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QUARTERLY

In comparison to progenitor platelets, microparticles are deficient in GpIb, GpIb-derived carbohydrates, glycerophospholipids, glycosphingolipids, and ceramides*

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Activated blood platelets shed microparticles with procoagulant activity that probably participate in normal hemostasis. We have isolated spontaneously formed microparticles from human blood and analysed them for ultrastructure, antigenic profile, and biochemical composition. In transmission electron microscopy microparticles appeared as regular vesicles with a mean diameter of 300 nm (50–600 nm). In flow cytometry almost all microparticles reacted with fluorescein isothiocyanate (FITC) labeled antibody to platelet glycoprotein complex IIb-IIIa (GpIIb-IIIa) and with FITC-annexin V but only 40–50% of microparticles reacted with FITC-antibody to platelet glycoprotein Ib (GpIb). The latter result was confirmed by double labeling of microparticles with FITC-antibody to GpIIb-IIIa and phycoerythrin (PE) labeled antibody to GpIb. Large microparticles reacted better with anti-GpIb than the small ones. A decreased level of GpIb was also demonstrated by SDS/polyacrylamide gel electrophoresis of microparticles. Compositional studies indicated, that in terms of cholesterol and protein contents, microparticles resembled platelets rather than platelet membranes as previously thought. They are, however, deficient in certain components. Thus, in comparison to platelets, microparticles had reduced contents of sialic acid (by 56.4%), galactosamine (by 48.2%), glucosamine (by 22.4%), galactose by (11.8%) and fucose (by 21.6%). Mannose content was increased by 11.8%. Total phospholipids in microplatelets were lower by 17.8%. Glycerophospholipids only were affected with phosphatidylserine being decreased as much as by 43.2%. Neutral glycosphingolipids, gangliosides and ceramides in microparticles were reduced by half.

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Abbreviations: GpIb, platelet glycoprotein Ib; GpIIb-IIIa, platelet glycoprotein complex IIb-IIIa; PRP, platet rich plasma; HPAEC, high pH anion exchange chromatography; PAD, pulse amperometric detection (or detector); FITC, fluorescein isothiocyanate; PE, phycoerythrin; TBS, Tris-buffered saline; PBS, phosphate buffered saline.

We conclude that the biochemical composition of microparticles probably reflects previous activation of progenitor platelets.

Platelet vesicles, known also under the name of platelet-derived microparticles or microplatelets, are shed by platelets under a variety of conditions. *Ex vivo* microparticles are released during blood coagulation or activation of isolated platelets with most agonists that cause their aggregation and secretion (reviewed in [1–3]). Vesiculation of platelets is also promoted by the action of the membrane attack complex C5b-9 of the complement system [4], Ca^{++} ionophore inducing calcium influx across the platelets plasma membrane [5, 6], and also echinocytogenic amphiphiles [7]. Release of platelet microparticles may be also brought about by mechanical disruption of platelets, like for instance by repeated freezing and thawing [8] or application of high shear stress [9]. Spontaneous release of microparticles, probably resulting from activation of platelets, occurs during storage of platelets at 22°C [10, 11]. *In vivo*, microparticles are present in circulating blood albeit at a low concentration [12]. The amount of microplatelets in blood was, however, increased in patients who had undergone major surgery [13], plasmapheresis [14], and generally in those suffering from thrombotic conditions. Interestingly, microparticles participate in clot formation [15, 16] and carry both procoagulant [17, 18] and anticoagulant functions [19, 20]. In addition they adhere to vessel subendothelium [21] bind fibrinogen and fibrin [22] and thus, they may contribute to normal hemostasis.

The mechanism of platelet vesiculation is unknown. The vesiculation is significantly associated with loss of plasma membrane phospholipid asymmetry that occurs during platelet activation (reviewed in [1] and [3]). In normal platelets, like in erythrocytes, anionic phospholipids, phosphatidylserine and phosphatidylethanolamine occupy the inner, cytoplasmic leaflet of the plasma membrane whereas choline containing phospholipids, phosphatidylcholine and sphingomyelin, are present at the outer leaflet. During activation of platelets, phosphatidylserine becomes exposed at the outer surface of the plasma membrane [18] and this localization is retained in microparticles [23, 24].

In spite of numerous studies on the formation and function of platelet-derived microparticles little is known about their composition. It has been reported that microparticles are carbohydrate-free protein-lipid vesicles [25]. Results of flow cytometry analyses indicate, however, that microparticles contain GpIb and GpIIb-IIIa glycoproteins [4, 26] the latter being most likely in the intact form [27]. Herein we report on ultrastructure, glycoprotein antigens, carbohydrates, protein, phospholipids, glycosphingolipids, cholesterol and ceramides in spontaneously formed microparticles. Preliminary results were presented at the XIV International Symposium on Glycoconjugates held in Zürich in September, 1997.

MATERIALS AND METHODS

***Chemicals*.** Phospholipid and carbohydrate standards were from Sigma Chemical Co. (St. Louis, U.S.A.). Sodium hydroxide for determination of carbohydrates by HPAEC was from J.T. Baker (B.V-Deventer, Holland). HPTLC plates and organic solvents were purchased from E. Merck (Darmstadt, Germany).

Isolation of platelets and platelet-derived microparticles. Human blood (450 ml) was collected into Quadrupole Blood Pack, Baxter, Health Care Corp. (Deerfield, U.S.A.), containing 63 ml of citrate-phosphate-dextrose-adenine-1 solution and immediately centrifuged in a J-6M/E centrifuge (Beckman-U.S.A.) at 1400 × g for 4.5 min at 22°C. The supernatant was centrifuged at 1500 × g for 20 min at 22°C and platelet sediment resus-

pended in about 1/3 volume of the original plasma in a PL-1240 container. Thus obtained platelet rich plasma (PRP) was subsequently stored for 18 h at 22°C with slow stirring in a platelet rotator (Helmer, Health Care Corp., Deerfield U.S.A.). Thereafter PRP was recentrifuged at 1500 × *g* for 20 min, and the plasma supernatant saved for isolation of microparticles. Platelets in the pellet were resuspended in and three times washed with 0.9% NaCl. Lastly platelets were counted in a Cell Dyn 1500 Hematology Analyser (Sequoia Turner, Mountain View, U.S.A). If remnants of other blood cells were present, platelet preparation was centrifuged once more at 80 × *g* for 4 min and the sediment discarded. Biochemical and other analyses were performed on platelets that contained less than 0.1% of leukocytes and erythrocytes.

Plasma supernatant containing microparticles was diluted with one volume of 0.9% saline and centrifuged at 1500 × *g* for 20 min at 22°C. Platelet pellet, if any, was washed with 0.9% NaCl, counted in a Hematology Analyser, and pooled with the main fraction of platelets. The supernatant was diluted again with one volume of 0.9% NaCl, centrifuged at 20000 × *g* for 15 min at 22°C and the sediment – containing platelet debris and residual platelets – discarded. The supernatant was centrifuged once more but at 33000 × *g* for 20 min at 22°C. The pellet contained microparticles.

Preparations of platelets and microparticles for biochemical analysis were dialysed overnight at 4°C against water and lyophilized.

Chemical analyses. Cholesterol was determined as described by Bhandaru *et al.* [29], lipid phosphorus according to Lowry *et al.* [30], sphingoid base as described by Higgins [31], and protein by the DC protein assay (Bio-Rad, U.S.A.) according to manufacturer's instruction.

Determination of carbohydrates. For determination of sialic acid, samples were hydrolysed with 0.2 M trifluoroacetic acid for 1 h at 80°C. To release neutral sugars and hexosamines, hydrolysis was performed with 2 M TFA for 4 h at 100°C. Monosaccharides including sialic acid were determined by high pH anion exchange chromatography with pulse amperometric detection (HPAEC with PAD) with a Dionex series of 4500i system (Dionex Co., Sunnyvale, U.S.A), a Carbopac PA-1 analytical column (4 mm × 250 mm) and a Carbopac PA-1 guard column (4 mm × 40 mm). The PAD had electrode potentials set on E_1 = +0.05V, E_2 = +0.6V, and E_3 = −0.6V with pulses of 300, 120 and 60 ms durations, respectively. Neutral sugars and hexosamines were eluted with 16 mM NaOH, and sialic acid with 150 mM NaOH. Separations were achieved isocratically at ambient temperature [32].

Determination of lipids and glycosphingolipids. Aliquots of freeze-dried platelets or microparticles (20–30 mg) were extracted sequentially with 10 ml portions of chloroform/methanol, 2:1 (v/v), chloroform/methanol 1:2 (v/v) and chloroform/methanol/water, 30:60:8 (by vol.). The extracts were pooled and dried under nitrogen. The residue was dissolved in chloroform/methanol (1:1, v/v) and divided into three portions *a*, *b*, and *c*, constituting of 0.1, 0.4 and 0.4 part of the initial volume, respectively. Fraction *a* was used for determination of total cholesterol and total lipid phosphorus. Fraction *b* was partitioned in a chloroform/methanol/water, 8:4:3 (by vol.) solvent mixture, and the lower (*bl*) and upper phase (*bu*) fractions were collected. In order to separate glycosphingolipids from phospholipids completely, fraction *bl* was acetylated and subsequently fractionated on SPE Florisil (6 ml) columns (J.T. Baxter, Deventer, Holland) according to Saito & Hakomori, [33]. Subsequently this material was deacetylated and fractionated on SPE Supelclean LC-Si (1 ml) columns (Supelco Inc., U.S.A.) into ceramides and glycosphingolipids [32]. The *bu* material was dialysed at 4°C against water, and freeze-dried. This material was subsequently fractionated on DEAE-Sephadex (Pharmacia, Sweden) in acetate form into residual neutral

glycosphingolipids and gangliosides, eluted, respectively with chloroform/methanol/water, 5:5:1 (by vol.) and then with the same solvent mixture but containing 0.2 M ammonium acetate. The residual neutral glycosphingolipids of fraction *bu* were pooled with neutral glycosphingolipids of fraction *bl* prior to the acetylation step. Total amounts of ceramides, neutral glycosphingolipids, and gangliosides in the respective fractions were quantitated from contents of sphingoid bases. To compare neutral and acidic glycosphingolipid composition of platelets and microparticles the extracts were analysed by thin-layer chromatography and HPAEC. Fraction *c* was used for determination of phospholipids after separation by one dimensional TLC on boric acid impregnated silica gel [34]. For localization of phospholipid spots the plates were sprayed with 5 mg% primuline in acetone/water, 4:1 (v/v) and inspected under UV lamp. Individual phospholipids were scraped off, extracted with chloroform/methanol, 1:1 (v/v) and analysed for phosphorus [30].

Flow cytometry. Platelets or microplatelets, previously washed with Tris-buffered saline, pH 7.4 (TBS), were incubated for 30 min with fluorescein isothiocyanate (FITC) labeled antibody CD41 (anti-GpIIb-IIIa) or FITC-antibody CD42b (anti-GpIb), at 4°C in the dark. After incubation, platelets or microparticles were washed twice with TBS and suspended in 1% paraformaldehyde in phosphate -buffered saline, pH 7.4 (PBS). In all washing steps platelets were centrifuged at 1500 × ***g*** for 10 min and microparticles at 33000 × ***g*** for 20 min. For double labeling we employed FITC-CD41 and phycoerythrin (PE) conjugated CD42b that were applied to platelets or microplatelets simultaneously. Murine FITC-IgG1 and PE-IgG1 were used as negative controls. All labeled antibodies were from Immunotech S.A. (France).

Phosphatidylserine on the surface of microparticles or platelets was detected with the recombinant annexin V (Annexin-V-Fluos) from Boehringer Mannheim (Germany). Platelets or microparticles (1×10^7) suspended in 200 μl aliquots of 10 mM Hepes buffer, pH 7.4, containing 140 mM NaCl, 5 mM $CaCl_2$ and 4 μl of annexin V were incubated for 15 min at room temperature. Thereafter samples were fixed for 15 min in 2% paraformaldehyde in PBS containing 3 mM $CaCl_2$, diluted with one volume of PBS, and analysed by flow cytometry. Fresh and ionophore A 23187 treated platelets were used as negative and positive controls, respectively. All samples were analysed with an Ortho Cytoron Absolute flow cytometer, version 1.7C from Ortho-Diagnostic System (Germany) equipped with an argon ion laser, wavelenght 488 nm.

Polyacrylamide gel electrophoresis and immunostaining for GpIb. Platelets or microparticles were boiled for 3 min in 0.062 M Tris/Cl buffer, pH 6.8, containing 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and electrophoresed on SDS/polyacrylamide slab gel by the method of Laemmli [35] using 4% concentrating gel and 10% separation gel. Staining for protein was made with Coomassie Brilliant Blue and that for carbohydrates with periodate-fuchsin. For immunostaining, bands were electroblotted onto nitrocellulose sheets (Hoefer-Pharmacia Biotech Inc. San Francisco, U.S.A) and stained with Ponceau S (Sigma) to confirm the efficiency of transfer. Thereafter blots were destained by washing with water, blocked with 3% bovine serum albumin (BSA) in TBS (BSA-TBS) and incubated with rabbit anti-GpIb in BSA-TBS for 20 h. We washed the blots five times for 10 min each with BSA-TBS and then incubated with mouse anti-IgG peroxidase conjugate (Sigma) for 3 h. After additional six washes, the enzymatic reaction was developed with 4-chloro-1 naphtol.

Electron microscopy. Microparticles were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 at 4°C for 1 h, centrifuged at 33000 × ***g*** for 20 min at 22°C and washed once with glutaraldehyde-free cacodylate buffer. Subsequently microparticles were postfixed with 1% osmium tetraoxide, dehy-

drated and embedded in Epon 812. Ultrathin sections were examined with a JEOL 1000 CX electron microscope after uranyl acetate and lead citrate staining.

Statistics. Statistical comparisons were made with the use of Student's *t*-test for unpaired samples.

RESULTS

In the present study microparticles were isolated by sequential centrifugation of saline diluted plasma. The last centrifugation at 33000 × ***g*** for 20 min resulted in sedimentation of practically all microparticles. Further centrifugation of the supernatant at 100000 × ***g*** for 1 h yielded no visible pellet. Ultrastructure of the microparticles, as shown in Fig 1, presents an array of rather regular vesicles with a mean diameter of 300 nm (50–600). Intact platelets were not observed. Large vesicles in 500–600 nm range were seen also in flow cytometry (see Fig. 2). Most vesicles reacted with FITC-CD41 (anti-GpIIb-IIIa) and FITC-annexin V but only 50–60% reacted with FITC-CD42 (anti-GpIb). Using double labeling with FITC-CD41 and PE-CD42 large microparticles reacted better than the small ones. In SDS/polyacrylamide gel electrophoresis microparticles exhibited a less intense protein staining in a high molecular mass region (Fig. 3a). This region contains cytoskeletal proteins and GpIb. Calpain proteolysis of the former has been postulated to be involved in the vesiculation mechanism [36, 37] but we have restricted our study to GpIb. Thus, the reduced content of the latter is shown by immunostaining of the electrophoregram with anti-GpIb (Fig 3b). There were significant differences in quantitative carbohydrate composition (Table 1) between microparticles and platelets, especially with respect to sialic acid, galactosamine, and glucosamine. Direct determinations of total lipid P, cholesterol and protein, calculated per dry mass, revealed that platelets contained 20.7% phospholipids and 66.7% protein while microparticles contained 17.2% phospholipids and 63.4% protein (in calculations of phospholipid content we assumed that 1 mg of lipid phosphorus is equivalent to 25 mg of phospholipid). Cholesterol content of platelets and microparticles amounted to 6.03% and 5.7%, respectively. Analysis of phospholipid classes showed a significant reduction of phosphatidylserine and phosphatidylcholine (see Table 2). On the other hand, sphingomyelin content of microparticles was

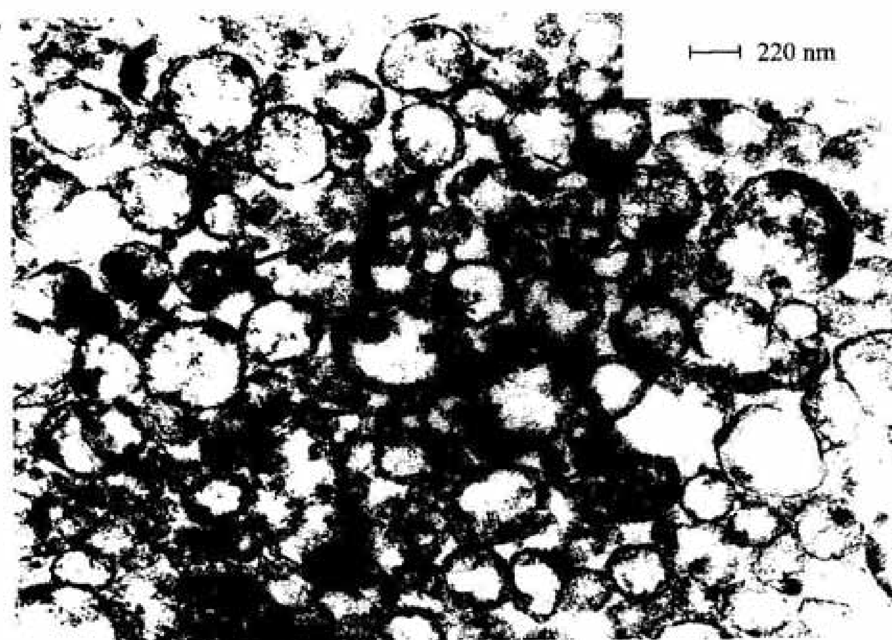

Figure 1. Electron micrograph of platelet-derived microparticles (×40000).

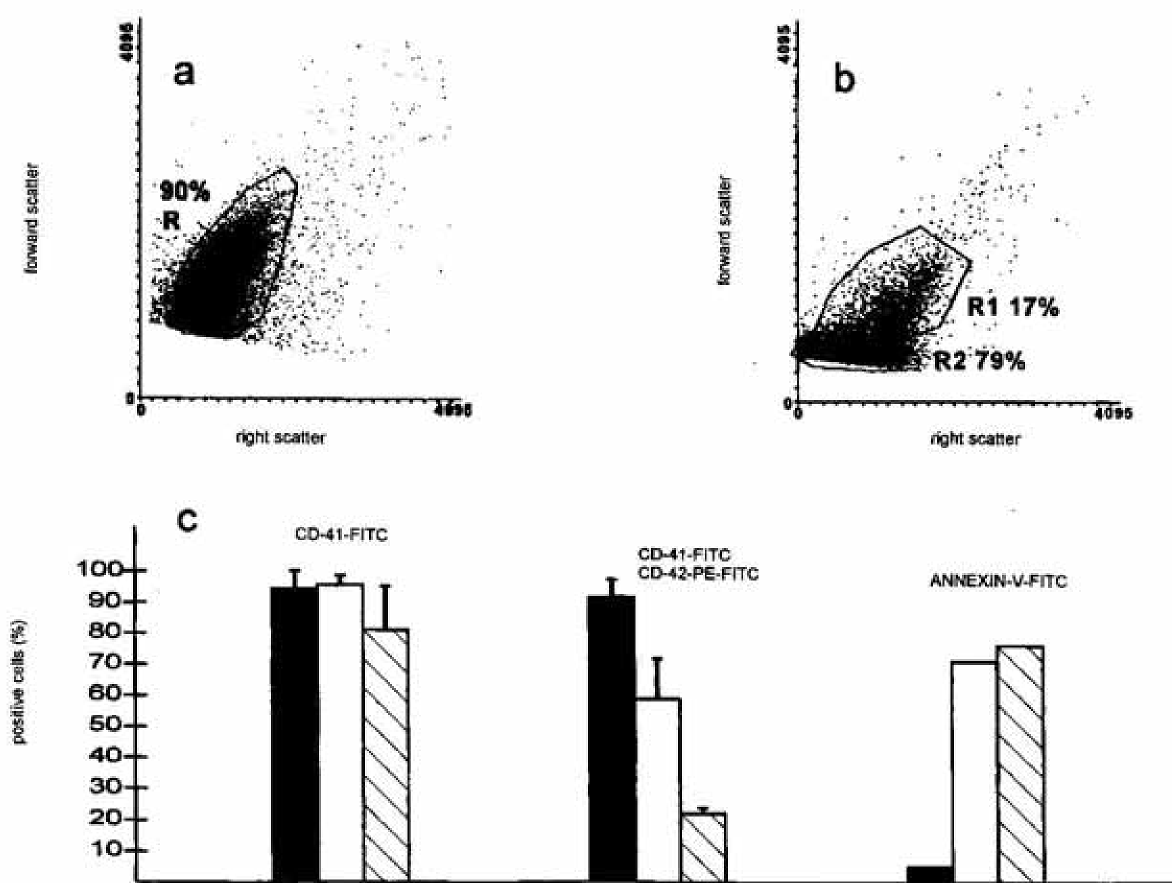

Figure 2. Flow cytometric analysis of platelets and platelet-derived microparticles.

(a, b) Dot plots of forward *versus* right scatter for platelets and platelet-derived microparticles. (a) Platelets, R; (b) large microparticles, R1; and small microparticles, R2; (c) reactivity of platelets, ■, large microparticles, □, and small microparticles, ▨ with FITC-CD41 (anti-GpIIb-IIIa), FITC-CD41, and PE-CD42 (double labeling with anti-GpIIb-IIa and anti-GpIb), and FITC-annexin V (reagent for phosphatidylserine). A 23187 ionophore-treated platelets reacted with FITC-annexin V in 61% (positive control, not showing in Fig. 2). T atop bars indicate standard deviation (n = 3). Bars without T represent means of two experiments.

almost exactly the same as in progenitor platelets. Contents in microparticles of neutral glycosphingolipids, gangliosides and ceramides were significantly reduced (Table 2). Qualitatively, however, the composition of neutral glycosphingolipids and gangliosides of platelets and microparticles, according to TLC, was similar, with predominant neutral glycosphingolipids being lactosylceramide, ceramide trihexoside (Galα1-4Galβ1-4Glcβ1-1Cer), and globoside (GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer), and the predominant ganglioside was hematoside (NeuAcα2-3Galβ1-4Glcβ1-1Cer) (not shown). Similarity of qualitative composition of glycosphingolipids in platelets and microparticles was supported by the identical molar ratios of carbohydrates in neutral and acidic fractions of platelets and microparticles (not shown). It should be pointed out that in both neutral and acidic fractions of glycosphingolipids glucose:sphingoid base molar ratios were close to 1:1.

DISCUSSION

Microparticles as obtained in this study had formed spontaneously partly *in vivo* and partly *ex vivo*. Formation of microparticles *ex vivo* occurred during short storage of platelets

Table 1. Carbohydrates in platelets and platelet-derived microparticles ± S.D.

	Platelets (n = 3)	Microparticles (n = 3)	Difference (%)
	nmoles/mg protein		
Total carbohydrates	293.5 ± 1.4 (292.2–294.9)	262.5 ± 21.2 (245.3–286.2)	–10.5
Fuc	11.1 ± 1.5 (10.2–12.9)	8.7 ± 2.4 (6.6–11.3)	–21.6
GalN	16.8 ± 2.9 (13.7–19.4)	8.7± 0.8 (7.9–9.4)	–48.2 ($P < 0.01$)
GlcN	96.1 ± 10.3 (84.4–103.8)	74.6 ± 1.8 (72.6–76.0)	–22.4 ($P < 0.02$)
Gal	55.0 ± 4.9 (50.1–59.8)	48.5 ± 0.4 (48.1–48.9)	–11.8 ($P < 0.05$)
Glc	44.3 ± 8.9 (38.0–54.4)	60.7 ± 22.3 (41.5–85.2)	+37.0
Man	45.1 ± 0.2 (43.1–47.7)	50.4 ± 1.2 (49.5–51.7)	11.8 ($P < 0.002$)
NANA	25.0 ± 4.5 (20.4–25.3)	10.9 ± 1.7 (9.9–12.9)	–56.4 ($P < 0.01$)

at 22°C before the separation of microparticles from platelets was carried out. Longer storage would produce higher yield of microparticles [11] but we have chosen the short storage time to minimize unphysiological conditions that might have affected the integrity of platelets and yield unspecific fragments. We prepared microplatelets by ultracentrifugation of platelet supernatants that had been already ultracentrifuged though at a lower speed. This might account for the fact that the preparations were practically free of intact platelets and platelet debris, as shown by electron microscopy.

Identity of microparticles was ascertained by their ultrastructure, reactions with antibodies to platelet specific glycoproteins GpIb and GpIIb-IIIa and the exposure of phosphatidylserine at the surface (reviewed in [2] and [3]), as demonstrated by binding of annexin V [24].

Practically all microparticles bound anti-GpIIb-IIIa but only some, predominantly the large ones, were positive with anti-GpIb. This was due to a reduced GpIb content in microparticles as shown by electrophoretic analysis. The reduction of GpIb content in microparticles resulted likely from partial degrada-

Table 2. Lipids and glycosphingolipids in platelets and platelet-derived microparticles.

Range in parentheses, ±S.D.

	Platelets (n = 3)	Microparticles (n = 3)	Difference (%)
	nmoles/mg protein		
Cholesterol	234.0 ± 1.7 (232–235)	233.0 ± 1.5 (232–235)	–0.4
Total phospholipids	428.0 ± 10.4 (422–440)	352.0 ± 7.6 (345–360)	–17.8 ($P < 0.001$)
Phosphatidylethanolamine	88.3 ± 5.6 (83.3–94.3)	77.7 ± 4.5 (72.5–82.4)	–12.0
Phosphatidylcholine	185.8 ± 8.4 (176.4–192.4)	143.7 ± 11.8 (131.2–154.8)	–22.6 ($P < 0.001$)
Phosphatidylserine	37.1 ± 0.8 (36.6–38.0)	21.1 ± 2.7 (18.0–22.9)	–43.2 ($P < 0.002$)
Phosphatidylinositol	27.7 ± 1.5 (26.2–29.7)	21.5 ± 4.6 (16.4–25.2)	–22.3
Sphingomyelin	89.0 ± 10.1 (78.1–98.0)	87.7 ± 17.4 (75.9–107.6)	–1.5
Free ceramides	1.9 ± 0.17 (1.8–2.1)	1.0 ± 0.1 (0.9–1.1)	47.0 ($P < 0.001$)
Neutral GSLs	2.0 ± 0.2 (1.9–2.2)	1.0 ± 0.2 (0.8–1.2)	–50.0 ($P < 0.001$)
Gangliosides	0.4 ± 0.03 (0.3–0.5)	0.2 ± 0.01 (0.8–0.2)	–50.0 ($P < 0.001$)

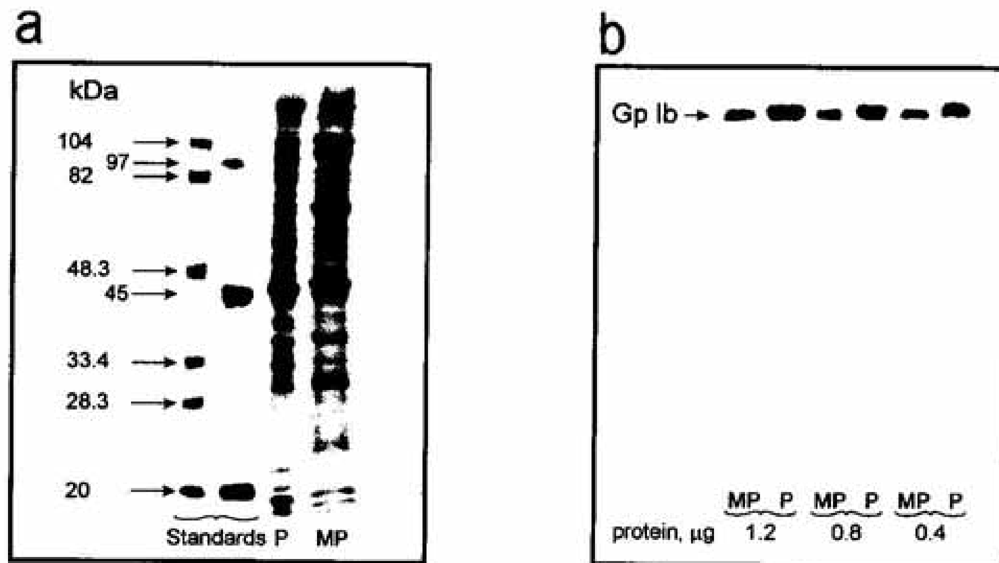

Figure 3. SDS/polyacrylamide gel electrophoresis of platelets (P) and platelet-derived microparticles (MP).

(a) Stained with Coomassie Blue; (b) immunostained for GpIb; nitrocellulose blot of gel slab was reacted sequentially with murine antibody against GpIb and rabbit anti-mouse IgG, conjugated to peroxidase.

tion of the glycoprotein in progenitor platelets during stimulation (reviewed in [38] and [39]). The degradation involves proteolytic cleavage of GpIb with a release of carbohydrate rich glycopeptide, glycocalicin that is a carrier of most of platelet sialic acid [40]. The glycopeptide contains mostly O-linked glycans and apart of sialic acid contains galactosamine, glucosamine, and galactose. Glycocalicin contains also some fucose [41]. The same sugars were found to be reduced in microparticles. Thus, it is reasonable to assume that they were lost from progenitor platelets as components of glycocalicin. The differences in anti-GpI binding between large and small microparticles could be due to a limited surface area of the latter that would accomodate fewer glycoprotein molecules; indeed, the calculated surface area of very small microparticles was 144 times lower than that of the largest ones. Thus, when GpIb content was reduced through proteolysis, the residual GpIb in very small microparticles might be insufficient to bind significant number of antibody molecules. On the other hand the mannose content of microparticles was sligtly higher than in platelets suggesting that N-linked glycoproteins, like GpIIb-IIIa were not significantly degraded in progenitor platelets. This conclusion is in keeeping with the results of flow cytometry analysis of microparticles.

With respect to protein and lipid contents microparticles resembled platelets rather than platelet membranes because the latter are enriched in cholesterol and phospholipids [42] whereas our preparation of microparticles were clearly depleted of phospholipids. Thus, our material was different from that analysed by Sandberg *et al.* [25], which in terms of lipid and protein contents was similar to platelet plasma membranes, and in addition was reported to be carbohydrate-free.

Apart of GpIb also glycerophospholipids are known to be partly degraded in stimulated platelets [43]. Low content of glycerophospholipid but not of sphingomyelin was found by us also in microparticles. This suggested again that a prior stimulation of progenitor platelets was a probable cause. GpIb is a signaling transmembrane glycoprotein (reviewed in [38] and [39]) that should activate glycerophospholipid hydrolysis because of its close contact with phospholipase A_2 (14-3-3 protein) on the cytoplasmic side of platelet

plasma membrane [44]. The binding site for the enzyme in GpIba has been recently identified [45].

Thus, we may conclude that signaling through GpIb is probably an important event in vesiculation mechanism. A disproportionally large loss of phosphatidylserine from microparticles is, however, intriguing and cannot be readily explained. According to the recent study on phospholipid hydrolysis in thrombin stimulated platelets, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol were degraded to a similar extent of 40–50% while hydrolysis of phosphatidylcholine was less extensive [43]. Further studies are required on this point but it should mentioned that a new phosphatidylserine-specific phospholipase has been recently identified in rat platelets [46].

The decrease in microparticles of ceramides and glycosphingolipids including gangliosides is also of interest. Ceramide is a bioactive substance that, among others, is implicated in regulation of signaling, immune response, apoptosis (reviewed in Ref. [47]) and senescense [48]. We do not think that glycosphingolipids or ceramides are degraded in progenitor platelets. A more probable explanation is that glycosphingolipid-rich domains of the plasma membrane, like for instance caveolae (reviewed in [49]) or glycosphingolipid-enriched microdomains [50], do not significantly participate in vesicle formation.

The overall conclusion of this paper is that in terms of composition, microparticles witness the past activation of progenitor platelets.

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