EFFECT OF SOME ACYL DERIVATIVES OF AMINO ACIDS ON CELL-FREE PROTEIN SYNTHESIZING SYSTEMS FROM EHRLICH ASCITES, RAT AND MOUSE LIVER CELLS

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1. Introduction

Intraperitoneal injections of carbobenzoxy (CBZ)-phenylacetyl and phenylpropionyl derivatives of amino acids, especially derivatives with more than one aromatic group, were found to markedly inhibit the growth of Ehrlich ascites tumor in mice [1–3].[1] Such derivatives also strongly inhibit a number of isolated enzyme systems (rat liver asparaginase and glutaminase [4, 5], rat liver and ovine brain glutamine synthetase [6, 7], rat liver NAD-synthetase [8] and the purine synthesizing system of pigeon and chicken liver [9]. Preliminary experiments with suspensions of Ehrlich ascites cells carried out in this department by G. Mor also revealed that α,ϵ-di-CBZ-L-lysine and *N*-CBZ-S-benzyl-L-cysteine markedly inhibit the incorporation of L-leucine and L-phenylalanine into ascites protein. The latter finding, however, does not necessarily indicate that these compounds inhibit the protein synthesis as such (e.g., they might affect some ATP generating enzyme systems). We decided therefore to investigate the effect of the above amino acid derivatives on cell-free protein synthesizing systems prepared from Ehrlich ascites cells and from rat and mouse liver. The present paper deals with this subject.

2. Materials and methods

CBZ-L-phenylalanine, *N*-CBZ-S-benzyl-L-cysteine and α,ϵ-di-CBZ-L-lysine were from Fluka A.G., Bucks, Switzerland. CBZ-L-glutamic acid γ-benzyl ester was prepared according to Hanby et al. [10]. The preparation of *N*-phenylacetyl and *N*-phenylpropionyl-S-benzyl-L-cysteine will be described elsewhere [7]. All derivatives were used in the form of their alkali salts. These were prepared as described previously [1] for the preparation of the sodium salt of CBZ-L-phenylalanine.

Free ribosomes from rat and mouse liver were prepared according to Bloemendal, Bont and Benedetti [11]. Free ribosomes from ascites cells were prepared as follows: Packed cells were washed and suspended in TKM buffer (Tris–HCl, 50 mM, pH 7.4; KCl, 25 mM and $MgCl_2$, 10 mM) containing 0.25 M sucrose, the suspension was passed through a French press (at a pressure of 8000 psi). The lysate was centrifuged at 17 000 *g* for 15 min, and from the supernatant free ribosomes were prepared according to Bloemendal et al. [11]. For incorporation experiments, crude enzyme preparations were prepared according to Bloemendal et al. [11]. For the preparation of fractions containing aminoacyl-tRNA synthetases the method of Geels et al. [12] was used. tRNA from the various sources was isolated essentially according to Bauer et al. [13]. In all cases the enzymes, tRNA and ribosomes used in one experiment were from one and the same source.

3. Results

As can be seen from table 1, the amino acid derivatives tested strongly inhibited the poly U directed incorporation of phenylalanine into polypeptide by a

Table 1
The effect of some amino acid derivatives on the poly U promoted incorporation of phenylalanine by incorporation systems frome Ehrlich ascites, mouse and rat liver cells

Inhibitor	Final (mM)	Ehrlich ascites (% Inhibition)	Mouse liver (% Inhibition)	Rat liver (% Inhibition)
N-CBZ-S-benzyl-L-cysteine	1.0	36	8	0
	2.5	87	49	16
α,ε-di-CBZ-L-lysine	0.25	68	10	0
	2.5	100	80	50
CBZ-L-glutamic acid γ-benzyl ester	2.5	71	44	13
N-phenylacetyl-S-benzyl-L-cysteine	2.5	41	0	0
	5.0	67	35	10
N-phenylpropionyl-S-benzyl-L-cysteine	5.0	95	33	36
CBZ-L-phenylalanine	5.0	82	–	22

The reaction mixtures contained in a final volume of 0.22 ml: Tris–HCl, pH 7.2, 0.06 M; KCl, 0.05 M; $MgCl_2$, 0.01 M; DTT, 0.001 M; GTP, 0.0005 M; ATP, 0.0005 M; amino acid mixture, 5×10^{-5} M (each amino acid); [^{14}C] phenylalanine, 0.2 μCi (sp.act. 513 μCi/μmole; PEP, 5×10^{-3} M; PEP kinase (Boehringer) 2 μg protein; poly U, 40 μg; tRNA, 20 μg; ribosomes 0.8–1.0 A_{260nm} units and aliquots of the corresponding enzyme fractions (necessary for optimal incorporation). The phenylalanine incorporation without poly U was negligible in the ascites system and was approximately 20% of the total incorporation in the presence of poly U in the systems derived from mouse and rat liver. 0.05 ml samples were counted for 5% hot TCA insoluble material.

ribosomal incorporation system derived from Ehrlich ascites cells. The inhibition caused by these compounds in similar incorporation systems derived from mouse or rat liver was much less pronounced. The most active inhibitor of the compounds tested in all three incorporation systems was α,ε-di-CBZ-L-lysine. The extent of inhibition was calculated from results obtained after 15 min of incubation. The rates of phenylalanine incorporation were linear during the first 15 min of incubation in all three ribosomal systems. The first step in the pathway leading from free phenylalanine to peptide bound phenylalanine is the activation of phenylalanine by the phenylalanyl-

Table 2
The effect of some amino acid derivatives on the phenylalanyl-tRNA synthetase of Ehrlich ascites, rat and mouse liver cells.

Inhibitor	Final conc. (mM)	Ehrlich ascites (% Inhibition)	Mouse liver (% Inhibition)	Rat liver (% Inhibition)
N-CBZ-S-benzyl-L-cysteine	0.25	3	27	16
	2.5	71	85	69
α,ε-di-CBZ-L-lysine	0.25	48	60	40
	2.5	92	96	92
CBZ-L-glutamic acid γ-benzyl-ester	0.25	26	24	25
	2.5	82	73	65
N-phenylpropionyl-S-benzyl-L-cysteine	2.5	57	72	59

The reaction mixture (37°C) contained in a final volume of 0.22 ml: Tris–HCl, pH 7.4, 0.1 M; $MgCl_2$, 0.01 M; ATP, 0.002 M; mercaptoethanol, 0.02 M; bovine serum albumin, 40 μg; [^{14}C] Phe, 0.2 μCi (sp. act. 513 μCi/μmole; tRNA from the various sources, 80–150 μg and aliquots of corresponding synthetases which give a linear reaction rate during the first 10 min of reaction. 0.1 ml samples were counted for 5% cold TCA insoluble material.

tRNA synthetase. We investigated therefore the effect of some of the above amino acid derivatives on the activation of phenylalanine. The results are shown in table 2.

As can be seen from table 2, the amino acid derivatives inhibit the phenylalanyl-tRNA synthetase from ascites cells and from mouse and rat liver to the same extent, contrary to their effect on the poly-phenylalanine formation as given in table 1. However, the Phe-tRNA synthetase is not the only step in the incorporation of phenylalanine into polypeptide inhibited by the amino acid derivatives. As shown in table 3, α,ε-di-CBZ-L-lysine and *N*-CBZ-S-benzyl-L-cysteine inhibited the poly U directed incorporation of phenylalanine from Phe-tRNA into polypeptide in an incorporation system obtained from Ehrlich ascites cells. Table 3 also shows that the extent of inhibition of phenylalanine incorporation depends upon the substrate used. With Phe-tRNA as substrate the inhi-

Table 3
The effect of *N*-CBZ-S-benzyl-L-cysteine and α, ε-di-CBZ-L-lysine on the poly U dependent phenylalanine by an incorporation system from Ehrlich ascites cells

Inhibitor	Final conc.	Substrate	
		Phe-tRNA	Phenylalanine
	(mM)	(% Inhibition)	
N-CBZ-S-enzyl-L-cysteine	2.5	54	71
α,ε-di-CBZ-L–lysine	2.5	47	95

For methods see legend to table 1. With [^{14}C] Phe-tRNA (18 000 cpm corresponding to 24 pmoles of Phe-tRNA), ATP, tRNA and free [^{14}C] phenylalanine were omitted.

bition was smaller than in a parallel experiment with free phenylalanine.

4. Discussion

N-CBZ-S-benzyl-L-cysteine and α,ε-di-CBZ-L-lysine inhibit the incorporation of leucine and phenylalanine into cellular protein of Ehrlich ascites cell suspensions. This inhibition may be due to the effect of the inhibitors on one or more systems directly or indirectly involved in the incorporation of amino acids into protein, such as penetration of amino acids into the cell, depletion of ATP or a direct influence on one or more of the reactions leading from intercellular amino acids to protein. The results described here show that one or more of the enzyme reactions directly involved in the amino acid incorporation is affected. Of the compounds tested *N*-CBZ-S-benzyl-L-cysteine and α,ε-di-CBZ-L-lysine strongly inhibited the phenylalanine incorporation in a cell-free system from ascites cells and to a much lesser degree in cell-free systems from mouse and rat liver. These compounds also exerted the strongest inhibitory effect on the growth of Ehrlich ascites tumor in mice. It is of course impossible at this stage to draw any conclusion about the correlation (if any) of these two inhibitory effects. It has to be taken into account that the in vitro conditions of our experiments on amino acid incorporation are greatly different from the in vivo conditions, such as ionic environment, the relative activity of the different enzymes involved and the use of synthetic messenger RNA and not natural messenger RNA. Phenylalanyl-tRNA synthetase from Ehrlich ascites cells and from rat and mouse liver is strongly inhibited by the different derivates used. However, not all aminoacyl-tRNA synthetases behave in this way; thus we found that valyl-tRNA synthetase was not inhibited at all under similar conditions by the same derivatives. From the results presented in tables 2 and 3 one can conclude that at least two of the amino acid derivatives, α, ε-di-CBZ-L-lysine and *N*-CBZ-S-benzyl-L-cysteine interfere with the phenylalanine incorporation at more than one step in a cell-free system from Ehrlich ascites cells. More detailed investigations on the effect of the above-mentioned and similar amino acid derivates on the various isolated enzyme systems involved in protein biosynthesis are being carried out at present in our laboratory.

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