

Thiol Methyltransferase

Thiol Methyltransferase Activity in Regenerating Liver after Partial Hepatectomy in Rats

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Abstract : Changes of thiol methyltransferase (TMT) activity, and its K_m and V_{max} values in cytosolic, mitochondrial and microsomal fractions in regenerating rat livers were determined over a period of ten days after 70% partial (median and left lateral lobes) hepatectomy. The enzyme activity in serum was also measured. Mitochondrial and microsomal TMT activities after partial hepatectomy were found to be significantly increased between the first and the second day, and between the twelfth hour and the third day of regeneration, respectively, whereas the cytosolic TMT activity increased at the second day after partial hepatectomy. On the other hand, the serum TMT activity did not change. The V_{max} values of the cytosolic, mitochondrial and microsomal TMTs were significantly increased at the second day after partial hepatectomy. However, the K_m values of the above hepatic enzymes did not vary in all the experimental groups. Therefore, the above results indicate that the biosynthesis of TMT was increased during regeneration of rat liver.

Key Words : Partial hepatectomy, Regenerating rat liver, Thiol methyltransferase

Thiol methyltransferase(S-adenosyl-L-methionine: thiol S-methyltransferase, EC 2.1.1.9) 7 -thiospirolactone, D-L-penicillamine[1], captopril, N-acetyl -cysteine, spironolactone[1], hydrogen sulfide[2], 2-thiouracil, 6-propyl-2-thiouracil, diethyldithiocarbamate, 2,3-dimercaptopropanol[3], mercaptoethanol, mercaptoacetic acid, methylmercaptan[3,4], dithiothreitol[4], 6-mercaptapurine[5], dimercaprol[6], thiourea, methimazole, thiamin tetrahydrofuryl disulfide[7] sulfhydryl S-adenosyl-L-methionine methyl

2 (phase xenobiotic biotransformation) [2,8]

[7,8]. 가 , [7].

[9]

[10],

가 가

가 [11-19] . Thiol methyltransferase(TMT)

가

TMT

12

10

Km Vmax

2

1.

-Chlorothiopenol, ethylenediaminetetraacetic acid disodium dihydrate, Triton X-100, potassium phosphate monobasic, potassium phosphate dibasic (10 g/100 mL bovine serum albumin) Sigma () [methyl-³H] S-adenosyl-L-methionine New England Nuclear () , PPO(2,5 -diphenyloxazole), bis-MSB{ -bis-(O -methylstyryl benzene) } ; toluene(scintillation grade) Packard ()

2.

4

320 350 g Sprague-Dawley

5

12

1) 가 : 가 12 , 1 , 2 , 3 , 6 10 (6)

2) : 12 , 1 , 2 , 3 , 6 10

(6)

가
 , 12
 2 cm
 %가 (original liver)
 가
 3.
 12
 4 0.25 M sucrose
 sucrose 가 4 0.25 M
 sucrose 가
 2 4
 5 g 9
 0.25 M sucrose , Teflon pestle
 glass homogenizer(chamber clearance
 0.005 0.007 inches, Thomas ,) 2
 4 400 rpm 5
 10% (w/v)

sucrose
 density gradient [20]
 2 4
 Du Pont Sorvall
 () RC-5B refrigerated superspeed
 centrifuge OTD-65B ultracentrifuge
 rotor Du Pont Sorvall SS-
 34 T865 rotor , sucrose linear
 density gradient gradient
 former(model 570, ISCO,)
 4. TMT
 70
 5 mg/mL
 가 0.25 M sucrose
 1% Triton X-100 2 4
 30
 mg/mL가 0.25 M sucrose 5
 5.
 TMT
 -chlorothiophenol [methyl
 -³H] S-adenosyl-L-methionine
 37 10
 methyl 4-chlorophenyl sulfide
 toluene
 Weisiger Jakoby
 [21] 1
 1 mL 1 mg
 methyl 4-chlorophenyl sulfide
 pmol
 2

liquid 8.
scintillation spectrometer (Tricarb 4530, Packard
,) .
Student's t-test
0.05 .
6. Km Vmax
2
TMT 1. TMT
1/vi 1/[S]
(double reciprocal plot)
Km Vmax TMT
2 가
(P 0.01) TMT
7. 1 2 ,
TMT 12 , 1 , 2
acid methanol-ether (3 : 1) 가 (Table 1).
Greenberg Rothstein [22] TMT (Table 2).
biuret .

Table 1. Activities of cytosolic, mitochondrial and microsomal thiol methyltransferase in regenerating rat liver after partial hepatectomy

Post-hepatectomy days	Thiol methyltransferase activity (pmol methyl 4-chlorophenyl sulfide min ⁻¹ mg protein ⁻¹)					
	Cytosol		Mitochondria		Microsome	
	Original liver	Regenerating liver	Original liver	Regenerating liver	Original liver	Regenerating liver
0.5	6.33 ± 0.63	6.68 ± 0.57	5.09 ± 0.68	6.04 ± 0.74	5.02 ± 0.52	6.04 ± 0.73 ^a
1	6.37 ± 0.57	6.89 ± 0.86	5.15 ± 0.64	6.84 ± 0.96 ^a	4.95 ± 0.57	8.36 ± 0.89 ^c
2	6.32 ± 0.49	8.47 ± 1.12 ^b	5.13 ± 0.59	6.78 ± 0.62 ^b	4.90 ± 0.54	8.15 ± 1.04 ^c
3	6.36 ± 0.55	6.70 ± 0.61	5.17 ± 0.62	5.63 ± 0.71	4.93 ± 0.67	6.53 ± 0.73 ^b
6	6.34 ± 0.53	6.42 ± 0.64	5.11 ± 0.72	5.31 ± 0.75	4.86 ± 0.58	5.67 ± 0.66
10	6.40 ± 0.51	6.48 ± 0.67	5.15 ± 0.74	5.18 ± 0.58	4.89 ± 0.63	4.81 ± 0.58

The data are expressed as mean ± SD with 5 rats in each group. Significant difference from original livers (a, P<0.05; b, P<0.01; c, P<0.001).

Table 2. Activity of serum thiol methyltransferase after partial hepatectomy in rats

Post-hepatectomy days	Thiol methyltransferase activity (pmol methyl 4-chlorophenyl sulfide min ⁻¹ mL ⁻¹)	
	Sham	Hepatectomy
0.5	16.10 ± 0.75	16.39 ± 1.21
1	16.16 ± 0.92	16.78 ± 1.32
2	16.13 ± 0.86	16.87 ± 1.37
3	16.07 ± 0.94	16.36 ± 1.46
6	16.09 ± 0.98	16.40 ± 0.92
10	16.11 ± 1.04	15.83 ± 0.86

The data are expressed as mean ± SD with 5 rats in each group; Sham: Sham operation, Hepatectomy: 70% hepatectomized rats.

Table 3. Thiol methyltransferase kinetic parameters from regenerating rat livers

Cell fractions	Km (mM)		Vmax (pmol methyl 4-chlorothiophenol sulfide min ⁻¹ mg protein ⁻¹)	
	Original liver	Regenerating liver	Original liver	Regenerating liver
Cytosol	64.3 ± 7.3	62.7 ± 6.5	17.9 ± 1.9	22.8 ± 2.7 ^b
Mitochondria	76.4 ± 4.5	71.6 ± 5.9	14.7 ± 1.5	20.6 ± 2.4 ^b
Microsome	72.1 ± 6.7	69.8 ± 7.2	13.6 ± 1.2	23.1 ± 3.1 ^c

Michaelis-Menten constants for thiol methyltransferase were determined using 4-chlorothiophenol and [(methyl-³H) S-adenosyl-L-methionine at 37°C for cytosolic, mitochondrial and microsomal fractions of original, and regenerating rat livers at 2nd day after partial hepatectomy. The data are expressed as mean ± SD with 5 rats in each group. Significant difference from original liver (b, P<0.01; c, P<0.001).

2. Km Vmax TMT
2 TMT Km Vmax [10].
가 가 .
(Table 3). 가

가 가
 monoamine oxidase[16], alcohol
 dehydrogenase, aldehyde dehydrogenase,
 microsomal ethanol oxidizing system[13],
 aryl sulfotransferase[18] arylamine N-
 methyltransferase[19] 가
 glutathione
 S-transferase, glutathione peroxidase[11],
 xanthine oxidase, superoxide dismutase[12],
 rhodanese[14], arylesterase, carboxylesterase
 [15] cholinesterase[17]

TMT 가
 TMT
 Km Vmax
 TMT
 2, 1 2
 가 가 가
 12 3 가 가
 2
 TMT Km Vmax Km
 가 Vmax 가 가
 Km 가 Vmax 가 가
 가 가 가
 가 가 가
 가 가

가
 TMT 가
 TMT
 Km Vmax
 TMT
 2, 1 2
 12 3 가
 2
 TMT Km Vmax 가
 TMT 가

가 [10]
 [23,24]
 가 [11,16]
 TMT
 가 가
 가가

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