml medium and resuspended in 400 ml untritiated medium. Samples of approximately 50 ml were taken into 5 ml 50% (w/v) trichloroacetic acid, weighed and 5 mg glutathione added as carrier. The glutathione present was isolated [20] and an aliquot used to measure the total-^3H. The ^3H at the C-2 of the γ-glutamyl

moiety of the remainder was determined by racemization with acetic anhydride [14]. The recovery was assayed by the method of Tietze [12]. We have shown this isolation method to produce glutathione of at least 95% radioactive purity [10].

^{3}H and ^{14}C were determined by liquid scintillation counting.

We have examined the use of this methodology when applied to glutathione. Evidence that it is valid and details of the experimental procedure followed are reported in ref. [10].

Uptake of L-*methionine.* Yeast were cultured on 400 ml medium lacking L-methionine. The rate of uptake was determined by the method of Grenson *et al.* [18] using Sartorius cellulose nitrate filters (pore dia 1.2 μm).

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