

PREFERENTIAL ADSORPTION OF HIGH DENSITY LIPOPROTEIN (HDL) IN BLOOD PLASMA/POLYMER INTERACTION

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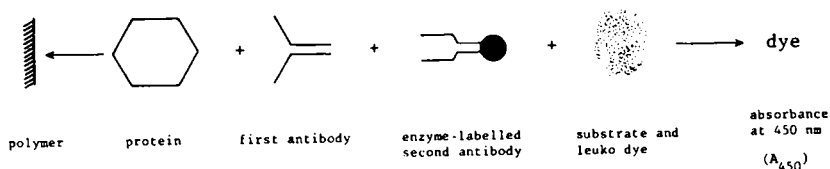
Abstract - A few studies on the adsorption of plasma proteins to polymeric surfaces show that major plasma proteins: albumin (Alb), fibrinogen (Fb) and immunoglobulin (IgG) are adsorbed in much smaller quantities from plasma than from protein solutions (1,2). Present results show that this difference in adsorption is due to the preferential adsorption of high density lipoprotein from plasma onto the material surfaces studied (PVC and PS).

INTRODUCTION

Exposure of a foreign material to blood generally leads to platelet adhesion and surface activation of blood coagulation. Both processes are said to be determined by the initial adsorption of proteins from the blood onto the foreign surface. An extensive amount of work has been done by many researchers to obtain insight in the adsorption behaviour of the major blood proteins. However, it was shown by Brash et al. (1) that protein adsorption from plasma is very small and that adsorption experiments with protein solutions fail to predict the adsorption from plasma.

EXPERIMENTAL

A two step enzyme immuno assay was developed by us to investigate the protein and lipoprotein adsorption from bloodplasma and from solutions onto polymeric surfaces:



MATERIALS

All experiments were carried out with multiwell PVC or PS microtitre plates. Human bloodproteins Alb, Fb and IgG were obtained from the Lab. of the NL Red Cross Blood Transfusion Service (CLB).

Lipoproteins (VLDL, LDL, HDL) were isolated from EDTA (3 mmol) plasma by density gradient centrifugation. Rabbit sera directed against Alb, Fb and

IgG and LDL were obtained from CLB, a serum against HDL from Behringerwerke A.G., Marburg.

Before use the sera were diluted 100 fold in 0.02M phosphate buffered saline containing 1% bovine albumin (PBS-BSA). Purified sheep antirabbit immunoglobulin G (ShaIgG) was covalently bound to horseradish peroxidase (Boehringer, Mannheim) by a modified thiolation according to Carlsson et al (3).

Before use the conjugate was diluted 1 : 2000 in 0.02M Trisbuffer (pH 7.4), containing 0.02M NaCl, 1% BSA, 1% polyethyleneglycol 6000 and 25 mg l⁻¹ ShaIgG. Urea peroxide (Hepanostika, Organon Teknika) was used in the enzyme substrate and 3,3', 5,5' tetramethylbenzidine (Fluka) as the leukodye.

METHOD

The wells of PVC or PS microtitre plates were contacted with 0.075 ml of a protein or plasma solution obtained by serial dilution. After 1 h contact time the wells were emptied and washed 4 times with 0.15 ml 0.01 M PBS solution, containing 0.05% Tween-20R using a multichannel syringe. Solutions (0.05 ml) of the first antibody were applied to each of the washed wells of one series.

To the wells of another series 0.05 ml PBS-BSA was added to obtain negative controls. After 1 h incubation at 20°C the wells were washed 4 times with PBS-Tw. Then 0.05 ml of the conjugate solution was added to each of the wells and incubated for 1 h at 20°C. The enzyme induced color reaction was stopped by adding 0.025 ml 2 M H₂SO₄ to the substrate solution in the wells and the absorbance at 450 nm was determined by using a colorimeter provided with an 80 µl flow cell (Vitatron).

RESULTS AND DISCUSSION

Normal adsorption isotherms were obtained for the adsorption of Alb, Fb or IgG from solutions containing a single protein on PVC or PS. The results of adsorption experiments of these proteins from a mixed solution (Alb : IgG : Fb = 10 : 5 : 1 w/w) are presented in Fig. 1. Fb reaches a plateau level as found for the adsorption from a single Fb solution, but the Alb and IgG adsorptions on both polymers show a maximum and a decrease from there at higher concentrations.

Obviously a preferential Fb adsorption takes place at plasma concentrations. When undiluted plasma was contacted with PVC or PS only a very small adsorption of Alb, IgG and Fb took place (Fig. 2). Especially the amount of Fb adsorbed from plasma is very small in comparison to the amount adsorbed from a solution in which the concentrations of Alb, Fb and IgG are equal to those in plasma.

These phenomena have to be attributed to the preferential adsorption of another plasma component. Therefore, adsorption experiments were carried out with fibronectin (FN), antithrombin III (AT-III) and various lipoproteins (VLDL, LDL, HDL) from solutions and plasma onto PVC and PS. The effect of the presence of these components on the Fb adsorption was investigated also. The adsorption isotherms of HDL (apo-A-1) and LDL (apo-B) from single solutions are given in Fig. 3.

Adsorption isotherms of these lipoproteins from plasma (Fig. 4) are quite different. LDL (apo-B) shows a low adsorption level, while HDL (apo-A-1) reaches a plateau value of 80% of the one obtained with single lipoprotein adsorption experiments.

Evidently the adsorption of HDL is not much influenced by the presence of high concentrations of other plasma proteins. The amount of Fb on the polymer surface reaches a maximum at low plasma concentrations and thereafter decreases to a low adsorption value. The adsorption isotherms obtained by serial dilution of a mixture of Fb and VLDL shows that the adsorption of Fb

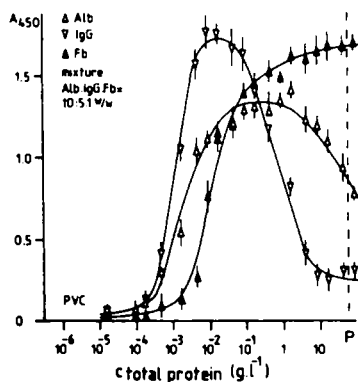


Fig. 1. Adsorption of albumin (Alb), immunoglobulin G (IgG) and fibrinogen (Fb) to PVC from solutions of a ternary mixture. P = concentration of the proteins in plasma.

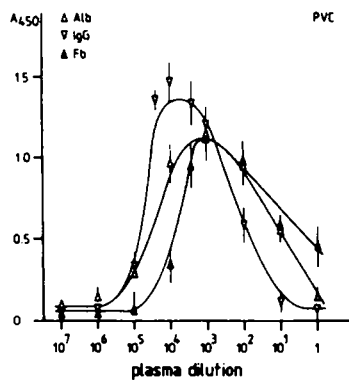


Fig. 2. Adsorption of Alb, IgG and Fb to PVC from plasma as a function of plasma concentration.

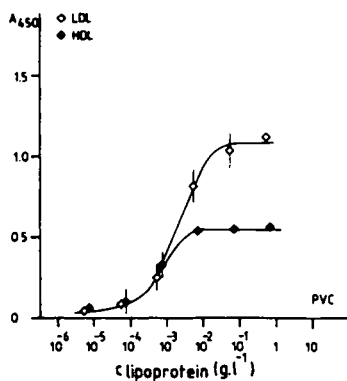


Fig. 3. Adsorption of LDL and HDL to PVC from single lipoprotein solutions. Concentrations of LDL and HDL are expressed as concentrations of the apoproteins apo-B and apo-A-1 respectively.

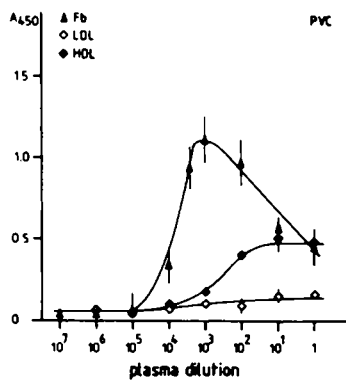


Fig. 4. Adsorption of Fb, LDL and HDL to PVC from plasma as a function of plasma concentration.

is not influenced (Fig. 5).

Similar experiments with Fb and HDL or Fb and LDL mixtures (resp. 3.3 : 1 w/w and 4.2 : 1 w/w) showed a reduced Fb adsorption compared with adsorption from single Fb solutions. When HDL is present the decrease in Fb adsorption is very pronounced and here exists a great similarity to the Fb adsorption from plasma. In Fig. 6 the effect of the presence of HDL on the Fb adsorption onto the polymer surface is illustrated. Here solutions with a fixed Fb concentration (2.5 g.l^{-1}) and an increasing HDL concentration were contacted with PS.

At low HDL concentration ($<2.5 \text{ g.l}^{-1}$) Fb adsorption is about the same as for a pure Fb solution. At higher concentrations of HDL the Fb adsorption is severely reduced. The adsorption behaviour of Fb is not affected by the presence of FN, AT-III or Alb.

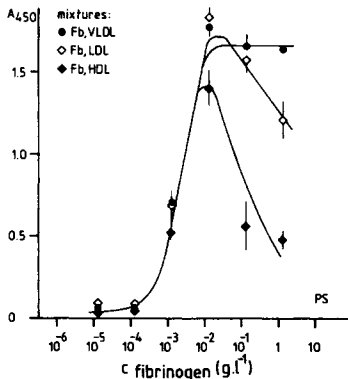


Fig. 5. Influence of different lipoproteins on the adsorption of Fb to polystyrene (PS). (Fb : LDL = 4.2 : 1 w/w and Fb : HDL = 3.3 : 1 w/w).

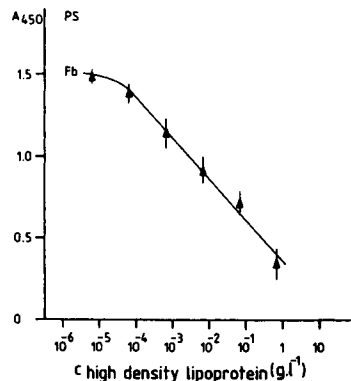


Fig. 6. Adsorption of Fb to PS from binary mixtures as a function of HDL concentrations. Fb concentration = 2.5 g.l^{-1} .

Precoating of a polymer surface with HDL leads also to a strongly reduced Fb adsorption.

The finding that the preferential adsorption of HDL from plasma leads to a much reduced protein adsorption has many implications for aspects leading to thrombus formation, such as surface activation of clotting factors and platelet adhesion and aggregation. These implications are currently being investigated by us.

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