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Activity of platelet α-6-fucosyltransferase is inversely related to blood platelet concentration

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The activity of serum α-6-fucosyltransferase, a platelet derived enzyme, determined in sera of 22 normal individuals and 86 patients with various disorders was positively correlated with platelet counts. When the enzyme activity in 1 μl serum was calculated per 1000 of platelets in blood (coefficient F/P) an inverse correlation became evident in that F/P was proportionally the higher the lower was platelet count in blood. The F/P values were in a good agreement with the results of direct assays of enzyme activities in isolated platelets. Neither granulocytes, lymphocytes nor red cells significantly contributed to serum enzyme activity though granulocytes enhanced the thrombin-induced enzyme release from platelets. In platelets separated by centrifugation in density gradients the enzyme was shown to be present in platelets of intermediate and high density but missing from the light ones. It is suggested that α-6-fucosyltransferase of platelets may be a marker of the ploidy level of megakaryocytes.

α-6-Fucosyltransferase is an enzyme of glycoprotein biosynthesis that transfers fucose to position 6 of the asparagine-linked *N*-acetylglucosamine residue of N-glycans (EC 2.4.1.68). In human serum the activity of α-6-fucosyltransferase is derived in about 95% from blood platelets [1, 2]. Platelets release the enzyme during coagulation of blood or after treatment with agonists that make them to change shape and secrete [3]. Biological significance of the enzyme release is unknown but the structure synthesized by α-6-fucosyltranferase is present in glycans of GPIIb-III a complex of platelets [4] that is essential for fibrinogen binding. Previously we have shown that serum activity of α-6-fucosyltransferase is positively correlated with blood platelet concentration [1, 2]. In this study we demonstrate yet another correlation, this time inverse, between platelet concentration in blood and the enzyme activity per platelet. In addition, we report that the enzyme is present only in some subpopulations of platelets of high buoyant density. Granulocytes of blood also contain the enzyme activity but do not release it under the action of thrombin.

[1] Abbreviations: AA, aplastic anemia; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogeneous leukemia; ET, essential thrombocythemia; IT, idiopathic thrombocytopenia; MF, myelofibrosis; MD, myelodysplastic syndrome; PV, polycythemia vera; T, thrombocytosis.

PATIENTS AND METHODS

The study was made on 22 first time blood donors (10 men and 12 women) and 86 patients (41 men and 45 women) of the Institute of Hematology and Blood Transfusion. The patients were selected for low, high and normal platelet counts in blood. Among them were: 33 patients with idiopathic thrombocytopenia (IT)[1], 15 with acute myelogenous leukemia (AML), four with chronic myelogenous leukemia (CML), four with acute lymphocytic leukemia (ALL), three with chronic lymphocytic leukemia (CLL), 12 with lymphoma; 5 with myelofibrosis (MF), one with myelodysplastic syndrome (MD), four with polycythemia vera (PV), two with aplastic anemia (AA), one with essential thrombocythemia (ET), two with thrombocytosis (T), that was not diagnosed at the time of examination. Of these patients 42 were treated with cytostatic or hormone drugs, 27 were new, non-treated cases and 14 have not been treated for at least four weeks before enzyme assays.

The activity of serum α-6-fucosyltransferase was determined as described previously [3] employing as a substrate a "stripped" IgG glycopeptide and GDP-[^{14}C]fucose (200 mCi/mmol), Amersham, as fucose donor, and expressed in pmoles of [^{14}C]fucose transferred to glycopeptide substrate/1 μl serum per 1 h. The F/P coefficient was calculated by dividing the latter value by the number of thousands of platelets in 1 μl of blood and expressed in fmoles of [^{14}C]fucose transferred to glycopeptide substrate/1 μl serum per 1000 platelets in blood. When the enzyme activity was determined in isolated platelets the cells were lysed beforehand in 0.5% Triton X-100. For determination of the activity of the enzyme in plasma, blood was collected on heparin (POLFA, Poland). Plasma enzyme determinations were routinely performed in patients with platelet counts below 20000. The enzyme activities in plasma were subsequently subtracted from those of serum. The activity of serum and platelet α-2-fucosyltransferase was assayed with β-phenylgalactoside as substrate [5].

Platelet counts and volumes were determined with the use of a hematology analyser Cell-Dyn 1500 (Sequoia Turner). Platelet concentrates were prepared by differential centrifugation. Separation of platelets into subpopulations was made by centrifugation in Percoll (Pharmacia) gradients (30 ml) as in [6] but blood cells were centrifuged for a longer time (10000 × *g* for 15 min). The gradients spanned the density range of 1.039–1.083 g/ml and were calibrated with density marker beads (Pharmacia). Leukocytes were isolated from blood of healthy donors by leukapheresis and subsequently separated into granulocytes and lymphocytes by centrifugation in Percoll gradients [7]. Platelets and other types of blood cells were treated with thrombin as in [3]. Statistical significance of differences between means was assessed by the Student's *t*-test.

RESULTS AND DISCUSSION

In keeping with our previous findings [1, 2] we found serum activities of α-6-fucosyltransferase to be proportional to platelet counts (see Fig. 1). The proportion was reversed, however, when platelet counts were related with serum enzyme activities per 1 000 platelets (coefficient F/P) (see Fig. 2). Differences between means for

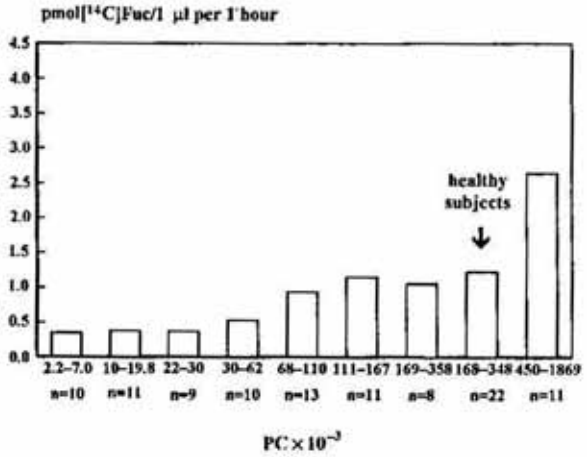

Fig. 1. Serum activities of α-6-fucosyltransferase and platelet counts (PC) in blood.
Blood samples were donated by healthy subjects or patients with the following diseases: platelets 2200–7000, IT-1, AA-2, CML-2, AML-1, ALL-2, MF-1, MD-1; platelets 10000-19800, IT-10, AML-1; platelets 22000–30000, IT-8, CML-1; platelets 30000–62000, IT-6, CLL-1, AML-1, lymphoma-2; platelets 68000–110000, IT-5, AML-4, MF-1, lymphoma-3; platelets 111000–167000, IT-1, CLL-1, AML-3, ALL-1, lymphoma 5; platelets 169000–358000, IT-1, CML-1, CLL-1, AML-4, ALL-1; platelets 168000–348000, healthy subjects; platelets 450000– 1869000, AML-1, MF-3, lymphoma-2, PV-2, ET-1, undiagnosed-2.

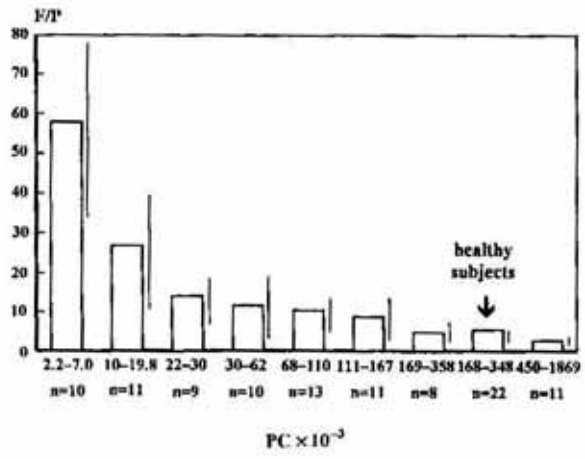

Fig. 2. Coefficient F/P plotted against blood platelet concentration.
Experimental groups are the same as in Fig. 1. Vertical lines to the right of bars indicate standard deviations.

F/P in the group of healthy subjects or that of patients with platelet counts of 450000–1869000 were significantly lower than mean F/P values for each of the other groups of patients ($p < 0.001$) except the patients with platelet count of 169000–358000. The mean for the latter group did not significantly differ from that of healthy subjects with comparable blood platelet concentration but was still higher (at $p = 0.05$) than the mean for patients with very high concentration of platelets in blood. The F/P values accurately described true enzyme activities in platelets as determined in platelet lysates (see Table 1). The F/P coefficient was not significantly altered by treatment of patients with cytostatics and steroid drugs providing that platelet counts were similar (not shown). The nature of the disease may affect F/P values though this cannot be proved with a limited number of patients examined in this study. Obviously variations of F/P values did not result from differences in the size of platelets because the volumes of platelets produced under conditions of thrombocytopenia and thrombocytosis varied by a factor of 3 whereas F/P values varied by a factor of 50.

To exclude a possible contribution to serum activity of α-6-fucosyltransferase by other types of blood cells, we determined enzyme activities in Triton X-100 lysates of granulocytes and lymphocytes. As shown by Tables 2 and 3 granulocytes exhibited some enzyme activity though this was not released by thrombin. Granulocytes enhanced, however, thrombin induced enzyme release from platelets, presumably through the action of cathepsin G [8]. Lymphocytes did not exhibit any enzyme activity under our conditions of assay. The latter cells were examined only in mixtures with platelets since we were unable to prepare platelet-free lymphocytes. α-6-Fucosyltransferase is also missing from erythrocyte membranes (not shown).

Lastly, we examined α-6-fucosyltransferase and in one instance also α-2-fucosyltransferase in platelet subpopulations of subjects with low, normal and high values of F/P. As shown in Fig. 3, numbers and densities of subpopulations varied from subject to subject. The distribution of the enzyme was not uniform, either, with most of the activity being present in subpopulations of platelets with high densities. The light and heavy subpopulations of platelets predominated in blood of subjects with low and high values of F/P, respectively (see Fig. 3 a and d). Thus, the enzyme-active platelets appear to represent only some subpopulations of platelets. It is likely that these platelets contain Golgi apparatus which, according to observations by electron microscopy, is present in only 10% of platelets [9]. It

Table 1
Comparison of F/P values with direct assays of the activity of α-6-fucosyltransferase in isolated platelets

Initials and diagnosis	F/P	Blood platelets	
		F/P_{is}	count (thousands)
J.K., control	4.5	5.1	220
A.D., polycythemia	6.3	7.6	265
K.K., polycythemia	9.6	11.6	450
J.R., thrombocytopenia	12.5	14.6	120

F/P_{is}, fmoles [^{14}C]fucose incorporated to the glycopeptide substrate by 1 µl of platelet lysate/1 h per 1 000 platelets.

Table 2

Activity of α-6-fucosyltransferase in Triton X-100 lysates of pure and mixed populations of platelets, lymphocytes, and granulocytes

Enzyme source (cell concentrations in thousands/μl)	Enzyme activity per 1000 cells
Platelets (590)	11.2[a]
Granulocytes (135)	2.6[b]
Mixture of lymphocytes (45) and platelets (369) (no enzyme in lymphocytes)	11.2[a]

[a]per 1000 platelets; [b]per 1000 granulocytes.

Table 3

Activity of α-6-fucosyltransferase in supernatants of thrombin treated platelets

Enzyme source (cell concentrations in thousands/μl)	Enzyme activity per 1000 cells
Platelets (369)	2.1[a]
Granulocytes (108)	0.0[b]
Platelets (146) in mixture with granulocytes (9 to 89)	5.9[a]
Platelets (92) in mixture with lymphocytes (29)	2.1[a]

[a]per 1000 platelets; [b]per 1000 granulocytes.

should be pointed out that F/P values were the lowest in subjects with thrombocythemia and became progressively higher the lower was the platelet count in blood. Thus, they behaved exactly like ploidy levels of megakaryocytes [10]. It is therefore our hypothesis that platelets with high activity of α-6-fucosyltransferase are derived from high-ploidy megakaryocytes. The latter arise in the bone marrow through the action of thrombopoietin [11]. It should be emphasized that a molecular mechanism by which platelets acquire high activity of α-6-fucosyltransferase is unknown. The Golgi apparatus, where α-6-fucosyltransferase should be lo-

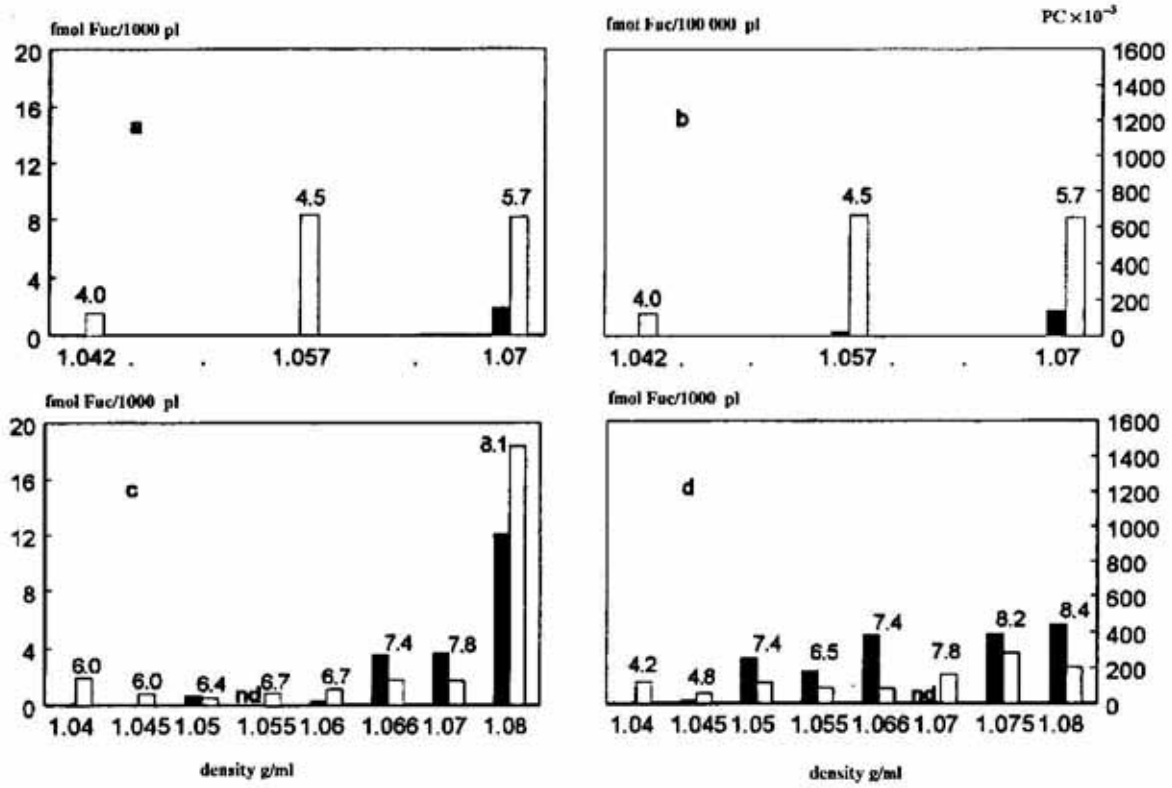

Fig. 3. Activities of α-2- and α-6-fucosyltransferases (filled-in bars) in subpopulations of platelets separated by centrifugation in Percoll gradients.

Each fraction is equivalent to the volume of 1 ml. Platelet counts (per 1 μl), (PC × 10^{-3}), open bars. The activity of α-6-fucosyltransferase expressed in fmoles of [^{14}C]fucose transferred to glycopeptide substrate /1 h per 1000 platelets, a, c, d; and that of α-2-fucosyltransferase in fmoles of [^{14}C]fucose transferred to β-phenylgalactoside/1 h per 1000000 platelets, b; a and b, patient K.S., with a low F/P value of 1.8; c, patient, M.C., with a high F/P value of 18.0; d, patient W.H. with an average F/P value of 8.0. Numbers above bars refer to platelet volumes (fl).

cated, is present in mammalian cells as a single copy [12]. It fragments into vesicles during mitosis and this is thought to ensure its equal division among daughter cells. After mitosis the vesicles reassemble into single-copy Golgi apparatus in each cell. Presumably, the new Golgi apparatus with its glycosyltransferases is made of both a pre-existing and newly-biosynthesized material. Megakaryocytes, however, expand not through mitosis but endomitosis i.e. the process in which nuclei divide without cytoplasmic division. Thus, our data suggest that under these conditions glycosyltransferases may accumulate in the cytoplasm.

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