Effect of Carbobenzoxy Derivatives of Amino Acids on Glutamine Synthesis and γ-Glutamyl Transfer

In a previous publication[1] the inhibitory effect of α-N-ethyl and α-N-propyl-L-glutamine on ovine brain glutamine synthetase and γ-glutamyl transferase was reported. Similar results were obtained with rat liver glutamine synthetase[2]. In the course of further investigations on the effect of α-N-derivatives of glutamine on enzyme systems connected with glutamine metabolism, we found that carbobenzoxy-L-glutamine weakly inhibits ovine brain glutamine synthetase and, to a greater extent, γ-glutamyl transferase. A comparison with carbobenzoxy (Cbz) derivatives of other amino acids revealed that approximately the same inhibitory effects are exerted by Cbz-L-asparagine, Cbz-L-alanine and Cbz-glycine. However, very much stronger inhibitions were obtained with Cbz-derivatives of amino acids bearing an aromatic ring in addition to that of the Cbz-group: Cbz-L-phenylalanine, Cbz-L-tyrosine, Cbz-β-N-benzyl-L-asparagine, N-Cbz-S-benzyl-L-cysteine and α, ε-di-Cbz-L-lysine. The present paper deals with this phenomenon.

Materials and methods. Cbz-glycine was prepared according to BERGMANN and ZERVAS[3], Cbz-L-phenylalanine according to GRASSMANN and WÜNSCH[4]. The preparation of Cbz-β-N-benzyl-L-asparagine will be described elsewhere. All the other Cbz-derivatives were purchased from Fluka AG, Buchs, Switzerland.

ATPase-free ovine brain and rat liver glutamine synthetase preparations were obtained as described previously[1,2]. The brain preparations were also used for the transfer reaction.

Assay procedure. Synthetase reaction: each reaction mixture contained in 5.3 ml 0.1 *M Tris* buffer (pH 7.2): L-glutamic acid, 25 μmoles; NH_2OH, 50 μmoles; $MgSO_4$, 40 μmoles; ATP, 10 μmoles; enzyme solution, 0.3–0.5 ml of ovine brain enzyme, 2 ml of rat liver enzyme; Cbz-derivatives, as indicated in the Tables. Transferase reaction: each reaction mixture contained in 5.3 ml 0.1 *M Tris* buffer (pH 7.2): L-glutamine, 75 μmoles; NH_2OH, 50 μmoles; $MgSO_4$, 40 μmoles; ADP, 0.25 μmoles; phosphate, 25 μmoles; enzyme solution, 0.3–0.5 ml; Cbz-derivatives, as indicated in the Table. The incubation was carried out for 15 min at 30 °C. At the end of the incubation 0.5 ml 50% CCl_3COOH was added and after some cooling the mixtures were centrifuged and – when necessary – filtered. The hydroxamic acid formed was estimated in aliquots by the method of LIPMANN and TUTTLE[5]. The values given in the Tables are calculated for the whole volume of the reaction mixtures plus that of CCl_3COOH added. In a part of the experiments with ovine brain synthetase ammonia (25 μmoles) was used instead of hydroxylamine and the synthesis was measured by estimating the inorganic phosphate liberated from ATP using the method of GOMORI[6]. In these cases the reaction mixture (5.5 ml) contained less ATP (5 μmoles) and less enzyme (0.2 ml).

Results. As can be seen from Table I, Cbz-derivatives of amino acids bearing an aromatic ring in addition to that contained in the Cbz-group inhibit the synthesizing reaction to a much greater extent than the corresponding Cbz-derivatives without such an additional aromatic ring. Thus Cbz-L-alanine in a molar ratio of 10:1 of inhibitor to substrate inhibited the synthesis only by 15%, whereas at the same molar ratio Cbz-L-phenylalanine gave over 80% inhibition. The inhibition by Cbz-β-N-benzyl-L-asparagine is many times greater than that by Cbz-L-asparagine and the inhibition by α, ε-di-Cbz-L-lysine is much greater than that exerted by α-Cbz-L-lysine. A very high inhibition is also obtained with N-Cbz-S-benzyl-L-cysteine.

Table I. Effect of Cbz-derivatives of various amino acids on ovine brain glutamine synthetase

Derivative added (μmoles)		Hydroxamic acid formed (μmoles)
None		4.03
Cbz-L-glutamine	250	3.42
Cbz-L-asparagine	250	3.61
Cbz-glycine	250	3.42
Cbz-L-alanine	250	3.42
Cbz-L-phenylalanine	250	0.52
None		4.32
Cbz-L-phenylalanine	125	2.03
Cbz-L-tyrosine	125	2.48
N-Cbz-S-benzyl-L-cysteine	125	0.00
	100	0.64
	50	2.26
None		4.90
Cbz-L-asparagine	188	4.53
Cbz-β-N-benzyl-L-asparagine	188	2.48
α-Cbz-L-lysine	100	4.71
α, ε-di-Cbz-L-lysine	100	1.94

Table II. Effect of various Cbz-derivatives of amino acids on rat liver glutamine synthetase

Derivative added (μmoles)		Hydroxymic acid formed (μmoles)
None		3.48
Cbz-L-phenylalanine	200	1.32
Cbz-L-tyrosine	200	1.68
N-Cbz-S-benzyl-L-cysteine	75	1.61
None		4.03
α-Cbz-L-lysine	188	3.48
α, ε-di-Cbz-L-lysine	100	1.93
	188	0.64

Table III. Effect of various Cbz-derivatives of amino acids on ovine brain glutamyl transferase

Derivative added (μmoles)		Hydroxamic acid formed (μmoles)
None		4.38
Cbz-L-glutamine	300	2.26
Cbz-L-asparagine	300	2.13
Cbz-L-alanine	300	2.28
Cbz-L-phenylalanine	300	0.0
Cbz-L-phenylalanine	75	1.64
Cbz-L-tyrosine	75	2.74
N-Cbz-S-benzyl-L-cysteine	75	0.52
None		3.74
α-Cbz-L-lysine	150	2.31
α, ε-di-Cbz-L-lysine	150	0.0
Cbz-L-alanine	150	2.51
Cbz-L-phenylalanine	150	0.90
Cbz-β-N-benzyl-L-asparagine	150	0.62

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A strong inhibition of the synthetase reaction by Cbz-derivatives with an additional aromatic ring was also observed in the case of rat liver glutamine synthetase (Table II).

Table III shows the effect of Cbz-derivatives of amino acids on ovine brain γ-glutamyl transferase activity. Although in this case the inhibition by Cbz-derivatives without an additional aromatic ring is more pronounced than in the synthetase reaction, here, too, the derivatives with an additional aromatic ring are considerably stronger inhibitors.

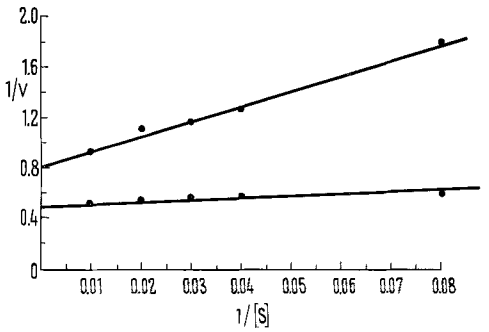

Fig. 1. Inhibition of ovine brain glutamine synthetase by Cbz-L-phenylalanine. (I) L-glutamic acid; (II) L-glutamic acid and Cbz-L-phenylalanine (75 µmoles). [S] is the concentration of L-glutamic acid (µmoles) in 5.5. ml reaction mixture. Velocity (V) is expressed in µmoles inorganic phosphate liberated in 15 min at 30 °C.

Figures 1 and 2 show that the inhibitions of ovine brain glutamine synthetase and γ-glutamyl-transferase by Cbz-L-phenylalanine are of a mixed type. Similar results, for both reactions, were obtained with Cbz-L-tyrosine and N-Cbz-S-benzyl-L-cysteine.

In the case of ovine brain glutamine synthetase, the extent of inhibition exerted by Cbz-L-phenylalanine (125 µmoles) or N-Cbz-S-benzyl-L-cysteine (75 µmoles) was the same when the respective concentrations of ATP, hydroxylamine or Mg^{++} were increased three-fold.

Discussion. The strong inhibitory activity of the derivatives containing 2 aromatic groups may be explained as follows. The 2 aromatic rings interact through hydrophobic bonds with 2 suitable sites on the enzyme molecule. This causes inhibition either by blocking the active site *per se* or by exerting an allosteric effect. It remains to be shown, by use of appropriate compounds, whether the carboxyl groups of the inhibitors play a role in the inhibition. Cbz-derivatives of amino acids bearing an additional aromatic group also strongly inhibit rat liver asparaginase[7] and rat liver glutaminase[8]. It was also reported[9] that Cbz-L-phenylalanine inhibits the proteolytic and the esterolytic activity of chymotrypsin. On the other hand, Cbz-L-phenylalanine does not inhibit the glutamine-requiring carbamyl phosphate synthetase of *E. coli*[8].

It is of interest to note that i.p. injections of the sodium salts of Cbz-L-phenylalanine and N-Cbz-S-benzyl-L-cysteine markedly inhibit the growth of Ehrlich ascites carcinoma in mice[10]. Since both compounds affect a multitude of enzymes, the exact nature of these tumour inhibitions require further elucidation.

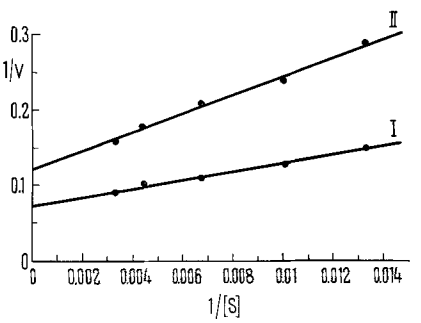

Fig. 2. Inhibition of ovine brain γ-glutamyl transferase by Cbz-L-phenylalanine. (I) L-glutamine; (II) L-glutamine and Cbz-L-phenylalanine (50 µmoles). [S] is the concentration of L-glutamine (µmoles) in 5.3 ml reaction mixture. Velocity (V) is expressed in µmoles hydroxamic acid formed in 15 min at 30 °C.

Zusammenfassung. Carbobenzoxyderivate von aromatischen Aminosäuren mit einer zusätzlichen aromatischen Gruppe hemmen die Glutaminsynthetase und γ-Glutamyl-Transferase aus Schafshirn sowie die Glutaminsynthetase aus Rattenleber stark.

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Purines and Cortisone in Lipid Mobilization

Lipolysis in adipose tissue is under a complex control system[1]. A number of hormones including epinephrine, glucagon, ACTH and adrenal cortical steroids have been shown to affect the release of fatty acids from adipose tissue in several species[2–6], and a variety of purines and purine derivatives have a similar affect[7–9]. Some of these substances, epinephrine and ACTH for instance, apparently act on the cell membrane in such a way as to activate adenyl cyclase, thus leading to increase in cyclic 3′5′-AMP[10,11]. Other compounds such as purine, theophylline and caffeine appear to have their effect by inhibiting the phosphodiesterase[7,12], thus increasing the level of cyclic AMP by a different mechanism. Cortisone has been shown to act by a third, entirely different mechanism which has been called the 'permissive effect', since it permits the lipolytic action of epinephrine to be expressed[6,13]. This is thought to occur through a decrease in uptake of glucose by the fat cell, which in turn causes a decrease in