

This paper is dedicated to Professor Włodzimierz Ostrowski
Short communication

The activity of 3-mercaptopyruvate sulfurtransferase in erythrocytes from patients with polycythemia vera

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The activity of 3-mercaptopyruvate sulfurtransferase in erythrocytes from patients with polycythemia vera is significantly increased compared to healthy subjects.

3-Mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2) is an enzyme widely distributed in animal tissues [1, 2]. The enzyme catalyzes the transfer of sulphur from 3-mercaptopyruvate to sulphur thiophile acceptors such as cyanide, sulphite or thiol compounds [3]. 3-Mercaptopyruvate is produced from cysteine by transamination [4]. Although much is known about molecular properties of MPST [5, 6] biological function of this enzyme has not been elucidated. The erythrocytes from rats and human beings show significant activity of MPST [2, 7]. Mårtensson & Sörbo [8] found that almost all of MPST activity in human blood cells is confined to erythrocytes, and assayed this activity in erythrocytes from the patients with some haematological disorders including polycythemia vera, which is a myeloproliferative disorder characterized by uncontrolled proliferation of erythroid, granulocytic and megakaryocytic cells in bone marrow [8, 9]. In this paper we have demon-

strated a significant increase in MPST activity in the erythrocytes from the patients with polycythemia vera at the initial stage of the disease before pharmacological treatment.

MATERIALS AND METHODS

Blood samples. Blood samples from twenty healthy human subjects of either sex, 19-49 years old were obtained from Blood Bank and blood samples from eight patients with polycythemia vera were from the Department of Hematology (Jagiellonian University).

Separation of blood cells. Blood for the enzyme assay was collected in tubes coated with Na₂EDTA and centrifuged at 1000 × *g* for 5 min at 4°C. Erythrocytes were washed with 3 vol. of isotonic saline and haemolysed in 12 vol. of distilled water for 15 min. The haemolysate was centrifuged at 3000 × *g* for 15 min at 4°C, and the enzyme activity was

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Abbreviations: MPST, 3-mercaptopyruvate sulfurtransferase

assayed in the supernatant. Erythrocytes were counted in a Bürker chamber.

Chemicals. Ammonium 3-mercaptopyruvate was synthesized according to Sprinson & Chargaff [10] in the Laboratory of Organic Synthesis of the Jagiellonian University (Cracow, Poland). Sodium sulphite, *N*-ethylmaleimide, NADH, dithiothreitol and lactate dehydrogenase (EC 1.1.1.27) from pig heart were provided by Sigma Chemical Company.

Enzyme assay. MPST activity was assayed according to Valentine & Frankelfeld [11]. The incubation mixture contained in a final volume of 1 ml: 0.5 ml, 0.12 M sodium phosphate buffer, pH 8.0, and 0.1 ml each: 0.5 M sodium sulphite, 0.15 M dithiothreitol, haemolysate, H₂O and 0.1 M ammonium 3-mercaptopyruvate added to start the reaction. The mixture was incubated for 15 min at 37°C, then the reaction was terminated by the addition of 0.5 ml 1.2 M perchloric acid followed by filtration. The subsequent pyruvate assay mixture included: 2.4 ml, 0.12 M sodium phosphate buffer, pH 8.0, 0.2 ml, 0.1 M *N*-ethylmaleimide, 0.1 ml, NADH 5 mg/ml and 0.2 ml perchloric acid filtrate. After equilibration at 37°C, 5 µl of lactate dehydrogenase (7 IU) was added, and the decrease in absorbance was measured at 340 nm.

The enzyme activity was expressed as µmoles of pyruvate formed during one minute per 10¹⁰ erythrocytes at 37°C.

The enzyme was stable in whole blood but not in haemolysate. Blood samples could be kept for 2 h at room temperature or 6 h at 4°C without significant changes of enzyme activity. The level of enzyme activity decreased by about 30% after two weeks storage at -20°C.

Statistical analysis of the results was performed by the Student's *t*-test.

RESULTS AND DISCUSSION

Variability of MPST activity in human erythrocytes from healthy subjects is rather low and ranged from 31.3 to 45.5 µmoles of pyruvate per 10¹⁰ erythrocytes per minute (average 40.0 ± 3.0) while in erythrocytes from patients with polycythemia vera it ranged from 50 to 90 µmoles (average 73.1 ±

14.1). The increase was significant at $P < 0.001$ (Fig. 1). Elevation of MPST activity in polycythemia patients was previously reported by Mårtensson & Sörbo [8], however, the differences found between healthy subjects and polycythemia patients were less evident.

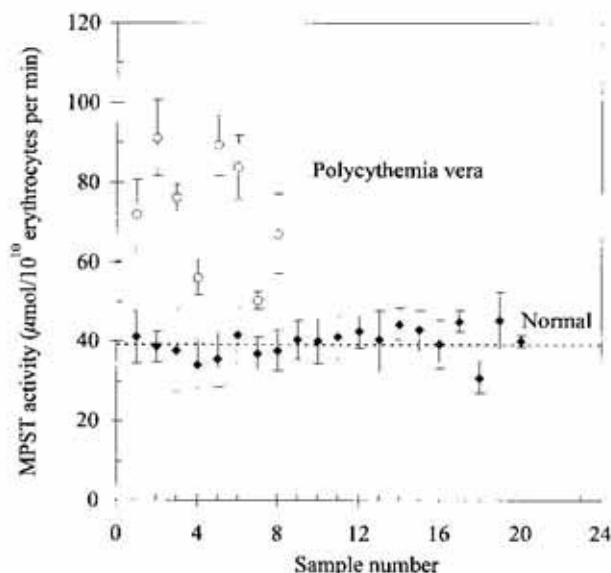


Figure 1. The activity of 3-mercaptopyruvate sulfrtransferase in erythrocytes from healthy human subjects and from patients with polycythemia vera.

The function of MPST in erythrocytes is still unknown. In other cells MPST participates in the formation of iron-sulphur enzymes [12]. Our results support the hypothesis that the metabolism of sulphur compounds in erythrocytes of patients with polycythemia vera is more active than in normal subjects. Although estimation of MPST activity in erythrocytes may throw some light on sulphur metabolism in erythrocytes in polycythemia vera, the differences in the metabolism of normal erythrocytes and those from polycythemia vera patients can not be of decisive diagnostic value.

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