

THE ROLE OF GLUTATHIONE IN AMINO ACID ABSORPTION BY YEAST

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Enzymes of the γ -glutamyl cycle [1] have been shown to be present in yeast [2]. Experimental results which have been interpreted to mean that the γ -glutamyl cycle is involved in the uptake of glycine by yeast (*Saccharomyces cerevisiae*) have been obtained using a mutant [3]. Osuji [4] has reported a direct test for the participation of the γ -glutamyl cycle in amino acid uptake by the yeast *Candida utilis*. The experiments seek to relate the rate of uptake of amino acids to the turnover of glutathione measured by equilibrating the glutathione present in yeast with $^3\text{H}_2\text{O}$ and measuring the loss of $2\text{-}^3\text{H}$ from glutathione on transfer to H_2O [5]. These experiments were initiated in this laboratory where Osuji obtained a half-life of 11 min for glutathione. These results could not be confirmed by us and a proposed paper was withdrawn.

We have changed the analytical methods and continued experiments to relate amino acid uptake to glutathione turnover. Our results are at variance with those in [4]. Under our growth conditions the half-life of glutathione is 230 min and the turnover is *not* significantly altered during the uptake of amino acids which Meister [6] suggests are good substrates for the γ -glutamyl cycle (glutamine and methionine). We will report the experimental details elsewhere, but it is necessary to establish the cause of the discrepancy in the results.

The yeast suspension used in [4] (5 g in 200 ml nutrient) is claimed to grow with a doubling time of 4 h. In our experience, yeast will not grow exponentially at this very high concentration. To measure a half-life of 39.2 h, it would be necessary to grow the yeast for ≥ 16 h, by which time the yeast concentration would be 40 g/200 ml! We are forced to the conclusion that the yeast could not have had a doubling

time of 4 h, were probably not growing at all, and would have been anaerobic.

The half-life of glutathione is reported to be reduced in the presence of those amino acids supplied. The values given are obtained by extrapolating to zero concentration the relationship between half-life and amino acid added. Not only do the curves fail to extrapolate to a common value at zero concentration but there can be no theoretical justification for this treatment. Furthermore, Osuji [4] claims that the half-life of glutathione is independent of the concentration of amino acid, a statement incompatible with a role for glutathione in amino acid absorption at non-saturating concentrations of amino acid.

What is needed is to relate the turnover of glutathione to the rate of uptake of amino acids. The experiments in [4] do not allow this to be done. In our experiments, the turnover of glutathione cannot account for more than a few % of the uptake of methionine. Our conclusions are thus the opposite of those in [4] – namely that the γ -glutamyl cycle could only account for an insignificant amount of amino acid uptake in yeast.

References

- [1] Meister, A. (1973) *Science* 180, 33–39.
- [2] Mooz, E. C. and Wigglesworth, L. (1976) *Biochem. Biophys. Res. Commun.* 68, 1066–1072.
- [3] Mooz, E. D. (1979) *Biochem. Biophys. Res. Commun.* 90, 1221–1228.
- [4] Osuji, G. O. (1979) *FEBS Lett.* 105, 283–285.
- [5] Humphrey, T. J. and Davies, D. D. (1976) *Biochem. J.* 156, 561–568.
- [6] Meister, A. and Tate, S. S. (1976) *Ann. Rev. Biochem.* 45, 559–603.