STUDY OF THE ENZYMES TAKING PART IN THE SYNTHESIS AND DISINTEGRATION OF GLYCOGEN IN THROMBOCYTES OF DONORS AND PATIENTS WITH CHRONIC MYELOSIS AND POLYCYTHEMIA

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It was established earlier that glycogen of the thrombocytes possesses considerable metabolic activity [1] and that thrombocytes from healthy people contain all the enzymes* necessary for the synthesis of this polysaccharide by uracil-glucoside-diphosphate (hexokinase, phosphoglucomutase, UGDP-pyrophosphorylase, and UGDP glycogen synthetase) and its decomposition by phosphorolysis [3].

The rate of renewal of glycogen differs in thrombocytes of healthy people and in certain diseases of the blood system (polycythemia and chronic myelosis) [1]. Therefore, there is considerable interest in the study of the individual enzymatic systems associated with the synthesis and decomposition of glycogen in thrombocytes, for the purpose of determining the cause of the changes in the glycogen metabolism of the platelets during pathology. Human thrombocytes are characterized by a high glycolytic ability, which indicates the presence of hexokinase in them [1, 2].

The aim of this work was to study the enzymes participating in the glycogen metabolism in thrombocytes of healthy people and those ill with certain diseases of the circulatory system.

PROCEDURE

In the work we used thrombocytes isolated from the blood of donors, the blood of polycythemia patients and chronic myelosis patients by a method of precipitation from a gelatin-citrate solution [2]. For the quantitative determination of UGDP, freshly separated thrombocytes, which were treated with chilled perchloric acid (final concentration 2%), were used. The nucleotides were isolated from the sediment by adsorption on charcoal, they were eluted with a 50% solution of ethanol, the eluates were evaporated under vacuum to a small volume, and the concentrated solution was applied on a paper chromatogram (VF II). The run was carried out in an alcohol-1M ammonium acetate system according to Paladini and Leloir [6] in the ratio of 37.5 : 15.0. In the extracts of the spots corresponding to the position of the reference spot (R_f = 0.23), UGDP was determined quantitatively in the enzymatic reaction with UGDP-pyrophosphorylase, produced in the laboratory according to the method of Munch-Petersen [5]. In a study of the enzyme activities of the thrombocytes, enzymatic systems were made up in such a way that the final link was the reduction of TPN to TPN-H, which was easily recorded spectrophotometrically by an increase in the optical density at 340 mμ. By this means the hexokinase activity (substrate glucose + ATP) glycose-6-phosphate dehydrogenase activity (substrate G-6-P), phosphoglucomutase activity (substrate G-1-P), UGDP-pyrophosphorylase activity (substrate UGDP + phosphoglucomutase), and phosphorylase activity (substrate glycogen + phosphate) were determined. The testing of the enzymatic activities was carried out with extracts prepared from acetone

* The following abbreviations are used in the work: ATP—adenosine triphosphate; ADP—adenosine diphosphate; UTP—uridine triphosphate; UDP—uridine diphosphate; UGDP—uracil-glucoside-diphosphate; P—P—pyrophosphate; G-1-P—glucose-1-phosphate; G-6-P—glucose-6-phosphate; TPN—triphosphopyridine nucleotide; TPN-H—reduced triphosphopyridine nucleotide.

TABLE 1. Activity of Hexokinase, Glucose-6-Phosphate Dehydrogenase, Phosphoglucomutase, UGDP, Pyrophosphorylase, and Phosphorylase in Donor Thrombocytes and in the Thrombocytes of Chronic Myelosis and Polycythemia Patients

Enzymes	Substrates added	Enzymatic activity in extracts of thrombocytes (in micromoles per mg of protein per min)		
		Donors	Polycythemia patients	Chronic myelosis patients
G-6-P dehydrogenase	0.1 ml 0.02 M G-6-P solution	0.061 ± 0.007	0.049 ± 0.005	0.055 ± 0.009
Hexokinase	0.1 ml 0.03 M glucose solution 0.1 ml 0.02 M ATP solution	0.016 ± 0.004	0.020 ± 0.007	0.016 ± 0.003
Phosphoglucomutase	0.1 ml 0.02 M G-1-P solution	0.050 ± 0.010	0.048 ± 0.005	0.044 ± 0.007
UGDP pyrophosphorylase	60 micrograms UGDP 0.1 ml 0.01 M sodium pyrophosphate solution	0.013 ± 0.001	0.011 ± 0.001	0.015 ± 0.006
Phosphorylase	0.1 ml (500 micrograms) glycogen 0.2 ml 0.015 M phosphate buffer solution	0.013 ± 0.002	0.013 ± 0.002	0.012 ± 0.002

Composition of sample: 2.5 ml 0.05 M tris buffer, pH 7.5; 0.1 ml 0.2 M $MgCl_2$ solution; 0.1 ml 0.05 M cysteine solution; 0.1 ml 0.01 M TPN solution; 0.05 ml acetone powder extract of thrombocytes. Total volume 3 ml; temperature 20°.

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powders by treatment of them with a 0.1% $NaHCO_3$ solution in a 1 : 100 ratio. The acetone powders were produced by treatment of the blood platelets with acetone cooled to −12° (in the ratio 1 : 20), followed by drying with peroxide-free ethyl ether.

The following preparations were used: hexokinase from the Light Co. (England); phosphoglucomutase from the Reanal Company (Hungary); G-6-P-dehydrogenase produced in the laboratory according to Kornberg [4]; UGDP preparation from the Sigma Co. 98-100% purity; C^{14}-glucose—98% purity; UTP and TPN from the Reanal firm (Hungary).

RESULTS

Surplus G-6-P-dehydrogenase should be considered the first necessary condition for the quantitative determination of those enzymatic activities which were studied, since they all effected the reduction of TPN to TPN-H using G-6-P-dehydrogenase. The experiments indicated that G-6-P-dehydrogenase is found in the thrombocytes in excess in relation to the other enzymes studied, and this permitted omission of the addition of the enzyme. Table 1 cites the results of the study of the activity of G-6-P-dehydrogenase in various types of thrombocytes. As is evident from the data cited in Table 1, all the types of thrombocytes investigated contained highly active G-6-P-dehydrogenase.. The detection of G-6-P-dehydrogenase activity in the thrombocytes of donors is in good agreement with the results obtained in the work of Wurzel et al. [8] in the determination of G-6-P-dehydrogenase in thrombocytes of healthy people. In our data, the highest G-6-P-dehydrogenase activity was found in the donor thrombocytes. The highest glycolytic ability was detected in the thrombocytes of polycythemia patients.

Phosphoglucomutase, which catalyzes the reversible conversion of G-1-P to G-6-P, plays an important role in glycogen synthesis by the uridine diphosphate pathway, by supplying the G-1-P essential for the formation of UGDP. The phosphoglucomutase content in all the types of thrombocytes considerably exceeded (by 2 to 3 fold) the activity of other enzymes in the system of glycogen synthesis, but proved to be approximately the same in the thrombocytes of various types.

TABLE 2. Determination of Synthetase Activity in Thrombocytes of Donors, and Thombocytes of Patients with Polycythemia and Patients with Chronic Myelosis

Thrombocytes	Transfer of C^{14}-glucose with UGDP-C^{14} to glycogen	
	Counts/min per mg of protein	Micromoles of glucose (per mg of protein) per min
Donors	7,041	0.008
Patients with polycythemia	5,639	0.006
Patients with chronic myelosis	4,718	0.005

Composition of the sample: 0.05 ml 0.5 M tris buffer, pH 7.5; 0.05 ml 0.2 M $MgCl_2$; 0.05 ml 0.02 M versene; 0.05 ml 0.5 M cysteine; 0.1 ml 0.02 M G-1P; 0.05 ml thrombocyte extract; 70 micrograms of UGDP-C^{14} with total radioactivity of 6300 counts/min. Incubation 20 min at 37°.

The study of UGDP pyrophosphorylase activity was of special interest, since UGDP is formed in this reaction —the direct transfer agent of glucose radicals to glycogen: UTP + G-1-P ⇌ UGDP + P-P. The greatest UGDP pyrophosphorylase activity was detected in the thrombocytes of patients with chronic myelosis; the least—in those with polycythemia. It was interesting that the somewhat larger activity of UGDP pyrophosphorylase in the thrombocytes of patients with chronic myelosis corresponds to an increased content of UGDP in the platelets of those patients. Thus, we detected 34 micrograms of UGDP per ml in the platelets of patients with chronic myelosis, 22 micrograms in the platelets of donors, and 24 micrograms in the thrombocytes of polycythemia patients.

Table 2 presents data characterizing the activity of the enzyme UGDP-glycogen transferase (synthetase)—the terminal enzymatic link in glycogen synthesis.

UGDP glycogen transferase catalyzes the following reaction: UGDP + $\text{glycogen}_{(n)} \rightarrow$ UDP + $\text{glycogen}_{(p+1)}$. The synthesizing activity was determined by the rate of transfer of C^{14} glucose from UGDP-C^{14} to glycogen. Radioactive UGDP-C^{14} was synthesized enzymatically in the system consisting of the following components: 1.0 ml 0.5 M tris buffer, pH 7.5; 2.2 ml water; 0.1 ml 0.2 M $MgCl_2$; 0.1 ml 0.05 M cysteine; 0.4 ml (8 micromoles) ATP; 0.4 ml (10 micromoles) UTP; 0.1 ml (5 micromoles) C^{14} glucose; 0.1 ml (500 micrograms) phosphoglucomutase; 0.4 ml (360 micrograms protein) UGDP pyrophosphorylase; 0.1 ml 0.01 M inorganic pyrophosphate. The synthesis of UGDP-C^{14} was carried out according to the following scheme: ATP + C^{14}-glucose $\xrightleftharpoons{\text{hexokinase}}$ C^{14}-G-6-P + ADP; C^{14}-G-6-P $\xrightleftharpoons{\text{phosphoglucomutase}}$ C^{14}-G-1-P; C^{14}-T-1-P + UTP $\xrightleftharpoons{\text{UGDP-pyrophosphorylase}}$ UGDP-C^{14} + P-P. The samples were incubated at 37° for one hour. UGDP-C^{14} was isolated by paper chromatography by the method described above. Under the conditions of our experiment UGDP-C^{14} was obtained with a specific radioactivity of 300 counts/min. One microgram of glucose, acquired in the same way, was used in the system cited in Table 2. As can be seen from Table 2, the highest amount of C^{14}-glucose was transferred in the extracts of donor thrombocytes. Thus, there is no proportional dependence between the synthetase activity and the glycogen content in the thrombocytes, as was observed in relation to the other tissues [8]. Despite the different glycogen content in the thrombocytes of donors and polycythemia patients, the synthetase activity was depressed in the thrombocytes of the patients.

During the determination of the phosphorylase activity in thrombocytes (see Table 2), no significant difference between the thrombocytes of donors and patients with chronic myelosis and polycythemia were found.

The results of the works do not permit a complete explanation of the differences in the rates of glycogen restoration of the thrombocytes, noted in the preceding investigations [1]. We have indicated that in thrombocytes of patients with chronic myelosis and polycythemia the rate of circulation of glucose residues in glycogen is higher

than in the norm. Although the increased metabolic activity of glycogen of the thrombocytes of patients with chronic myelosis may be explained by the higher content of UGDP than in the thrombocytes of patients with polycythemia this explanation is unsuitable, since the UGDP content is not increased in them. The activity of the enzymes of the UGDP pathway of glycogen synthesis also did not deviate from the norm. Thus, the reason for the increase in the metabolic activity of the glycogen of the thrombocytes of polycythemia patients is not entirely clear. However, not excluded is the possibility that together with UGDP there exist both another pathway for glycogen synthesis in the platelets and other synthetic pathways. Data in favor of such a hypothesis were also obtained during the study of the influence of certain inhibitors on the enzymes of the UGDP mechanism of glycogen synthesis in experiments with whole thrombocytes.

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