Increased β-aminoisobutyric acid in rat liver with 6-azauracil and its enantiomer

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Received 16 August 1985; revised version received 2 September 1985

When 6-azauracil was subcutaneously injected, β-aminoisobutyric acid and β-alanine contents were increased 22 and 61-fold, respectively, in rat liver. Incorporation of [methyl-^{14}C]thymine into β-aminoisobutyric acid was increased to 42-fold by 6-azauracil treatment. The absolute configuration of this amino acid was proved to be the (*R*)-form by means of a gas-chromatographic technique. 6-Azauracil inhibited β-alanine-pyruvate aminotransferase activity with an I_{50} of approx. 2.5 mM.

β-Aminoisobutyric acid *β-Alanine* *6-Azauracil* *Thymine* *β-Alanine-pyruvate aminotransferase*

1. INTRODUCTION

It is well known that 6-azauracil and its derivatives act as a metabolic inhibitor for RNA synthesis. 6-Azauridine 5′-phosphate is a competitive inhibitor of orotidine-5′-phosphate decarboxylase [1]. Moreover, the mode of action of 6-azauridine in its capacity as a growth inhibitor has been attributed to inhibition of UTP biosynthesis by 6-azauridine 5′-phosphate [2] or incorporation of it into RNA [3]. In [4–6], we found that β-alanine content in rat liver was remarkably increased by injection of 6-azauracil and that 6-azauracil was a competitive inhibitor of purified β-alanine-2-oxoglutarate aminotransferase (EC 2.6.1.19) from rabbit liver. Slavik et al. [7] also proposed that 6-azauridine triacetate induced hyper β-alaninemia.

β-AIB is an end product of the metabolic degradation of thymine [8,9], its absolute configuration being of the (*R*)-form [10,11]. Solem et al. [11,12] found the (*S*)-isomer present in serum, which must have a different metabolic origin than from thymine degradation. The (*R*)- and (*S*)-enantiomers of β-AIB were transaminated by different enzymes such as β-AIB-pyruvate aminotransferase (β-alanine-pyruvate aminotransferase, EC 2.6.1.18) and β-AIB-2-oxoglutarate aminotransferase (L-3-aminoisobutyrate aminotransferase, EC 2.6.1.22), respectively [13,14].

This report describes the increase of β-AIB caused by 6-azauracil with the concomitant decrease of the activity of β-alanine-pyruvate aminotransferase in vivo and the absolute configuration of this amino acid was proved to be the (*R*)-enantiomorph.

Abbreviation: β-AIB, β-aminoisobutyric acid

2. MATERIALS AND METHODS

All chemicals used were of analytical grade and purchased from Nakarai Chemicals, Kyoto, if not otherwise stated. 6-Azauracil was obtained from Sigma. [*methyl*-^{14}C]Thymine was purchased from Commissariat à l'Energie Atomique, France, and β-[2-^{14}C]alanine from New England Nuclear, USA. Pure (*R*)- and (*S*)-isomers of β-AIB were kindly provided by Professor Y. Kakimoto of School of Medicine, Ehime University, Japan.

Male Sprague-Dawley rats, weighing 120–150 g, were housed in individual screen-bottomed cages in a room maintained at 23 ± 1°C with 50%

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00145793/85/$3.30

humidity and light regulation (12 h light per day). The animals were fed on a commercial stock diet (Oriental Kobe) and water ad libitum for 1 week before the experiment to acclimatize them to the new environment.

2.1. *Preparation of sample for DNA-RNA and amino acid analysis*

Preparations of a DNA-RNA fraction and a sample for amino acid analysis from rat liver were according to [4,15].

2.2. *Amino acid and gas-chromatographic analysis*

The quantitative determination and fractionation of amino acids were performed with a preparative amino acid autoanalyzer (Hitachi KLA-3B type) and an aliquot of the β-AIB fraction was saved for estimation of radioactivity and determination of the absolute configuration of the amino acid. The radioactivity was measured with a Packard liquid scintillation counter equipped with an external standard for compensating counting efficiency. The β-AIB fraction from the preparative amino acid analyzer was desalted as shown by Ogawa et al. [16] and converted to the *N*-trifluoroacetyl-(*S*)-prolyl derivative of the methyl ester of β-AIB by the method of Bonner [17]. The enantiomers of the dipeptides were separated isothermally at 185°C using a 3 mm × 2 m column containing 2% OV 17 on gas chromatography (Yanagimoto).

2.3. *Assay of β-alanine-pyruvate aminotransferase*

β-Alanine-pyruvate aminotransferase activity was assayed by following the rate of formation of malonic semialdehyde according to [18].

3. RESULTS

Table 1 shows the incorporation of radioactivity into β-AIB in rat liver 20 min after [*methyl*-^{14}C]thymine administration. The position of the radioactivity on paper chromatography (*n*-butanol : acetic acid : water = 4 : 4 : 1) was the same as that of β-AIB. The conversion of radioactive thymine into β-AIB was increased very significantly by 6-azauracil. In livers of rats treated with 6-azauracil the radioactivity of β-AIB was 37% of the total radioactivity injected, while that in control rat liver was 0.9%. On the other hand, the radioactivities of the DNA + RNA fraction in liver of control and 6-azauracil-treated rat were 0.32 and 0.11%, respectively, of [*methyl*-^{14}C]thymine administered.

Table 1

Incorporation of [*methyl*-^{14}C]thymine into β-AIB in rat liver

Treatment	dpm/g wet tissue	% of injection
Control	$10^3 \times (23.9 \pm 1.2)$	0.86 ± 0.05
6-Azauracil	$10^3 \times (1004.2 \pm 55.5)^a$	37.61 ± 1.59[a]

Five rats were used in each experimental subgroup. 10 mg 6-azauracil was dissolved in 0.4 ml of 20% propylene glycol and subcutaneously injected into the rats 5 times at intervals of 2 h. Control animals were injected with 20% propylene glycol in the same manner. 2 h after the last injection of 6-azauracil, 10 μCi [*methyl*-^{14}C]thymine (50 mCi/mmol) was injected into the femoral vein and the animals killed 20 min later. The liver was quickly removed and provided as the sample preparations. Each value is the mean ± SE. [a] $p < 0.01$ compared to control

Table 2

Effect of 6-azauracil on β-AIB and β-alanine contents in rat liver

Treatment	μmol/g wet tissue	
	β-AIB	β-Alanine
Control	0.040 ± 0.003	0.180 ± 0.028
6-Azauracil	0.849 ± 0.037[a]	10.950 ± 0.572[a]

6-Azauracil treatment and autopsy were the same as in table 1. Each value is the mean ± SE. [a] $p < 0.01$ compared to control

Table 2 shows the contents of β-AIB as well as β-alanine in rat liver and the effect of 6-azauracil. When 6-azauracil was consecutively injected 5 times at intervals of 2 h, the levels of β-AIB and β-alanine were increased very significantly, 22- and 61-fold, respectively.

By application of gas-liquid chromatography, the enantiomorphs of β-AIB in liver could be determined. Authentic *N*-trifluoroacetyl-(*S*)-prolyl-(*R*)-β-AIB methyl ester was separated from the

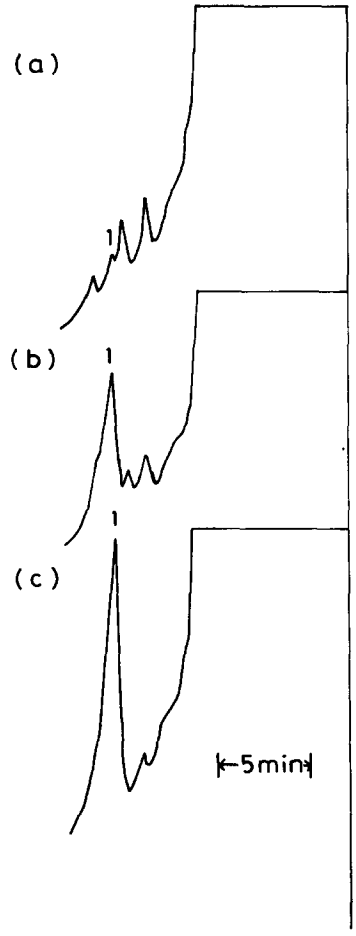

Fig.1. Gas-chromatographic determination of the β-AIB enantiomorph in liver of rat administered with 6-azauracil. 6-Azauracil was injected as described in table 1. Sample preparation and gas-chromatographic conditions are described in section 2. (a) Control rat liver, (b) 6-azauracil-treated rat liver, (c) (b) + authentic *N*-trifluoroacetyl-(*S*)-prolyl-(*R*)-β-AIB methyl ester.

derivative of the (*S*)-isomer (not shown). As shown in fig.1, the striking accumulation of the (*R*)-enantiomer of β-AIB was observed in liver of rat injected with 6-azauracil and the peak of the derivative of the (*R*)-isomer was increased by the addition of the authentic one. Thus, it is apparent that the increase of liver total β-AIB was caused by the (*R*)-enantiomorph only.

Fig.2 shows the inactivation of β-alanine-pyruvate aminotransferase activity by 6-azauracil. The enzyme activity in raw extract of rat liver was

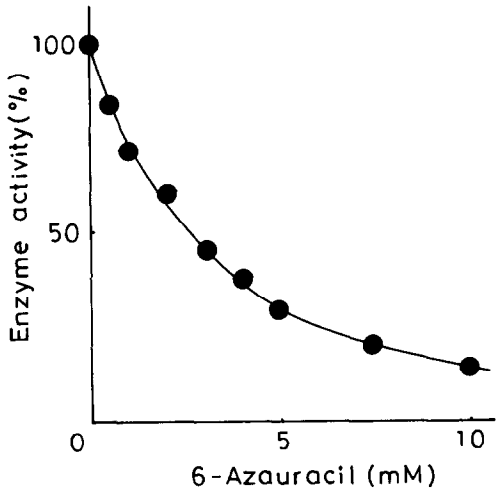

Fig.2. Effect of 6-azauracil on β-alanine-pyruvate aminotransferase activity in rat liver. Rat liver was homogenized in 10 mM potassium phosphate, pH 7.5, containing 1 mM EDTA, 2 mM 2-mercaptoethanol and 40 μM pyridoxal 5′-phosphate. The homogenate was centrifuged for 20 min at 27000 × *g* and the supernatant used for the estimation of enzyme activity. Relative activity is defined as the percentage of the activity compared to that in the absence of 6-azauracil.

0.17 ± 0.02 nmol/min per mg protein under the standard assay conditions. In the presence of 1 mM β-alanine and 10 mM pyruvate, 6-azauracil inactivated β-alanine-pyruvate aminotransferase activity with an apparent I_{50} of 2.5 mM.

4. DISCUSSION

Here, the (*R*)-isomer of β-AIB was accumulated in rat liver by 6-azauracil treatment (table 2, fig.1). These results show that 6-azauracil increased one of the metabolites of thymine. The incorporation of radioactivity from [*methyl*-^{14}C]thymine into β-AIB in liver was increased 42-fold by 6-azauracil. Furthermore, 6-azauracil inhibited uridine 5′-phosphate synthesis [1] and decreased the incorporation of [*methyl*-^{14}C]thymine into DNA. From these results, it may be proposed that 6-azauracil does not enhance pyrimidine biosynthesis, but that it inhibits the metabolism of (*R*)-β-AIB. As shown in fig.2, 6-azauracil inactivated β-alanine-pyruvate aminotransferase activity in rat liver.

In [6,18], we found that β-alanine was mainly transaminated by β-alanine-2-oxoglutarate ami-

notransferase in liver and that 6-azauracil inhibited the purified enzyme activity. In this paper we also found that 6-azauracil inactivated β-alanine-pyruvate aminotransferase activity to accumulate (*R*)-β-AIB. Thus, 6-azauracil may facilitate the study of pyrimidine metabolism including β-alanine and (*R*)-β-AIB.

ACKNOWLEDGEMENTS

We are grateful to Professor K. Tanaka for helpful discussions. We are also indebted to Professor Y. Kakimoto for providing (*R*)- and (*S*)-isomers of β-AIB.

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