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Low-Density Lipoprotein Specifically Binds Glycoprotein IIb/IIIa: A Flow Cytometric Method for Ligand-Receptor Interaction

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Primary platelet aggregation requires agonist-mediated activation of membrane receptor glycoprotein (GP) IIb/IIIa, binding of fibrinogen to GpIIb/IIIa, and cellular events after fibrinogen binding. This study investigated whether fibrinogen receptor GpIIb/IIIa is also the binding site for low-density lipoprotein (LDL) in platelets by using GpIIb/IIIa-coated polystyrene microbeads incubated with various concentrations of fluorescein isothiocyanate (FITC)-labeled ligands. Binding was assayed by flow cytometry. Binding of fibrinogen (Fg)-FITC and LDL-FITC to GpIIb/IIIa coated microbeads was concentration dependent, reaching saturation. Binding of LDL-FITC to GpIIb/IIIa coated microbeads was inhibited by fibrinogen. Binding of LDL-FITC or Fg-FITC to freshly isolated platelets gave similar results as those of GpIIb/IIIa coated microbeads. Glycoprotein IIb/IIIa, the fibrinogen receptor on platelets is also one of the binding sites of LDL on platelets. This rapid and reliable flow cytometric technique using coated microbeads may be used as a first step for the study of all ligand receptor interactions.

Keywords: low-density lipoprotein; glycoprotein IIb/IIIa; platelets; flow cytometry; microbeads

Flow cytometry has been used as a powerful technique for obtaining the structural and functional information about intact cells. Multiplex assays using fluorescent microspheres are exciting techniques that have been gaining popularity in areas of research.[1]

Circulating platelets are important in hemostasis and have also been implicated in the pathogenesis of atherosclerosis, arterial thrombosis, and cardiovascular diseases.[2,3] The human platelet membrane receptor glycoprotein (Gp) IIb/IIIa binds fibrinogen and is known to mediate fibrinogen binding, leading to platelet aggregation.[4-6] Upon activation of platelets, GpIIb/IIIa-related changes occur where ligand-binding sites become available on the platelet membrane.[7,8] The interactions of platelets with plasma lipoproteins, monocyte-derived macrophages, and the arterial wall are very important in hemostasis.[9,10] Activated platelet aggregates and cholesterol-rich lipoproteins are present in atherosclerotic plaque and they participate in atherosclerotic lesion formation.[11-14]

The atherogenic lipoproteins, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), alone induce platelet activation and have stimulatory effects on the actions of strong agonists such as thrombin.[15-17] Oxidized lipoproteins, particularly oxidized LDL, are thought to play a major role in atherogenesis.[18-20] Studies have demonstrated that the gold-labeled native LDL binds to washed human platelets and they appear on the platelet membrane and on the open canalicular system.[21,22] Preincubation of the platelets with polyclonal antibodies against GpIIb/IIIa inhibits the binding of native LDL and fibrinogen.[21]

These competitive experiments suggest specificity of the interaction between LDL and possibly its binding site GpIIb/IIIa on platelets. However, contradictory

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to this observation are reports stating that the platelet LDL receptor is different from the classic LDL receptor and fibrinogen receptor and that GpIIb/IIIa is not implicated in the binding of LDL to intact resting platelets.[23,24] There are other observations indicating that CD36 (antibody for GpIV, IIIb) is related to the binding of both oxidized and native LDL.[25-27]

The aim of this study was to find an answer to the question of whether or not platelet GpIIb/IIIa is also the binding site for LDL on platelets. For this purpose, flow cytometry was used to investigate ligands labeled with fluorescein isothiocyanate (FITC), such as fibrinogen, LDL, bovine serum albumin (BSA, as a negative control), and monoclonal antibody specific for GpIIIa, as well as CD61 (as a positive control) bindings to both GpIIb/IIIa-coated microbeads and isolated platelets.

Materials and Methods

Peptide gly-arg-gly-asp-ser-pro (GRGDSP) and commercial GpIIb/IIIa were obtained from Calbiochem (EMD Biosciences, San Diego, Calif). Cyanogen bromide-activated Sepharose was purchased from Pharmacia (Piscataway, NJ), polystyrene microbeads (1µ diameter) were purchased from Bangs Laboratories (Fishers, Ind), and CD61-FITC was purchased from Becton Dickinson (Franklin Lakes, NJ). Fibrinogen, LDL, FITC, BSA, fetal calf serum (FCS), Tyrode's buffer, phenyl methyl sulfonyl fluoride (PMSF), phosphate-buffered saline (PBS), and dimethylsulfoxide (DMSO) were obtained from Sigma (St Louis, Mo). The other chemicals were reagent grade and purchased from Sigma.

Purification of Glycoprotein IIb/IIIa From Platelets

Glycoprotein IIb/IIIa was purified from octylthioglucoside extracts of human platelets, followed by affinity chromatography using heptapeptide GRGDSP-Sepharose according to the method of Pytela, with some modifications (column size, 1 × 17 cm).[28,29] Isolated platelets obtained by platelet apheresis were washed with Tyrode's buffer containing 1 mM of calcium chloride, 1 mM of magnesium chloride, and 50 mM octylthioglucoside in phosphate-buffered saline (PBS; pH 7.3). After centrifugation at 2200 rpm (Universal K2S centrifuge, Andreas Hettich Gmbh, Tuttlingen, Germany), the pellet was suspended in the solution of 10 mM of phosphate, 150 mM of sodium chloride, 1 mM of calcium chloride, and 1 mM of phenylmethylsulfonyl fluoride (PMSF). The suspension was centrifuged at 14 250 rpm (Ultracentrifuge, Sigma 3K30, Steinheim, Germany) for 20 minutes at 4°C, and the supernatant (crude platelet extract) was added to GRGDSP-Sepharose, which was prepared by incubating 100 mg of GRGDSP peptide with 3.5 grams of cyanogen bromide–activated Sepharose.

After overnight incubation at 4°C, the affinity material was packed into a column and washed with equilibrium buffer (PBS containing 1 mM of PMSF). The protein bound to the matrix was eluted with the equilibrium buffer containing 1 mg/mL GRGD peptide. Fractions of 1 mL were collected with a flow rate of 0.96 mL/min. The amount of protein was measured by the method of Lowry et al.[30] Each fraction was applied to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 7.5% polyacrylamide.[31]

Preparation of Polystyrene Microbeads Coated With Glycoprotein IIb/IIIa

Polystyrene microbeads (5 uL, 100 µg/mL) were centrifuged at 13 000 rpm in an Eppendorf Centrifuge 5415R (Westbury, NY) at 4°C for 5 minutes, washed twice with 1 mL of 1M sodium bicarbonate ($NaHCO_3$)/sodium carbonate (Na_2CO_3) buffer (pH 9.6) and suspended in 1 mL of the same buffer. Then, 5 µL, 2.5 µL, or 1 µL from the suspension of washed microbeads was added to 1 mL of purified or commercial GpIIb/IIIa (80 µg/mL) and incubated at 4°C overnight with gentle mixing.[32]

Conjugation of Ligands With Fluorescein Isothiocyanate

Low-density lipoprotein, fibrinogen, and BSA were conjugated with FITC according to the protocol described by Xia et al.[33] Briefly, 100 µL of ligand (1 mg/mL), 10 µL of FITC (1 mg/mL DMSO), and 900 µL of 1M $NaHCO_3$/Na_2CO_3 buffer (pH 9.6) were mixed gently at 4°C. Fluorescein isothiocyanate-conjugated ligands were isolated by Sephadex G-25 gel filtration (column size, 10 × 30 mm; elution buffer, PBS). To verify the FITC labeling of ligands, spectrophotometric measurements were performed at 280 nm and 495 nm.[33]

Flow Cytometric Measurements

A Becton Dickinson FACScan analyzer was used to quantify fluorescence (excitation wavelength, 488 nm; emission wavelength, 530 nm) at the single-cell level, and data were analyzed using CellQuest 3.3 software (Becton Dickinson). In each sample, the mean fluorescence intensity of the analyzed GpIIb/IIIa-coated microbeads or platelets was determined. After gating the platelets or microbeads population by forward and side light scatter, signals were recorded on a dot plot. In total, 60 000 cells were acquired, and debris (located on the bottom left corner of the dot plot) was excluded by prior gating, thereby limiting platelet or microbead populations, which usually contained 10 000 populations. Fluorescence intensity of FITC-labeled ligand bound to GpIIb/IIIa-coated microbeads or platelets was recorded on a frequency histogram. The percentage of gating was assumed as the percentage of bound ligand–FITC.

Isolation of Platelets

Platelet-rich plasma was obtained by platelet apheresis from 12 healthy donors (200 000-300 000/mm^3) who had not ingested any medication for at least 10 days. To obtain platelet-containing supernatant, platelet-rich plasma was centrifuged at 1500 rpm at room temperature for 10 minutes. To prepare diluted FCS solution, 1 mL of commercial FCS was diluted to 100 mL with PBS. For binding studies, 1 mL of platelet-containing supernatant was added to 1 mL of diluted FCS solution and then centrifuged at 3000 rpm at room temperature for 10 minutes. The platelet pellet was suspended in 1 mL of diluted FCS solution and used for ligand-binding studies within 2 hours.

Ligand-Binding Assays

Increasing volumes (0-100 µL) of BSA-FITC, fibrinogen (Fg)-FITC, and LDL-FITC were prepared from stock solution (10 mg/mL, 1 M $NaHCO_3$/Na_2CO_3 buffer, pH 9.6) and 10 µL of CD61-FITC was added separately into the tubes containing 5-µL suspensions of microbeads coated with GpIIb/IIIa. Then this solution was diluted to 100 µL with PBS and incubated at room temperature in the dark for 30 minutes. Ligand-bound microbeads were centrifuged at 10 000 rpm for 5 minutes, washed with 1 mL of PBS, and suspended in 1 mL of PBS before flow cytometric measurements. A similar procedure was used for detection of binding of ligands to isolated platelets, which were washed and suspended in diluted FCS. Then, 0 to 100 µL of FITC-labeled ligands (10 mg/mL), completed to 100 µL with diluted FCS solution, was added to 100 µL of platelet suspension at room temperature, incubated in the dark for 30 minutes, and washed with diluted FCS solution. It was then centrifuged at 10 000 rpm for 5 minutes, and the pellet was suspended in 1 mL of diluted FCS solution. Fluorescein intensity was measured by flow cytometry and the percentage of gating was recorded (see "Flow Cytometric Measurements"). The 12 samples were studied in duplicates.

Competition Assays of Ligand Binding to Glycoprotein IIb/IIIa–Coated Microbeads

To detect the effects of unlabeled ligands on FITC-labeled ligand-binding to GpIIb/IIIa-coated microbeads, maximum binding concentrations of unlabeled fibrinogen (12.14 µg/mL) or unlabeled LDL (14.74 µg/mL) were added to 5 µL of the GpIIb/IIIa-coated microbead solution, and diluted with 1 mL PBS, and preincubated in the dark at room temperature for 30 minutes. Increasing concentrations of LDL-FITC or Fg-FITC were added to each tube and incubated for 30 minutes at room temperature, in the dark. The suspension was centrifuged at 10 000 rpm at room temperature for 5 minutes using the Eppendorf centrifuge. The pellet was washed and completed to 1 mL with PBS, and flow cytometric analysis was performed.

Competition Assays on Ligand Binding to Isolated Platelets

To detect the effect of purified unlabeled GpIIb/IIIa (10 µL) on the binding of LDL-FITC or Fg-FITC to isolated platelets, 100 µL (500 µg/mL) of isolated platelet suspension in diluted FCS was added to a series of tubes containing 10 µL of purified GpIIb/IIIa (1.82 µg/mL). Then the suspension was completed to 200 µL with diluted FCS. After incubation at room temperature for 30 minutes in the dark, increasing volumes (10-100 µL) of LDL-FITC (10 mg/mL) or Fg-FITC (10 mg/mL) were added and incubated for 30 minutes. The suspensions were diluted with 1 mL of diluted FCS and centrifuged at 10 000 rpm at room temperature for 5 minutes.

Flow cytometric measurements were performed within 2 hours.

To detect the effect of unlabeled LDL on the binding of Fg-FITC to isolated platelets, 80 µL (14.74 µg/mL) of unlabeled LDL was added to 100 µL (500 µg/mL) of isolated platelet suspension in the presence of diluted FCS. This solution was incubated at room temperature for 30 minutes in the dark. Increasing concentrations of Fg-FITC were added, and incubations were continued for 30 more minutes. Samples were diluted to 1 mL with diluted FCS and centrifuged at 10 000 rpm for 5 minutes at room temperature. Flow cytometric measurements were done within 2 hours.

Statistical Analysis

The results for the 12 samples were expressed as mean value ± the standard deviation. Statistical analyses were performed with the Student *t* test for paired data, and values of $P < .05$ were considered significant.

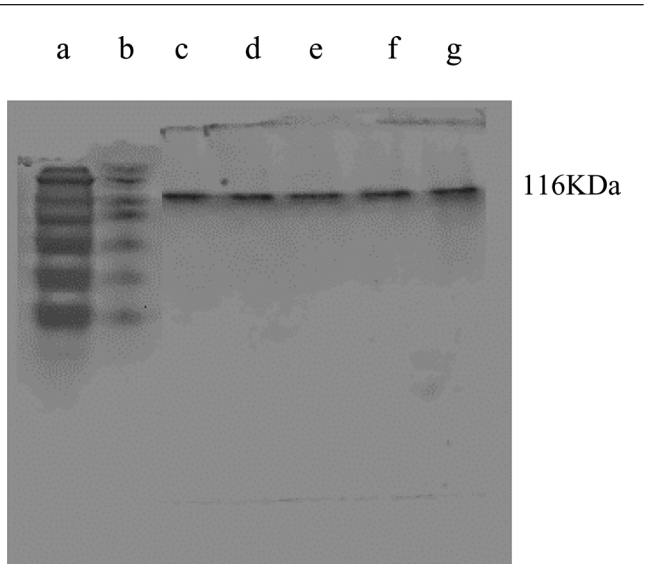

Figure 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of purified glycoprotein IIb/IIIa. a: molecular weight markers myosin, 205K; β-galactosidase, 116K; phosphorylase b 97K; bovine serum albumin, 66K; ovalbumin, 45K; and carbonic anhydrase, 29K. b: crude platelet extract before affinity chromatography. c-g: fractions from the affinity chromatography.

Results

Purified GpIIb/IIIa complex obtained by affinity chromatography was determined to have 116 KDa molecular weight by SDS-PAGE (Figure 1).

Because CD61-FITC specifically recognizes GpIIIa, it was used as a positive control to detect the fibrinogen-receptor activation and ligand-binding specificity of GpIIb/IIIa from affinity chromatography, and BSA-FITC was the negative control. Figure 2A shows gating of GpIIb/IIIa-coated microbeads in flow cytometry. Flow cytometric results showed 90% binding of CD61-FITC to the purified GpIIb/IIIa coated microbeads, indicating that the purified protein was GpIIb/IIIa (Figure 2B), whereas BSA-FITC showed no binding to GpIIb/IIIa-coated microbeads (Figure 2C).

Figure 3A shows gating of isolated platelets in flow cytometry and CD61-FITC binding to isolated platelets is shown in Figure 3B.

At different volumes of GpIIb/IIIa-coated microbead suspensions, the percent bound Fg-FITC remained constant, whereas the percentage of bound CD61-FITC differed significantly. No binding was observed with BSA-FITC. In all of the other experiments, 5 µL of GpIIb/IIIa coated microbeads was used (Table 1).

Similar results were found between purified GpIIb/IIIa-coated microbeads and commercial GpIIb/IIIa-coated microbeads, as shown in Table 2. Purified GpIIb/IIIa was used in all of the binding studies. The binding of Fg-FITC to GpIIb/IIIa-coated microbeads was dependent on temperature (Figure 4) and incubation time (Figure 5). A 30-minute incubation time and a 22°C incubation temperature were selected for all of the binding studies.

Binding of Fg-FITC or LDL-FITC to GpIIb/IIIa-coated microbeads was found to be concentration dependent, reaching saturation. The concentrations of 12.14 µg/mL of Fg-FITC and 14.74 µg/mL of LDL-FITC were evaluated as maximum binding concentrations (Figure 6). Beyond these concentrations, self-inhibitions of FITC-labeled ligand binding were observed.

Figure 7 shows the unlabeled fibrinogen loading effect on LDL-FITC binding to GpIIb/IIIa-coated microbeads. The binding of LDL-FITC to GpIIb/IIIa-coated microbeads was inhibited 50% on preincubation with fibrinogen (12.14 µg/mL). Fibrinogen-FITC binding to GpIIb/IIIa-coated microbeads was inhibited 30% upon preincubation with unlabeled LDL (14.74 µg/mL). Inhibition of LDL-FITC ($P < .0001$) and Fg-FITC bindings ($P < .001$)

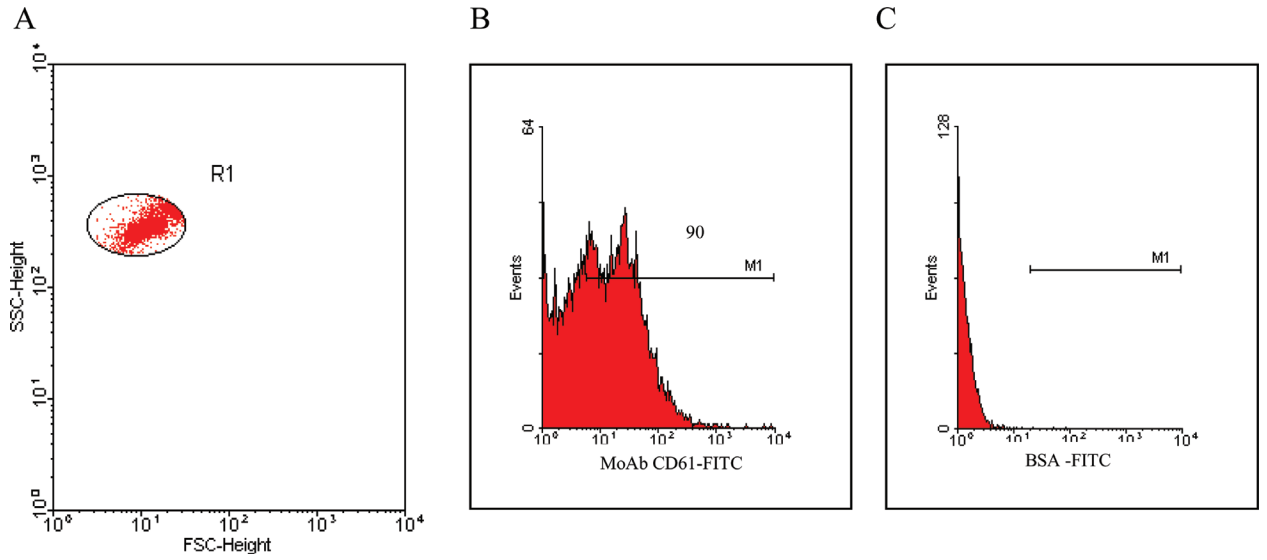

Figure 2. FACScan analysis of monoclonal antibody for GpIIIa (CD61)-fluorescein isothiocyanate (FITC) and bovine serum albumin (BSA)-FITC binding to glycoprotein (Gp) IIb/IIIa-coated microbeads. (A) Typical dot plot of GpIIb/IIIa-coated microbeads (R1) shows the spread of the total recorded "events" calculated by their forward and side light scatter. The eclipse-shaped R1 area represents the gated cell population (or GpIIb/IIIa-coated microbead). (B) Binding of CD61-FITC to GpIIb/IIIa coated microbeads. Analysis of CD61-FITC fluorescence was performed on the gated GpIIb/IIIa-coated microbeads. (C) BSA-FITC binding to GpIIb/IIIa-coated microbeads. Analysis of BSA-FITC fluorescence was not detected on the gated cell populations. SSC = side scatter; FSC = forward scatter; MoAb = monoclonal antibody.

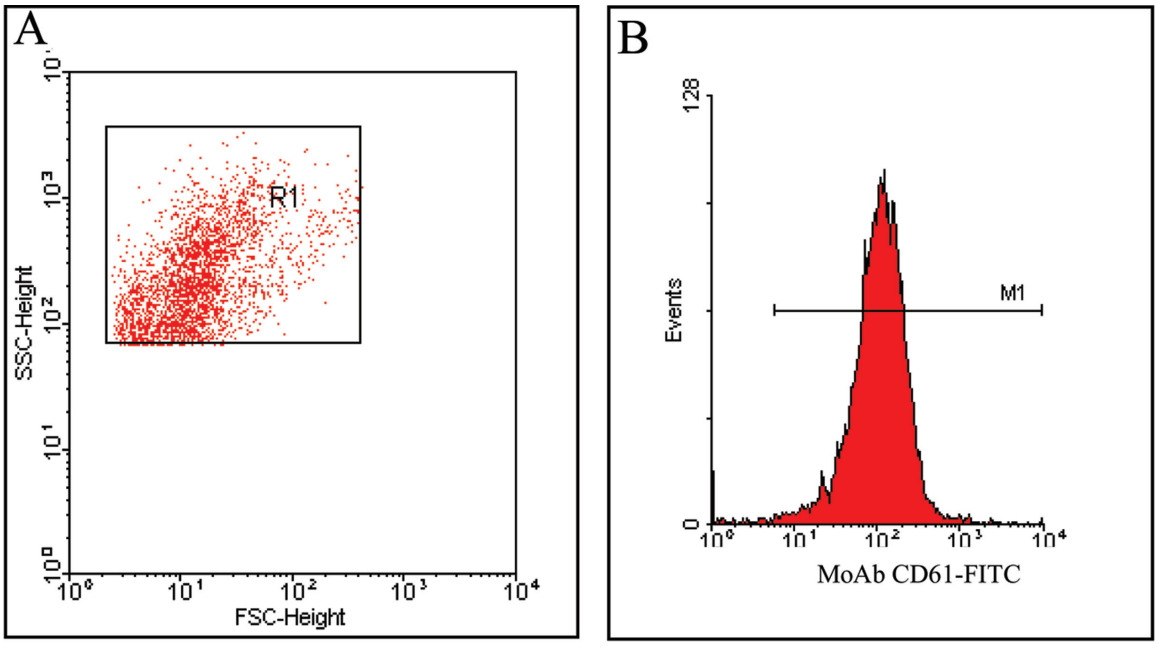

Figure 3. Binding of monoclonal antibody for glycoprotein (Gp) IIIa (CD61)-fluorescein isothiocyanate (FITC) to isolated platelets. (A) Typical dot plot of platelets (R1) shows the spread of the total recorded "events" calculated by their forward (FSC) and side (SSC) light scatter. The shaded quaternary-shaped R1 represents the gated cell population. Gated cell populations usually contain 10 000 platelets. (B) Binding of CD61-FITC to isolated platelets. Analysis of monoclonal antibody (MoAb)-CD61-FITC fluorescence was performed on the gated cell populations.

Table 1. Percentage of Binding to Different Concentrations of Glycoprotein IIb/IIIa-Coated Microbeads[a]

GpIIb/IIIa-Coated Microbead Solution	CD61-FITC % Bound	Fg-FITC % Bound	BSA-FITC % Bound
1 µL	55 ± 14	60 ± 15	0
2.5µL	82 ± 20	62 ± 18	0
5 µL	78 ± 16	62 ± 16	0

NOTE: Gp = glycoprotein; FITC = fluorescein isothiocyanate; Fg = fibrinogen; BSA = bovine serum albumin.
[a]Concentrations were CD61-FITC, 10 µL; Fg-FITC, 70 µL; and BSA-FITC, 100 µL.

Table 2. Percentage of Binding to Purified and Commercial Glycoprotein IIb/IIIa-Coated Microbeads

GpIIb/IIIa	Fg-FITC % Bound (12.14 µg/mL)	LDL-FITC % Bound (14.74 µg/mL)
Commercial	68.13 ± 4.13	70.52 ± 2.20
Purified	63.51 ± 6.27	67.65 ± 5.74

NOTE: Gp = glycoprotein; Fg = fibrinogen; FITC = fluorescein isothiocyanate; LDL = low-density lipoprotein.

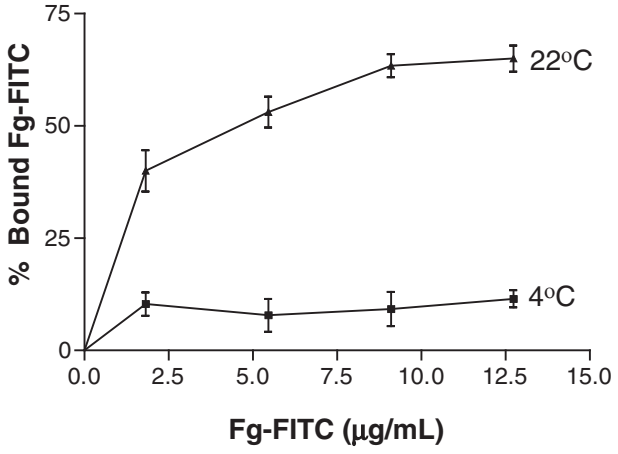

Figure 4. Effect of incubation temperature on fibrinogen (Fg)-fluorescein isothiocyanate (FITC) binding to glycoprotein (Gp) IIb/IIIa-coated microbeads (incubation time, 30 minutes). Results are presented with the standard deviation (range bars).

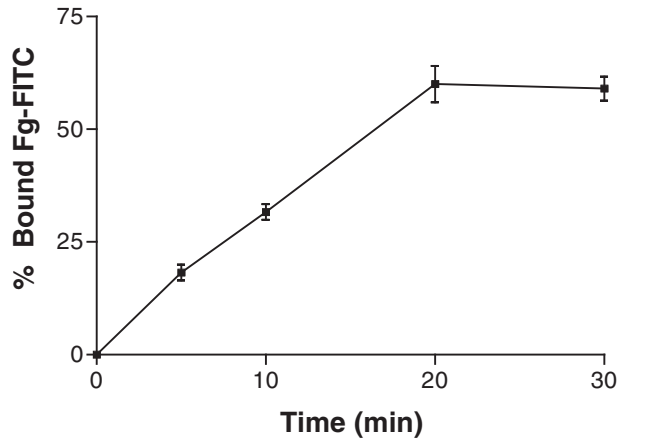

Figure 5. Effect of incubation time on fibrinogen (Fg)-fluorescein isothiocyanate (FITC) binding to glycoprotein (Gp) IIb/IIIa-coated microbeads at 22°C (Fg-FITC, 12.14 µg/mL). Results are presented with the standard deviation (range bars).

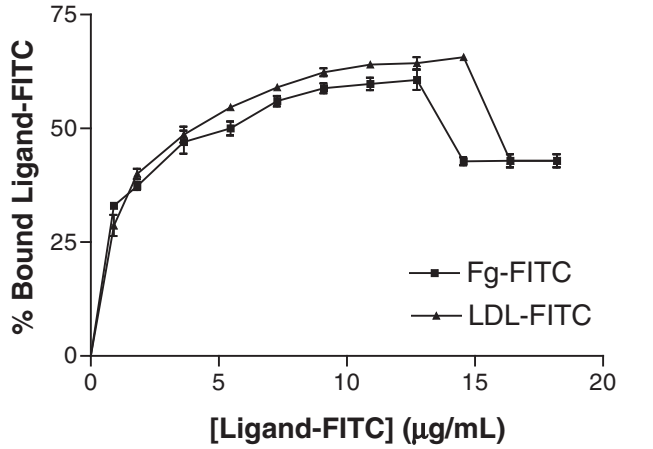

Figure 6. Ligand concentration dependency of the percentage of bound ligand fluorescein isothiocyanate (FITC) to purified glycoprotein (Gp) IIb/IIIa-coated microbeads is shown. Results are presented with the standard deviation (range bars). Fg = fibrinogen; LDL = low-density lipoprotein.

at 9.1 µg/mL ligand-FITC concentrations were found highly statistically significant.

Figure 8 shows Fg-FITC and LDL-FITC binding to isolated platelets. Ligand-FITC bindings were found to be concentration dependent and reached saturation. Preincubation with unlabeled GpIIb/IIIa blocked LDL-FITC binding to isolated platelets 100% but had no effect on Fg-FITC binding to platelets.

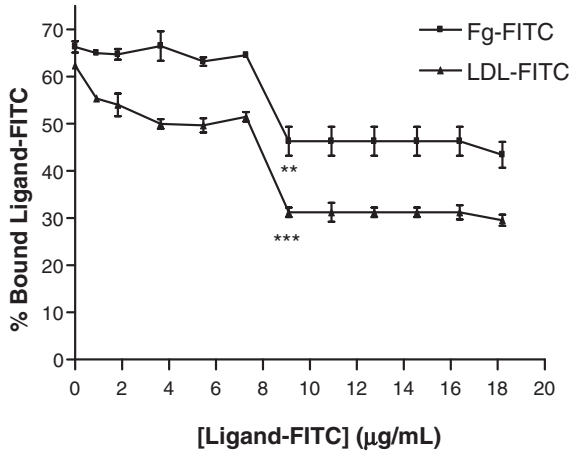

Figure 7. Effect of preincubation with unlabeled ligand on ligand-fluorescein isothiocyanate (FITC) binding to glycoprotein (Gp) IIb/IIIa-coated microbeads. Results are presented with the standard deviation (range bars). $^{**}P < .001$, $^{***}P < .0001$. LDL = low-density liporotein; Fg = fibrinogen.

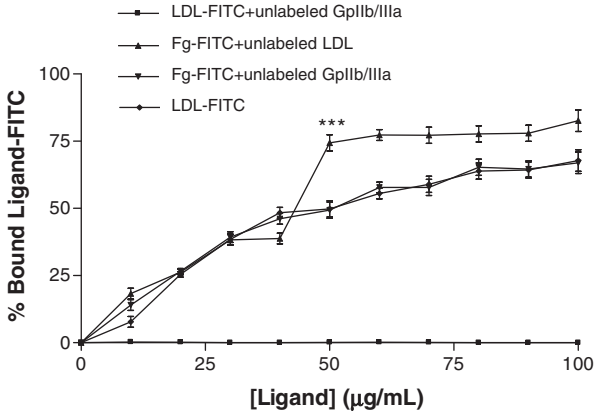

Figure 8. Ligand concentration dependency on the percentage of bound ligand fluorescein isothiocyanate (FITC) to isolated platelets, the effect of glycoprotein (Gp) IIb/IIIa on low-density lipoprotein (LDL)-FITC and fibrinogen (Fg)-FITC bindings, and the effect of LDL on Fg-FITC binding to platelets. Results are presented with the standard deviation (range bars). $^{***}P < .0001$.

Preincubation with unlabeled LDL stimulated Fg-FITC binding to isolated platelets at Fg-FITC concentrations exceeding 50 µg/mL.

Discussion

The interaction between the platelet surface and lipoproteins is important in platelet functions. Platelets cannot synthesize cholesterol, and they use megacaryocytes or lipoproteins as cholesterol sources.[34] Binding of lipoproteins to platelets is reported in the literature.[34,35] The studies are concentrated on the idea that platelet fibrinogen receptor GpIIb/IIIa is the binding site for lipoproteins.[36]

Glycoprotein IIb/IIIa complex purification is the primary step for ligand-binding experiments. According to the purification techniques, different molecular weights are reported for GpIIb, GpIIIa, and GpIIb/IIIa complex.[36-39] Koller at al[36] reported that 2 protein bands were obtained by affinity chromatography with 140 000 and 114 000 MW. They showed fibrinogen binding only to 114 000 MW protein and named it GpIIb/IIIa.[36]

In this work, GpIIb/IIIa complex was purified by affinity chromatography and its MW was determined as 116 000 KDa by SDS-PAGE. Because it is reported that dissociated GpIIb and GpIIIa subunits have altered ligand-binding characteristics, this 1-step affinity chromatography method is advantageous in obtaining biologically active protein.[39]

Various investigations have used pure monoclonal antibodies specific to this protein.[40] In this work, CD61-FITC antibody specific for GpIIIa was used as the positive control for detecting GpIIb/IIIa complex activity, and BSA-FITC was used as the negative control.

There are reports on the specific binding of lipoproteins to isolated activated platelets. The binding studies showed 1500 binding sites for LDL and 3200 binding sites for high-density lipoprotein (HDL),[34,41] and HDL and LDL also had different binding sites on platelets.[42] The major HDL lipoprotein, apolipoprotein A-I, binds specifically to platelets.[43] A group of investigators also showed that proteases inhibit ^{125}I-LDL binding to fibroblasts, but have no effect on platelets.[24] Fibrinogen binding to platelets was stimulated by LDL in protease stimulated platelets. These researchers concluded that the LDL receptor was different than the fibrinogen receptor.[24]

In recent decades, flow cytometry has become an extremely useful technique and has growing importance in the study of platelets for clinical applications.[44-46] Basically, preparations of whole blood, platelet-rich plasma, and washed platelets are commonly suitable for flow cytometric analyses.[47]

A method using directly purified receptors in ligand receptor–binding studies by flow cytometry is not described in the literature so far. The use of receptor-coated polystyrene microbeads in ligand-binding

assays, as established in this study, is a direct method that seems to be safe and dependable. We have successfully conjugated ligands such as LDL, fibrinogen, and BSA with FITC and conducted their binding studies to GpIIb/IIIa-coated microbeads or to platelets with flow cytometry,[33] because a linear relationship exists between the relative fluorescein intensity and the number of bound FITC-ligands in the flow cytometric method. Our results also show that FITC-conjugated fibrinogen and LDL bind both to GpIIb/IIIa-coated microbeads and to isolated platelets in a concentration-dependent manner, reaching saturation. In our other ligand-binding studies such as with CD61-FITC, HDL-FITC, and BSA-FITC binding to GpIIb/IIIa, we observed similar results showing specificity of this flow cytometric method.[48,49] This method, in addition to showing quantitative ligand-binding to the receptor, also reflects binding conditions in the circulating platelets.

We observed that isolated GpIIb/IIIa-coated microbeads and whole platelets differed in their interaction with LDL. Low-density lipoprotein acted as an inhibitor on fibrinogen binding to GpIIb/IIIa-coated microbeads, showing competition in binding to isolated GpIIb/IIIa, but high concentrations of LDL stimulated fibrinogen binding to platelets. The interaction of LDL with whole platelets could be realized in 2 ways. Because LDL is an inducer for platelet activation, it stimulates fibrinogen binding to whole platelets[50,51] and also induces atherosclerotic plaque formation by transferring lipids to the platelet membrane.[52] Contrary to this result, other investigations have reported that binding of LDL to GpIIb/IIIa is not involved in its platelet activation effect.[50]

We observed in this work that preincubation of platelets with GpIIb/IIIa inhibited LDL-FITC binding to platelets completely but had no effect on fibrinogen binding to platelets. This shows that fibrinogen and LDL-binding mechanisms or sites to GpIIb/IIIa are different. Conversely, incubation of platelets with LDL stimulated fibrinogen binding to platelets acting as a platelet activator, indicating that LDL increases the exposure of fibrinogen-binding sites on platelets. Our results are in accordance with the literature.[21,50]

Conclusion

Glycoprotein IIb/IIIa is also the receptor for LDL on platelets, as seen in the specific binding of LDL-FITC and Fg-FITC to purified GpIIb/IIIa-coated microbeads. The binding of LDL-FITC and Fg-FITC to both platelets and purified GpIIb/IIIa-coated microbeads was dependent on concentration and temperature. Unlabeled fibrinogen inhibited both LDL-FITC binding and Fg-FITC binding to GpIIb/IIIa-coated microbeads.[53]

Glycoprotein IIb/IIIa is one of the binding sites for LDL. This site is most probably different than the fibrinogen-binding site. Low-density lipoprotein may have another binding site in addition to GpIIb/IIIa that is involved in platelet activation. Further research on signal transduction would better explain the mechanism of this activation.

Acknowledgment

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