

**Zusammenfassung.** N-terminale Sequenzanalyse der an SDS-Hydroxyapatit im präparativen Maßstab getrennten  $\alpha$ - und  $\beta$ -Untereinheiten zeigte in den letzten 11 Amino-

säureresten geringe, in den folgenden 17 Aminosäureresten aber starke Sequenzhomologie zwischen den beiden Polypeptidketten. Starke Unterschiede bestehen zwischen beiden Untereinheiten in den tryptischen Peptiden.

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### Increase in Cystathionine $\beta$ -Synthase Activity in the Liver of Rats Treated with L-Ethionine

Cystathionine  $\beta$ -synthase (EC 4.2.1.21) is a pyridoxal-P-dependent enzyme catalyzing the formation of cystathionine from L-homocysteine and L-serine. Recently it has been found that both the cystathionine  $\beta$ -synthase and L-serine sulphydrase (EC 4.2.1.22) activities are due to a single enzyme<sup>1,2</sup>. Deficiency, or absence, of cystathionine  $\beta$ -synthase activity caused by a genetic defect, results in a serious inborn disease called homocystinuria<sup>3-6</sup>.

Ethionine is known to produce an inhibition of hepatic protein synthesis<sup>7-10</sup> and increase in the concentration of free amino acids in plasma and tissues<sup>11</sup>. This analog of methionine also causes an inhibition of phospholipid biosynthesis<sup>12</sup> as well as a fatty liver<sup>13,14</sup>. Treatment with ethionine induces a rapid fall in the liver ATP concentration<sup>15-19</sup> and causes a depression of RNA synthesis<sup>20-23</sup>. It has been demonstrated that ethionine causes an alteration in hepatic ionic composition<sup>24-26</sup>, then a diminution of NAD and NADP levels<sup>27</sup> and a change in the polyamines content<sup>28</sup>.

In view of the importance of transsulfuration in mammals, the effect of ethionine on cystathionine  $\beta$ -synthase activity is reported in this paper.

**Materials and methods.** Female and male albino rats weighing 180-220 g were used. L-ethionine, actinomycin D, cycloheximide and ATP were injected i.p. in doses of 400 mg, 1 mg, 20 mg and 200 mg per kg of body weight, respectively. DL-methionine, L-methionine-DL-sulfoximine

Table I. Specific activities of cystathionine  $\beta$ -synthase measured as L-serine sulphydrase (in nmoles cysteine/mg protein/h)

Treatment	Cystathionine $\beta$ -synthase
NaCl	75.8 $\pm$ 4
L-Ethionine	371.6 $\pm$ 18 <sup>a</sup>
Actinomycin D	73.3 $\pm$ 9
Actinomycin D + L-Ethionine	359.2 $\pm$ 12 <sup>a</sup>
Cycloheximide	72.8 $\pm$ 6
Cycloheximide + L-Ethionine	354.7 $\pm$ 21 <sup>a</sup>
DL-Methionine	71.9 $\pm$ 8
DL-Methionine + L-Ethionine	89.9 $\pm$ 6
DL-Methionine sulfone	76.5 $\pm$ 4
DL-Methionine sulfone + L-Ethionine	363.5 $\pm$ 11 <sup>a</sup>
L-Methionine-DL-sulfoximine	74.6 $\pm$ 5
L-Methionine-DL-sulfoximine + L-Ethionine	367.4 $\pm$ 15 <sup>a</sup>
ATP	77.1 $\pm$ 8
ATP + L-Ethionine	366.8 $\pm$ 20 <sup>a</sup>

Results are expressed as mean  $\pm$  standard error for 12 rats. <sup>a</sup> Differs from NaCl-group at  $p < 0.001$ .

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Table II. The effect of L-ethionine on cystathionine  $\beta$ -synthase in vitro

Rats treated with	Medium for incubation	No. of experiments	Enzyme activity	
NaCl (0.15 M)	Liver homogenates + H <sub>2</sub> O	8	74.3 $\pm$ 5	
	Liver homogenates + ethionine (1 mM)	8	73.8 $\pm$ 9	$p > 0.05$
Ethionine (400 mg/kg)	Liver homogenates + H <sub>2</sub> O	8	377.5 $\pm$ 16	
	Liver homogenates + ethionine (1 mM)	8	371.6 $\pm$ 19	$p > 0.05$

and DL-methionine-sulfone were administered by the same route in an amount equimolar to the quantity of ethionine. Control rats received an equal volume of 0.15 M NaCl. Sulfur-containing amino acids, as well as ATP, actinomycin D and cycloheximide, were given 15 min before ethionine. Animals were killed 3 h after ethionine injection. Determination of cystathionine  $\beta$ -synthase activity was made according to the method of STEPIEN and PIENIAZEK<sup>29</sup>. The quantity of protein was determined by the method of LOWRY et al.<sup>30</sup>.

**Results and discussion.** As can be seen in Table I, ethionine in a dose of 400 mg/kg of body weight causes an increase in cystathionine  $\beta$ -synthase activity in rat liver. Enzyme activity is approximately 5 times above the normal level 3 h after ethionine injection. There is no sex difference in this ethionine effect. Pretreatment with ATP, actinomycin D, cycloheximide, methionine sulfoximine or methionine sulfone do not change the ethionine effect significantly, but methionine does prevent the increase in enzyme activity. Given without ethionine, these substances do not modify the basal cystathionine synthase activity significantly.

It is well known from previous publications that treatment with ethionine causes a rapid depletion of hepatic ATP<sup>15-19</sup>. The data presented in this paper indicate that ATP administration is without effect on cystathionine synthase activity. The failure of ATP to affect the ethionine action seems to exclude the possibility that this effect is related to hepatic ATP deficiency induced by ethionine. The inhibitors of RNA and protein synthesis, actinomycin D and cycloheximide, do not prevent the ethionine action. These findings show that the increase in cystathionine synthase activity in rat liver caused by ethionine is not a result of de novo enzyme synthesis.

The effect of ethionine on the enzyme in vitro was examined also (Table II). Control rat liver tissue-homogenates were used in the study, as well as liver homogenates of rats which had received ethionine (400 mg/kg) 3 h before they were killed. To these homogenates ethionine was added to the final concentration of 1 mM. The incubation was carried out at 37°C for 30 min. No difference in the enzyme activity between control and ethionine-treated homogenates was found. Adding ethionine to the liver homogenates of rats which had received ethionine did not cause any additional increase in cystathionine synthase activity. These data suggest that ethionine influence on enzyme is not direct: it is possible to suppose that some ethionine metabolite causes this effect. Also the possibility that cystathionine synthase activity increase, caused by ethionine, was helped by some mediator is not exclude.

Cystathionine synthase has been reported to possess 2 additional catalytic activities: serine dehydratase (EC 4.2.1.13)<sup>31</sup> and threonine dehydratase (EC 4.2.1.16)<sup>32</sup>.

However, later it was demonstrated that purified cystathionine synthase is entirely different from serine dehydratase<sup>33</sup>. SAYRE et al.<sup>34</sup> studied the substrate induction of threonine dehydratase in vivo and in perfused livers. They found that ethionine blocks the induction of enzyme. Findings given in this paper support the conclusion that cystathionine synthase and threonine dehydratase activities are in relation with 2 different enzymes.

Since numerous studies have shown that the induction of many enzymes is blocked by ethionine<sup>35-40</sup>, a highly significant increase ( $p < 0.001$ ) cystathionine synthase activity following ethionine injection may be of interest for investigations of biochemical mechanisms of enzyme induction.

**Résumé.** Une seule injection d'éthionine (400 mg/kg) provoque une augmentation significative de l'activité de la cystathionine  $\beta$ -synthase du foie de rat. La méthionine empêche l'effet de l'éthionine, tandis que l'actinomycine D, le cycloheximide, la méthionine sulfoximine, la méthionine sulfone et l'ATP ne le modifient pas. In vitro, l'éthionine n'a aucune influence sur l'activité de la cystathionine synthase hépatique.

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