GLUTATHIONE TURNOVER AND AMINO ACID UPTAKE IN YEAST

Evidence for the participation of the γ-glutamyl cycle in amino acid transport

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1. Introduction

The γ-glutamyl cycle, consisting of a series of 6 enzyme-catalyzed reactions has been proposed to function in amino acid transport [1]. Evidence derived from the activities of the enzymes of the cycle in vitro have been advanced in support of the operation of the cycle in a number of mammalian tissues [2], in yeast [3] and also recently in plant tissues (G.O.O., in preparation). There is however insufficient evidence [4–6] to confirm unequivocally the physiological role of the cycle in vivo. The aim of the present study was to obtain some direct data concerning the function of the cycle in vivo. To achieve this, the turnover rate of glutathione, the key reactant in the γ-glutamyl cycle was determined in the absence and then presence of external amino acid, the argument being that if the cycle functions in the transport of amino acids, the turnover rate of glutathione would become more rapid in the presence than in the absence of amino acids. Yeast was chosen as the experimental organism because it contains a lot of glutathione and the 6 enzymes of the γ-glutamyl cycle are present [3].

The uptake by yeast of the 4 amino acids investigated here caused the yeast glutathione to turnover more rapidly thus proving that the γ-glutamyl cycle is operating in the yeast and that the cycle functions in amino acid transport. The results also show that the cycle is almost switched off in the absence of amino acids but switched on in response to added amino acids.

2. Methods

2.1. *The composition of the yeast nutrient solution*

The nutrient solution was prepared by mixing 500 ml mineral solution, 500 ml glucose solution and 2 ml trace element solution after they had been autoclaved. The mineral solution contained in 500 ml 0.5 g Na_2HPO_4, 8 g KH_2PO_4, 1.5 g K_2SO_4 and 2 g NH_4Cl. The glucose solution contained 10 g D-glucose in 500 ml. The trace element solution contained in 500 ml 60 mg $MgCl_2$, 20 mg $CaCl_2$, and 50 mg of each of $FeCl_3$, boric acid, $ZnCl_2$, $MnCl_2$ and KI.

2.2. *Labelling of the yeast glutathione (GSH)*

Candida utilis was tritiated by culturing in a nutrient solution containing 100 μCi tritiated water (Radiochemical Centre, Amersham) /ml for ~16 h at 29°C. The labelled yeast was centrifuged (7000 × *g* for 5 min) and washed thoroughly with sterilized distilled water to remove the external tritiated nutrient solution. Yeast so tritiated was not stored but used immediately for GSH turnover experiments.

2.3. *Determination of the turnover rate of yeast GSH*

For GSH turnover in the absence of amino acids, 5 g washed tritiated yeast was placed in 200 ml nutrient solution and kept in a rotary shaker at 29°C. At varying time intervals, 20 ml was removed and the yeast centrifuged at 7000 × *g* for 5 min. The yeast was lysed with 20 ml boiling 60% (v/v) ethanol. The yeast residue

was centrifuged out and to the supernatant was added 10 mg AnalaR GSH to facilitate purification of the yeast GSH by precipitation using the method in [7]. The isolated GSH was acid hydrolysed to amino acids and the hydrolyzate subjected to the acetic anhydride racemization process [8] in order to abstract the tritium on the C-2 of the amino acids. Scintillation counting was done with 0.2 ml of the 1.25 ml racemized distillate in 15 ml scintillation fluid [8].

For GSH turnover in amino acid-enriched nutrient solution, a known amount of the amino acid was added to 200 ml nutrient solution. The glutamic acid experiments contained 0.5, 5, 10, and 20 μmol L-Glu; the histidine experiments contained 1, 10, 20, and 40 μmol L-His; the leucine experiments contained 0.3, 3, 15, and 30 μmol L-Leu; while the arginine experiments contained 5, 15, 25, and 50 μmol L-Arg. To each amino acid-enriched nutrient solution was then added 5 g freshly tritiated yeast and the experiment kept at 29°C in a rotary shaker. At intervals, 20 ml was withdrawn, the yeast centrifuged out, and lysed with 20 ml boiling 60% (v/v) ethanol. The purification of the yeast GSH by precipitation, acid hydrolysis of the GSH to its amino acids, abstraction of the C-2 tritium from the resulting amino acids and the evaluation of radioactivity of the racemized distillate were as above.

In these GSH turnover experiments, blank experiments with unlabelled yeast were processed as the main experiments and their radioactivities subtracted from those of the main experiments.

3. Results and discussion

From the radioactivities of the GSH in the turnover experiments (absence and presence of amino acids), the first order rate constants (k) were calculated. From these rate constants the half-life ($0.693/k$) of GSH was calculated for each experimental condition. The results show that the half-life of yeast GSH in amino acid-free nutrient solution is 39.2 h. The half-lives determined in amino acid-enriched nutrient solution were plotted against the amounts of amino acids present in the nutrient solutions to obtain the results shown in fig.1.

All the 4 amino acids tested caused the half-life of

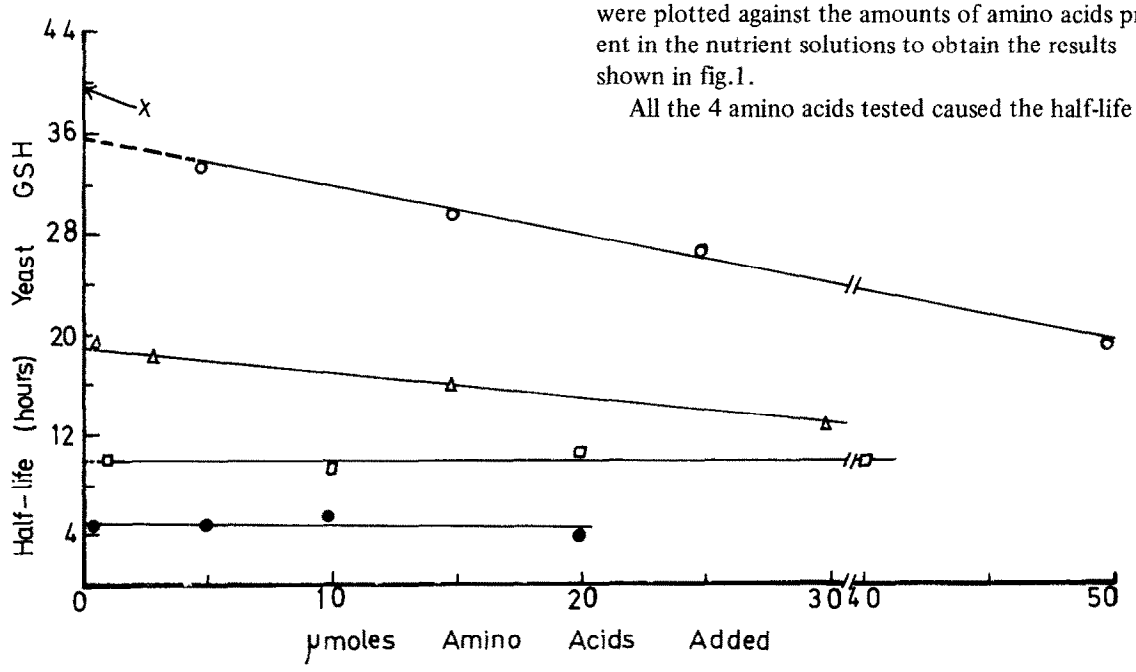

Fig.1. The effect of some amino acids on the half-life of yeast GSH. Tritiated *C. utilis* was grown in nutrient solution containing known amounts of amino acids and at intervals, the yeast GSH was isolated and its radioactivity determined. From the results, the half-life ($0.693/k$) was calculated for each experimental condition. X=39.2 h, the half-life of yeast GSH in the absence of external amino acids; (—○—) is the effect of external Arg; (—△—) is the effect of external Leu; (—□—) is the effect of external His; (—●—) is the effect of external Glu, on the half-life of yeast GSH.

the yeast GSH to decrease. This is therefore direct experimental evidence in support of the functioning of the γ-glutamyl cycle in amino acid transport in vivo. If it were the contrary, the half-life of the GSH would have remained unaffected by the added amino acids. Thus it could be said that the amino acids stimulated the γ-glutamyl transpeptidase present in the yeast cell wall to degrade the yeast GSH more rapidly leading to the absorption of the amino acids. In order to restore the steady state concentration of the GSH within the yeast cell the other enzymes of the γ-glutamyl cycle become equally stimulated and the overall result is the acceleration of the operation of the cycle. The results therefore also give support to the operation of the γ-glutamyl cycle in yeast in vivo. The presence of all the 6 cycle enzymes in yeast was reported [3] but no further evidence was presented to show that the cycle actually operates in vivo. The fact also that the 4 amino acids studied affected the half-life of yeast GSH to different degrees (cf. fig.1) is consistent with the observed different specificities of the γ-glutamyl transpeptidase for amino acids as acceptors of the γ-glutamyl group.

If the half-life curves in fig.1 are extrapolated to intersect the vertical axis, it is seen that Glu caused the half-life of the GSH to drop from 39.2 to 5 h, His caused it to drop to 10 h, Leu caused it to drop to 19 h while Arg caused it to drop to 36 h. Figure 1 is therefore a convenient method for expressing the ease of absorption of various amino acids through the γ-glutamyl cycle. The most rapidly absorbed amino acid being that which causes the greatest drop in the half-life of the GSH. In the absorption of Arg and Leu however, other secondary factors are involved because their absorption increases as their concentrations increase.

The GSH half-life of 39.2 h in the absence of amino acids shows that the GSH is almost metabolically static because the time taken by *C. utilis* to double in cell number is ~4 h under optimal growth conditions as in these experiments. Thus the γ-glutamyl cycle appears to be switched off in the absence of amino acids but switched on in the presence of amino acids. The cycle therefore appears to be primarily designed for the absorption of amino acids, and yeast is usually grown in media containing amino acids as nutrients.

The results presented here are the closest to proving the exact physiological role of the γ-glutamyl cycle because they have emerged as a result of all the reactions of the cycle functioning as a unit unlike many studies by others which dealt with isolated reactions of the cycle. So these results do contribute significantly to supporting the amino acid transport function of the γ-glutamyl cycle. Since amino acids affect the turnover rate of GSH, it follows that previous estimates of GSH turnover [9] which were determined by labelling the GSH with amino acids did reflect the accelerated turnover rates caused by the absorption of the labelled amino acids. This problem was avoided in the present investigation by using the tritium labelling procedure [8] which also minimizes recycling of metabolites.

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