INHIBITORS OF RAT LIVER ASPARAGINASE

G.MOR and N.LICHTENSTEIN
Department of Biological Chemistry,
The Hebrew University, Jerusalem, Israel

Received 25 April 1969

1. Introduction

An intense interest in asparaginase has developed in recent years due to the discovery that this enzyme exhibits anti-tumor activity [1,2]. Various α-*N*-alkyl derivatives of D,L-asparagine were found to inhibit L-asparaginase of *Mycobacterium phlei* [3] and rat liver [4]. It was also found that carbobenzoxy-L-asparagine markedly inhibits L- and D-asparaginase activity of Saccharomyces cerevisiae [5]. The present paper deals with the inhibition of rat liver asparaginase by carbobenzoxy derivatives of some amino acids bearing an aromatic group in addition to that of the carbobenzoxy group: carbobenzoxy-L- and D,L-phenylalanine, *N*-carbobenzoxy-L-tyrosine and *N*-carbobenzoxy-*S*-benzyl-L-cysteine.

2. Materials and methods

Carbobenzoxy-L-phenylalanine was prepared according to Grassmann and Wünsch [6]. All the other carbobenzoxy derivatives were purchased from Fluka A.G., Buchs, Switzerland.

Rat liver enzyme was prepared as described earlier [4], the homogenation being carried out in 0.1 M phosphate buffer, pH 7.4, instead of water.

Assay procedure: The reaction mixtures contained in 3 ml 0.1 M phosphate buffer, pH 7.4: L-asparagine, 18 μmoles; rat liver enzyme, 0.6 ml; inhibitors, as indicated in table 1. Incubation was carried out at 37° for 30 min. At the end of the incubation 1 ml of 5% trichloroacetic acid, 3 ml of borate buffer, pH 10.1 [7], and 0.5 ml tributyl phosphate were added. Ammonia was transferred by aeration at 50° for 15–20 min into 0.2 M citrate buffer, pH 5.0, and estimated colorimetrically by the ninhydrin method of Moore and Stein [8].

3. Results and discussion

As can be seen from table 1, carbobenzoxy-L-phenylalanine and *N*-carbobenzoxy-*S*-benzyl-L-cysteine strongly inhibit rat liver asparaginase. Fig. 1 shows that the inhibition is competitive. Table 1 also shows that the asparaginase activity is inhibited by *N*-carbobenzoxy-L-tyrosine, although to a lesser de-

Table 1
Effect of carbobenzoxy derivatives of phenylalanine, tyrosine and *S*-benzylcysteine on rat liver asparaginase.

Carbobenzoxy (Cbz) derivative added μmoles	Ammonia liberated (μmoles*)	Per cent inhibition
None	5.30	
Cbz-L-phenylalanine, 67	1.30	75
Cbz-L-phenylalanine, 90	0.20	98
None	5.47	
Cbz–l-phenylalanine, 65	1.44	74
Cbz-D,L-phenylalanine, 65	0.86	84
N-Cbz-L-tyrosine, 65	3.03	45
N-Cbz-*S*-benzyl-L-cysteine, 65	0.12	98
None	5.55	
N-Cbz-S-benzyl-L-cysteine, 18	2.73	51
N-Cbz-*S*-benzyl-L-cysteine, 24	1.95	65
N-Cbz-*S*-benzyl-L-cysteine, 30	0.66	88

* All blanks subtracted.

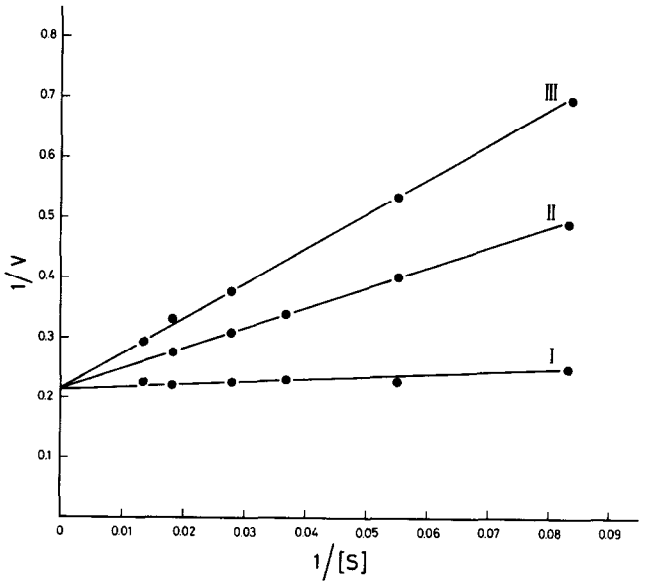

Fig. 1. Competitive inhibition of rat liver asparaginase by carbobenzoxy L-phenylalanine and *N*-carbobenzoxy-*S*-benzyl -L-cysteine. I, L-asparagine; II, L-asparagine and carbobenzoxy-L-phenylalanine (50 μmoles); III, L-asparagine and *N*-carbobenzoxy-*S*-benzyl-L-cysteine (20 μmoles). [S] is the concentration of L-asparagine (μmoles) in 3 ml reaction mixture. Velocity, *V*, is expressed in μmoles ammonia liberated in 30 min at 37°.

gree than by the two other carbobenzoxy derivatives. Carbobenzoxy-D,L-phenylalanine inhibits somewhat more than the corresponding L-compound. Under the same conditions, 0–9% inhibition was obtained with L-phenylalanine (90 μmoles) or with 90 μmoles of the carbobenzoxy derivatives of the following amino acids: glycine, L-alanine, L-aspartic acid, L-asparagine, L-glutamic acid and L-glutamine. These results seem to indicate that for an efficient inhibition of rat liver asparaginase activity the inhibitor must possess an aromatic ring in addition to that contained in the carbobenzoxy group. The distance between the two aromatic groups seems also to be of importance since *N*-carbobenzoxy-*S*-benzyl-L-cysteine is a more effective inhibitor than carbobenzoxy-L-phenylalanine. As to the mechanism of the strong inhibition exerted by the derivatives containing two aromatic rings, one might postulate that the carboxyl group of the inhibitor binds to the active site of the enzyme. This binding brings the aromatic groups in close juxtaposition for further interaction, by hydrophobic bonds, with two additional sites onthe enzyme molecule. Addition of larger amounts of the substrate abolishes the inhibition by displacing the carboxyl group of the inhibitor.

Anti-tumor activity of carbobenzoxy-L-asparagine was described recently [9]. It was also found that interperitoneal injections of the sodium salt of carbobenzoxy-L-phenylalanine inhibit the growth of Ehrlich ascites carcinoma in mice [10]. Carbobenzoxy-L-phenylalanine also markedly inhibits the following enzyme systems connected with glutamine metabolism: rat liver glutaminase, rat liver glutamine synthetase, ovine brain glutamine synthetase and γ-glutamyl transferase [11]. In view of these findings, it appears desirable to extend the studies on the effect of carbobenzoxy derivatives of amino acids (or of compounds related to amino acids) on various enzyme systems and tumors. Experiments in this direction are under way.

References

[1] J.D.Broome, Nature 191 (1961) 1114.
[2] L.T.Mashburn and J.C.Wriston, Arch. Biochem. Biophys. 105 (1964) 450.
[3] Y.S.Halpern and N.Grossowicz, Biochem. J. 65 (1957) 716.
[4] N.de Groot and N.Lichtenstein, Biochim. Biophys. Acta 40 (1960) 92.
[5] O.Yaron-Gizri, M.Sc.Thesis, The Hebrew University, Jerusalem (1963).
[6] W.Grassmann and E.Wünsch, Ber. Chem. Ges. 91 (1958) 462.
[7] R.M.Archibald, J. Biol. Chem. 151 (1943) 141.
[8] S.Moore and W.H.Stein, J. Biol. Chem. 176 (1948) 367.
[9] M.Schlesinger, N.Grossowicz and N.Lichtenstein, Experientia 25 (1969) 14.
[10] M.Schlesinger, N.Grossowicz and N.Lichtenstein, to be published.
[11] G.Mor, H.Spiegelstein-Klarfeld, N.Lustig and N.Lichtenstein, to be published.