INHIBITORS OF PURINE BIOSYNTHESIS

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1. Introduction

It was previously found that some α-*N*-alkyl derivatives of L-glutamine markedly inhibit the growth of glutamine-requiring strains of hemolytic streptococci [1] and of experimental mouse tumours [2]. It was also found that such compounds inhibit ovine brain and rat liver glutamine synthetase in vitro [3, 4]. On the other hand, they could substitute for glutamine in the synthesis of NAD by rat liver preparations [5]. It was recently observed that carbobenzoxy (Cbz) derivatives of various amino acids inhibit several enzyme systems connected with the metabolism of glutamine and asparagine [5–8] as well as the growth of some transplantable murine tumours [9, 10]. In view of these findings it was of interest to study the effect of α-*N*-alkyl derivatives of L-glutamine and of Cbz-amino acids on purine biosynthesis. The present paper deals with this problem.

2. Materials and methods

α-*N*-Alkyl derivatives of L-glutamine were prepared as previously described [1, 3, 11]. They contained less than one percent free glutamine as tested chromatographically and by the colour reaction with aqueous ninhydrin. The preparation of Cbz-β-*N*-benzyl-L-asparagine and Cbz-D,L-homocysteine (5 mg/ml) and will be described elsewhere. All other Cbz-derivatives and L-glutamine were purchased from Fluka A.G., Buchs (Switzerland). D-Ribose-5-phosphate (Na_2), ATP and D-3-phosphoglyceric acid (Na) were from Sigma Chemical Company, St.Louis, Missouri. $H^{14}COONa$ (10.8 mCi/nmole) was obtained from Radiochemical Centre, Amersham (England).

2.1. *Enzyme preparations*

Homogenates of pigeon livers were prepared by the procedure of Flanks and Lukens [12]. After the first centrifugation at 100,000 *g* in a Spinco model L centrifuge for 30 min, the supernatant fluid was lyophilized with D,L-homocysteine (5 mg/ml) and kept at −22°. For enzymatic experiments the lyophilized preparations were dissolved in the homogenizing medium (50 mg/ml) described by Franks and Lukens [12] and dialized against this medium for 16–18 hr at 4°.

2.2. *Assay procedure*

The reaction mixture contained (in μmoles/1.5 ml): phosphate buffer, pH 7.4, 50; $NaHCO_3$, 50 (pH adjusted to 7.4 by bubbling CO_2 through); $MgCl_2$, 5; glycine, 5; L-aspartate, 5; ATP, 2; D,L-homocysteine, 5; D-ribose-5-phosphate, 5; D-3-phosphoglycerate, 28; $H^{14}COONa$ (20,000 cpm/μmole), 5; L-glutamine and various inhibitors, as indicated in the tables; enzyme (dialized as described above), 50 mg. Incubation was carried out at 38° for 60 min, terminated with 0.2 ml of 50% trichloroacetic acid and the tubes cooled and centrifuged. Aliquots (0.8 ml) of the supernatants were evaporated to dryness at 130° after addition of 0.4 ml 6 N HCl. 6 N HCl (0.2 ml) was added again and the solutions were again evaporated to dryness. The solid residue was dissolved in 0.2 ml water and was then diluted to

Table 1
Effect of a α-*N*-alkyl derivatives of L-glutamine on purine biosynthesis.

L-Glutamine derivatives added (μmoles)	Formate-^{14}C incorporated (nmoles)	% Inhibition	Radioactive hypoxanthine formed (nmoles)*	% Inhibition
None	105	–	88	–
α-*N*-Ethyl (50)	63	40	55	38
α-*N*-Ethyl (100)	32	69	38	57
α-*N*-Propyl (50)	65	38	62	30
α-*N*-Propyl (100)	39	63	42	52
α-*N*-Butyl (50)	74	30	66	25
α-*N*-Butyl (100)	60	43	57	35
α-*N*-Amyl (50)	66	37	62	30
α-*N*-Amyl (100)	47	55	51	42
α, α′-*N*-Dimethyl (100)	14	–	88	–

All reaction mixtures contained 10 μmoles of L-glutamine.
* Hypoxanthine was determined chromatographically in solvent 1.

10 ml with scintillation fluid prepared according to Brey [13]. Radioactivity was measured in Packard, model 3320, scintillation counter. Blanks without enzyme or L-glutamine were very low and were subtracted.

2.3. *Determination of radioactive hypoxanthine by paper chromatography*

Aliquots of the reaction mixtures were evaporated as above, but the evaporation was carried out at 100° (under these conditions purine nucleotides are hydrolized to free bases without significant damage to the latter [14]). The residues were dissolved in 0.1 ml water and aliquots were taken for chromatography. Descending chromatography was carried out on Whatman no. 3 paper, using two different solvents – solvent 1: butanol sat. with water (NH_3 atmosphere); solvent 2: butanol–formic acid–water (77:10:13, v/v) [15] – and run for 22–24 hr. At the end of this time the chromatogram was dried, the place of the markers was located by the use of a UV lamp and the radioactive material was detected with a Packard, model 7201, radiochromatogram scanner. Most of the radioactivity obtained with the two different solvents corresponds to hypoxanthine. The area of the peaks were cut out, put into 10 ml scintillation fluid prepared according to Brey [13], and the radioactivity was estimated in a Packard, model 3320, scintillation counter.

3. Results and discussion

As can be seen from table 1, α-*N*-alkyl derivatives of L-glutamine inhibit the incorporation of radioactive formate and hypoxanthine formation by pigeon liver preparation. The most effective inhibition was given by α-*N*-ethyl and α-*N*-propyl-L-glutamine. Under the same condition α-*N*-dimethyl-L-glutamine showed no inhibition. When higher amounts of L-glutamine were added to the incubation mixtures (table 2), the inhibition by α-*N*-ethyl and α-*N*-propyl-L-glutamine was markely diminished. No significant decrease of the extent of inhibition was observed when the concentration of glycine or L-aspartate in the reactions mixtures was increased.

As can be seen from table 3, Cbz-L-glutamine and Cbz-derivatives of other aliphatic amino acids strongly inhibit purine biosynthesis. All these compounds inhibit approximately to the same extent. On the other hand, Cbz-amino acids with an additional aromatic group (e.g. Cbz-L-phenylalanine, Cbz-*S*-benzyl-L-cysteine are much stronger inhibitors than

Table 2
Effect of high concentrations of L-glutamine on the inhibition of purine biosynthesis by α-*N*-alkyl derivatives of L-glutamine.

L-Glutamine added (μmoles)	L-Glutamine derivative added	Formate-^{14}C incorporated (nmoles)	% Inhibition
(10)	None	195	–
(200)	None	200	–
(10)	α-*N*-Ethyl	59	70
(200)	α-*N*-Ethyl	115	41
(10)	α-*N*-Propyl	86	56
(200)	α-*N*-Propyl	136	30

Cbz-derivatives of aliphatic amino acids. The most effective inhibitor of this kind was Cbz-*S*-benzyl-L-cysteine. There was no difference in inhibition by Cbz-L- or D, L-phenylalanine. An increase of the concentration of either L-glutamine, glycine or L-aspartate in the reaction mixture did not affect the inhibition by Cbz-L-glutamine, Cbz-glycine, Cbz-L-aspartic acid and Cbz-L-phenylalanine.

The effect of α-*N*-alkyl derivatives of L-glutamine and Cbz-derivatives of amino acids on the biosynthesis of purine by chicken liver preparations was also studied. Essentially the same inhibitory effects as with pigeon liver preparation were obtained.

As to the mechanism of the inhibition, the similarity of the α-*N*-alkyl derivatives of glutamine and the fact that the inhibition by these derivatives

Table 3
Effect of Cbz derivatives of amino acids on purine biosynthesis.

Cbz-derivatives added (μmoles)	Formate-^{14}C incorporated (nmoles)	% Inhibition	Radioactive hypoxanthine formed (nmoles)*	% Inhibition
None	182	–	150	–
Cbz-L-glutamine (100)	55	70	53	65
Cbz-L-asparagine (20)	152	17	120	20
Cbz-L-asparagine (100)	49	73	47	69
Cbz-β-*N*-benzyl-L-asparagine (20)	100	45	75	50
Cbz-L-alanine (20)	115	37	97	35
Cbz-L-alanine (100)	22	88	30	80
Cbz-L-phenylalanine (20)	14	92	15	90
None	112	–	91	–
Cbz-L-aspartic acid (100)	35	69	34	63
Cbz glycine (20)	78	30	66	27
Cbz glycine (100)	43	62	30	67
Cbz-D, L-phenylglycine (10)	62	45	52	43
Cbz-D, L-phenylglycine (20)	7	94	9	90
Cbz-D, L-phenylalanine (10)	62	45	52	43
Cbz-L-phenylalanine (10)	60	47	49	46
Cbz-L-tryptophan (10)	44	61	32	65
Cbz-*S*-benzyl-L-cysteine (5)	62	45	46	49
Cbz-*S*-benzyl-L-cysteine (10)	0	100	0	100

All reaction mixtures contained 10 μmoles of L-glutamine

* Hypoxanthine was determined chromatographically in solvent 1.

diminished with increasing glutamine concentration may indicate that they act as structural analogs of glutamine. On the other hand, it may be assumed that the mechanism of inhibition by Cbz-amino acids is similar to that suggested for the inhibition of other enzyme systems by such compounds [7]. The aromatic groups of the inhibitors are thought to bind to the enzyme molecule by hydrophobic interactions causing inhibition by conformational changes. It was recently found [16] that some Cbz-derivatives of amino acids strongly inhibit the proteolytic activity of trypsin and chymotrypsin, hence the inhibitory effect of such compounds is not restricted to enzymes connected with the metabolism of glutamine and asparagine. Purine biosynthesis is the result of action of a multitude of enzymes and it is therefore difficult to decide which is inhibited by the Cbz-compounds. An investigation of the effect of such compounds on the isolated enzymes participating in purine biosynthesis therefore appears desirable.

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