Antibody-Detectable Conformational Change of Human Fibrinogen Adsorbed on Modified Polyurethane Surface

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1. Introduction

Polyurethane has been used in many blood-contacting devices including vascular prostheses, blood filter catheter, vascular assist devices, and total artificial heart because it has an excellent blood and tissue compatibility. And the various modified polyurethane has been developed to date in order to improve its physical and biochemical characteristics.

However, the exposure of artificial surface including modified polyurethane, to blood leads to a thin layer of adsorbed plasma proteins within seconds, which determines the thrombogenecity of the specific artificial surface. Although this protein film consists of a variety of plasma proteins, it has been known that fibrinogen molecules of theses constituents play an critical role in giving rise to the thrombogenecity of the surfaces. The thrombogenecity in this process does not depend only on the amount of fibrinogen adsorbed but also its molecular organization (i.e., distribution, orientation, and conformation).[1] It is also believed that the conformational state of fibrinogen is correlated intimately with platelet reactivity and eventually thrombus formation. Many investigators have reported that conformational intact fibrinogen but not altered fibrinogen interacts with platelets, which leads to activation of platelets- aggregation and secretion.[1]

We studied the pattern that the conformational changes of human fibrinogen molecules was altered on the each surface and how much these changes were influenced by the surface characteristics[2,3]. For this purpose, we utilized the antibodies that recognize the conformational intact site of the fibrinogen chain. We believed that the number of antibodies detecting the intact site indicate the amount of the fibrinogen molecules with native structure-bearing site.[1]

2. Materials and Methods

Three kinds of surface modified polyurethane

The beads made of unmodified polyurethane, poly(ethylene oxide) (PEO) grafted polymer, sulfonated polyurethane(PU- SO3), and sulfonated PEO- grafted PU (PU-PEO-SO3) were processed and supplied by polymer chemistry laboratory, KIST.[3]

Antibodies

Goat polyclonal anti-human fibrinogen antibody and mouse polyclonal anti-goat Ig G antibody(Fc specific) were purchased from Sigma Immuno chemicals. Mouse Human fibrinogen domain specific monoclonal antibodies (155B and Z69) were donated from Dr. Ruggeri, Scripps Laboratory, CA [8]and polyclonal anti- mouse Ig G goat antibody (Fc specific) was purchased (Sigma Immunochemical, St, Louis, MO).

Labeling of antibodies with ^{125}I

Secondary antibodies [mouse anti-goat Ig G and goat anti-mouse Ig G] were labeled with ^{125}I by chloramine T method. (specific activity about 1000 cpm/ng).[4]

Human fibrinogen adsorption

Quantities of surface modified PU beads(700mg, surface area of 12 cm^2) were weighed into disposable 5 ml syringe. Human fibrinogen(Sigma chemical) was dissolved in buffer(PBS: 125 mM NaCl, 25 mM NaH_2PO_4, pH 7.2). The stock of fibrinogen solution was diluted into different concentrations of solution(0, 1, 10, 20, 50, 100, 200,

and 500 ug/ml). 1.4 ml of this fibrinogen solution was introduced into syringes and incubated for 2 hours at 37°C After being adsorbed at each concentrations, the solution was expelled from syringe columns, leaving the bead inside syringe. The syringe was filled and extensively washed with PBS.

Measurement of binding of antibody-detectable fibrinogen

To measure total fibrinogen binding, the syringe was filled with solution(1 ml) of goat polyclonal antibody of 1 ug/ml and incubated for 2 hours and then washed with PBS. The syringe was filled with 1 ml of buffer containing ^{125}I- labeled mouse anti-goat antibody and incubated for 2 hours at room temperature. After washing with PBS, its radioactivity was measured. To quantitize the amount of fibrinogen with intact conformation on the specific domain, we prepared the bead syringes with the adsorbed fibrinogen molecules as above. And 1 ml of PBS containing 155B and Z69 antibodies(1 ug/ml) was added and incubated for 2 hours at 37°C. After washing extensively, ^{125}I- labeled goat anti-mouse Ig G antibody was added and incubated for 2 hour at room temperature. After another washing, the retained radioactivity was measured. First and second antibody solution contained bovine serum albumin(3 mg/ml, Sigma chemical) to block nonspecific binding of antibody. All the experiments were performed in three parallel.

3. Results

1) Polyclonal antibody binding for determination of total fibrinogen

Fibrinogen adsorption was quantitized in syringe column with surface-modified beads at different concentrations of purified fibrinogen. This measurement depended on two kinds of antibodies(Goat polyclonal anti-human fibrinogen antibody and I^{125}-labeled antibody interacting with Fc fragment of goat antibody). As shown in Figure 1, binding of fibrinogen increased as the fibrinogen concentration was raised up to 50 ug/ml except on the PU-PEO-SO3 surface where the increase of fibrinogen binding was kept until 200ug/ml. Above these concentrations, only a slight increase in adsorption was observed. The adsorbed amount of fibrinogen was largest on the PU-SO3 surface and similar profiles were obtained on both PU and PU-PEO surfaces

2) Anti-γ chain(400-411) detecting antibody binding

We determined the amount of antibody-detectable fibrinogen molecules, using monoclonal antibody against the COOH-terminal dodecapeptide(Fig.2). The level of antibody binding is intimately correlated with the amount of intact-structure bearing fibrinogen molecules. In the case of SO3 surfaces, the binding of monoclonal antibody increased with the saturation concentration of fibrinogen(200 ug/ml). PEO-SO3 and PEO showed initial increase and then decline in the antibody binding. In the other hand, only a little antibody binding was observed on PU surface.

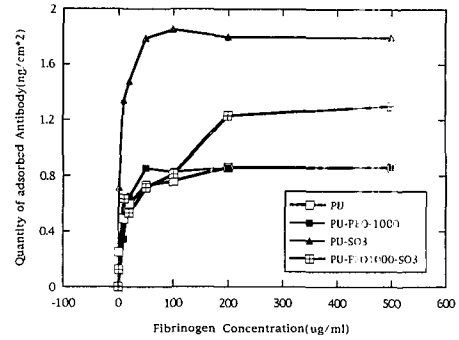

Figure 1. Total Fibrinogen Adsorption

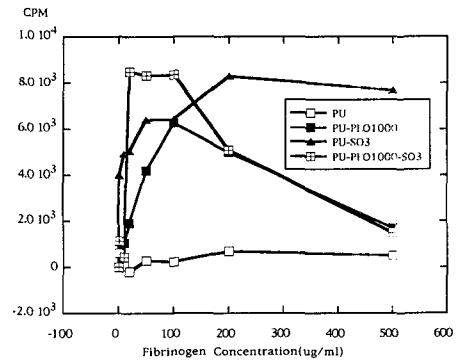

Figure 2. Z69(specific to 400-411)
Antibody-detectable Fibrinogen Measurement

3) Anti-α(89-100) chain detecting antibody binding

Similar experiments were performed with the monoclonal antibody against the epitope of 89-100 in alpha chain of fibrinogen. As depicted in figure 3, PU-SO3 surface also showed the highest antibody binding with a little decrease beyond the saturation concentration(>100ug/ml). In the structure-detecting pattern of PU and PEO surfaces, the amount of monoclonal antibody recognizing adsorbed fibrinogen was greater on PU than on PEO surface. But at lower concentrations of fibrinogen solution, more fibrinogen molecules with intact conformation were observed on PEO surface than on PU surface.

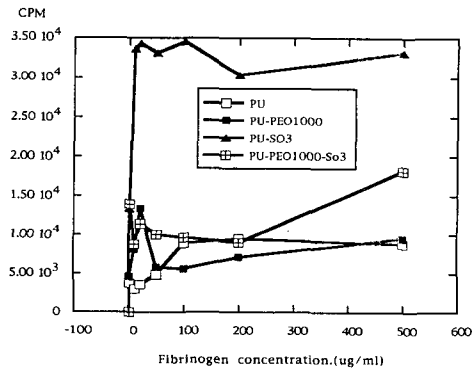

Figure 3.155B(specific to 89-100)
Antibody-detectable Fibrinogen Measurement

4. Discussion

Plasma protein adsorption to artificial surface depends upon the surface characteristics of polymer, which affects their blood compatibility.[2] It was known that the hydrophobicity and hydrophilicity of surface is very important in this protein adsorption.[3] In this view, we had expected that PU surface would show the highest level in the adsorbed fibrinogen molecules. However in the total fibrinogen binding experiment, we observed that PU surface showed the similar fibrinogen adsorption profile with PU-PEO surface.[5]

That PU-SO3 adsorbed much more fibrinogen than PU-PEO, can be explained by its very strong affinity to fibrinogen due to negatively charged SO3 group of this surface.[6] And intermediate level of fibrinogen adsorption to the PEO-SO3 surface may be derived from the non adhesive property of PEO chains and the high affinity of sulfate group toward fibrinogen molecules[6].

In experiment with mAb against γ-chain(400-411), fibrinogen molecules adsorbed on PU-SO3 provided the profiles similar to those of the whole fibrinogen binding pattern(Fig.1 and Fig.2)-suggesting that on PU-SO3 surface, only small a portion of adsorbed fibrinogen molecules go through their conformational changes in this dodecapeptide. A different results came out on PU-PEO-SO3 and PU-PEO. In spite of much more fibrinogen on PU-PEO-SO3 than on PU-PEO at high fibrinogen concentrations, PU-PEO-SO3 show the similar antibody detecting pattern to PU-PEO. This suggests that fibrinogens adsorbed to PU-PEO-SO3 get more conformational changes in this epitope.

Using monoclonal antibody 155B, we observed that fibrinogen on PU-SO3 surface retained much intact structure although there are a little decrease beyond 100 ug/ml. But on PEO-SO3 surface, the increase in the profile accompanied the concentration. Differently from mAb Z69 results, this result informed that the fibrinogens on PEO-SO3 get more or similar break-out of conformational change in this region of fibrinogen compared with on PU-PEO surface.

We suggest that the conformational changes of fibrinogen epitopes occur differently depending on the surface characteristics of the respective polymer. In γ chain,400-411, the fibrinogen molecules on PU-PEO-SO3 have the highest proportion of change in three kinds of surfaces. And in the alpha chain,89-100, the fibrinogen molecules on PU-PEO surface and PU-PEO-SO3 surface provide similar profiles of conformational change of fibrinogen each other.

In order to explore which domain of fibrinogen plays a major role in the interaction with platelet and which is more biocompatible surface, the experiment in plasma system and actual platelet interaction experiments are required.

5. Reference

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