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3-mercaptopyruvate sulfurtransferase and rhodanese activities in human myometrium and leiomyomas of the uterus

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Background. 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) and rhodanese (thiosulfate: cyanide sulfurtransferase, EC 2.8.1.1) participate in L-cysteine desulfuration – the main source of metabolically active sulfane sulfur atoms, which possibly influence the proliferation of malignant cells. It has been demonstrated that 3-mercaptopyruvate sulfurtransferase and rhodanese activity can decrease in some transplanted neoplasms.
Aim. To examine tumour tissue of the human uterus and to investigate the myometrium from which the growth developed.
Methods. The activity of 3-mercaptopyruvate sulfurtransferase and rhodanese was assayed in myometrium and leiomyoma samples immediately after hysterectomies.
Results. The activities of two sulfurtransferases are higher in the leiomyoma than in the myometrium.
Conclusion. An increase in the activity of both sulfurtransferases in leiomyomas of the uterus is surprising in light of present day understanding of sulfur compound metabolism in neoplasms, and may be due to the fact that leiomyoma is a benign neoplasm.

Aktywność transferazy siarkowej 3-merkaptopirogronianu i rodanazy w mięśniu i mięśniaku macicy ludzkiej

Transferaza siarkowa 3-merkaptopirogronianu (EC 2.8.1.2) i rodanaza (transferaza siarkowa tiosiarczan: cyjanek, EC 2.8.1.1) uczestniczą w procesie desulfuracji L-cysteiny, prowadzącym do tworzenia związków zawierających metabolicznie aktywną zredukowaną siarkę, tak zwaną siarkę sulfanową, mającą wpływ na proliferację komórek nowotworowych. W przypadku niektórych tkanek nowotworowych obserwowano znacznie obniżoną aktywność transferazy siarkowej 3-merkaptopirogronianu i rodanazy, jak również śladową ilość siarki sulfanowej. Podjęto badania porównawcze aktywności obydwu enzymów w tkance mięśniaka ludzkiego oraz mięśnia macicy, z którego się rozwinął. Oznaczenia wykonywano natychmiast po otrzymaniu tkanek, uzyskanych w trakcie zabiegu chirurgicznego usuwania nowotworu. Niespodziewanie stwierdzono, że aktywność obydwu badanych enzymów jest wyższa w mięśniaku, w porównania do tkanki niezmienionej nowotworowo, co można tłumaczyć tym, że jest to nowotwór łagodny.

Key words: 3-mercaptopyruvate sulfurtransferase; rhodanese; leiomyoma; myometrium
Słowa kluczowe: transferaza siarkowa 3-merkaptopirogronianu; rodanaza; mięśniak macicy

Introduction

Many animal tissues are able to convert 3-mercaptopyruvate, a product of L-cysteine transamination, into pyruvate (Figure 1). The enzyme involved in this process is known as 3-mercaptopyruvate sulfurtransferase (3-MPST, EC 2.8.1.2). It is different from rhodanese (thiosulfate: cyanide sulfurtransferase, EC 2.8.1.1), which exhibits particular affinity towards certain sulfur donors of either inorganic (e. g. thiosulfate) or organic (e. g., polysulfides, such as thiocystine, persulfides, e.g. thiocysteine) origin and effects transfer of a sulfur atom to various nucleophilic acceptors via an enzyme-sulfane "transition state" [1]. Thus, it participates in cyanide detoxification [2], iron-sulfur (FeS) clusters formation [3] or enzymatic activity regulation through a mechanism that involves the incorporation of sulfur [4] (Figure 1). In rats the highest activity of 3-MPST was found in the liver and kidney [5]. 3--MPST is present in the mitochondria and cytosol, as opposed to rhodanese, which, in mammals is found only in the mitochondria [6]. The activity of 3-MPST in man has only been examined in red blood cells, whereas rhodanese has been examined in other tissues [7, 8]. Acc. to Jarabak and Westley catalysis by this enzyme involves a single-displacement mechanism: a sulfur atom from 3-

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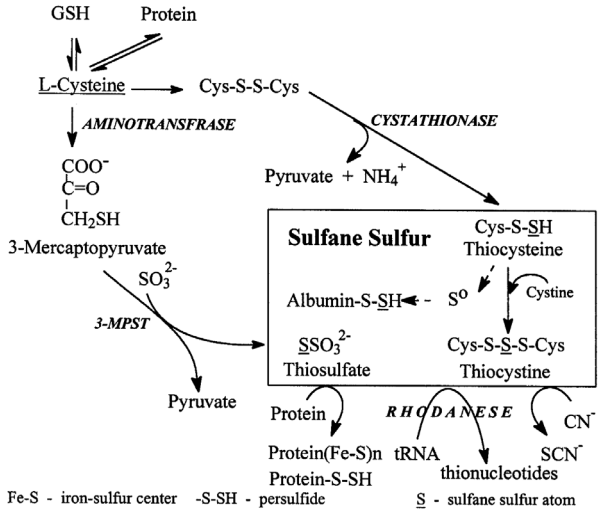

Figure 1. L-Cysteine desulfuration
Fe-S – iron-sulfur center -S-SH – persulfide S – sulfane sulfur atom

-mercaptopyruvate is transferred to a suitable acceptor (e.g. sulfite, cyanide) or to a second molecule of 3-mercaptopyruvate in case a different acceptor is not present [9]. From the results of Finazzi – Agro et al. it has been concluded that one of the possible biological functions of both these sulfurtransferases is to participate in the formation of active iron-sulfur proteins, which in turn take part in electron transfer [10]. It has been demonstrated that the activity of 3-MPST and rhodanese can decrease in some transplanted neoplasms [5, 7]. Therefore it seemed logical to examine tumour tissue of the human uterus and to investigate the myometrium, from which the tumour developed.

Materials and methods

Myometrium and leiomyoma samples were obtained immediately after hysterectomies from myoma patients from the Department of Gynecology of the Jagiellonian University in Cracow. The samples were transported on ice to the laboratory, where the enzyme activities were examined. The age of the patients varied between 32 and 50 years. All patients were premenopausal. Recognition of myoma was confirmed by histopathological examination. Myometrium and leiomyoma samples were homogenized with 0.1 M phosphate buffer, pH 7.45, using 10 ml of cold buffer per 1 g of tissue, by a warring blender and centrifuged at 650 x g for 5 min.

3-MPST activity was assayed according to Kun and Fanshier using the modification of Kasperczyk, Koj and Wasylewski [11]. Enzyme activity was expressed in μmoles of pyruvate produced during 15 min at 37°C and calculated per 1 mg of protein (arbitrary units).

The activity of rhodanese was assayed accordingly to Sörbo [12]; results were expressed in μmoles of SCN^- formed during 5 min at 20°C and calculated per 1 mg of protein.

Protein concentration was determined by the method of Lowry, Rosebrough, Farr and Randal [13], using serum bovine albumin as the standard.

Mann-Whitney's test was applied to confirm statistical significance.

Results

The activity of 3-mercaptopyruvate sulfurtransferase in human myometrium homogenate amounted to 1.4 units; range: 0.078 – 1.9 6 units. The activities of 3-MPST significantly increased in leiomyoma samples to 2.26 units; range: 1.16 – 3.09 units (Table I).

Rhodanese activity in the myometrium was small and amounted to 0.037 units; range: 0.024 – 0.056 units. The activity of this enzyme increased in samples of leiomyoma, where it amounted to 0.076 units; range: 0.035 – 0.162 (Table I).

3-Mercaptopyruvate sulfurtransferase and rhodanese activities were 61 % and 105 % higher, respectively, in leiomyoma samples versus the amount found in myometrium samples.

The difference in activities of both enzymes in leiomyoma, as compared to the myometrium, was statistically significant ($p<0.05$).

Discussion

Few studies have reported decreases in enzyme activity in sulfur-compound metabolism, particularly that of cystathionase, rhodanese and 3-MPST in neoplasm cells [14--16]. Many aspects of sulfur metabolism remain unclear, including details of the incorporation of sulfur into modified bases in RNA. Thiopyrimidines and methylthiopurines are normal constituents of tRNA in all organisms, and are thought to have important regulatory functions in translation. Enzyme preparation from rat liver and several other tissues transfer the sulfur from 3-mercaptopyruvate to tRNA and, as described by Wong, Harris and Morris [17], in fast growing Morris hepatoma cells the tRNA sulfurtransferase activity is decreased. Recently Palenchar, Buck, Cheng et al. [18] have reported the sequence similarity of an enzyme from E. coli that plays a role in the biosynthesis of 4-thiouridine in bacterial tRNA to rhodanese. However 3-MPST and rhodanese activities in Morris hepatoma 51230 and 7777 are only slightly decreased in relation to hepatic tissue [5].

Table I. 3-Mercaptopyruvate sulfurtransferase (3-MPST) and rhodanese activity in myometrium and leiomyoma samples

Tissue	Number of cases	3-MPST μmoles of pyruvate /15 min · mg	Rhodanese μmoles of SCN^- / 5 min · mg
Myometrium	15	1.40 ±0.037	0.037 ±0.009
Leiomyoma	14	*2.26 ±0.067	*0.076 ±0.039

*$p<0.05$

A decrease in 3-mercaptopyruvate sulfurtransferase activity can lead to an accumulation of 3-mercaptopyruvate in cells, which, as already demonstrated, can in turn lead to polyploidy [19]. The process of L-cysteine desulfuration is the main source of metabolically active sulfane sulfur (Figure 1). The biological role of reduced sulfur, such as sulfane sulfur, is not completely understood, although according to Toohey (1989) malignant cell proliferation may be related to a deficiency of sulfane sulfur and the uncontrolled operation of a set of enzymes normally inactivated by sulfane sulfur.

Demonstrating an increase in the activity of 3-MPST in leiomyomas of the uterus is surprising, in light of the present understanding of the metabolism of sulfur compounds within the neoplasm. The difference in activities of 3-MPST and rhodanese in leiomyomas and fast growing transplanted neoplasms, may be caused by the fact that leiomyoma is a benign neoplasm. Examining enzyme activity in sarcomas, a malignant neoplasm, should prove to be interesting. On examining one case of this neoplasm, the authors found trace activity of both enzymes.

The works of Maggivilli, Gustafson, Rector and Hilf [20] on enzyme activity in carbohydrate metabolism is interesting. Myometrium and leiomyoma samples were examined and no substantial difference in activity was found. Therefore no generalization of enzyme activity in leiomyomas of the uterus can be made.

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References

1. Cooper AJ. Biochemistry of sulfur-containing amino acids. *Annu Rev Biochem* 1983; 52: 187-222.
2. Sörbo B. Thiosulfate sulfurtransferase and mercaptopyruvate sulfurtransferase. In: Greenberg DM (ed.) *Metabolic Pathways*. New York: Academic Press; 1975; 7: 433- 437.
3. Bonomi F, Pagani S, Cerletti P. Insertion of sulfide into ferredoxins catalyzed by rhodanese. *FEBS Letters* 1977; 84: 149-152.
4. Toohey JL. Sulphane sulphur in biological systems: a possible regulatory role. *Biochem J* 1989; 264: 625-632.
5. Koj A, Michalik M, Kasperczyk H. Mitochondrial and cytosolic activities of three sulphurtransferases in some rat tissues and Morris hepatomas. *Bull Acad Pol Sci* 1977; 25: 1-6.
6. Koj A, Frendo J, Wojtczak L. Subcellular distribution and intramitochondrial localization of three sulfurtransferases in rat liver. *FEBS Letters* 1975; 57: 42-6.
7. Frendo J, Koj A, Górnik A. Aktywność desulfhydrazy cysteiny i rodanazy w tkankach ludzkich. *Pol Med J* 1963; 5: 655-660.
8. Valentine WN, Frankenfeld JK (1974) 3-Mercaptopyruvate sulfurtransfease (EC 2.8.1.2): A simple assay adapted to human blood cells. *Clin Chim Acta* 1974; 51: 205-210.
9. Jarabak R, Westley J. Steady-state kinetics of 3-mercaptopyruvate sulfurtransferase from bovine kidney. *Arch Biochem Biophys* 1978; 185: 458-465.
10. Finazzi-Agro A, Cannella C, Graziani MT et al. A possible role for rhodanese: the formation of "labile" sulfur from thiosulfate. *FEBS Lett* 1971; 16: 172-174.
11. Kasperczyk A, Koj A, Wasylewski Z. Similarity of some molecular and catalytic parameters of mitochondrial and cytosolic mercaptopyruvate sulphurtransferase from rat liver. *Bull Acad Pol Biol* 1977; 25: 7-13.
12. Sörbo B. Rhodanese. In: Colowick SP, Kaplan NO (eds.). Methods in Enzymology, New York: *Academic Press*; 1955, 334-337.
13. Lowry O, Rosebrough NJ, Farr AL et al. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.
14. Jackson JF, Morse PA. Desulfurase activity and chromosome analysis of cultured rat hepatoma cells. *Rev Eur Etud Clin Biol* 1970; 15: 906-910.
15. Koj A, Frendo J, Borysiewicz J. Cysteine desulfhydrase and rhodanese activity in cells of tissue cultures and in transplantable tumors. *Acta Med Pol* 1964; 2: 109-117.
16. Włodek L, Wróbel M, Czubak J. Transamination and transsulphuration of L-cysteine in Ehrlich ascites tumor cells and mouse liver. The nonenzymatic reaction of L-cysteine with pyruvate. *Int J Biochem* 1993; 25: 107-112.
17. Wong T-W, Harris MH, Morris HP. The presence of an inhibitor of tRNA sulfurtransferase in Morris hepatomas. *Biochem Biophys Res Commun* 1975; 65: 1137-1145.
18. Palenchar PM, Buck CJ, Cheng H, Larson TJ, Mueller EG. Evidence that ThiI, an enzyme shared between thiamin and 4-thiouridine biosynthesis, may be a sulfurtransferase that proceeds through a persulfide intermediate. *J Biol Chem* 2000; 275: 8283-8286.
19. Jackson JF, Lindhal-Kieseling K. Polyploidy and endoreduplication in human lekocyte cultures treated with mercaptopyruvate. *Science* 1963; 141: 423-425.
20. Maggiulli MJ, Gustafson JC, Rector WD et al. Hilf R. Enzyme activities in human endometrium, myometrium and leiomyomas of the uterus. *Enzyme* 1977; 22: 13-8.

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