THE KINETICS OF THE γ-GLUTAMYL CYCLE-MEDIATED UPTAKE OF AMINO ACIDS

Considerations explaining the bifurcation of the γ-glutamyl cycle

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Received 4 December 1979

1. Introduction

Meister and his associates proposed the existence of the γ-glutamyl cycle and have accumulated evidence mostly indirect to show the participation of the cycle in the transport of amino acids [1]. Some evidence has emerged which disputes the functioning of the cycle in amino acid translocation [2]. However, amino acids have been shown to modulate the turnover rate of glutathione [3], and the γ-glutamyl cycle to bifurcate into the glutathione utilization and the accelerated diffusion pathways in the yeast [4]. Thus, that the γ-glutamyl cycle functions in amino acid transport, at least in the yeast, is no longer in dispute. This clearer understanding of the operation of the γ-glutamyl cycle now permits a reappraisal of the kinetics of the absorption process using whole organisms. In vitro kinetic studies [5,6] of the absorption process had suggested a ping-pong mechanism for the formation of the transpeptidation complex but suggested nothing about the kinetics of the accelerated diffusion process.

The results of this reappraisal of the kinetics of amino acid uptake show that the carrier enzyme, γ-glutamyl transpeptidase, has two amino acid sites. At very low external amino acid concentration, amino acid binds preferentially at the transpeptidation site to give the transpeptidation complex. This amino acid is absorbed via the glutathione utilization pathway [4,6]. But at higher amino acid concentrations, amino acid also binds at the accelerated diffusion site and the two molecules of amino acids are then absorbed via the accelerated diffusion pathway.

2. Methods

The turnover rates of yeast (*Candida utilis*) glutathione (GSH) in the presence of varying amounts of L-glutamic acid, L-histidine, L-leucine and L-arginine, were determined by the tritiation method in [3].

The rates of uptake of varying amounts of the 4 amino acids by 0.75 g *C. utilis* were determined as in [4], except that many different concentrations of each amino acid up to 50 μmol/70 ml nutrient solution were used. The amounts absorbed were plotted against time, and from the curves, the amounts absorbed in the first 30 min were used for calculating the initial rates of uptake.

The GSH content of *C. utilis* was determined as in [7].

3. Results

From the degree of involvement of the GSH utilization pathway [4], the amount of amino acid absorbed in the first 30 min at each external amino acid concentration was resolved into the fraction absorbed via the GSH pathway, and that via the accelerated diffusion pathway. The initial velocities of amino acid uptake via each pathway were then calculated for each external amino acid concentration. Double reciprocal plots of initial velocities versus external concentrations of amino acid gave straight lines from which V_{max} and K_m were calculated. Subjecting the initial velocities and external amounts of amino acids to the Hanes-Woolf plot [8] also gave straight

 Elsevier/North-Holland Biomedical Press

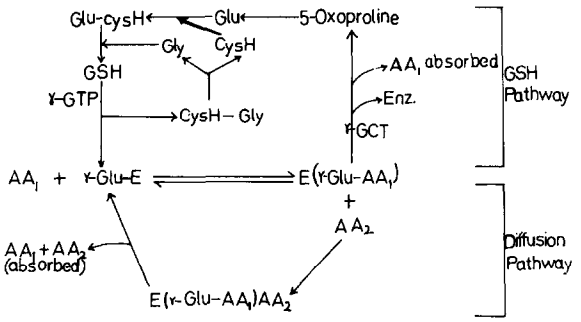
lines from which the K_m and V_{max} derived from the reciprocal plots were confirmed. Table 1 contains the K_m and V_{max} of the absorption of each amino acid.

4. Discussion

Table 1 shows that each of the 4 amino acids studied has 2 K_m values: one for the GSH utilization pathway, and the other for the accelerated diffusion pathway. Therefore γ-glutamyl transpeptidase (γ-GTP) has two binding sites for amino acids (not one [5,6]) and this suggests a mode of operation of the pathways of the γ-glutamyl cycle explained as follows:

The Michaelis constant for the absorption of each amino acid via the GSH pathway is lower than that for absorption via the diffusion pathway. Thus the amino acid binding site for absorption via the GSH pathway is the first site. The binding of amino acid at this site (transpeptidation site) leads to the formation of the transpeptidation complex, enzyme–γ-glutamyl–amino acid, (E–[γ-Glu–AA_1]), which is metabolized by γ-glutamyl cyclotransferase (γ-GCT) to effect the uptake of the bound amino acid via the GSH pathway. This therefore confirms Meister's proposal for amino acid uptake via the GSH utilization pathway [1].

Since the K_m for the diffusion pathway is greater than that for the GSH pathway, the binding of the two molecules of amino acid takes place consecutively but non-cooperatively. In addition the second molecule of amino acid is an activator because the V_{max} of the absorption process increases following its binding at the accelerated diffusion site of the transpeptidation complex. The binding of amino acid to the transpeptidation complex forms a specially



Scheme 1

The full γ-glutamyl cycle of the yeast *Candida utilis*, showing its bifurcation into the GSH and the accelerated diffusion pathways. For the enzymes catalysing the different reactions of the GSH pathway refer to [1]

activated complex, the diffusion complex, (E–[γ-Glu–AA_1]AA_2), which triggers on the functioning of the diffusion pathway. In Meister's model of the diffusion pathway, the transpeptidation complex was also the diffusion complex. As there was no kinetic reason to justify such a situation, it raised some uncertainty about the operation of the diffusion pathway. The specially activated diffusion complex revealed by this study lends some credence to the operation of the accelerated diffusion pathway in the yeast. From these considerations, scheme 1 is proposed for the mechanism of uptake of the amino acids studied.

Table 1 shows that in the GSH pathway, the V_{max} for the uptake of Arg and Leu are lower than those for Glu and His since they are poorer substrates of γ-GTP and γ-GCT, which are key enzymes in the GSH pathway. Conversely in the diffusion pathway, the V_{max} for the uptake of Arg and Leu are higher

Table 1

The kinetic constants (K_m and V_{max}) for the uptake of some L-amino acids via the two pathways of γ-glutamyl cycle, by the yeast *C. utilis*

Amino acids	GSH pathway		Diffusion pathway	
	K_m (mM)	V_{max} (μmol . min^{-1} . l^{-1})	K_m (mM)	V_{max} (μmol . min^{-1} . l^{-1})
Glutamic acid	0.04	0.17	0.2	0.5
Histidine	0.05	0.15	0.17	0.8
Leucine	0.08	0.08	0.16	1.6
Arginine	0.1	0.05	0.12	2.3

than those for Glu and His. These inverse relationships, also exhibited by the K_m values, are consistent with the bifurcation of the γ-glutamyl cycle [4], whose operation depends largely on the specificities of the different amino acids for the two key enzymes, γ-GTP and γ-GCT. Those amino acids which are good substrates of γ-GTP and γ-GCT are more rapidly absorbed via the GSH pathway, while those that are poor substrates are more rapidly absorbed via the accelerated diffusion pathway. The diffusion pathway is therefore useful because if the γ-glutamyl cycle consisted of only the GSH pathway, yeast would have been unable to absorb sufficient quantities of amino acids especially those which are poor substrates of γ-GTP and γ-GCT.

The mechanism proposed in scheme 1 gives support to the degree of involvement of the GSH pathway in the uptake of amino acids as observed in [4], because since amino acids first bind at the transpeptidation site it follows that at very low external concentrations of amino acids only the GSH pathway operates. In fact, at concentrations lower than V_{max} for the GSH pathway, amino acids are absorbed entirely via the GSH pathway. But as the concentration increases, amino acid molecules start binding to the accelerated diffusion site so that absorption via the diffusion pathway commences while the involvement of the GSH pathway drops. If the K_m for the diffusion pathway were equal to that of the GSH pathway, it would have contradicted the increased involvement of the GSH pathway in the uptake of low concentrations of amino acids [4].

These 4 amino acids are therefore absorbed entirely via the γ-glutamyl cycle. This conclusion has been reached understanding the effects of amino acids on the turnover rates of GSH [3] and the functional pathways of the full γ-glutamyl cycle [4]. A study of the kinetics of the absorption of amino acids based only on their observed rates of uptake would have given results, suggesting the non-involvement of the γ-glutamyl cycle in their uptake [2]. Also an ordinary kinetic study of the properties of purified γ-GTP [5,6] would have been unable to detect the bifurcation of the full cycle. Finally these results show that Meister's full γ-glutamyl cycle [1] refers to the GSH utilization pathway. The full cycle is more elaborate, comprised of the GSH utilization and the accelerated diffusion pathways as in scheme 1.

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