SUBCELLULAR LOCALIZATION OF CYSTATHIONINE SYNTHASE IN RAT BRAIN

S. KASHIWAMATA

Department of Biochemistry, Nagoya University School of Medicine, Tsuruma-cho, Showa-ku, Nagoya, Japan

Received 23 August 1971

1. Introduction

The presence of cystathionine synthase (L-serine hydro-lyase (adding homocysteine), EC 4.2.1.21 [sec 1]) in the brain tissue was first shown by Hope [2], who presented the direct evidence by isolating cystathionine after incubating DL-homocysteine and L-serine in the rat brain homogenate. Despite extensive study on the relationship between cystathionine or cystathionine synthase and brain function [3–5], little has been attempted as to biochemical characterization of brain cystathionine synthase, unlike the liver enzyme [6–9]. The report from this laboratory [10] showed that in the rat brain the developmental pattern of cystathionine synthase activity was considerably different from that in the liver, and also suggested a possible attachment of approximately half the enzyme activity to the subcellular particles in the brain tissue. The present communication reports on the localization of cystathionine synthase activity of rat brain, both in the mitochondrial and the soluble fractions, and also on the attempts to solubilize the mitochondrial enzyme.

2. Materials and methods

The brain tissue rostral to the inferior colliculi was used throughout the experiments. Subcellular fractions were prepared at 0–4° from the brain homogenate of rats (250–350 g in body weight) as described by Eichberg et al. [11]. The identity of the fractions was confirmed by electron-microscopic examination carried out by Dr. J. Asai.

Cystathionine synthase activity was assayed as described previously [6, 10] with 60 min incubation as a routine. Cupric sulfate was introduced into the incubation mixtures, as was the case also in crude liver preparations [12]. Succinate dehydrogenase was used as a mitochondrial marker [13] and was assayed as succinate-tetrazolium reductase [14]. A unit was defined as the amount that yielded one μmole of cystathionine or formazan per min. Protein was determined by a biuret method [15, 16] with bovine serum albumin as a standard.

3. Results and discussion

Volpe et al. [4], on the basis of the studies on the regional distribution of cystathionine and cystathionine synthase in monkey brain, suggested that cystathionine might be synthesized in the perikaryon of neurons and then migrate along the axons to accumulate in areas of white matter. In this laboratory it was found that in rat brain tissue approximately half the enzymic activity of cystathionine synthase was associated with particulate fraction [10]. Table 1 confirms this observation. When the brain homogenate was fractionated into four fractions, i.e., nuclear (P_1), mitochondrial (P_2), microsomal (P_3) and soluble (S) fractions, the activity of cystathionine synthase was distributed mainly in two fractions. One was P_2 fraction (37%) and the other S fraction (43%). The activity of succinate dehydrogenase, a mitochondrial marker, was exclusively found in P_2 fraction (92%). When the crude mitochondrial fraction (P_2) was then subfractionated into myelin (P_2A), synaptosomal (P_2B) and mitochondrial (P_2C) fractions in a discontinuous sucrose density gradient, more than 80% of the activity of cystathionine synthase was detected in the P_2C fraction (table 2). The pattern of the distribution was nearly similar to

Table 1
Subcellular localization of cystathionine synthase and succinate dehydrogenase in rat brain I. Fractionation of homogenate.

Fraction	P_1		P_2		P_3		S	
Conditions	Precipitate at 1100 *g* 10 min		Precipitate at 17,500 *g* 60 min		Precipiatte at 105,000 *g* 60 min		Supernatant at 105,000 *g* 60 min	
	(%)	(RSA)	(%)	(RSA)	(%)	(RSA)	(%)	(RSA
Protein	11		64		4		21	
CTS	17	1.5	37	0.6	3	0.8	43	2.0
SDH	7	0.6	92	1.4	1	0.3	0	0.0

Recoveries for the fractions from homogenate were 91% for protein, 111% for cystathionine synthase (CTS) and 86% for succinate dehydrogenase (SDH). The results were means of four separate experiments and expressed as percentages of the sum of the fractions. RSA (relative specific activity) was a ratio of (% of recovered activity)/(% of recovered protein).

that of succinate dehydrogenase activity, approximately 75% of which was found in P_2C fraction. Mitochondria thus separated came from nerve endings, neuronal perikarya and glia cells. The present findings can not show in which mitochondria the activity of cystathionine synthase mainly localizes. However, this enzyme is reported more abundant in grey matter than in white matter in primate brain [4]. It is, therefore, possible to consider that the high concentration of the enzyme in the P_2C fraction might be contributed by neuronal perikarya and/or synaptic endings, not by glia cells.

Table 2
Subcellular localization of cystathionine synthase and succinate dehydrogenase in rat brain II. Fractionation of crude mitochondrial fraction (P_2).

Fraction	P_2A		P_2B		P_2C	
Conditions	Subfractions of P_2 in a gradient 53,000 *g*, 2 hr					
	(%)	(RSA)	(%)	(RSA)	(%)	(RSA)
Protein	21		45		34	
CTS	9	0.4	8	0.2	83	2.4
SDH	3	0.1	22	0.5	75	2.2

Recoveries for the fractions from crude mitochondrial fraction were 71% for protein, 74% for cystathionine synthase (CTS) and 57% for succinate dehydrogenase (SDH). The results were means of four separate experiments and expressed in the same manner as in table 1.

Table 3 shows the effects of various treatments on release of cystathionine synthase in a crude mitochondrial fraction (P_2). A freshly prepared P_1-free supernatant was divided equally into 5 portions and centrifuged at 17,500 *g* for 60 min. The P_2 thus obtained was treated as shown in the table. The percent distribution of the enzymic activity was approximately 80 and 20 in 105,000 *g* supernatant and precipitate, respectively, with treatments of Triton X-100 and sonic oscillation. Interestingly the activities in the supernatants increased approximately twofold, as compared with non-treated P_2. High specific activities of the enzyme were also obtained in the supernatants (7.1×10^{-3} for Triton X-100 and 10.3×10^{-3} for sonic oscillation). Since both Triton X-100 and sonic oscillation treatments disrupt mitochondrial membranes, it is possible to assume that the enzyme localizes in the mitochondrial matrix in a loosely bound form. This assumption may be supported by the finding that the osmotic shock at a final sucrose concentration of 0.064 M, which was reported to reduce the solubility of the enzyme contained in the matrix of mitochondria [17] and to rupture the synaptic endings without irreversible damage of mitochondria [18], resulted in some release of cystathionine synthase with relatively high specific activity (4.4×10^{-3}). This may be due to mechanical disruption of mitochondria during homogenization. The osmotic shock at a sucrose concentration of 0.032 M caused more release of the enzyme from mitochondria than that at 0.064 M, and gave a higher specific activity (8.5 ×

Table 3
Effects of various treatments on cystathionine synthase activity of crude mitochondrial fraction.

Treatment	105,000 *g* supernatant		105,000 *g* precipitate			
	Specific activity (units/mg protein × 1000)	Activity (units × 1000)	Specific activity (units/mg protein × 1000)	Activity (units × 1000)	Sum of activity (units × 1000)	Recovery of activity (%)
None	–	–	2.1	132	132	100
% Distribution				100		
Triton X-100	7.1	253	2.3	63	316	239
% Distribution		80.1		19.9		
Sonic oscillation	10.3	305	2.6	87	392	297
% Distribution		77.9		22.1		
0.064 M sucrose	4.4	48	2.5	131	179	136
% Distribution		26.6		73.4		
0.032 M sucrose	8.5	101	1.6	82	183	139
% Distribution		55.4		44.6		

Crude mitochondrial fractions were treated at a protein concentration of 13 mg per ml by 0.2% Triton X-100 in 0.32 M sucrose, sonic oscillation (20 Kc for 7 min in 0.32 M sucrose), 0.064 M sucrose and 0.032 M sucrose, and centrifuged at 105,000 *g* for 60 min. Each precipitate was resuspended in 0.32 M sucrose with brief homogenization.

10^{-3}) in the same degree as Triton X-100 and sonic oscillation treatments. As was the case of aspartate aminotransferase in rat brain [18], the observation that the recoveries of cystathionine synthase after various treatments were always in excess of 100% (table 3) may show the possibility that the enzyme in the mitochondrial fraction is an "occluded" or "latent" enzyme and protected from the access of the exogenous substrates, homocysteine and/or serine. At the present stage it is not clear whether the mitochondrial and the soluble enzyme are the same, or are isozymes.

Acknowledgements

I am extremely grateful to Dr. J. Asai, Department of Pathology, Nagoya University School of Medicine, for carrying out the electron-microscopic studies on brain subcellular fractions.

References

[1] H.H. Tallan, T.A. Pascal, K. Schneidman, B.M. Gillam and G.E. Gaul, Biochem. Biophys. Res. Commun. 43 (1971) 303.
[2] D.B. Hope, Federal Proc. 18 (1959) 249.
[3] M.K. Gaitonde, in: Handbook of Neurochemistry, III, ed. A. Lajtha (Plenum Press, New York, 1970) p. 225.
[4] J.J. Volpe and L. Laster, J. Neurochem. 17 (1970) 425.
[5] T.L. Perry, K. Berry, S. Hansen, S. Diamond and C. Mok, J. Neurochem. 18 (1971) 513.
[6] S. Kashiwamata and D.M. Greenberg, Biochim. Biophys. Acta 212 (1970) 488.
[7] H. Kimura and H. Nakagawa, J. Biochem. (Tokyo) 69 (1971) 711.
[8] F.C. Brown and P.H. Gordon, Can. J. Biochem. 49 (1971) 484.
[9] F. Pan, S.F. Chin and S.H. Pai, Endocrinol. 88 (1971) 723.
[10] S. Kashiwamata, Brain Res. 30 (1971) 185.
[11] J. Eichberg, Jr., V.P. Whittaker and R.M.C. Dawson, Biochem. J. 92 (1964) 91.
[12] S. Kashiwamata, Anal. Biochem. 42 (1971) 293.
[13] V.P. Whittaker, Biochem. J. 72 (1959) 694.
[14] R.J. Pennington, Biochem. J. 80 (1961) 649.
[15] A.G. Gornal, C.J. Bardawill and M.M. David, J. Biol. Chem. 177 (1949) 751.
[16] K.W. Cleland and E.C. Slater, Biochem. J. 53 (1953) 547.
[17] D.S. Bendall and C. De Duve, Biochem. J. 74 (1960) 444.
[18] L. Salganicoff and E. De Robertis, J. Neurochem. 12 (1965) 287.