BIOSYNTHESIS OF CYSTATHIONINE FROM HOMOSERINE AND CYSTEINE BY RAT LIVER CYSTATHIONASE

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1. Introduction

Among the inborn errors of metabolism, those related to sulfur containing amino acids (homocystinuria, cystathioninuria) have been actively investigated during the last years and the results so far obtained have been simultaneously reviewed by Berlow [1] and Chatagner [2] in 1967.

As far as homocystinuria is concerned, Mudd et al. [3] showed that the enzymatic block is located at the level of cystathionine synthetase which is lacking or whose activity is very low in the liver of homocystinuric patients. Recently Wong et al. [4,5] observed a production of cystathionine from homoserine and cysteine by homogenates of rat liver, and of homocystinuric as well as normal human liver, and they suggested that this reaction is catalyzed by cystathionase, the level of which is, according to Mudd et al. [6] unaltered in homocystinuria. This observation was of interest from, at least, two points of view. Firstly this finding may contribute towards a treatment against homocystinuria: Wong et al. [4,5] suggested that supplementation of homoserine in the diet of homocystinuric patients may be beneficial; secondly it affords some insights in the possibility of a "double" active site in cystathionase, one for the binding of homoserine, and the other one for the binding of cysteine. It seems therefore of interest to ascertain whether the production of cystathionine from homoserine and cysteine was due to cystathionase and to investigate this reaction.

The present paper deals with the results obtained until now; a preliminary report of some of these findings has appeared [7].

2. Materials and methods

The enzymic preparations used in these experiments were: (a) supernatants obtained from rat liver homogenates prepared in 8.5% saccharose, by centrifugation at 40,000× *g* for 1 hr 30 min in the cold (HR_1 International Refrigerated Centrifuge) and dialysed, in the cold, for 4 hr against two changes of phosphate buffer 0.02 M pH = 7.8 containing EDTA (final concentration 0.0001 M). (b) highly purified cystathionase prepared from rat liver as already described [8] and which we refer to a "CM-enzyme"; in some cases, a further step [9] was included in the purification procedure: a run of the "CM-enzyme" through a column of DEAE-cellulose equilibrated with phosphate buffer 0.01 M pH 7.3, which removes a contaminant protein occasionally observed in "CM-enzyme". These samples were referred to as "DEAE-enzyme". The incubations were carried out in Warburg cells, under nitrogen atmosphere, at 37° for 20 min in phosphate buffer containing the enzymic preparation, pyridoxal phosphate (PLP; Koch and Light, Colnbrook, England) and the compounds tested: L-homoserine (Calbiochem, Los Angeles, USA), L-cysteine (Nutritional Biochemicals Corporation, Cleveland, USA), and, in some cases, adenosine 5′ monophosphate (5′ AMP; Boehringer, Mannheim, West Germany). The technical details are given in tables 1 and 2. The reaction was stopped by addition of ethyl alcohol (threefold the volume of the incubated mixture).

After 20 hr in the cold, the precipitate was discarded by centrifugation and the supernatant was dried under reduced pressure. The residue was dissolved

Table 1
Formation of L-cystathionine from L-homoserine and L-cysteine by supernatants from rat liver homogenates.

Supernatant	L-homoserine μmoles	L-cysteine μmoles	5'AMP μmoles	L-cystathionine μmoles
a	30	15	0	2.30
a	30	15	15	2.00
b	30	15	0	4.36
b	30	15	15	4.80
c	30	15	0	2.77
c	30	15	15	2.88

Each Warburg cell contains, for a final volume of 3 ml: 0.6 ml of phosphate buffer 0.2 M (containing EDTA 0.001 M) pH 7.8; 0.1 ml of a solution of pyridoxal phosphate (100 μg); 2 ml of supernatant (a,b,c were different preparations); 0.1 ml of a solution of L-homoserine; 0.1 ml of a solution of L-cysteine; 0.1 ml of a solution of AMP or 0.1 ml of water. Incubations were carried out for 20 min at 37°C under nitrogen.

in 2 ml of 0.1 N HCl. Analysis of the amino acids was performed on the automatic Technicon Auto Analyzer, according to the method of Hamilton [10]; norleucine was used as internal standard.

3. Results and discussion

In preliminary experiments we have investigated the production of cystathionine from homoserine and cysteine by dialyzed supernatants and we have observed that such a production occurred. The examination indeed of the amino acid profile obtained by the automatic Analyzer revealed a peak in the position for cystathionine, after that of methionine and before that of isoleucine, in the assays containing both homoserine and cysteine ("full" assays). This peak was absent in the assays containing homoserine alone, cysteine alone or the enzymic preparation alone ("control" assays). Addition of pure L-cystathionine (Calbiochem) to the amino acids solution obtained from "full" assays increased the peak of cystathionine, and addition of L-cystathionine to the amino acids solution obtained from "control" assays yielded a new peak located at the position identical with that occupied by the characteristic peak of the "full" assays. Furthermore, the amount of cystathionine thus produced changed with the enzymic preparation used and with the concentration of homoserine and of cysteine.

Table 1 shows the results obtained in experiments in which the incubations were carried out in presence of 30 μmoles of L-homoserine and 15 μmoles of L-cysteine. In these experiments also the effect of the inclusion of 5' AMP in the incubated mixture was investigated.

From these findings it is clear that supernatants from rat liver homogenates produced cystathionine when incubated in presence of homoserine and cysteine, and that 5' AMP was without effect on the amount of cystathionine formed. Thus our results were similar to those described by Wong et al. [4,5].

In another series of experiments purified cystathionine was used. Firstly we observed that these experiments yielded similar results to those obtained with supernatants, and secondly that identical results have been achieved using "CM-enzyme" or "DEAE-enzyme". In other words, purified cystathionase catalyzed the production of L-cystathionine from L-homoserine and L-cysteine.

Table 2 shows some of the results obtained with diluted samples of four separate (P1, P2, P3, P4) preparations of cystathionase. In each case the amount of cystathionase present in the diluted samples was calculated by means of the spectrophotometric method described by Matsuo and Greenberg [11] and is indicated in table 2.

From the results in table 2 it appears, firstly, that, under the same conditions, there is a relation between the amount of cystathionase and the amount of cystathionine produced, secondly that 5' AMP markedly enhances this production, whereas it did not

Table 2
Formation of L-cystathionine from L-homoserine and L-cysteine by purified rat liver cystathionase.

Enzyme (μg)	L-homoserine μmoles	L-cysteine μmoles	5′ AMP μmoles	L-cystathionine μmoles
100 (P_1)	15	15	0	0.08
100 (P_1)	15	15	15	0.19
200 (P_1)	15	15	0	0.13
200 (P_1)	15	15	15	0.33
200 (P_2)	15	15	0	0.14
200 (P_2)	15	15	15	0.29
? (P_3)	15	15	0	0.23
? (P_3)	15	15	15	0.39
450 (P_4)	15	15	0	0.36
450 (P_4)	15	15	15	0.73

P_1, P_2, P_3, P_4 were different preparations. Other details as described in table 1.

modify the formation of cystathionine by supernatants. Thus, although we have, at the moment, no explanation for this behaviour and although the significance of this finding is far from clear, 5′ AMP appears to be a mediator in modulation of cystathionase activity as far as the production of cystathionine is concerned.

In conclusion these results furnish conclusive evidence that cystathionase promotes formation of L-cystathionine from L-homoserine and L-cysteine, and they strongly support the earlier suggestion of Wong et al. [4,5].

A more detailed paper will be published elsewhere.

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