Acta Medica Okayama

Volume 43, *Issue* 6 1989 *Article* 3

DECEMBER 1989

Effect of cysteine on the inactivation of cystathionine gamma-lyase by D,L-propargylglycine.

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Abstract

In vivo inactivation of cystathionine gamma-lyase by D,L-propargylglycine, a suicide inhibitor, was found to be less profound in rat kidney than in the liver. We investigated the cause of this difference using rat tissues. We fractionated kidney extract to characterize the substance which protected enzyme, and found that cysteine exhibits protecting action. Addition of 0.3 mM L-cysteine to the incubation mixture containing dialyzed kidney supernatant and 0.5 mM D,L-propargylglycine resulted in the protection of cystathionine gamma-lyase from the inactivation by the inhibitor. The content of cysteine in the kidney was six-fold higher than that in the liver. Thus, we have concluded that one of the reasons why the in vivo inactivation of cystathionine gamma-lyase in rat kidney was less than that in the liver is the presence of a higher concentration of cysteine in the kidney. S-Carboxymethylcysteine, a cysteine derivative, exhibited a similar, but weaker, protective effect.

KEYWORDS: cystathionine ?-lyase, D, L-propargylglycine, cysteine

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*PMID: 2624140 [PubMed - indexed for MEDLINE]

Acta Med Okayama 43 (6) 329-335(1989)

Effect of Cysteine on the Inactivation of Cystathionine γ-Lyase by D,L-Propargylglycine

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In vivo **inactivation of cystathionine γ-lyase by D,L-propargylglycine, a suicide inhibitor, was found to be less profound in rat kidney than in the liver. We investigated the cause of this difference using rat tissues. We fractionated kidney extract to characterize the substance which protected enzyme, and found that cysteine exhibits protecting action. Addition of 0.3 mM L-cysteine to the incubation mixture containing dialyzed kidney supernatant and 0.5 mM D,L-propargylglycine resulted in the protection of cystathionine γ-lyase from the inactivation by the inhibitor. The content of cysteine in the kidney was six-fold higher than that in the liver. Thus, we have concluded that one of the reasons why the *in vivo* inactivation of cystathionine γ-lyase in rat kidney was less than that in the liver is the presence of a higher concentration of cysteine in the kidney. S-Carboxymethylcysteine, a cysteine derivative, exhibited a similar, but weaker, protective effect.**

Key words : **cystathionine γ-lyase, D,L-propargylglycine, cysteine**

The affinity labeling technique has been used widely in studying the active site of enzymes. D, L-propargylglycine is an affinity labeling reagent which inactivates rat liver cystathionine γ-lyase (EC 4. 4. 1. 1.), as first reported by Abeles and Walsh (1). The mechanism of the inactivation was later described in detail (2), and it was shown that the compound also inactivated alanine aminotransferase (EC 2. 6. 1. 2.) (3, 4), aspartate aminotransferase (EC 2. 6. 1. 1) (5) and D-amino acid oxidase (EC 1. 4. 3. 3) (6) *in vitro*.

We have reported on the accumulation of cystathionine and N-acetylcystathionine in various tissues, excretion of cystathionine and its metabolites into the urine, and changes in the enzyme activities related to cystathionine metabolism in propargylglycine-treated rats (7). During these studies, we noticed that there is a difference between liver and kidney in the degree of inactivation of cystathionine γ-lyase after the intraperitoneal administration of D,L-propargylglycine. The activity of the enzyme in the liver was almost completely lost at a dose of 1mg of D,L-propargylglycine per 200g of body weight. However, about 20% of the enzyme activity in the kidney remained even at a dose of 20mg/200g of body weight.

The difference between the inactivation of

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enzymes in the liver and kidney was observed by Shinozuka *et al*. in mice injected intraperitoneally with L-propargylglycine (8). They concluded that the difference was due to the degree of incorporation of L-propargylglycine into the various tissues.

The present paper presents evidence to support the hypothesis that the presence of a high concentration of cysteine in the kidney protects cystathionine γ-lyase from inactivation by propargylglycine *in vivo*.

Materials and Methods

Chemicals. D,L-propargylglycine was purchased from Sigma Chemical Company, St. Louis, MO, USA. S-(carboxymethyl)-L-cysteine and S-(carboxymethyl)-L-homocysteine were synthesized by the method of Ubuka *et al*. (9) and Kodama *et al*. (10), respectively. All other chemicals used were obtained from commercial sources and were of analytical grade.

Animals. Male Wistar rats weighing about 200 g were used in all experiments. All animals were fed a commercial laboratory diet and water *ad libitum*.

Enzyme preparations. Rats were killed by decapitation. The liver and other tissues were removed and homogenized in 3 volumes of cold 0.01 M potassium phosphate buffer (pH 7.5) containing 0.15 M KCl, 0.1 mM pyridoxal phosphate and 0.5 mM EDTA. The homogenates were centrifuged at 10,000 × *g* for 10 min, and the resulting supernatants were used for the assay. All these procedures were performed at 0 to 4 °C.

Enzyme assays. The activity of cystathionine γ-lyase was determined by a modification of the method of Matsuo and Greenberg (11) as follows. The reaction mixture contained 32 mM D,L-homoserine, 0.05 mM pyridoxal 5'-phosphate, 7.5 mM 2-mercaptoethanol, 7.0 mM EDTA, 0.1 M potassium phosphate buffer (pH 7.5) and enzyme solution in a final volume of 1.0 ml. After preincubation of the reaction mixture lacking D,L-homoserine at 37 °C for 5 min, the reaction was initiated by the addition of 0.2 ml of 0.16 M D,L-homoserine. Enzyme activity was expressed as absorbance at 515 nm of the alkaline solution of the 2, 4-dinitrophenylhydrazone of the α-ketobutyrate formed.

Fractionation of kidney extract by Sephadex G-25. Kidney was homogenized with 3 volumes of 0.01 M potassium phosphate buffer (pH 7.5), and the homogenate was centrifuged at 10,000 × *g* for 10 min. About 20 ml of the supernatant was applied to a Sephadex G-25 column (3 × 40 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 7.5) and elution was performed with the same buffer at a flow rate of 0.9 ml per min, collecting 3.5-ml fractions.

Dialysis of kidney supernatant. Kidney supernatant (20 ml) was dialyzed against three changes of one liter each of 20 mM potassium phosphate buffer (pH 7.5) containing 0.01 mM pyridoxal 5'-phosphate, 1 mM 2-mercaptoethanol and 0.5 mM EDTA for 3 h.

Determination of cysteine. Cysteine was determined by the method of States *et al.* (12).

Results and Discussion

Effect of D,L-propargylglycine on cystathionine γ-lyase activity in the rat tissue supernatants. Fig. 1 shows the effect of D,L-propargylglycine on cystathionine γ-lyase activity in the supernatants of liver, kidney and pancreas. The enzyme activities in the liver and pancreas were completely lost when incubated with 0.5 mM D,L-propargylglycine at 37 °C for 5 min. On the contrary, about 30 % of the enzyme activity in the kidney supernatant remained even when treated with 1.6 mM D,L-propargylglycine. From these observations, we suspected that kidney might contain a substance which protected the enzyme from inactivation by D,L-propargylglycine.

Separation of the substance which protects cystathionine γ-lyase with a Sephadex G-25 column. As described above, cystathionine γ-lyase activity in the liver supernatant was completely lost when incubated with 0.5 mM D,L-propargylglycine. We used this concentration of D,L-propargylglycine for the detection of the substance in the kidney extract which protects cystathionine γ-lyase from inactivation by propargylglycine. The preparation and fractionation of renal extracts were carried out with a Sephadex G-25 column as described above. A portion of each fraction (0.34 ml) was added to the mixture containing 0.46 ml of liver extract and 0.5 mM

propargylglycine, and the mixture was preincubated at 37°C for 5 min. Then, D,L-homoserine was added, and cystathionine γ-lyase activity was determined as described above.

As shown in Fig. 2, the enzyme activity was partially protected when fractions 55 to 70 were added to the preincubation mixture. The figure also shows that the enzyme activity was partially inhibited by the addition of the same fraction to the preincubation mixture without D,L-propargylglycine.

Separation of the substance which protects cystathionine γ-lyase with an ion-exchange resin column. From the results obtained above, the enzyme protecting substance was assumed to be a small molecular weight compound. Therefore, we tried to separate the substance with a column of strongly acidic ion-exchange resin (Diaion SK-1). Fractions 55 to 70 obtained by Sephadex G-25 chromatography were pooled. About 50 ml of the solution was evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of water, and the solution was adjusted to pH 4.0 with acetic acid. It was applied to a Diaion SK-1 column (H^+-form, 2 × 16 cm). The column was washed with 250 ml of water, and then eluted with 250 ml of 2 M ammonia. The eluate was collected, dried as above and dissolved in 10 ml of water. A portion of the solution (0.34 ml) was used to examine the protective effect on cystathionine γ-lyase activity. About 13% of the cystathionine γ-lyase activity remained when 0.34 ml of the solution was added to the preincubation mixture containing liver extract and 0.5 mM D, L-propargylglycine. Again this solution inhibited (about 44%) the enzyme activity when added to the preincubation mixture without D,L-propargylglycine.

Effect of various amino acids on cystathionine γ-lyase activity in liver supernatant. Chromatographic behavior suggested that the enzyme protecting substance was an amino acid(s). Thus, we examined the effect of various amino acids on cystathionine γ-lyase activity. Among usual amino acids, only cysteine protected cystathionine

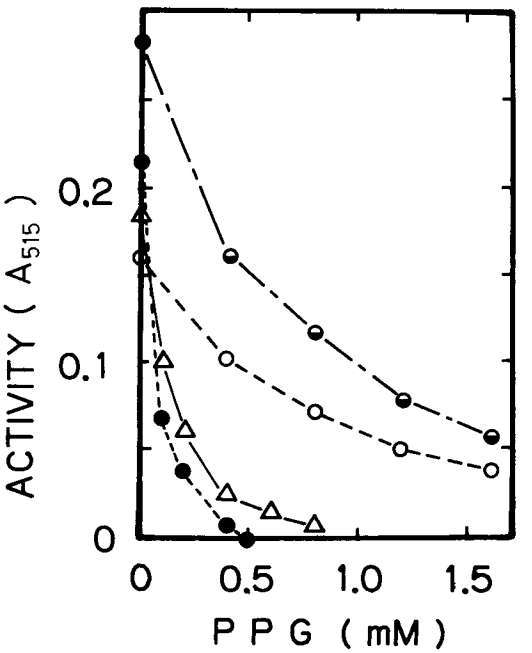

Fig. 1 Effect of D,L-propargylglycine on cystathionine γ-lyase activity in the supernatants of liver, kidney and pancreas. Supernatant fractions were prepared as described under Materials and Methods. As enzyme sources, 0.05 ml of liver (●), 0.30 ml of kidney (○), a mixture of 0.05 ml of liver plus 0.30 ml of kidney (◒), and 0.30 ml of pancreatic supernatant (△) were used, and preincubated with various concentrations of D, L-propargylglycine at 37°C for 5 min. Each preincubation mixture (0.8 ml) contained 0.06 mM pyridoxal phosphate, 9.4 mM 2-mercaptoethanol, 8.8 mM EDTA, 0.1 M potassium phosphate buffer (pH 7.5), one of the above supernatant and various concentrations of D,L-propargylglycine. Then, the enzyme reaction was started by the addition of 0.20 ml of 0.16 M D, L-homoserine and continued for 5 min at 37°C. The concentration of D, L-propargylglycine (PPG) is shown as the final concentration in 1.0 ml of the reaction mixture.

γ-lyase activity. Fig. 3-A shows the protective effect of L-cysteine on the inactivation of cystathionine γ-lyase activity in the liver supernatant by D,L-propargylglycine. The enzyme activity was completely lost when the extract was preincubated with 0.5 mM D,L-propargylglycine in the absence of L-cysteine. When L-cysteine was added to the preincubation mixture together with D,L-propargylglycine, the enzyme was partially protected from inactivation. The protection by L-cysteine was maximum at a concentration of

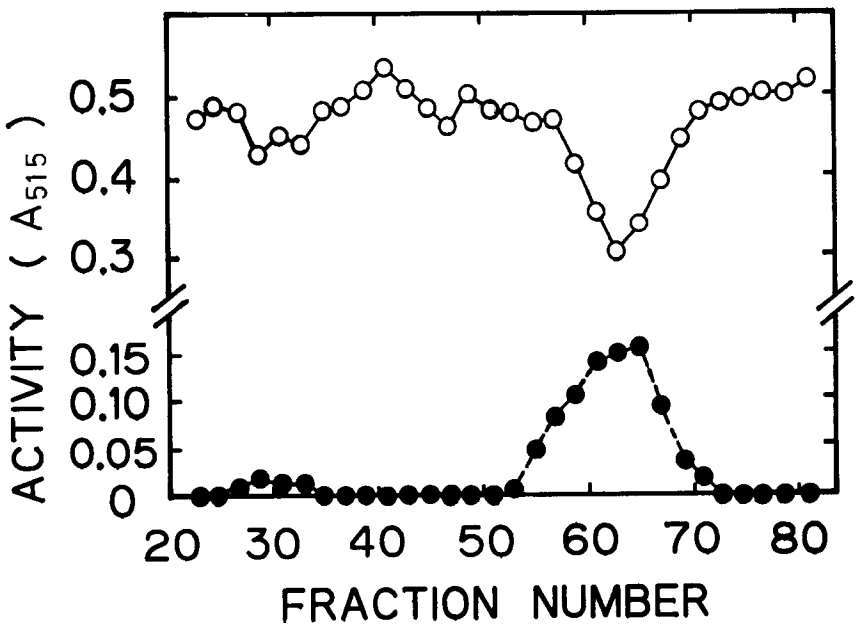

Fig. 2 Fractionation of cystathionine γ-lyase protecting substance with a Sephadex G-25 column. Rat kidney was homogenized with 3 volumes of 0.01 M potassium phosphate buffer (pH 7.5), and about 20 ml of the supernatant was applied to a Sephadex G-25 column (3 × 40 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 7.5) and eluted with the same buffer at a flow rate of 0.9 ml per min, collecting 3.5-ml fractions. A portion of each fraction (0.34 ml) was mixed with liver supernatant (0.05 ml), and the mixture was preincubated with (●) or without 5 mM D,L-propargylglycine (○) at 37 °C for 5 min. The enzyme reaction was then started by the addition of 0.20 ml of 0.16 M D, L-homoserine. The reaction time was 20 min for the reaction with D, L-propargylglycine and 10 min for that without D, L-propargylglycine.

1 mM, and the protective effect decreased gradually at concentrations greater than 1 mM.

Fig. 3-A also shows that cysteine inhibits the enzyme activity when added to the preincubation mixture containing liver extract without D,L-propargylglycine. Among amino acids tested, L-cysteine and L-alanine inhibited the activity of this enzyme. At a concentration of 5 mM, the rate of inhibition was about 80 % and about 40 %, respectively. Thus, L-cysteine exhibited the dual effects of protection and inhibition on cystathionine γ-lyase. The inhibitory action of L-cysteine and L-alanine are consistent with the reports that deamination of L-homoserine by this enzyme was inhibited competitively by L-cystine and noncompetitively by L-alanine and that L-homoserine inhibited desulfhydration of L-cysteine when the concentration of the latter was small (17).

Fig. 3-B shows that S-(carboxymethyl)-L-cysteine exhibited a protective action to a lesser extent than L-cysteine. The contents of S-(carboxymethyl)cysteine in various tissues were very much lower than those of cysteine. Therefore, it would not seem to be a significant protective agent *in vivo*. S-(carboxymethyl)-L-homocysteine did not show any protective action.

Among non-protein sulfur-containing amino acids, S-(carboxymethyl)-L-homocysteine and S-(carboxymethyl)-L-cysteine were effective inhibitors. The rate of inhibition was about 80 % and 60 % at a concentration of 5 mM, respectively as shown in Fig. 3-B. Yao reported that S-(carboxymethyl)-L-cysteine and S-(carboxymethyl)-L-homocysteine were competitive to cystathionine in the cystathionine γ-lyase reaction (15). On the other hand, when homoserine was used as a substrate, S-(carboxymethyl)-L-cysteine

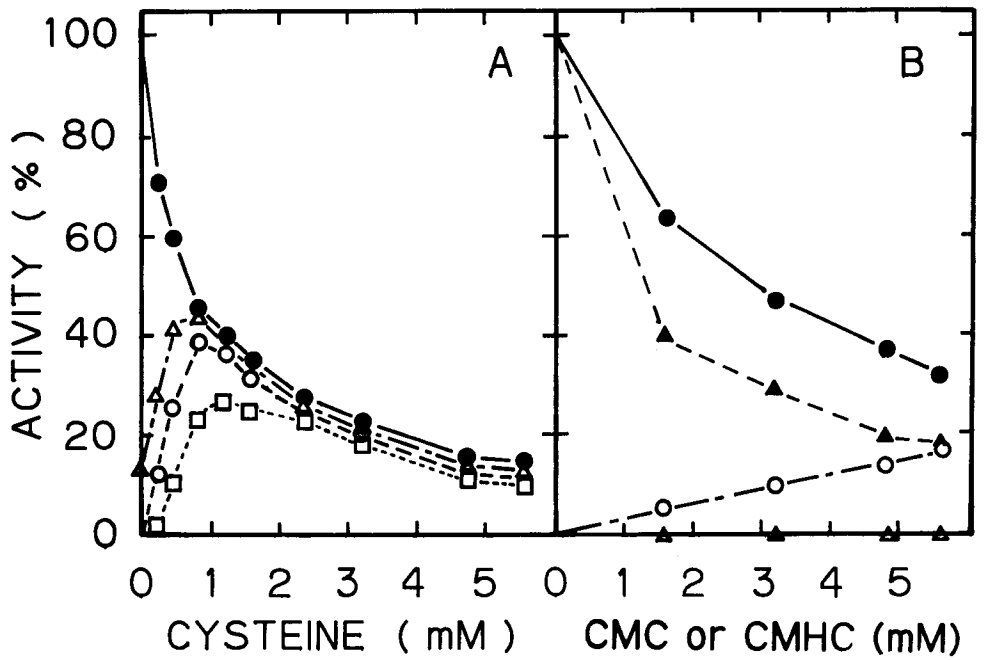

Fig. 3 Protective effect of cysteine, S-(carboxymethyl)-L-cysteine (CMC) and S-(carboxymethyl)-L-homocysteine (CMHC) on the inactivation of cystathionine γ-lyase activity by D, L-propargylglycine. (A) Liver supernatant (0.05 ml) and various concentrations of cysteine were preincubated with or without D, L-propargylglycine at 37°C for 5 min. The enzyme reaction was started by the addition of 0.2 ml of 0.16 M D, L-homoserine, and was carried out at 37°C for 5 min. Final concentrations of D, L-propargylglycine were 0 (●), 0.25 (△),0.5 (○) and 1.0 mM (□). (B) In the presence of S-(carboxymethyl)-L-cysteine or S-(carboxymethyl)-L-homocysteine, liver supernatant was preincubated with or without 0.5 mM D, L-propargylglycine. Then the enzyme reaction was carried out as in (A). ●, S-(carboxymethyl)-L-cysteine ; ○, S-(carboxymethyl)-L-cysteine with D, L-propargylglycine ; ▲, S-(carboxymethyl)-L-homocysteine ; △, S-(carboxymethyl)-L-homocysteine with D, L-propargylglycine.

noncompetitively inhibited the enzyme activity, while S-(carboxymethyl)-L-homocysteine competitively inhibited the activity (15). Deme *et al.* have hypothesized that the enzyme has an active site with "double" binding sites, one site being involved in the binding of homoserine and the other participating in the binding of cysteine (16, 17). The present results seem to be consistent with their reports, and suggest that the cysteine-binding site can bind with propargylglycine or S-(carboxymethyl)-L-cysteine, and the homoserine-binding site can bind with S-(carboxymethyl)-L-homocysteine.

Contents of cysteine in liver, kidney and pancreas. Table 1 shows the contents of cysteine, cystine and total cysteine in some rat tissues. The content of total cysteine was about six-fold higher in kidney than in liver. From these results, we estimated that the total cysteine concentrations in the assay systems using homogenates of liver, kidney and pancreas were about 0.01mM, 0.33mM and 0.13mM, respectively.

Fig. 3-A shows that 0.01 mM cysteine scarcely affected the enzyme activity after preincubation with 0.5 mM D,L-propargylglycine. On the contrary, 0.13 mM and 0.33 mM cysteine showed some protection, and the activity was about 15 % and 25 % of the control, respectively.

Effect of cysteine on the inactivation of cystathionine γ-lyase in dialyzed kidney supernatant by D,L-propargylglycine. The supernatant fraction obtained from kidney homogenate was dialyzed as described under Materials and Methods to remove cysteine in order to examine its effect on the inactivation of cystathionine

Table 1 Contents of cysteine and cystine in rat liver, kidney and pancreas[a]

Tissue	Cysteine	Cystine	Total cysteine[b]
Liver	0.55 ± 0.06	0.07 ± 0.01	0.69 ± 0.08
Kidney	3.77 ± 0.44	0.33 ± 0.12	4.43 ± 0.52
Pancreas	1.46 ± 0.17	0.11 ± 0.05	1.68 ± 0.27

a : Values represent means ± SD (μmol/g tissue). n = 8.
b : Cysteine + 2 × cystine.

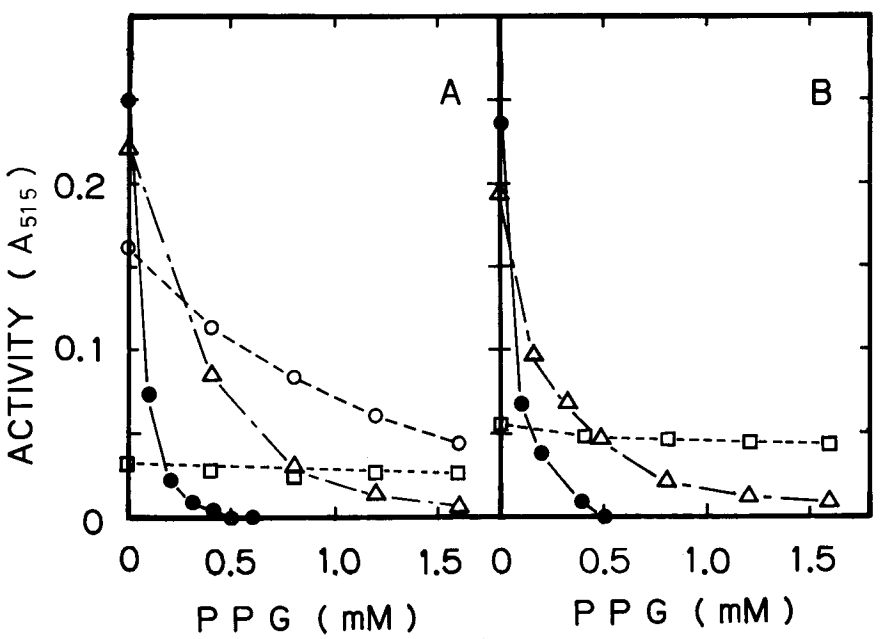

Fig. 4 Effect of L-cysteine on the inactivation of cystathionine γ-lyase activity in dialyzed kidney supernatant by D,L-propargylglycine (PPG). (A) Kidney supernatant was dialyzed as described under Materials and Methods. The dialyzed supernatant (0.30 ml) and various concentrations of D, L-propargylglycine were preincubated with or without L-cysteine at 37°C for 5 min. Then the enzyme reaction was started by the addition of 0.2 ml of 0.16 M D, L-homoserine, and carried out at 37°C for 5 min. ○, Undialyzed kidney supernatant ; ●, dialyzed kidney supernatant ; △, dialyzed kidney supernatant + 0.3 mM L-cysteine (final concentration); □, dialyzed kidney supernatant + 3.0 mM L-cysteine (final concentration). (B) Liver supernatant (0.05 ml) and various concentrations of D, L-propargylglycine were preincubated with or without L-cysteine. Final concentrations of L-cysteine added were 0 (●), 0.3 (△) and 3.0 mM (□).

γ-lyase by D,L-propargylglycine. Dialysis decreased the concentration of cysteine in the supernatant to 0.06 mM.

As shown in Fig. 4, the level of inactivation by D,L-propargylglycine of cystathionine γ-lyase with the dialyzed kidney supernatant was the same level as that with the liver supernatant. When cysteine was added to these supernatants, a similar protective effect on the enzyme against D,L-propargylglycine was observed.

Fig. 4 also shows that, in the absence of D, L-propargylglycine, L-cysteine exhibited an inhibition both to liver supernatant and dialyzed kidney supernatant essentially to the same degree.

The results presented in this paper seem to indicate that the presence of a high concentration of cysteine in the kidney is one of the reasons why the *in vivo* inactivation of cystathionine γ-lyase was less in the kidney than in the liver.

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Received March 13, 1989 ; accepted September 5, 1989