INHIBITION OF BRAIN γ-GLUTAMYL TRANSPEPTIDASE BY α-KETOGLUTARATE

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γ-Glutamyl transpeptidase of mouse brains was isolated and partially purified. The effect of pH on the acceleration by L-glutamine of the reaction catalyzed by this enzyme was observed. It was suggested that α-ketoglutarate was most probably accumulated in the reaction mixture at pH 9. The significant inhibition of brain γ-glutamyl transpeptidase activity by α-ketoglutarate was detected. The results are discussed from the point of view of proposed role of γ-glutamyl transpeptidase in the brain.

γ-Glutamyl transpeptidase catalyzes the transfer of γ-glutamyl residue from glutathione or other γ-glutamyl peptides to amino acids as acceptors[1,2]. The enzyme is widely distributed in animal tissues[3-10] and is a part of the γ-glutamyl cycle which was proposed as a transport system for amino acids[11-13]. It was also suggested that the γ-glutamyl transpeptidase activity is associated with the movement of amino acids between the blood and the brain[9].

If the activity of the isolated enzyme is measured *in vitro*, the acceleration of the reaction can be obtained by the addition of an acceptor amino acid or dipeptide. Of free amino acids, L-glutamine can serve as the most potent acceptor of γ-glutamyl group in a reaction catalyzed by purified rat kidney γ-glutamyl transpeptidase[14]. But it was found[15] that the acceptor activity of higher concentrations of L-glutamine was very low if the partially purified γ-glutamyl transpeptidase of the mouse brain was used. Our report deals with the explanation of this striking finding and shows that it was most probably α-ketoglutarate, which was the cause of the latter phenomenon.

EXPERIMENTAL

Material and Methods

γ-Glutamyl-*p*-nitroanilide, L-glutamine and α-ketoglutarate were purchased from Sigma Chemical Co.

γ-Glutamyl transpeptidase was isolated from brain cortices of 6 to 7-week-old white male mice (inbred strain A), and partially purified by the procedure used for isolation of the kidney enzyme[14].

Ten grammes of tissue were homogenized in 40 ml of 0·9% NaCl. The homogenate was treated with 0·5 volume of acetone (precooled to −30°C) and the mixture was centrifuged at 16000 g for 30 min. The pellet was suspended in 22·9 ml of 0·1M Tris-HCl buffer (pH 8) containing 1% sodium deoxycholate and then homogenized for 2 min. The suspension was stirred at 4°C for 4 h and then centrifuged at 16000 g for 60 min. Solid ammonium sulphate was added to the supernatant solution (4·72 g per 10 ml) and after standing overnight the precipitate was spun off, suspended in a small volume of 0·01M Tris-HCl (pH 8) and dialyzed against 5 liters of the same buffer for 24 h. The dialyzed solution was frozen and stored at −30°C.

γ-Glutamyl transpeptidase activity was assayed with γ-glutamyl-*p*-nitroanilide as a γ-glutamyl donor[14,16]. The reaction mixture (1 ml) contained 2·5 mM γ-glutamyl-*p*-nitroanilide, 80 mM Tris-HCl buffer (pH 9 or 6·5), 75 mM-NaCl, L-glutamine, α-ketoglutarate (in concentrations as shown in figures) and the enzyme (about 0·5 mg of protein). The rate of release of *p*-nitroaniline was followed at 410 nm in a Specord UV-VIS model spectrofotometer (Zeiss, Jena). The specific activity of the enzyme was expressed in units per mg of protein. One unit of γ-glutamyl transpeptidase liberates one nanomole of *p*-nitroaniline per minute at 37°C under the condition specified above.

The protein content in the enzyme preparations was determined by the method of Lowry and coworkers[17].

The results in this paper represent the means of a least 5 experiments.

RESULTS AND DISCUSSION

The activity of a partially purified preparation of γ-glutamyl transpeptidase from mouse brain cortices was gradually increased by raising the concentration of various amino acids in the reaction mixture[15]. When the increasing concentrations of L-glutamine were used as an acceptors of γ-glutamyl residue, the enzyme activity increased progressively only at pH 6·5. If the reaction was running at pH 9, the activity of γ-glutamyl transpeptidase barely increased up to the 10 mM concentration of L-glutamine. The further increasing of the concentration of L-glutamine caused a conspicuous decrease in its stimulatory capacity (Fig. 1). This finding gave rise to the assumption

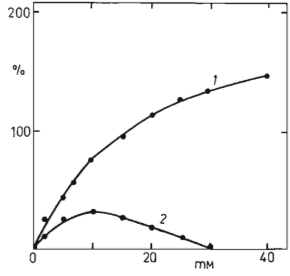

FIG. 1

Influence of Raising Concentration of L-Glutamine on Brain γ-Glutamyl Transpeptidase Activity at pH 6·5 and 9·0

Activity was determined as described under Experimental. *1* pH 6·5, *2* pH 9·0.

that some compound which inhibits the transpeptidation was arising from L-glutamine during the reaction at pH 9. One of the possible inhibitory mechanisms could consist in the accumulation of α-ketoglutarate in the reaction mixture. Therefore the influence of α-ketoglutarate on the reaction catalyzed by brain γ-glutamyl transpeptidase was studied. Fig. 2 shows that the addition of α-ketoglutarate inhibits the brain γ-glutamyl transpeptidase activity either in the absence or in the presence of 10 mM-L-glutamine. The inhibition occurs at both pH 6·5 and pH 9.

Lineweaver–Burk plots obtained with the two concentrations of α-ketoglutarate (Fig. 3) show that the inhibition of the enzyme activity by lower concentration of α-ketoglutarate is competitive. But the intersection of straight line *1* and *3* in Fig 3 does not fall on either axis, what is typical for mixed inhibition. In this case the presence of a higher concentration of the inhibitor on an enzyme, prevents the breakdown of the active complex, but also interferes to some extent with the binding of a substrate amino acid[18].

The data presented here support the idea that the decrease of the enhancing effect of higher concentrations of L-glutamine on the activity of brain γ-glutamyl transpeptidase shown in Fig. 1 is due to the appearance of α-ketoglutarate in the incuba-

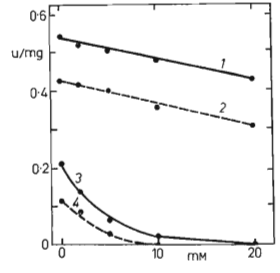

FIG. 2

Effect of α-Ketoglutarate on the Activity of Brain γ-Glutamyl Transpeptidase at pH 9·0 and pH 6·5

Enzyme activity was determined as described under Experimental. Full lines represent the activity stimulated by 10 mM-L-glutamine. Dashed lines represent the activity in the absence of acceptor amino acid. pH: *1*,*2* 9·0; *3*,*4* 6·5.

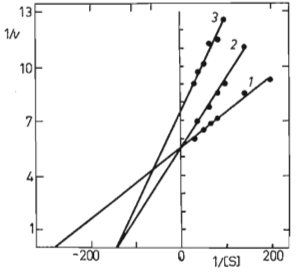

FIG. 3

Lineweaver–Burk Plots Obtained with α-Ketoglutarate as an Inhibitor of Brain γ-Glutamyl Transpeptidase

The velocity (v) of the reaction was expressed as units per mg of enzyme protein. The concentration of substrate ([S]) varied between 5 mM and 30 mM-L-glutamine. Enzyme activity was determined at pH 6·5 as described under Experimental. *1* No inhibitor, *2* 1·25 mM-α-ketoglutarate, *3* 2·50 mM-α-ketoglutarate.

tion mixture. It is probable that the conversion of L-glutamine into α-ketoglutarate is an enzymatic process which is activated by higher concentrations of L-glutamine at pH 9. There are at least two ways by which L-glutamine can be converted into α-ketoglutarate. As it has been reported[19], even purified preparations of the rat kidney γ-glutamyl transpeptidase exhibit glutaminase activity. It should therefore be admitted that some glutaminase or glutamine-keto acid transaminase activity is associated with the brain γ-glutamyl transpeptidase preparation. Both of these additional enzymatic activities can lead directly, or *via* formation of glutamate, to the appearance of α-ketoglutarate during the reaction catalyzed by brain γ-glutamyl transpeptidase. There is some direct evidence of the formation of a α-keto acid from L-glutamine in the medium during the γ-glutamyl transpeptidase reaction at pH 9. When the reaction was performed at pH 6·5, it was not possible to detect any α-keto acid[20]. These preliminary findings also support the idea about the inhibitory effect of α-ketoglutarate in case of the γ-glutamyl transpeptidase reaction.

It was described that free α-keto acids did not influence the kidney γ-glutamyl transpeptidase activity at all[14]. Therefore the specificity of the inhibitory effect of α-ketoglutarate with respect to mouse brain enzyme seems to be probable.

It is possible to conclude that the activity of mouse brain γ-glutamyl transpeptidase is remarkably depressed by α-ketoglutarate, especially at pH 6·5. This finding is particularly important from the point of view of an *in vivo* function of γ-glutamyl transpeptidase in the brain. It is possible to expect that changes of tissue levels of α-ketoglutarate can participate in the regulation of amino acid transport in the brain and through the blood-brain barrier.

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