ON THE ROLE OF GLUTATHIONE IN THE TRANSPORT OF AMINO ACID IN THE YEAST *SACCHAROMYCES CEREVISIAE*: CONTRADICTORY RESULTS

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1. Introduction

Despite its ubiquitous occurrence in cells, the metabolic role of glutathione has remained obscure. During the preceding decade a considerable debate has been centered around the hypothesis of Meister, according to whom glutathione is implied in the renal transport of amino acids [1]. However, such an intervention has not been corroborated; in fact many observations contradict the hypothesis [2,3]. In the case of yeast, a close relationship was found between the glutathione turnover and the rate of uptake of amino acids [4]. However, in [5,6] it was shown that the conclusions in [4] were based on a deficient methodology and that no such role could be ascribed to glutathione.

In [7] the phenotype of a mutant strain of *Saccharomyces cerevisiae*, impaired in the general amino acid permease, was related to a depletion in the glutathione pool, resulting from a deficiency in glutathione synthetase (EC 6.3.2.3). In a preliminary study of γ-glutamyltranspeptidase (EC 2.3.2.2) in several mutant strains of yeast, defective in amino acid transport, we reported that the problem was of considerable complexity and that a role of glutathione in the amino acid transport cannot be delineated [8]. Here we show that to the contrary of [7] no linkage between glutathione and amino acid transport can be demonstrated in *Saccharomyces cerevisiae*.

2. Materials and methods

All *Saccharomyces cerevisiae* strains used were from this laboratory and described in [8].

The mineral standard medium for growing yeast was as in [9]. Glucose as carbon source was added at 3% final conc. Vitamins and trace amounts of metal salts were added in [9]. When used as nitrogen source, ammonium sulfate was added at 50 mM final conc. Amino acids were added at 1 mg/ml final conc. Aerobic cultures were grown at 29°C under constant mixing and aeration. Growth was monitored at 660 nm and exponentially growing cells were harvested at a maximum A_{660} 0.3–0.4 for the determination of the glutathione intracellular concentration and the glutathione synthetase level.

Glutathione synthetase, in crude extracts obtained with the French press, was estimated according to the radioactive assay in [10]. Further modifications, required for obtaining reliable estimation of the *Saccharomyces cerevisiae* enzyme, are described in the text. Proteins were estimated as in [11] with bovine serum albumin as a standard. Glutathione intracellular level was estimated after cold extraction of the cells with trichloroacetic acid or extraction with water at 100°C [12]. The two methods gave essentially the same yield of glutathione. Thiol was estimated according to [13]. The values obtained were then expressed on the basis of the cellular dry wt and a mM level as in [12].

[U-^{14}C]Glycine (4.22 GBq/mmol) was obtained from the Radiochemical Centre, Amersham. L-γ-Glutamyl-L-aminobutyric acid was synthesized according to [14]. All the other products were of reagent grade quality and obtained from one of the following: Sigma; Fluka; Aldrich; Merck; Vega Biochemicals.

3. Results and discussion

3.1. *The problem of glutathione synthetase cellular level estimation in the yeast*

Fig.1a shows that the culture medium of *Saccha-*

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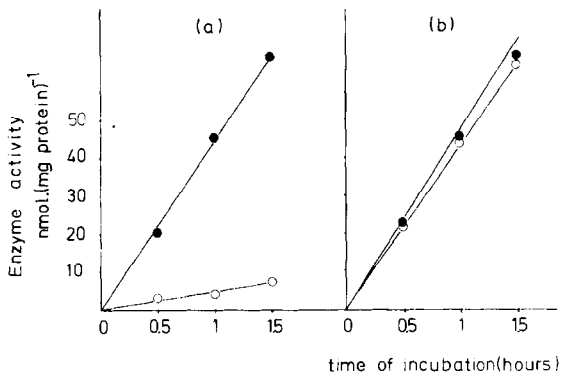

Fig.1. (a) Influence of the culture medium on the estimation of glutathione synthetase in *Saccharomyces cerevisiae* Σ1278b: the enzyme activity was measured in the time course in section 2 with 1 mg protein coming from *S. cerevisiae* crude extracts after growing on proline (○) or ammonium (●) as nitrogen sources. (b) As in (a) but in the presence of 10 mM serine borate.

romyces cerevisiae has a marked influence on the response obtained when using the standard method in [10]. In fact, a linear and finite response was only obtained when using extracts coming from cultures on ammonium sulfate as nitrogen source. In contrast, no significant activity was measured with glutamate or proline as nitrogen sources. Considering the fact that glutathione intracellular concentration was not markedly affected by the nitrogen source (table 1), we suspected that, in vitro, the reaction product was destroyed by the γ-glutamyltranspeptidase present in the extract. In fact, we had previously shown that a high cellular level of this enzyme was obtained in the presence of glutamate or proline as nitrogen sources and that it was repressed by ammonium [8]. That γ-glutamyltranspeptidase creates interferences in the estimation of glutathione synthetase was further confirmed by the addition, to the reaction medium, of 10 mM L-serine and 10 mM Na-borate, a combination inhibiting the transpeptidation reaction [15]. Indeed, a noticeable activity of glutathione synthetase in extracts grown on proline was obtained in the presence of the inhibitor (fig.1b).

3.2. *Role of the nitrogen source and amino acid permeation mutations on the intracellular level of glutathione synthetase and of the free thiol*

Table 1 shows that only minor changes were observed in the parameters of glutathione biosynthesis when growing the wild-type reference strain on

Table 1

Strain code	Characterization of the permeation mutation	Nitrogen source	Enzyme spec. act. (nmol . h^{-1} . mg protein^{-1})	Cellular glutathione (mM)
Σ1278b	Wild-type	Ammonium	45	4.2
Σ1278b	Wild-type	Proline	44	4.6
Σ1278b	Wild-type	Urea	53	5.2
2512c	*gap*$^-$	Ammonium	46	5.5
2512c	*gap*$^-$	Proline	53	4.7
RA68	*apf*	Ammonium	43	5.3
RA68	*apf*	Urea	60	6.0
MH168	*argp*$^-$	Ammonium	41	5.7
MG168	*argp*$^-$	Urea	42	6.0
MG168K3	*argp*$^-$, *gap*$^-$	Ammonium	36	6.0
MG168K3	*argp*$^-$, *gap*$^-$	Urea	35	6.5
RA309	*argp*$^-$, *lysp*$^-$	Ammonium	41	4.5
RA309	*argp*$^-$, *lysp*$^-$	Urea	46	4.9

Effect of amino acid permeation mutations on the *S. cerevisiae* gluathione synthetase and the free thiol cellular levels. The two parameters were estimated on exponential phase cells of *S. cerevisiae* which have grown on the nitrogen sources mentioned. Glutathione synthetase specific activity, expressed as nmol opthalmic acid formed . h^{-1} . mg protein^{-1}, was determined as in section 2 but in the presence of 5 mM serine borate (see fig.1b). Glutathione intracellular concentration was expressed in mM as in [12]

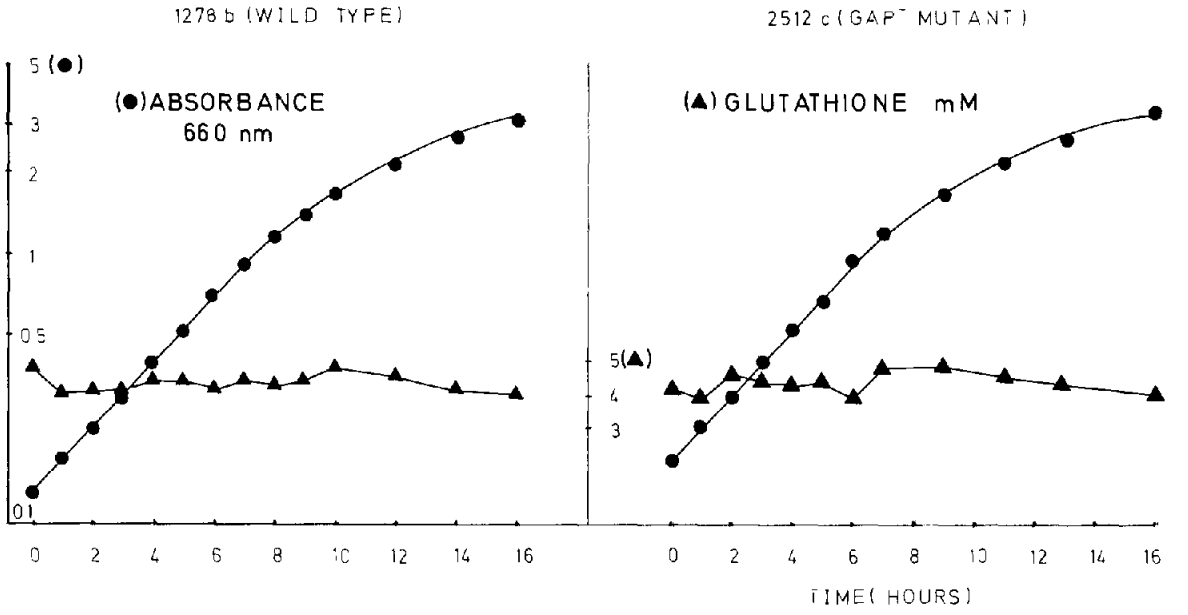

Fig.2. Evolution of the glutathione intracellular concentration during the growth on *S. cerevisiae* wild-type Σ1278b and of the *gap*⁻ mutant on proline as nitrogen source: the growth of the cells was monitored at 660 nm and the glutathione pool determined as in section 2.

various nitrogen sources. It thus appears that the tripeptide physiological role is not solely linked to the mode of nitrogenous nutrition of the yeast, that in contrast to the degredative pathway initiated by γ-glutamyltranspeptidase [8]. Moreover, we observed (table 1) that various mutations, affecting amino acid permeation systems, were not linked to any deficiency in the glutathione biosynthesis ability of *Saccharomyces cerevisiae*. These observations are inconsistent with the report [7] that the *gap*⁻ mutant, a strain impaired in the general amino acid permease [16], has a strongly reduced glutathione pool, consequent to a deficiency in glutathione synthetase. In [7] the effect seemed to be linked to the growth phase of the yeast, a view not shared by us after a detailed analysis of glutathione pool in relation to the growth phase of the microorganism (fig.2). However, in [7] obstacles in the estimation of glutathione synthetase by the standard method [10] were not encountered. This is puzzling due to the high intracellular level of γ-glutamyltranspeptidase when growing the wild-type strain and the *gap*⁻ mutant on proline [8]. This enzyme creates serious interferences in the quantitative estimation of the glutathione biosynthesis in various biological samples [17]. Finally, a strong discrepancy was found between the glutathione synthetase levels determined by us and those independently reported on the same strains in [7]. Our levels, which are comparable to those reported for a commercial baker's yeast [10], are 60-fold lower than those in [7]. In a parallel investigation on several commercial baker's yeast strains (C. J., M. P., unpublished), we found comparable levels of glutathione synthetase (40–70 units/mg protein) but often trace amounts of γ-glutamyltranspeptidase. This observation could explain the apparent ease with which glutathione biosynthesis is detected in baker's yeast. We conclude that, like the counterpart in animal tissues, a serious methodological debate is now open on the role of glutathione in microbial amino acid transport. We hope that the question will be resolved by our current study of mutants of the glutathione metabolism.

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References

[1] Meister, A. and Tate, S. S. (1974) Annu. Rev. Biochem. 45, 559–604.
[2] Horiuchi, S., Inoue, M. and Morino, Y. (1978) Biochem. Biophys. Res. Commun. 80, 873–878.
[3] McIntyre, T. M. and Curthoys, N. (1979) J. Biol. Chem. 254, 6499–6504.
[4] Osuji, G. O. (1980) FEBS Lett. 110, 192–194.
[5] Robins, R. J. and Davies, D. D. (1980) FEBS Lett. 111, 432.
[6] Robins, R. J. and Davies, D. D. (1981) Biochem. J. 194, 63–70.
[7] Mooz, E. D. (1979) Biochem. Biophys. Res. Commun. 90, 1221–1228.
[8] Penninckx, M., Jaspers, C. and Wiame, J. M. (1980) Eur. J. Biochem. 104, 119–123.
[9] Messenguy, F. (1976) J. Bacteriol. 128, 49–55.
[10] Mooz, E. D. and Meister, A. (1967) Biochemistry 6, 1722–1734.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Ramos, F., Thuriaux, P., Wiame, J. M. and Béchet, J. (1970) Eur. J. Biochem. 12, 40–47.
[13] Tietze, F. (1969) Anal. Biochem. 27, 502–522.
[14] Nishimura, J. S., Dodd, E. A. and Meister, A. (1964) J. Biol. Chem. 239, 2553–2558.
[15] Tate, S. S. and Meister, A. (1978) Proc. Natl. Acad. Sci. USA 75, 4806–4810.
[16] Grenson, M., Hou, C. and Crabeel, M. (1970) J. Bacteriol. 103, 770–777.
[17] Orlowski, M. and Meister, A. (1971) Methods Enzymol. 17B, 495–500.