THE PATHWAYS OF THE γ-GLUTAMYL CYCLE-MEDIATED UPTAKE OF AMINO ACIDS IN YEAST

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1. Introduction

The γ-glutamyl cycle proposed by Meister [1] for amino acid translocation has recently attracted new attention following the experimental demonstration that the turnover rate of glutathione in vivo in yeast is affected by amino acids [2]. The determination of the turnover rate of glutathione opens the stage for comparing the rates of amino acid uptake with the rates of glutathione turnover.

The results of such a comparison would throw light on important steps in the mechanism of the γ-glutamyl cycle. Meister et al. [1,3,4], in describing the mechanism of operation of the γ-glutamyl cycle proposed a number of pathways and models of the cycle to account for the absorption of amino acids. It is not known which of these pathways are functional in vivo. The aim of the present investigation was to determine which of the pathways proposed by Meister operate in vivo. To achieve this, the degree of the involvement of the glutathione utilization cycle in amino acid uptake was determined in the presence of varying concentrations of amino acids using *Candida utilis* as the experimental organism. The participation of more than one absorption pathway would be indicated by the characteristics of the curve obtained by plotting the degree of involvement of the glutathione utilization cycle against the amount of amino acid supplied.

The results show that for Glu, His, Arg and Leu, two absorption pathways operate: the glutathione utilization pathway is responsible for absorbing low concentrations of the amino acids; while in higher concentrations of the amino acids, a second pathway operates and the best candidate here is the accelerated exchange diffusion pathway also proposed in [4]. The results further show that the two pathways are linked probably by a common intermediate, and so together they constitute a functional unit, the γ-glutamyl cycle.

2. Methods

2.1. *Absorption of amino acids by yeast*

The rates of uptake by *C. utilis* of the following L-amino acids: glutamic acid, histidine, leucine and arginine were determined as follows:

Four concentrations of Glu were used: 0.5, 5, 10 and 20 μmol/ 70 ml nutrient solution. To each was added 1 μCi L-[^{14}C]glutamic acid (290 mCi/mmol). All radiochemicals were purchased from the Radiochemical Centre, Amersham.

Three concentrations of His were used: 1, 10, and 20 μmol/ 70 ml nutrient solution. Into each was added 20 μCi L-[2,5-^{3}H]histidine (55 Ci/mmol).

Four concentrations of Leu were used: 0.3, 3, 15 and 30 μmol/ 70 ml nutrient solution. Into each was added 20 μCi L-[4,5-^{3}H]leucine, (1 Ci/mmol).

Four concentrations of Arg were used: 5,15,25 and 50 μmol/70 ml nutrient solution. Into each solution was added 20 μCi L-[5-^{3}H]arginine (16 Ci/mmol). The nutrient solution was prepared as in [2].

After adding 0.75 g fresh *C. utilis* into each solution, the experiments were left at 29°C in a rotary shaker and at intervals, 3 ml of each culture solution was removed, centrifuged at 7000 × *g* for 5 min, and 200 μl supernatant removed for scintillation counting [2].

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2.2. *Determination of the turnover rate of yeast glutathione*

This was done as in [2] using tritiated yeast in the presence of the specified amino acid concentrations above, but the radioactive amino acids were not added.

3. Results and discussion

The four amino acids studied were rapidly absorbed by yeast. A plot of the rates of absorption against the external concentrations of the amino acids gave hyperbolic curves, thus confirming the amino acid absorption to be membrane mediated.

From the turnover rate of the yeast glutathione (GSH) in the presence of each amino acid concentration [2] and from the pool size of GSH in the yeast (~6.5 mmol GSH/kg fresh *C. utilis*) the theoretical rate of absorption of the amino acid through the GSH utilization pathway was calculated; and this value was expressed as percentage of the experimentally derived rate of absorption of the amino acid, to obtain the degree of involvement of the GSH pathway in the uptake of the amino acid. The percentage involvement was plotted against the external concentration of the amino acid to get the curves in fig.1.

Figure 1 shows that for the two amino acids, Glu and His, the turnover rates of GSH adequately account for their absorption at the lower concentrations of the amino acids. Thus for the first time, the rate of amino acid uptake has been shown to be directly proportional to the rate of GSH turnover by in vivo experiment. The estimates in [3] failed to establish this direct proportionality. For higher concentrations of Glu and His however, the turnover rates of GSH account for only a fraction of their amounts absorbed. The turnover rate of GSH accounts for ~50% of the absorption of low concentrations of Leu, while at higher concentrations, the participation of the GSH pathway drops to ~4%. For Arg, the degree of involvement of the GSH pathway remained very low, being ~2% at high concentrations of Arg. The uptake of very low concentrations of Arg was not studied experimentally in this investigation, but calculations based on the expected rate of Arg absorption at external concentrations < 2.5 μmol show that at these low levels of Arg the involvement of the GSH pathway increases.

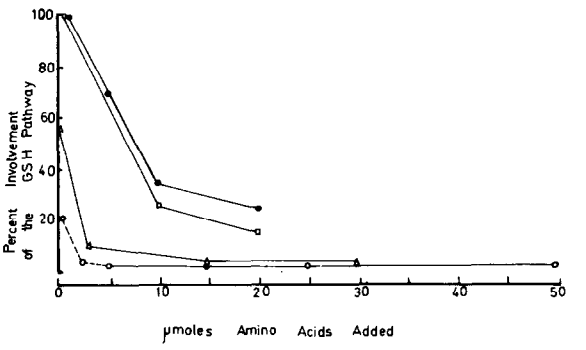

Fig.1. The degree of the involvement of two of the pathways of the γ-glutamyl cycle in the uptake of some L-amino acids by *C. utilis*. At each amino acid concentration, the GSH turnover rate [2] and the GSH pool size in 0.75 g *C. utilis*, were used to calculate the theoretical rate of the amino acid uptake through the GSH utilization pathway. This value was then expressed as percentage of the experimentally determined rate of the amino acid uptake, and plotted against the external concentration of the amino acid. The extrapolation in the Arg curve was theoretically calculated. The GSH content of *C. utilis* was determined by the method in [5]. (-●—●-) Glu, (-□—□-) His, (-△—△-) Leu, (-○—○-) Arg.

All the amino acid curves in fig.1 have similar shapes. Each curve drops from the area of low external amino acid concentrations to the area of high concentrations of amino acid. This change in the characteristics of the curves signifies that more than one pathway is involved in the uptake of the four amino acids. Meister and Tate [4] have proposed two main pathways of the γ-glutamyl cycle to account for the absorption of amino acids. The first pathway is the GSH utilization cycle, where GSH is the major γ-glutamyl donor in transpeptidation; while the second pathway is the accelerated exchange diffusion cycle where γ-glutamyl amino acids are the major γ-glutamyl donors. The results of the present investigation suggest that these two pathways are operating in *Candida*. At low concentrations of amino acid outside the yeast, amino acid is absorbed mainly through the agency of the GSH utilization pathway, but at higher concentrations, the absorption mechanism switches over to the accelerated exchange diffusion pathway. The drop in each curve in fig.1 is consistent with the switch over of pathway for the amino acid uptake.

The two pathways appear to be linked because the drop in each curve is gradual and in the case of the Glu curve a situation was obtained in the slope of the drop where at 5 μmol external concentrations of Glu, 70% of it was absorbed through the GSH pathway while 30% was absorbed through the second pathway. A sharp drop in the curves would have signified the absence of any linkage between the two pathways. The point of linkage is most probably the common intermediate, the enzyme–γ–glutamyl amino acid [E (γ-glu AA)] complex, proposed [4] which can be further metabolised either through the GSH utilization pahtway in the presence of very low concentrations of external amino acids, or through the exchange diffusion pathway in the presence of higher concentrations of external amino acids.

The exchange diffusion process for the uptake of amino acid is dependent on the concentrations of the amino acid outside the cell membrane [4] and the results in fig.2 satisfy this situation because the amounts absorbed by yeast increased as the external concentrations of the amino acids were increased.

During the exchange diffusion process, the GSH utilization cycle serves as a reservoir of the γ-glutamyl group from where it is released to 'charge' the amino acid uptake process [4]. This release of the γ-glutamyl group is represented by the 'residual' levels of operation of the GSH cycle in the area of high amino acid concentrations observed in fig.1. This 'residual' activity of the GSH cycle during the operation of the exchange diffusion pathway is essential because in the uptake of very high concentrations of Arg (50 μmol) where one would have expected a complete cessation of the operation of the GSH cycle, it, on the contrary, persisted and even increased slightly from 2% at 15 μmol external Arg to 2.5% at 5C μmol external Arg. The persistence of this 'residual' activity of the GSH cycle during the uptake of high concentrations of Arg is ensured by the steady increase in the turnover rate of GSH with increasing concentrations of Arg as was reported [2].

The results of this investigation therefore give further support to the functioning in vivo of the γ-glutamyl cycle in amino acid uptake in yeast. More importantly, the results have revealed how two of the

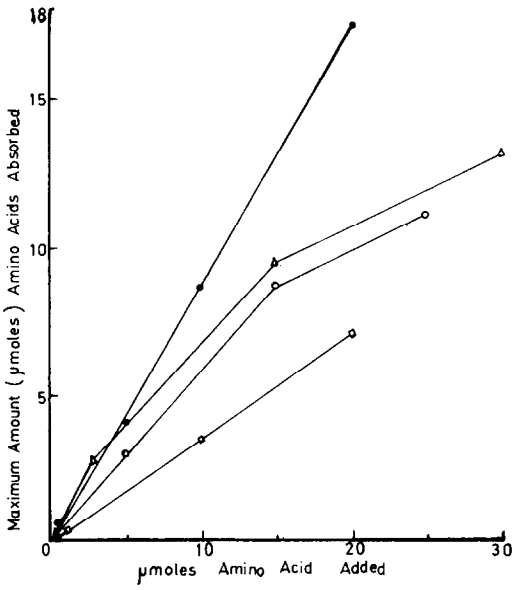

Fig.2. The relationship between the total amount of amino acid absorbed by *C. utilis* and the external concentration of the amino acid. The yeast was allowed to grow in the amino acid-enriched nutrient solutions for up to 10 h to ensure maximum absorption of the amino acid. Then the amount of amino acid left unabsorbed was determined from which that absorbed was calculated. (●———●) Glu, (□———□) His, (△———△) Leu, (○———○) Arg.

proposed pathways of the γ-glutamyl cycle are operated under different conditions to ensure uninterrupted uptake of amino acids. The results also suggest that the γ-glutamyl cycle is virtually the only route through which the four amino acids examined here are absorbed by yeast under the optimal growth conditions used in these experiments.

References

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