S-ADENOSYLHOMOCYSTEINE HYDROLASE IN HUMAN AND RAT LIVER IS LOCALIZED TO THE CYTOSOL FRACTION OF THE TISSUE HOMOGENATE

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1. Introduction

S-Adenosylhomocysteine (SAH) is formed from *S*-adenosylmethionine (SAM) upon transmethylation using SAM as a methyl donor [1]. SAH is a potent inhibitor to a wide group of methyl-transfer reactions and has been suggested to be a regulator of biological methylations [2–7]. The tissue level of SAH is regulated by the enzyme S-adenosylhomocysteinase (EC 3.3.1.1). This enzyme catalyzes the reversible synthesis of SAH from adenosine and L-homocysteine and the equilibrium of the reaction is far in the direction of condensation [8]. The metabolic flow has been suggested to be in the hydrolytic direction [9] and the enzyme catalyzes the first step in the degradation of SAH.

No study has been devoted to the subcellular localization of *S*-adenosylhomocysteinase. Data have been presented [10] suggesting that the post-microsomal supernatant fraction contains almost all of the *S*-adenosylhomocysteine synthase activity, but a substantial amount was associated with the 'nuclear' fraction. This finding seems interesting in the light of the finding that SAH is a potent inhibitor of DNA methylation in isolated rat liver nuclei [11]. However, in this report data are presented showing that *S*-adenosylhomocysteine hydrolase activity is localized exclusively to the soluble fraction of tissue homogenates from human and rat liver.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid; PMS, phenazine methosulfate; SAH, *S*-adenosyl-L-homocysteine; SAM, *S*-adenosyl-L-methionine

2. Experimental

2.1. *Materials*

Sources of most reagents used are in [12,13]. [8-^{14}C]Adenosyl-L-homocysteine (0.59 mCi/mmol) was synthesized enzymatically from [8-^{14}C]adenosine and D,L-homocysteine using homogenous *S*-adenosylhomocysteinase from mouse liver as enzyme [14]. Details on the synthesis and purification of *S*-[8-^{14}C]-adenosylhomocysteine are in [15]. The radioactive material (>99%) was identified as SAH in three chromatographic systems [12].

2.2. *Preparation of subcellular fractions*

The subcellular fractions from human liver (obtained from biopsy and which appeared normal upon histological examination) and rat liver were prepared essentially as in [16] with the modifications in [17].

2.3. *Assay for enzyme activities*

S-Adenosylhomocysteine hydrolase activity was assayed by incubating samples from the subcellular fractions with *S*-[8-^{14}C]adenosylhomocysteine (50 μM) at 37°C for increasing times. The incubation buffer was 15 mM Hepes (pH 7.0) containing 0.25% bovine serum albumin, 150 mM KCl, 5 mM Mg-acetate, 2 mM 2-mercaptoethanol and adenosine deaminase (50 units/ml). The incubations were terminated and the reaction products separated by thin-layer chromatography and determined as in [12].

Malate dehydrogenase (EC 1.1.1.31) [18], rotenone-insensitive NADPH-cytochrome *c* reductase (EC 1.6.2.4) [19], acid phosphatase (EC 3.1.3.2)

[20], succinate–PMS-oxidoreductase (EC 1.3.99.1) [21,22] and lactate dehydrogenase (EC 1.1.1.27) [23] were assayed as described.

2.4. *Determination of protein*

Protein was measured using a Folin-Ciocalteu reagent [24].

3. Results and discussion

The *S*-adenosylhomocysteinase activity here was determined in the hydrolytic direction. In this way the measurement of the enzyme activity is not obscured by the presence of other enzymes competing for the same substrate as hydrolysis of SAH to adenosine and l-homocysteine catalyzed by the enzyme *S*-adenosylhomocysteinase is the only known pathway for the degradation of SAH in crude extracts from rat liver [9]. In contrast, measurement of *S*-adenosylhomocysteine synthase activity in crude extract is hampered by the simultaneous deamination of adenosine to inosine catalyzed by the enzyme adenosine deaminase [25].

There was linearity of the *S*-adenosylhomocysteine hydrolase activity with respect to time for $\geqslant 5$ min under conditions of the experiment (data not shown) and the enzyme activities listed in table 1 are determined from the linear parts of the curves.

Table 1 shows the distribution of *S*-adenosylhomocysteine hydrolase compared with marker enzymes in fractions prepared from homogenates of human and rat liver. The recovery of enzymes and protein from both tissues was 90–103%. Mutual contamination of

Table 1
Distribution of *S*-adenosyl-L-homocysteine hydrolase and some marker enzymes in the homogenate of human (panel A) and rat (panel B) liver

		Percentage values					
	Absolute values	Nuclear fraction	Mitochondrial fraction	Light mitochondrial fraction	Microsomal fraction	Particle free supernatant	Recovery (%)
A *S*-Adenosylhomocysteine hydrolase	0.037	2.5	0.6	0.6	0.5	91.9	96.1
Succinate-PMS-oxidoreductase	0.72	14.0	56.1	23.2	4.3	1.2	98.8
Acid phosphatase	2.8	8.7	13.1	25.2	14.2	34	95.2
Malate dehydrogenase	23.1	6.8	14.0	9.3	1.1	58.4	89.6
NADPH-cytochrome *c* reductase	0.110	3.2	4.4	7.4	41.9	44.2	101.1
Lactate dehydrogenase	82.99	3.8	2.3	2.1	3.5	79.8	91.5
Protein	91.0	10.7	12.1	7.2	16.4	55.8	102.2
B *S*-Adenosylhomocysteine hydrolase	3.95 (3.81)	3.9 (4.1)	0.07 (0.08)	0.1 (0.2)	1.1 (1.5)	86.2 (85.0)	91.4 (90.9)
Succinate-PMS-oxidoreductase	60.5	19.2	73.2	8.9	1.2	0.5	103.0
Acid phosphatase	7.8	13.0	10.7	28.2	13.0	30.7	95.6
Malate hydrogenase	132.4	13.1	16.1	6.5	18.9	47.9	102.5
NADPH-cytochrome *c* reductase	0.54	6.0	1.8	4.4	56.5	26.7	95.4
Lactate dehydrogenase	952	11.8	0.6	0.8	3.3	77.2	93.7
Protein	2067	26.3	10.2	9.1	10.9	46.2	102.7

The absolute values for enzyme activity in total homogenate are expressed in μmol.min^{-1}. Protein is given in mg. The enzyme activities and the protein content in the fractions are expressed as % of the total value in whole homogenate (i.e., cytoplasmic extract + nuclear fraction). The values given in parentheses (panel B) represent the *S*-adenosylhomocysteine hydrolase activity measured in the rat liver fraction in the presence of 0.05% Triton X-100

the various fractions was moderate. The marker enzyme for the mitochondrial fraction, succinate–PMS-oxidoreductase [21,22], lysosomes, acid phosphatase [26], microsomes, NADPH-cytochrome *c* reductase [26] and soluble fraction, lactate dehydrogenase [26] were distributed as expected. Malate dehydrogenase was localized both to the mitochondrial and soluble fraction [26,27].

From the data presented it is evident that *S*-adenosylhomocysteine hydrolase is localized exclusively to the soluble fraction of both human (table 1A) and rat liver (table 1B). The small amount of enzyme activity associated with the nuclear fraction could be explained by contamination from the cytosol fraction. This statement is based on the observation that the nuclear fractions from human and rat liver are contaminated to the same or even higher degree with lactate dehydrogenase than *S*-adenosylhomocysteine hydrolase. The former enzyme is localized mainly to the soluble fraction [26]. No *S*-adenosylhomocysteine hydrolase activity could be solubilized from the tissue particles from rat liver homogenate by including 0.05% Triton X-100 in the incubation mixture (table 1B).

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