THE INHIBITION OF AMINOACYL-tRNA SYNTHETASES BY ACYL DERIVATIVES OF AMINO ACIDS

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1. Introduction

The different aminoacyl-tRNA synthetases are enzymes with a high degree of specificity towards both amino acid and tRNA [1]. It is known that aminoacyl-tRNA synthetases in mammalian cells are organised in complexes in the cell sap [2–5]. The mechanism of action of the aminoacyl-tRNA synthetases was studied thoroughly by several investigators who have reported a number of inhibitors of these enzymes [6].

Carbobenzoxy, phenylacetyl and phenylpropionyl derivatives of amino acids, especially those with more than one aromatic group, were found to inhibit growth of Ehrlich ascites tumor in mice [7,8]. These compounds also inhibit several enzymes from different sources [9–14]. It was reported earlier [15] that some of these acyl derivatives of amino acids inhibit the poly U promoted incorporation of phenylalanine into 5% hot TCA insoluble material in cell free protein synthesizing systems prepared from Ehrlich ascites cells and from rat and mouse liver. These inhibitors were shown to inhibit the formation of phenylalanyl-tRNA but did not inhibit the synthesis of valyl-tRNA [15]. The effect of acyl derivatives of amino acids on the different aminoacyl-tRNA synthetases from mouse liver and Ehrlich ascites tumor cells is described in this communication.

2. Materials and methods

^{14}C-labeled amino acids were purchased from Radiochemical Centre, Amersham, England. Specific activities (mCi/mmole) as follows: Ala 156; Asp 229; Asn 218; Glu 285; Gly 108; Ile 342; Leu 342; Met 56; Phe 475; Pro 265; Ser 160; Thr 208; Val 280. [^{3}H] Val, sp.ac. 4000 mCi/mmole was purchased from Kamag, Israel.

N-CBZ-*S*-benzyl-L-cysteine and α, ε-di-CBZ-L-lysine were purchased from Fluka A. G. Buchs, Switzerland. CBZ-L-glutamic acid γ-benzyl ester was prepared according to Hanby et al. [16]. *N*-phenylacetyl and *N*-phenylpropionyl-*S*-benzyl-L-cysteine were prepared according to Lustig et al. [12]. All the inhibitors were used in the form of their alkali metal salts [15].

Mouse liver was homogenized 1:1 (w/v) in TKMS buffer containing: 50 mM Tris–HCl (pH 7.4), 25 mM KCl, 10 mM $MgCl_2$, 0.25 M sucrose. Ehrlich ascites cells were crushed with a French press at a pressure of 8000 psi. Aminoacyl-tRNA synthetases were prepared from post microsomal supernatant as described by Geels [2]. tRNA from mouse liver and Ehrlich ascites cells were prepared according to Bauer et al. [17]. The purity of tRNA isolated from mouse liver and Ehrlich ascites cells was checked by microgel electrophoresis [18]. 90% of the RNA moved as 4 S material. Protein content was measured by the method of Lowry et al. [19].

The reaction mixture contained in final volume of 0.22 ml the following: 0.1 M Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 2 mM ATP, 20 mM mercaptoethanol, 20 μg BSA, ^{14}C-labeled amino acid: 0.2 μCi each (5.6–50 μM), 100–800 μg tRNA, synthetases from Ehrlich ascites cells: 4.6 μg protein, synthetases from mouse liver: 8.0 μg protein. All incubations were performed at 37°C. In all reaction mixtures the tRNA and the enzyme were from one and the same source. The reaction was started by adding enzyme preparation, 0.05–0.1 ml of sample was withdrawn, washed and

counted as '5% cold TCA-insoluble material' as described. Time of the reaction (2–10 min) was chosen in the constant velocity range. In all cases the reaction rate was linear with the enzyme concentration and was not dependent on the substrates' concentrations.

3. Results and discussion

As can be seen from table 1, the activities of the crude synthetases preparations both from Ehrlich ascites cells and mouse liver vary in respect to different amino acids. This may be explained by the different distribution of the synthetases in the cell sap or by artifacts induced during isolation [20]. The activity of most of aminoacyl-tRNA synthetases from Ehrlich ascites cells is significantly higher than those from mouse liver. This fact can be explained in terms of higher activity (or greater stability) of synthetases derived from Ehrlich ascites tumor, or by the inherently greater purity of Ehrlich ascites enzyme preparation.

Table 1
The activity of aminoacyl-tRNA synthetases from mouse liver and Ehrlich ascites cells.

Amino acid	E. ascites cells	Mouse liver
Methionine	782	10
Aspartic acid	449	40
Isoleucine	409	–
Valine	400	120
Glutamic acid	280	20
Serine	204	60
Threonine	196	90
Glycine	160	–
Phenylalanine	120	40
Leucine	102	11
Asparagine	102	–
Alanine	36	43
Proline	–	85

Activities of enzyme: pmole aminoacyl-tRNA synthesized · mg^{-1} · min^{-1}.

From tables 2 and 3 one can see that different aminoacyl-tRNA synthetases show different response to the same inhibitor. 5 mM *N*-CBZ-*S*-benzyl-L-cysteine strongly (above 60%) inhibits Phe-tRNA, Glu-tRNA and Gly-tRNA synthetases, while Asp-tRNA, Leu-tRNA, Met-tRNA, Asn-tRNA, Ile-tRNA are inhibited by 40–60% and Thr-tRNA, Ala-tRNA, Val-tRNA, Ser-tRNA, Pro-tRNA synthetases are affected much less. In general, the synthetases that are most sensitive to *N*-CBZ-*S*-benzyl-L-cysteine are also sensitive to the

Table 2
The percentage activity of aminoacyl-tRNA synthetases from Ehrlich ascites cells in the presence of inhibitors

	Inhibitor						
	1		2		3	4	5
Amino acid	2.5 mM	5.0 mM	1.0 mM	2.5 mM	2.5 mM	2.5 mM	2.5 mM
Phenylalanine	26	27	22	6	35	60	41
Glutamic acid	47	–	–	35	46	74	61
Aspartic acid	62	42	–	52	52	–	50
Leucine	72	43	–	38	56	78	73
Glycine	74	33	81	75	67	78	47
Asparagine	80	50	85	22	82	81	70
Isoleucine	65	43	89	68	40	85	62
Methionine	81	64	–	79	70	110	101
Threonine	76	76	81	70	77	71	74
Alanine	76	76	93	77	96	77	72
Valine	76	76	–	1–8	93	104	119
Serine	73	73	–	90	97	94	89

1) *N*-CBZ-*S*-benzyl-L-cysteine. 2) α, ε-di-CBZ-L-lysine. 3) CBZ-L-glutamic acid γ-benzyl ester. 4) *N*-phenylacetyl-*S*-benzyl-L-cysteine. 5) *N*-phenylpropionyl-*S*-cysteine.

Table 3
The percentage activity of aminoacyl-tRNA synthetases from mouse liver in the presence of inhibitors

	Inhibitor						
	1		2		3	4	5
Amino acid	2.5 mM	5.0 mM	1.0 mM	2.5 mM	2.5 mM	2.5 mM	2.5 mM
Phenylalanine	40	19	14	5	39	64	36
Glutamic acid	41	21	76	42	28	68	69
Aspartic acid	61	60	90	69	61	86	104
Leucine	85	53	85	52	89	92	73
Methionine	79	41	96	89	88	89	103
Threonine	83	87	100	97	98	92	91
Alanine	104	112	106	124	90	98	79
Valine	95	98	94	91	91	101	101
Serine	102	90	94	89	88	108	97
Proline	89	71	110	93	93	116	102

1) *N*-CBZ-*S*-benzyl-L-cysteine. 2) α-ε-di-CBZ-L-lysine. 3) CBZ-L-glutamic acid γ-benzyl ester. 4) *N*-phenylacetyl-*S*-benzyl-L-cysteine. 5) *N*-phenylpropionyl-*S*-benzyl-L-cysteine.

other inhibitors, and synthetases that are unaffected or only slightly affected by *N*-CBZ-*S*-benzyl-L-cysteine are not inhibited by the other compounds tested. There are, however, exceptions: Asn-tRNA synthetase from Ehrlich ascites cells is strongly inhibited by α, ε-di-CBZ-L-lysine and Gly-tRNA synthetase from the same source is almost unaffected by this compound.

It is known that formation of aminoacyl-tRNA is competitively inhibited by structural analogues of natural amino acids [6]. There is no structural correlation between the inhibitor tested and the amino acid whose aminoacyl-tRNA synthetase was inhibited. Moreover, phenylalanyl-tRNA and glutamyl-tRNA synthetases are inhibited to the same extent with *N*-CBZ-*S*-benzyl-L-cysteine (table 3).

It is interesting that the same inhibitors that affect aminoacyl-tRNA synthetase derived from Ehrlich ascites tumor cells inhibit the corresponding synthetase derived from mouse liver despite the difference in activities of these synthetases.

Since synthetases in mammalian cells exist in complexes [2–5], it was interesting to see if inhibition of the synthesis of an aminoacyl-tRNA has any effect on the synthesis of another aminoacyl-tRNA. We, therefore, checked inhibition of synthesis of ^{14}C-labeled Phe-tRNA and [^{3}H] Val-tRNA by 2.5 mM α, ε-di-CBZ-L-lysine in the Ehrlich ascites system. As shown in table 2, when checked individually, Phe-tRNA synthesis is 94% inhibited while Val-tRNA is not affected at all. When checked simultaneously, synthesis of

Table 4
The activity of Phe-tRNA synthetase from Ehrlich ascites cells after preincubation with inhibitor

System	Activity cpm/mg prot.
non-dialized synthetase without inhibitor	$168 \cdot 10^3$
non-dialized synthetase + α,ε-di-CBZ-L-lysine, 2.5 mM	$28 \cdot 10^3$
non-dialized synthetase + dialized α,ε-di-CBZ-L-lysine, 2.5 mM	$161 \cdot 10^3$
dialized synthetase without inhibitor	$250 \cdot 10^3$
synthetase dialized after preincubation for 20 min with α,ε-di-CBZ-L-lysine, 2.5 mM	$189 \cdot 10^3$

[^{14}C] Phe-tRNA is inhibited by 82% and synthesis of [^{3}H]Val-tRNA is not inhibited at all. From these data it seems likely that, even if the synthetases exist in complex in the experimental conditions, they act independently.

In order to investigate whether the action of the inhibitors tested affected the cofactors of the reaction, the concentrations of $MgCl_2$, ATP and mercaptoethanol were raised 4–6 fold. In all cases 2.5 mM *N*-CBZ-*S*-benzyl-L-cysteine inhibited the synthesis of Phe-tRNA in Ehrlich ascites systems to the same degree.

Table 4 gives the results of experiments designed to test the binding of an inhibitor to synthetase. Dialysis by itself did not inactivate the synthetase, and the inhibitor easily passed through the dialysis membrane. Dialysis of the enzyme after preincubation with inhibitor restores enzyme activity to 75% of the activity of the enzyme dialysed without preincubation with inhibitor, indicating that there is no irreversible association between the synthetase and the inhibitor.

It can be concluded that the synthetase preparation from Ehrlich ascites cells, in the conditions described above, is more active than the preparation from mouse liver; the degree of inhibition of an aminoacyl-tRNA synthetase does not depend on the source of the synthetase; the inhibitors affect some synthetases while others are not inhibited; and the inhibition is not irreversible. More detailed investigations on the effect of acyl derivatives of amino acids on the individual amino acyl-tRNA synthetases are being carried out at present in our laboratory.

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