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Effects of thiazolidine-4(R)-carboxylates and other low-molecular-weight sulfur compounds on the activity of mercaptopyruvate sulfurtransferase, rhodanese and cystathionase in Ehrlich ascites tumor cells and tumor-bearing mouse liver

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Summary. Changes of the specific activity of 3-mercaptopyruvate sulfurtransferase (MPST), rhodanese and cystathionase in Ehrlich ascites tumor cells (EATC) and tumor-bearing mouse liver after intraperitoneal administration of thiazolidine derivatives, L-cysteine, D,L-methionine, thiocystine or thiosulfate were estimated. Thiazolidine derivatives used were: thiazolidine-4-carboxylic acid (CF), 2-methyl-thiazolidine-2,4-dicarboxylic acid (CP) and 2-methyl-thiazolidine-4-carboxylic acid (CA). In the liver, the activity of MPST was significantly increased by all the studied compounds, whereas the activity of rhodanese was by CF and thiocystine and that of cystathionase was by the administration of cysteine and CP. On the other hand, cysteine lowered the rhodanese activity and the activity of cystathionase was decreased by the administration of methionine and thiocystine. Activities of MPST and rhodanese were even lower in EATC than those in the liver of tumor-bearing mouse and the activity of cystathionase in EATC was not be detected. The thiazolidine derivatives significantly increased the level of MPST activity in EATC, but decreased the rhodanese activity. Thiosulfate also increased the activity of MPST to a lesser degree, but cysteine, methionine and thiocystine gave little change in the activity. The rhodanese activity in EATC was slightly increased only by thiocystine. These findings suggest that the sulfur metabolism in the tumor-bearing mouse liver is different from that in the normal mouse liver, and that sulfur compounds are minimally metabolized to sulfane sulfur, a labile sulfur, in EATC.

Keywords: Amino acids – Thiazolidine-4(R)-carboxylates – Mercaptopyruvate sulfurtransferase – Rhodanese – Cystathionase Ehrlich-ascites tumor cell – Mouse liver

Introduction

3-Mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2), rhodanese (thiosulfate sulfurtransferase, EC 2.8.1.1) and cystathionase (cystathionine γ-lyase, EC 4.4.1.1) are well known enzymes participating in the formation of sulfane sulfur compounds and in transferring their labile sulfur atoms to various acceptors. In neoplastic cells, activities of these enzymes are even lower than those in the liver of normal mouse, suggesting that little synthesis of sulfane sulfur compounds from L-cysteine may occur in the cells due to deficiency of enzymes for anaerobic metabolism of L-cysteine (Koj et al., 1964; Jackson and Morse, 1970; Włodek et al., 1971, 1993a). The biological role of reduced sulfur, such as sulfane sulfur, is not completely understood, although the malignant proliferation of cells is possibly caused by a deficiency of sulfane sulfur and consequential disorder in regular control systems of enzymes with sulfane sulfur, as reviewed by Toohey (1989).

In our previous studies it was demonstrated that L-cysteine reacted nonenzymatically with formaldehyde, pyruvate and acetaldehyde to form thiazolidine-4-carboxylic acid (CF), 2-methyl-thiazolidine-2,4-dicarboxylic acid (CP) and 2-methyl-thiazolidine-4-carboxylic acid (CA), respectively (Włodek, 1984; 1988; Włodek et al., 1993b). Effects of these thiazolidine derivatives and low-molecular-weight sulfur compounds such as L-cysteine, D,L-methionine, thiocystine and thiosulfate, on changing activities of MPST and rhodanese in the liver of normal mouse were estimated previously (Wróbel and Frendo, 1992; Włodek et al., 1993c). In the present work responses of activities of MPST, rhodanese and cystathionase in the liver of tumor-bearing mouse, as well as in Ehrlich ascites tumor cells (EATC), to intraperitoneal administrations of the above mentioned thiazolidine derivatives and other sulfur compounds have been studied in order to examine the usefulness of these compounds for a delivery system of cellular sulfane sulfur.

Materials and methods

Animals and chemicals

Female Swiss mice weighing approximately 20g were grouped into 4–10 animals per a cage, and fed a standard diet and water *ad libitum* for at least one week before the experiment. CF and CP were made by the reaction of L-cysteine with formaldehyde and pyruvate, respectively, according to the method of Schubert (1936). CA was synthesized by the reaction between L-cysteine and acetaldehyde as reported by Nagasawa et al. (1984). Thiocystine (cystine persulfide) was made by the Fletcher and Robson's method (1963) and 3-mercaptopyruvate ammonium salt was prepared by the method of Sprinson and Chargaff (1946) in the Laboratory of Organic Synthesis of the Jagiellonian University (Krakow, Poland). L-Cysteine hydrochloride was purchased from Sigma Chemical Company (U.S.A.); D,L-methionine and thiosulfate were from Roenal (Budapest) and BDH Chemicals (England), respectively. Other chemicals used were from the Polish Chemical Reagent Company P.O.Ch. (Poland).

General methods

Intraperitoneal inoculation of EATC was performed to mice, and the mice were maintained on the laboratory diet and water *ad libitum* for 7 days. Each thiazolidine compound

was injected intraperitoneally to tumor-bearing mice at a dose of 1.2 mmol per kg of body weight once a day for 3 days. Other sulfur compounds were injected in the same manner except for a dose (mmol per kg of body weight) as follows: L-cysteine hydrochloride, 4.3; D,L-methionine, 9.9; thiocystine, 1.0; thiosulfate, 3.0. Control mice were injected with the same volume of 0.9% sodium chloride solution. On the fourth day, the mice were killed, and the liver was washed with cold 0.9% sodium chloride solution. The EATC were collected and then washed repeatedly by suspension into 0.9% sodium chloride solution followed by centrifugation at 650 × g for 5 min. The washed liver and EATC were kept in a freezer at −30°C until used. After the frozen liver had been thawed, 1 g of the liver was homogenized with 5 ml of 0.1 M potassium phosphate buffer, pH 7.4. The EATC were homogenized with two volumes of the phosphate buffer, pH 7.4. Each homogenate was centrifuged at 650 × g for 15 min to obtain a supernatant, and the supernatant was used for enzyme assay.

Enzyme assay

Contents of proteins were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The activity of MPST was determined by measuring the amount of pyruvate formed during 15 min of incubation at 37°C in accordance with the method of Kun and Fanshier (1959) modified by Kasperczyk et al. (1977). The activity of rhodanese was assayed by Sörbo's method (1955) measuring the amount of SCN^- formed during 5 min of incubation at 20°C. The activity of cystathionase was determined by Matsuo and Greenberg's method (1958) using homoserine as a substrate; the amount of 2-ketobutyrate formed during 30 min of incubation at 37°C was measured. Specific activities of these enzymes were expressed in nmols of the product formed per one min per one mg of protein.

Results

Changes in the activities of the enzymes in the tumor-bearing mouse liver are shown in Table 1. The activity of MPST in the liver was increased significantly by intraperitoneal administration of all the compounds; especially for CP and CA, the activity level was elevated to 240% and 170% of the control value, respectively.

As for the activity of rhodanese, CF and thiocystine increased the activity to 130% and 109% of the control level, respectively. On the contrary, L-cysteine decreased the activity of this enzyme to 62% of the control level, and other compounds showed little effect on the rhodanese activity.

In the case of cystathionase activity, D,L-methionine and thiocystine lowered the level of the activity to 74% and 42% of the control level, respectively, whereas the activity was elevated to 140% of the control level by the administration of either L-cysteine or CP. Other compounds had little influence on the activity of cystathionase.

As shown in Table 2, EATC triggered only 9% and 6.5% of the activity of MPST and rhodanese, respectively, in comparison to the tumor-bearing mouse liver (Table 1). The activity of cystathionase in EATC was not detected by the present study.

In EATC, the administration of all the thiazolidine derivatives enhanced the activity of MPST significantly: CP, 160%; CA, 120%; CF, 127% of the control level. Administration of thiosulfate also increased the activity to

Table 1. Activities of 3-mercaptopyruvate sulfurtransferase (MPST), rhodanese and cystathionase in the liver of tumor-bearing mouse on the fourth day after intraperitoneal administration of sulfur compounds[a]

Compounds	Number of animals	Activity (nmol/min/mg of protein)		
		MPST	Rhodanese	Cystathionase
Control	8	780 ± 30	370 ± 30	0.583 ± 0.090
Cysteine	8	1,010 ± 100***	230 ± 14***	0.820 ± 0.167**
Methionine	8	1,080 ± 50***	376 ± 40	0.433 ± 0.067*
CP	5	1,870 ± 240***	362 ± 28	0.813 ± 0.107**
CA	4	1,330 ± 60***	374 ± 10	0.593 ± 0.070
CF	4	910 ± 50**	476 ± 8***	0.690 ± 0.133
Thiocystine	7	1,020 ± 50***	400 ± 12*	0.243 ± 0.053***
Thiosulfate	8	1,070 ± 50***	360 ± 34	0.703 ± 0.147

[a] Sulfur compounds were intraperitoneally injected once a day for 3 days to Ehrlich ascites tumor-bearing mice as described under Materials and methods. Control mice were injected intraperitoneally with the same volume of 0.9% sodium chloride solution. Values represent the mean ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

Table 2. Activities of 3-mercaptopyruvate sulfurtransferase (MPST) and rhodanese in Ehrlich ascites tumor cells on the fourth day after the intraperitoneal administration of sulfur compounds to the tumor-bearing mouse[a]

Compounds	Number of animals	Activity (nmol/min/mg of protein)	
		MPST	Rhodanese
Control	8	70.7 ± 5.3	24.0 ± 0.2
Cysteine	8	77.3 ± 6.7	19.2 ± 0.6***
Methionine	8	69.3 ± 3.3	22.4 ± 2.0
CP	5	113.3 ± 14.0***	15.0 ± 0.2***
CA	4	85.3 ± 6.7**	19.4 ± 0.6***
CF	4	90.0 ± 4.7***	21.2 ± 0.4***
Thiocystine	7	71.3 ± 2.7	25.2 ± 0.6**
Thiosulfate	8	78.7 ± 6.0*	22.4 ± 1.6

[a] Sulfur compounds were injected intraperitoneally and enzyme activities were assayed as in Table 1. Control mice were injected intraperitoneally with the same volume of 0.9% sodium chloride solution. Values represent the mean ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

111% of the control level. However, neither L-cysteine, D,L-methionine nor thiocystine administration resulted in any change in the activity of MPST.

The activity of rhodanese in EATC was lowered significantly by the administration of all the thiazolidine derivatives and L-cysteine: CP, 63%; CA, 81%; CF, 88%; L-cysteine, 80% of the control level. On the other hand, only

thiocystine administration enhanced slightly but significantly the rhodanese activity: 105% of the control level.

Discussion

The activity of MPST in the liver of tumor-bearing mouse was enhanced by the administration of all the tested compounds as shown in Table 1 and this was consistent with the previous results obtained for the normal mouse liver except for the lesser effect of methionine (Wróbel and Frendo, 1992; Włodek et al., 1993c). On the other hand, the response of the rhodanese activity in the liver of tumor-bearing mouse to the administration of the sulfur compounds (Table 1) was not similar to that in the liver of normal mouse reported previously (Wróbel and Frendo, 1992). In the normal liver, the activity of rhodanese was remarkably enhanced by administration of methionine and thiocystine, and a slighter enhancement of the enzyme activity was obtained as a result of cysteine or thiosulfate administration. In addition, the activity of cystathionase in the liver of normal mouse was enhanced by the administration of methionine as demonstrated previously (Włodek et al., 1971). On the contrary, the administration of methionine to tumor-bearing mouse resulted in a significant decrease in the activity of cystathionase as shown in Table 1. These findings seem to suggest that formation and transfer of sulfane sulfur formed from methionine by catalysing actions of cystathionase and rhodanese may occur at an even lesser degree in the liver of tumor-bearing mouse than in the liver of normal mouse.

The thiazolidine derivatives, especially CP, seem to be useful for stimulating the formation of sulfane sulfur compounds such as thiocystine and thiosulfate in the liver of tumor-bearing mouse because of the increasing tendency in activities of MPST and cystathionase as shown in Table 1. However, the activity of MPST in the tumor-bearing mouse liver was only one half of that in the normal liver (Wróbel and Frendo, 1992). Moreover, no simultaneous change of the activity of rhodanese was obtained by the administration of thiosulfate as well as CP to tumor-bearing mouse as shown in Table 1. Therefore, it seems that sulfane sulfur compounds can be formed from thiazolidine derivatives in the liver of tumor-bearing mouse, but in the contrast to the normal mouse (Włodek et al., 1993c), the extent of the activity as donors of their labile sulfur atoms may be limited. A reason here may be found in the change of mitochondrial membrane permeability to sulfane sulfur compounds in tumor-bearing liver cells in comparison to normal liver cells.

In EATC, little conversion of sulfur compounds into sulfane sulfur may occur because of deficient activities of MPST, rhodanese and cystathionase in these cells, although the administration of thiazolidine derivatives enhanced the activity of MPST as shown in Table 2.

The present work demonstrates a difference in sulfur metabolism between the liver of tumor-bearing mouse and of normal mouse. The difference is possibly due to the intraperitoneal proliferation of the neoplastic cells, although sulfur metabolism in the liver of tumor-bearing mouse has not been established.

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