Monoclonal Anti-Human Platelet GMP-140 (P-selectin/CD62)

Clone WGA-1 (Cat.# M062) and Clone PL7-6 (Cat. # M063)

Application: Using Anti-GMP-140 Antibodies to Measure Thrombin-Mediated Platelet Activation

GMP-140 (also known as P-selectin or CD62P) is a cell adhesion molecule that is important in platelet recruitment and aggregation at wound sites. In unstimulated platelets, GMP-140 is located in the inner wall of the cell membrane. Platelet activation (through molecules such as thrombin) results in a "membrane flip flop" during which molecules on the inner wall are translocated to the outside surface of the cell.

Takara Bio offers monoclonal antibodies that recognize GMP-140 (Cat. # M062 and M063). In this experiment, these antibodies were used for flow cytometry, western blot, and a radiolabelled antibody binding assay to assess GMP-140 expression after stimulation by thrombin.

Methods

Platelet Preparation

Platelets were isolated from human fresh citrated plasma, washed, and suspended in PBS/0.1% NaN_3. Then, thrombin (0-1 units) was added to $1x10^7$ platelets in suspension, and the mixture was incubated for 5 minutes at room temperature. Paraformaldehyde (1% final) was added to fix the cells, and the reaction was allowed to proceed at room temperature for 2 minutes.

Flow Cytometry

Platelets were incubated with monoclonal anti-human platelet GMP-140 antibodies (clone PL7-6, Cat. # M063; or WGA-1, Cat. # M062) at a concentration of 1 µg IgG / 1×10^7 platelets at room temperature for 30 minutes. Then, the platelets were washed with PBS and treated with a FITC-labeled anti-mouse IgG secondary antibody at room temperature for 30 minutes. After staining, the cells were isolated by centrifugation and re-suspended in PBS. Fluorescence intensity was measured by flow cytometry.

Western Blotting

Protein lysates from thrombin-treated platelets were prepared using SDS to solubilize the platelets. Proteins were separated by SDS-PAGE under both reducing (+ β-mercaptoethanol) and non-reducing conditions. After electrophoresis, proteins were blotted to PVDF membrane, and the blots were incubated with each monoclonal antibody (10 µg/ml of antibody used as primary antibody).

Radiolabelled Antibody Binding Assay

The surface expression of GMP-140 was analyzed by a radiolabelled antibody binding assay. Thrombin-stimulated platelets were incubated with anti-platelet GMP-140 antibodies (clones PL7-6 and WGA-1) labeled with ^{125}I. Bound and free ^{125}I were measured and the results were plotted (Scatchard plot analysis) to determine the K_D and the number of GMP-140 binding sites/platelet.

Results

After thrombin stimulation there was an increase in fluorescence intensity in a thrombin concentration-dependent manner (Fig. 1), indicating an increase in cell surface GMP-140. In addition, GMP-140 could be detected by western blot under non-reducing conditions (Fig. 2). Finally, Scatchard plot analysis indicated that there were approximately 8000 GMP-140 sites per stimulated platelet cell (Fig. 3) and that clone WGA-1 had a stronger affinity for GMP-140.

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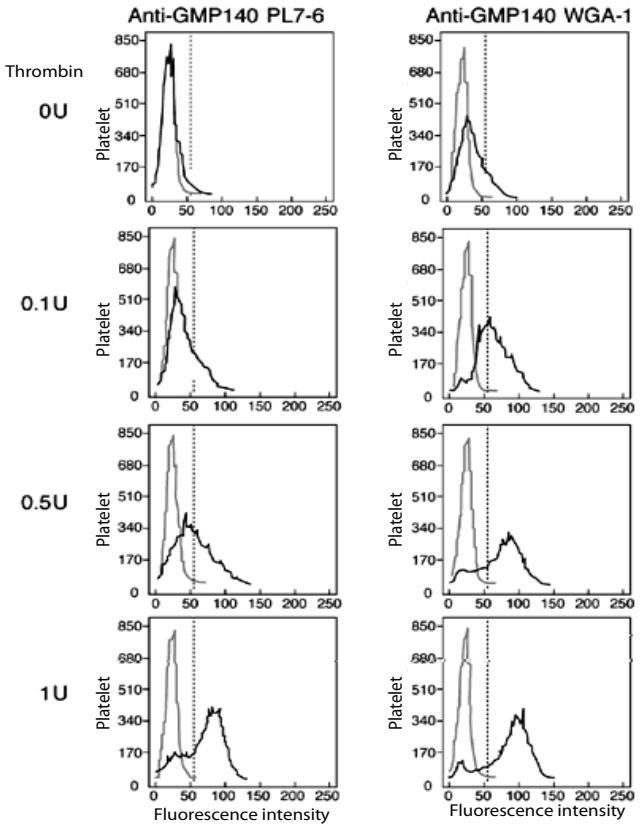

Figure 1. Fluorescence intensity of thrombin-treated platelets as measured by flow cytometry. Isolated human platelets were treated with increasing concentrations of thrombin (0, 0.1, 0.5, or 1 unit). After thrombin stimulation, platelets were stained with anti-platelet GMP-140 antibodies (clone PL7-6, Cat. # M063; or clone WGA-1, Cat. # M062) and a FITC-labeled secondary antibody. Fluorescence intensity was measured by flow cytometry and plotted as a histogram (gray trace- untreated; black trace- treated with antibody).

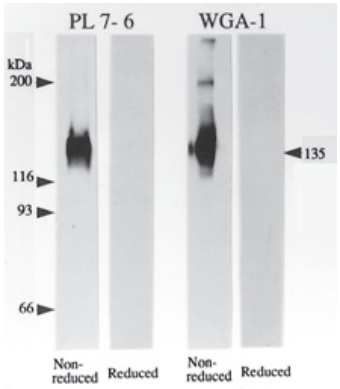

Figure 2. Western blot detection of GMP-140 from activated platelets. Protein lysate was prepared from thrombin-stimulated platelets, and proteins were separated by both reducing and non-reducing SDS-PAGE. GMP-140 was detected by western blot using monoclonal anti-GMP-140 primary antibodies (clones PL 7-6 or WGA-1).

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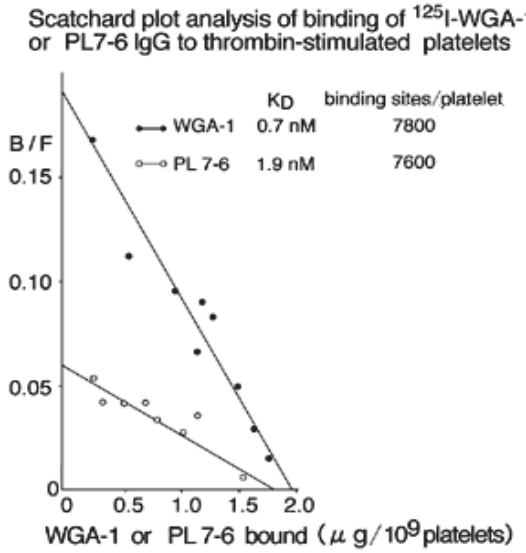

Figure 3. GMP-140 antibody binding assay. Thrombin-stimulated platelets were incubated with radiolabelled (^{125}I) GMP-140 antibodies (clones WGA-1 and PL 7-6), and the amount of bound and free ^{125}I was measured. A Scatchard plot (y-axis, Bound/Free; x-axis, amount of labeled antibody) was used to estimate the K_D and the number of GMP-140 binding sites per platelet.

Conclusions

The anti-human platelet GMP-140 antibodies (Cat. # M062 and M063) can be used for a variety of applications. These antibodies react with activated platelets, such as those stimulated with thrombin (flow cytometry). They can also be used for detection of human GMP-140 by western blot analysis under non-reducing, non-heating conditions. Finally, the PL7-6 and WGA-1 antibody clones recognize different epitopes on the GMP-140 molecule and have different affinities for GMP-140.