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Human platelets release α-6-L-fucosyltransferase upon activation

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Previously we have shown that human platelets release α-6-L-fucosyltransferase (EC 2.4.1.68) during coagulation of blood [(1987) Glycoconjugate J. 4, 43–49]. Here we report that agonists which induce platelet aggregation bring about release of the enzyme. In quantitative terms the release of α-6-L-fucosyltransferase by washed, aggregated platelets was very similar to that occurring during blood coagulation.

Platelet; Glycoprotein 6-α-L-fucosyltransferase; Release reaction

1. INTRODUCTION

Recently we have shown that serum α-6-L-fucosyltransferase (EC 2.4.1.68) originates from platelets to an extent of 90% [1,2]. The enzyme is released from platelets during coagulation of blood. In contrast, serum α-3-L-fucosyltransferase (EC 2.4.1.52) does not originate from platelets [2,3]. Here, we report that α-6-L-fucosyltranferase is liberated by agonists which cause platelets to change shape, aggregate, and secrete.

2. MATERIALS AND METHODS

2.1. *Chemicals and reagents*

GDP-[^{14}C]fucose (200 mCi/mmol) and [^{3}H]serotonin (creatinine sulphate) (10 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, England), ADP from Reanal (Hungary), ristocetin sulphate from H. Lundbeck (Copenhagen), adrenalin from Polfa (Warsaw), human fibrinogen from Kabi (Sweden), bovine thrombin from Biomed (Lublin, Poland) and collagen from Sigma. Other chemical reagents were from standard commercial sources.

2.2. *Preparation of washed platelets*

Blood withdrawn from regular blood donors was collected into 1/10 vol. of 3.8% sodium citrate. Platelets were isolated by the method of Mustard et al. [4] but the first washing solution contained 2 mM Na_2EDTA instead of heparin. The concentration of apyrase amounted to 50 mg/l. Following washings the platelets were suspended in solution A.

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2.3. *Labelling of platelets with [^{3}H]serotonin*

Labelling was essentially according to [5]. Briefly, citrated platelet-rich plasma (PRP, 15 ml) was treated with 130 μl of 10 μM serotonin in 0.15 M NaCl. Following incubation at 37°C for 40 min with occasional stirring the platelets were isolated as described [4].

2.4. *Aggregation of platelets and release reaction*

Portions of washed platelets (1 ml) were treated with 100-μl aliquots of aggregating agents and incubated at 37°C for 15 min with continuous stirring. The reaction was stopped by transferring the samples to an ice bath. Thereafter, the samples were centrifuged in a refrigerated centrifuge and the supernatants assayed for α-6-L-fucosyltransferase activity. If [^{3}H]serotonin-labelled platelets were used portions of the same supernatants were tested for radioactivity by liquid scintillation counting. Agonists employed and their final concentrations were: ADP (20 μM), with fibrinogen (1 mg/ml); collagen (0.2 mg/ml); adrenalin (50 μmol/ml); ristocetin (1.5 mg/ml); thrombin (0.4 U/ml or as indicated in section 3). In all experiments fractions of enzyme activities released were calculated relative to total enzyme activities determined in Triton X-100 lysates of platelets. Changes in light transmission were recorded in a model 169 aggregometer (Evans Electronic).

2.5. *α-6-L-Fucosyltransferase*

The enzyme activity was determined essentially as in [2] but using another batch of substrate (stripped IgG glycopeptide) and a simplified technique for product isolation. Incubation mixtures contained, in 29.25 μl: 25 μg IgG glycopeptide, 1 μmol Na-cacodylate buffer (pH 6.0), 0.2 μmol EGTA, 0.2 μmol $MgCl_2$, 61 pmol GDP-[^{14}C]fucose, 6 μl serum or supernatant of aggregated platelets. When washed platelets were assayed for

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enzyme activity they were lysed beforehand with 0.5% final concentration of Triton X-100. After 2 h at 37°C reactions were terminated by addition of 5 μl of 0.2 M EDTA. Aliquots were then directly spotted on DE-81 Whatman paper and separated by descending chromatography with water. The solvent was allowed to run to a distance of 16 cm from the starting line (marked previously with pencil). Thereafter, the papers were dried and developed once again with methanol/ethanol (3:2, v/v) down to 26 cm from the start. Radioactivity present on four 1 × 4 cm adjacent paper strips at 15–19 cm from the starting line was determined by liquid scintillation counting.

3. RESULTS

Table 1 lists the effects of different aggregating and activating agents upon release of α-6-L-fucosyltransferase from isolated platelets. All agonists brought about significant liberation of the enzyme. Blood donors for these experiments were preselected so that their platelets gave a good response to 20 μM ADP in the presence of 1 mg/ml fibrinogen as recorded by the aggregometer (minimum 20% change in light transmission at 20 s after addition of aggregant).

Platelets from donor 4 were treated with graded concentrations of thrombin, i.e. 0.08, 0.16, 0.4 and 5.0 U/ml. The response of platelets was dose-dependent and amounted to 15.6, 21.8, 28.7 and 28.1%, respectively, of the enzyme liberated. A similar experiment performed on platelets of donor 5 revealed that 5.0 U/ml of thrombin brought about the release of more enzyme (62.3%) than the standard concentration of 0.4 U/ml (see table 1). The data in table 1 plus plasma enzyme activity (not shown) also allow one to calculate the extent of enzyme released during coagulation of blood. Thus, after 30 min incubation of whole blood at 37°C, platelets of donors 1–3 released 17.8, 23.3 and 41.1%, respectively, of the total enzyme present in washed platelets. In the calculation corrections were made for the partial volume of erythrocytes in blood samples taken for preparation of the sera (during coagulation of blood the enzyme is released into a smaller volume than in the aggregated platelet concentrate), the enzyme present in blood plasma outside platelets, and different counts of platelets in blood and washed platelet concentrates.

Table 1

Release of α-6-L-fucosyltransferase activity (Ftr) by activated platelets

	Donor				
	1	2	3	4	5
Platelet count in blood × 1000	284	218	370	nd	nd
Platelet count in washed platelets × 1000	213	240	410	672	668
FTr in serum	1.2	1.05	4.45	nd	nd
FTr in washed platelets dissolved in 0.5% Triton X-100	2.5	2.45	6.05	2.65	3.2
Release (%) by:					
Thrombin	22.7 (58.1)	24.9 (23.7)	43.5 (64.7)	28.7	37.9
Collagen	25.7 (62.1)	23.5 (99.0)	48.2 (46.1)	35.6	51.9
ADP	2.6	4.8	4.8	11.9	44.6
Adrenalin	nd	nd	nd	18.8	42.3
Ristocetin	nd	nd	nd	14.3	46.2

Enzyme activity expressed as pmol [^{14}C]fucose incorporated into stripped IgG glycopeptide/μl per h. % release of enzyme activity was calculated relative to the total enzyme activity in washed platelets, dissolved in 0.5% Triton X-100; values in parentheses show % release of [^{3}H]serotonin; nd, not determined

4. DISCUSSION

α-6-L-Fucosyltransferase is the enzyme of glycoprotein biosynthesis which conveys L-fucose in α1–6 glycosidic linkage to asparagine-linked *N*-acetylglucosamine residues of *N*-glycans [6]. The resulting structure ..GlcNAcβ1–4[Fucα1–6]GlcNAcβ1–4-*N*-Asn-R. is present in the GPIIb-IIIa complex on the platelet surface [7]. The complex is an integrin for fibrinogen, von Willebrand factor and fibronectin but does not bind the ligands in unstimulated platelets [8]. Although limited in number, our experiments show quite clearly that the aggregated platelets release α-6-L-fucosyltransferase. Thrombin and collagen were good inducers of enzyme release while ADP was the least effective. The latter produced a good response only from platelets of donor 5. Under conditions of blood coagulation the release of α-6-L-fucosyltransferase into blood serum was quite similar to that caused by the action of either thrombin or collagen upon isolated platelets. Previously we have shown that the soluble α-6-L-fucosyltransferase activity peaks at 15 min when PRP is recalcined and subsequently incubated at 37°C [9]. The released enzyme was in a true soluble form and did

not sediment after centrifugation of serum at 100000 × *g* for 60 min. As shown in this paper thrombin at 0.08 U/ml, i.e. a concentration which produces only a transient, reversible aggregation of platelets, brings about significant release of the enzyme.

Superficially, liberation of α-6-L-fucosyltransferase from platelets by different agonists resembles the release reaction in which low- and high-M_r materials contained in a soluble form in platelet granules are secreted. This similarity may be misleading. Although glycosyltransferases are present in a soluble form in blood serum and other body fluids, these enzymes are located primarily within cells and are membrane-bound.

Recently, it has been shown that rat liver α-2,6-sialyltransferase (EC 2.4.99.1) is solubilized by proteolytic cleavage between membrane-binding and catalytic domains [10]. It is well known that the aggregation of platelets is accompanied by the activation of endogenous proteases (calpains) [11]. Thus, the release of α-6-L-fucosyltransferase from platelets may result either from secretion of a soluble enzyme contained within granules or proteolysis of a membrane-bound form or both. Experimental distinction between these mechanisms may be difficult.

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