개질 폴리우레탄표면의 단백질 흡착거동

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Protein adsorption to modified polyurethane surfaces

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I. INTRODUCTION

When blood is placed in contact with foreign materials, it forms the thrombus. Although the detailed process of such thrombus formation has not been yet completely clarified, it has been known that the protein adsorption is occurred first and followed by platelet adhesion and cascade reaction of coagulation factors and their subsequent activations, which lead to thrombus formation. Therefore, a study of protein adsorption is valuable in understanding blood response to materials and developing blood compatible polymers. In particular, fibrinogen is found to be a protein of high surface activity so that plays a leading part to result into thrombus formation.

The molecular properties of proteins possibly influencing their surface activity are known to be the size, the charge, structural factors such as stability and unfolding rates, and other chemical properties such as amphipathicity and solubility (1). In charged polymer surfaces, the isoelectric points (IP) of proteins is very important because charge-charge repulsion among the adsorbed molecules is minimized at or near the isoelectric pH. Generally, proteins nearer their isoelectric pH may adsorb more easily.

In our previous studies (2, 3), surface-modified polyurethanes (PUs) grafted by hydrophilic polyethyleneoxide (PEO) (PU-PEO) and further sulfonated by propane sultone (PU-PEO-SO_3) have showed enhanced thromboresistance. Particularly, PU-PEO-SO_3 exhibited the most excellent blood compatibility by means of a synergistic effect of the dynamic mobility of pendent PEO chains and electrical repulsion of negatively charged sulfonate (SO_3) groups. Also, from the results of fibrinogen adsorption, PU-PEO-SO_3 showed a considerably increased fibrinogen adsorption to compare with PU-PEO, where the first adsorption was nearly same regardless of plasma concentration and adsorption time, suggesting the high affinity of pendant sulfonate group to fibrinogen (4).

In this work, the adsorption of fibrinogen, albumin, and IgG from plasma onto modified PU surfaces were performed to investigate the adsorption behaviors of proteins in blood-material interaction and to elucidate further the high affinity of fibrinogen to sulfonate groups.

II. MATERIALS AND METHODS

Surface Modification of PUs

Polyurethane (PU) beads (Pellethane 2363-80A,: Dow Chemical Co.) was used in this experiment. The preparation methods of hydrophilic PU-PEO, directly sulfonated PU-SO_3, and PEO-sulfonated PU-PEO-SO_3 have been previously described in detail (5, 6), as shown in Fig. 1.

The surface of PU sheet was treated with hexamethylene diisocyanate (HMDI) to introduce free isocyanate (-NCO) groups in toluene with stannous octoate at 40 ℃ for 1 h under nitrogen to prepare PU-HMDI. Polyethyleneoxide (PEO, MW=1000) was grafted onto PU-HMDI in benzene with stannous octoate for 24 h at 40 ℃ to yield PU-PEO. Sulfonations were accomplished onto modified PU surfaces by two different methods using propane sultone (PST). PST was coupled directly onto PU-NH_2 via PU-HMDI in acetonitrile for 4 h at room temperature to produce PU-SO_3. The hydroxyl end groups of pendant PEO chains of PU-PEO were also sulfonated by PST in a mixture of isopropanol, Na_2CO_3, and dimethyl sulfoxide for 20 h at 45 ℃ to obtain PU-PEO-SO_3.

Proteins and Plasma

Bovine fibrinogen (Sigma Chem., 95 % clottable), bovine serum albumin (BSA, fraction V, Sigma Chem.), and bovine serum immunoglobulin G (IgG, Sigma Chem.) were dialyzed against 0.04 M potassium phosphate buffer (pH 7.0), lyophilized and stored at -70 ℃. All proteins were

radiolabeled using reductive alkylation with ^{14}C-formaldehyde (Amersham Int., 500 uCi) and sodium cyanoborohydride using the method of Dottavio-Martin and Ravel (7). Protein solutions for adsorption experiment from plasma were prepared by adding tracer amounts of radiolabeled proteins, in amounts corresponding to approximately 0.2-10 % of protein concentration, to freshly prepared bovine plasma. For experiment, labeled proteins in plasma were diluted with phosphate buffered saline (PBS, pH 7.4), respectively.

Protein Adsorption

Quantities of surface modified PU beads (800 mg, surface area of 13.80 cm^2) were carefully weighed into plastic disposable 10 ml syringes and equilibrated with 5 ml of PBS overnight. Prior to adsorption studies, the buffer was removed and 1.6 ml of protein solution introduced into the syringe system. Sets of syringes were arranged for varied adsorption time intervals (1-120 min) as well as different concentrations (0.2-10 %) of protein solution to produce the profiles of adsorption kinetic and adsorption as a function of plasma concentration. After adsorbed at each time and concentration, the plasma was expelled from the syringe, leaving the beads inside the syringe. The syringe was filled with PBS and washed extensively with PBS until no further activity was detected in the eluent. Finally, a 2 % (w/v) solution of sodium dodecyl sulfate (SDS) in PBS was added to the syringes and agitated for 48 h to dissolve any bound proteins. Aliquots of this washings were assayed for radioactivity and compared to the eluent and stock solutions for quantifiable depletion of radioactive species.

To compare each adsorption behavior of ^{14}C-labeled and unlabeled proteins onto modified PU surfaces, the amounts of adsorbed proteins were measured as a function of the percentage (5-100 % of labeled protein) of each protein present in plasma. No preferential adsorptions of labeled proteins were found. A control sample of plasma adsorbed without beads was used as a reference for each experiment and all data were taken to values measured at least in three separate adsorption experiments. Each point represented an average with standard deviations ranging from $\pm$ 2.0 % to $\pm$ 3.5 %.

III. RESULTS AND DISCUSSION

It is known that fibrinogen is a protein of high surface activity and also exhibits the initial behavior of transient adsorption and then the displacement by trace proteins, which has been called the "Vroman effect" (8). Albumin and IgG have been extensively studied because of their abundance in blood and their ability to prevent cell attachment.

Fig. 2 shows fibrinogen adsorption on modified polymer surfaces from diluted plasma after 5 minutes incubation. Adsorbed amount of fibrinogen at steady state increased in the order: PU-PEO < PU-PEO-SO_3 < PU < PU-SO_3, regardless of plasma concentration as well as adsorption time. It is interesting to note that the directly sulfonated PU-SO_3 showed the highest fibrinogen adsorption among modified PUs due to high affinity to sulfonate groups. The adsorption level of fibrinogen on the most blood compatible PU-PEO-SO_3 displayed the intermediate between PU-PEO and PU-SO_3. Such adsorption behavior of PU-PEO-SO_3 is attributed to both effect of the nonadhesive property and the highly dynamic motion of PEO chains and high affinity of pendant sulfonate group toward fibrinogen (4).

Fig. 3 shows 5 minutes adsorptions as a function of plasma concentration for albumin from plasma on different surfaces. Albumin adsorption increased with increasing plasma concentration and then leveled off. The amount of albumin adsorption increased as following order: PU-PEO < PU < PU-SO_3 < PU-PEO-SO_3. High albumin adsorption of PU-PEO-SO_3 seems to be closely related to the isoelectric point (IP) of albumin and the local pH of its sulfonated surface. As mentioned in Introduction, The local pH of PU-PEO-SO_3 in plasma solution may be possible to be similar to the isoelectric point of albumin and then albumin adsorbs to its sulfonate acid group more readily.

Fig. 4 shows IgG adsorption on modified PU surfaces from diluted plasma after 5 minutes incubation. The general adsorption behavior of IgG showed similar trend to that of albumin. However, as expected, PU-PEO-SO_3 surface demonstrated the least IgG adsorption probably due to the synergistic effect of the dynamic mobility of PEO chains and electrical repulsion of negatively charged sulfonate groups.

Fig. 5 shows the adsorption kinetics of fibrinogen, albumin, and IgG from plasma to PU-PEO-SO_3. The adsorption order of proteins is as follows: IgG < fibrinogen < albumin. This coincides well with the results of Figs. 2-4. In the case of fibrinogen, somewhat transient adsorption was observed.

In conclusion, although PU-PEO-SO_3 surface is the most blood compatible, the adsorption patterns on each proteins to its surface are different. It means that protein adsorption greatly depends on the surface characteristics of polymers and this affect their blood compatibility.

IV. REFERENCES

1. Proteins at Interfaces, J.L. Brash and T.A. Horbett (Eds), in ACS Symposium Series 343, ACS Press, Washington,

DC (1987).

2. D.K. Han, S.Y. Jeong, and Y.H. Kim, J. Biomed. Mater. Res.: Appl. Biomater., 23(A2), 211 (1989).
3. D.K. Han, S.Y. Jeong, K.-D. Ahn, Y.H. Kim, and B.G. Min, J. Biomater. Sci. Polym. Edn, in press (1992).
4. D.K. Han, G.H. Ryu, K.D. Park, S.Y. Jeong, Y.H. Kim, and B.G. Min, J. Biomed. Mater. Res., 25, 561 (1991).
5. D.K. Han, K.D. Park, K.-D. Ahn, S.Y. Jeong, and Y.H. Kim, J. Biomed. Mater. Res.: Appl. Biomater. 23(A1), 87 (1989).
6. D.K. Han, S.Y. Jeong, K.-D. Ahn, Y.H. Kim, and B.G. Min, J. Biomater. Sci. Polym. Edn, in press (1992).
7. D. Dottavio-Martin and J. M. Ravel, Anal. Biochem. 87, 562 (1978).
8. L. Vroman, A.L. Adams, G.C. Fischer, and P.C. Munoz, Blood, 55, 156 (1980).

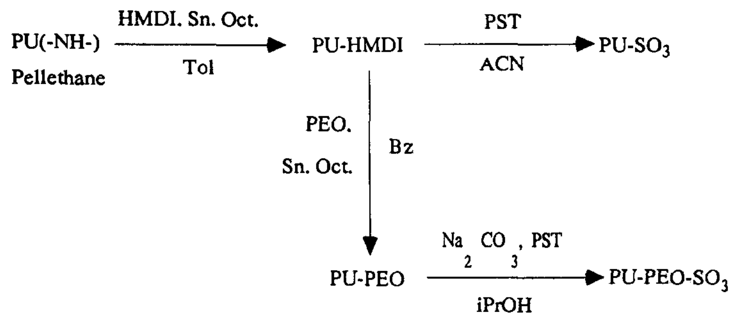

Fig. 1. Modification scheme of polyurethane (PU) surfaces: HMDI = $OCN(CH_2)_6NCO$, PEO = $HO(CH_2CH_2O)_nH$, PST = $(CH_2)_3SO_3$.

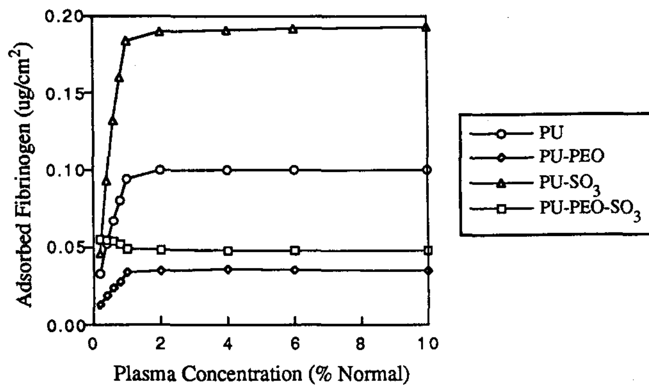

Fig. 2. Fibrinogen adsorption on modified polymer surfaces from diluted plasma after 5 minutes incubation.

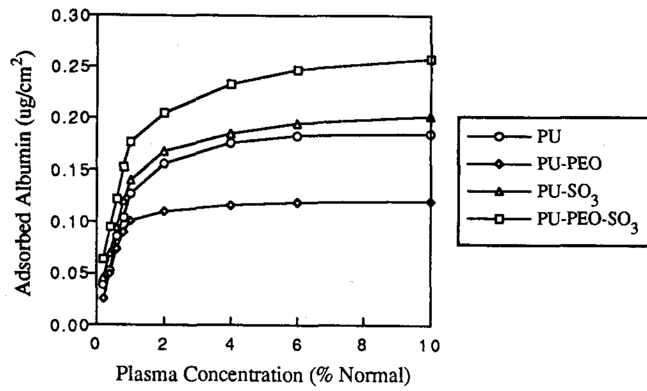

Fig. 3. Albumin adsorption on modified polymer surfaces from diluted plasma after 5 minutes incubation.

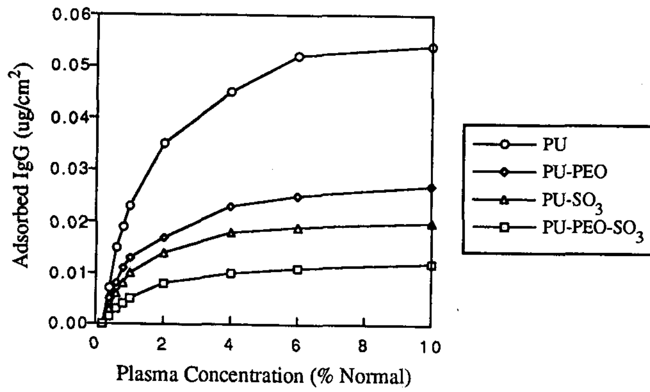

Fig. 4. IgG adsorption on modified polymer surfaces from diluted plasma after 5 minutes incubation.

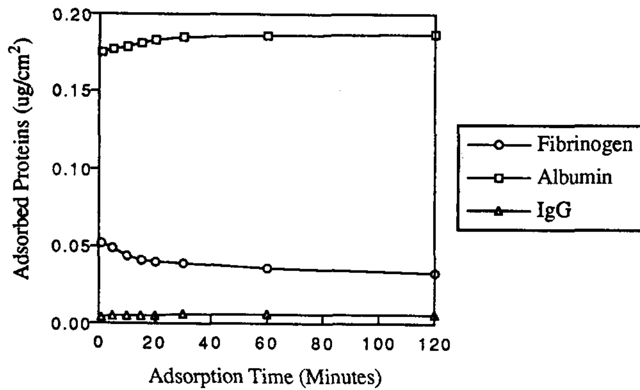

Fig. 5. Adsorption kinetics of fibrinogen, albumin, and IgG from plasma to PU-PEO-SO_3.