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Citation for published version (APA):

Document status and date:
Published: 01/01/1986

Document Version:
Version created as part of publication process; publisher's layout; not normally made publicly available

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

Download date: 03 Jun. 2020

Protein adsorption at the solid-liquid interface measured by
ellipsometry

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Organisation of the university

In 1974 the University of Maastricht was founded and the
mechanism of the regulation of the bloodclotting process was
chosen as a central field of research. In this research
different departments, like biophysics, biochemistry, cel-
biology, electronmicroscopy and physiology work close to-
gether offering multidisciplinary research possibilities.

The process of bloodclotting

The regulation of blood clotting is controlled by inter-
actions between a great number of components. These com-
ponents, i.e. proteins, calcium and complex phospholipid
structures (e.g. blood platelet membranes) are circulating
in the blood/and/or reside in the vessel walls. A number of
the proteins have been found to be serine proteases which
are present in their unactivated (zymogen) forms. The clot-
ting process consist of a series of enzyme activations and
in each step of this sequence, the activated zymogen acti-
vates another zymogen to an active protease.
Different clotting factors are purified in our departments
and their interactions are studied (1,2,3). Much effort has
been spent on the last part of this proces, the conversion

of prothrombin (II) to thrombin (II$_a$). More specifically the role of the phospholipid surface in this process is the main focus of our research. In order to study the interaction of the different proteins of this prothrombinase complex with the phospholipids we modified an existing optical technique, ellipsometry, in such a way that we could follow the adsorption of proteins on phospholipid mono- or multilayers (4,6,7,8).

Ellipsometry

The ellipsometer is an optical instrument that measures the changes in polarization of light due to reflection (fig. 1). The changes are measured by two adjustable polarizers indicated as polarizer (P) and analyzer (A). The measurement consists of finding the positions of P and A corresponding to minimal light intensity reaching the photodiode. The change in polarization due to reflection is dependent on the optical properties of the reflecting surface. If the surface is covered with phospholipidlayers, polymer films or a protein layer, then the optical properties of the reflecting surface are changed. This change in optical properties results in changes in the positions of P and A. From these P and A values the thickness and the refractive index of the adsorbed layer can be calculated. The formulas to calculate the mass from the refractive index n, and thickness, d, of the adsorbed layer where derived and experimentally validated (6).

A complete description of the instrument as well as the method of computation is given in ref. (6,7).
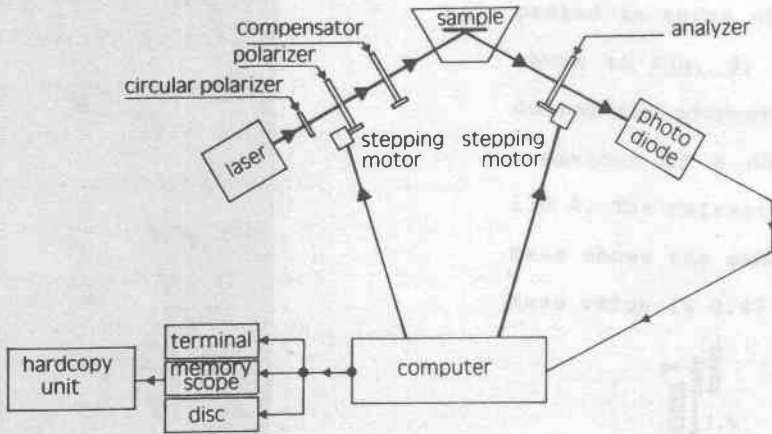
Figure 1   Schematic representation of the automated ellipso-
meter.

Preparation of the sample (4)

Adsorption of proteins was measured on a chromium coated
glass slide placed in a well stirred, temperature controlled
cuvette filled with buffer. In most of our studies these
chromium slides were covered with stacked mono- or multi-
layers of phospholipids.

This stacking was done by using a preparative Langmuirtrough
according to the method of Langmuir and Blodgett.

Different types of protein adsorptions, (9) structural aspects of the adsorbed layer.

Fibrinogen Adsorption on hydrophilic Chromium.

The cuvette was filled with buffer and the optical proper-
ties of the chromium surface, positions of P and A, were
measured. After about 200 seconds fibrinogen was added.

After the moment of addition, the polarizer and analyzer change fast and an end level is reached after about 2000 seconds for both analyzer and polarizer. The results interpreted in terms of thickness, refractive index and mass are shown in Fig. 2. The refractive index does not change much during the adsorption. The thickness follows the saturation behaviour of a monolayer adsorption and stabilizes around 130 Å. The refractive index is then n = 1.38. The calculated mass shows the same behaviour as the thickness does. The end mass value is 0.47 µg/cm^2.
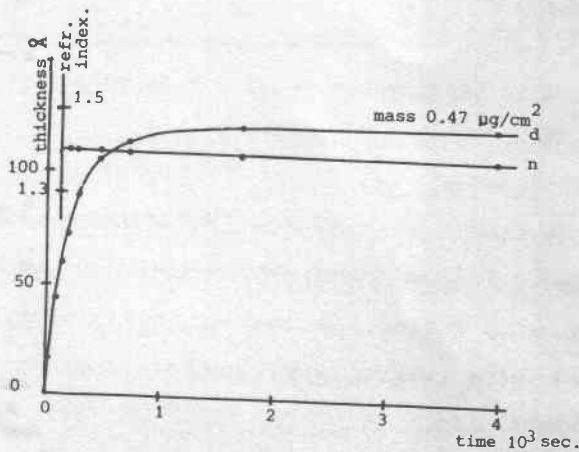

Figure 2 Adsorption of fibrinogen on hydrophilic chromium surface. Buffer 0,01 M Tris-HCl pH=7.0. Bufferconcentration of fibrinogen was 10 µg/ml.

Fibrinogen Adsorption on hydrophobic Chromium. Fig. 3 shows that during the first 150 seconds the thickness grows at constant refractive index n = 1.8. Then the refractive index

drops while the thickness still increases. The thickness grows until about 68 Å while the refractive index drops to n = 1.48. From this point on the layer gets thinner and optically denser. The layer stabilizes at d = 35 Å and n = 1.72. The calculated mass shows a regular monolayer type adsorption which reaches its maximum before the layer gets thinner m = 0.56 μg/cm^2.
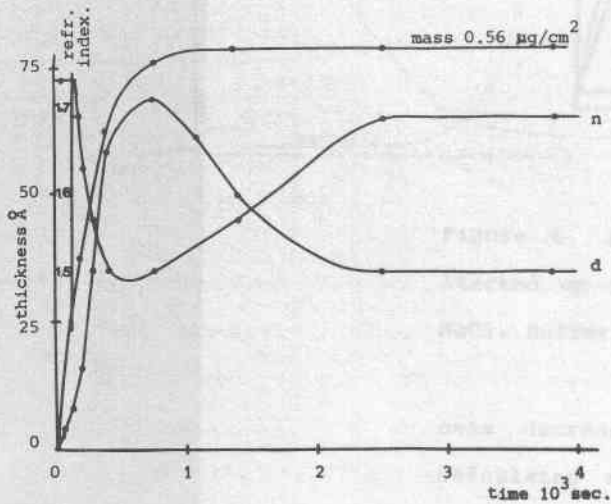

Figure 3   Adsorption of fibrinogen on hydrophobic chromium surface exp. conditions as in fig. 2.

Adsorption of clotting factor v$_a$ on dops, stacked on chromium.

Fig. 4 shows that the refractive index is changing slightly during the first 5000 seconds. The thickness increases during the first 2000 seconds. After this point the thick-
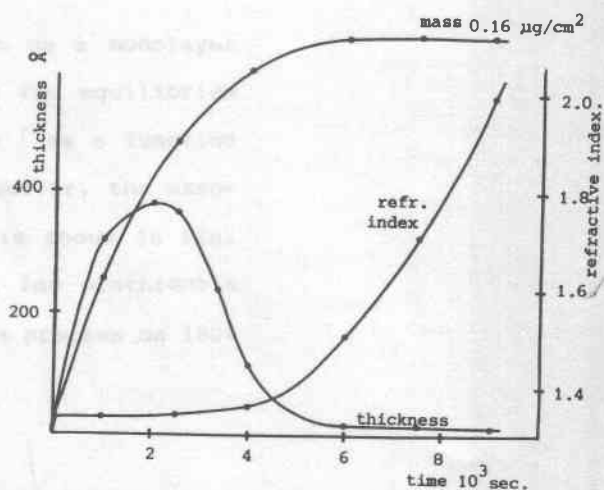
Figure 4   Adsorption of factor V_a on one layer of DOPS stacked on chromium buffer 0.05 m. Tris-HCl pH=7.5, 0.1 M NaCl. Buffer concentration of factor V_a was 2 μg/ml.

ness decreases and the refractive index increases. The calculated mass adsorbed shows a regular monolayer type adsorption. The refractive index of the layer increases even after the total mass adsorbed has reached an equilibrium value m=0.16 μg/cm^2. The value of the refractive index of the adsorbed layer is, n=2.3 after 10^4 seconds.

These three examples show respectively an adsorption with a constant refractive index, a changing refractive index indicating a reorganisation of the molecules after adsorption and a very high refractive index n=2.3 indicating penetration of the proteins into the phospholipid layer (9).

Binding constants of the proteins to phospholipids membranes
(4,5,10).

Fig. 5 shows the adsorption of prothrombin on a monolayer
containing 80% DOPS/20% DOPC. By measuring the equilibrium
values the adsorbed quantity of prothrombin Γ as a function
of the prothrombin concentration c in the buffer, the asso-
ciation constant K_a can be obtained. This is shown in Fig.
6. From these data it was calculated that for prothrombin
concentrations up to 10 μg/ml the adsorption process on 100%
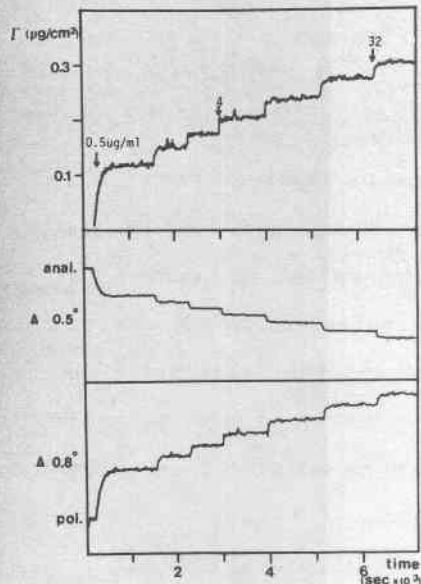
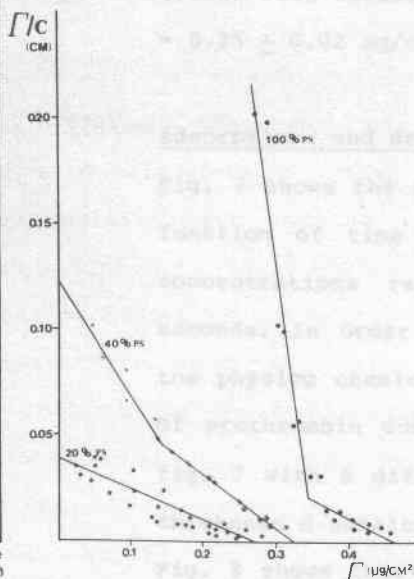Fig. 5 Stepwise adsorption of
prothrombin on 80% DOPS/20%
DOPC.

Fig. 6 Scatchard plot for
the adsorption of prothrom-
bin on DOPS/DOPC mixtures.
●=100% DOPS, 0=40% DOPS;
★=20% DOPS.

DOPS can be described by a single class of binding sites with $K_a = (2.01 \pm 0.02) \times 10^8$ M^{-1} (mean $\pm$ SD) and $\Gamma_{max} = 0.35 \pm 0.01$ $\mu g/cm^2$. For high prothrombin concentrations an extra amount of prothrombin is adsorbed. If the percentage of DOPC in the monolayer is increased, this biphasic behaviour becomes initially more pronounced with decreasing values of Γ_{max}. The adsorption on 20% DOPS/80% DOPC monolayers is still somewhat biphasic but can globally be described with values of $K_a = (1.11 \pm 0.34) \times 10^7$ M^{-1} and $\Gamma_{max} = 0.25 \pm 0.02$ $\mu g/cm^2$.

Adsorption- and desorption rates

Fig. 7 shows the adsorption on a double layer of DOPS as a function of time for different concentrations. All these concentrations reach an equilibrium value in about 300 seconds. In order to establish the relative importance of the physico chemical adsorption rate and the transport rate of prothrombin during adsorption, we analysed the data of fig. 7 with a diffusional model with an unstirred layer of thickness d obtained from the same data.

Fig. 8 shows that the binding kinetics of prothrombin is in the beginning independent of the amount of protein adsorbed i.e. diffusional limited up to 30% of maximum binding-capacity $\Gamma_{max} = 0.38$ $\mu g/cm^2$. The thickness of the unstirred layer is d = 5 μm. The adsorption of fibrinogen is diffusional limited at the iso-electric point pH=6 in 0.01 M Tris-HCl. After addition of 0.1 M NaCl or changing the pH to
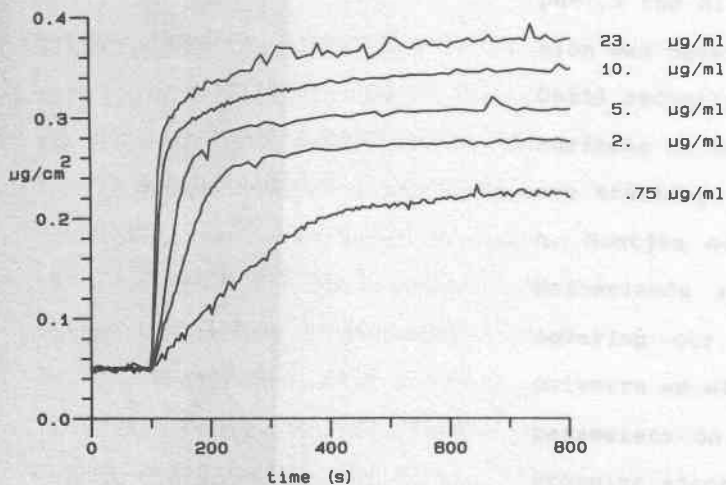
Fig. 7 Adsorption of Prothrombin on 2*18:1 PS. Buffer: Tris/HCl (.05M pH=7.5), NaCl (.1M),Ca^{++} (1.5 mM)
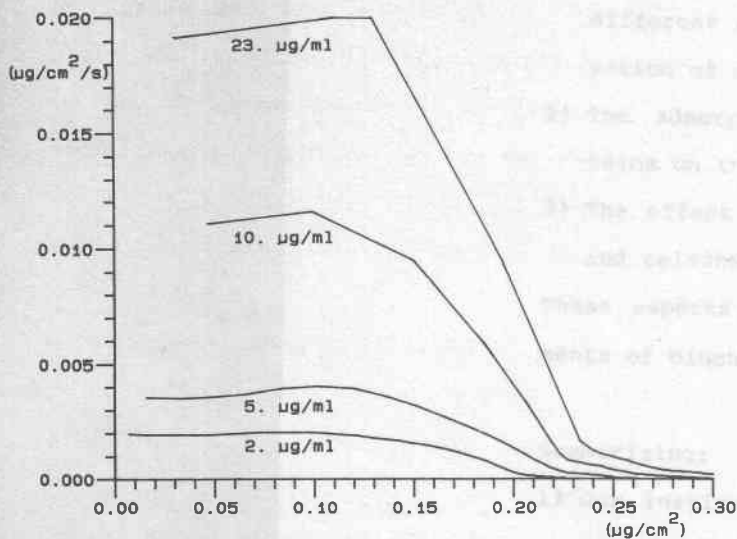

Fig. 8 Adsorption velocity of Prothrombin on 2*18:1 PS. Buffer: Tris/HCl (.05M pH=7.5), NaCl (.1M), Ca^{++} (1.5 mM).

pH=7.5 the diffusional limitations vanishes. Albumin adsorption was never diffusional limited in this system.

Until recently we mainly studied protein adsorption on metal surfaces or metal surfaces coated with phospholipids. Now we are starting in cooperation with the groups of J. Feyen and A. Bantjes of the Twente University of Technology in the Netherlands research on polymer protein interactions. By covering our reflecting surfaces with homolog series of polymers we will investigate the effect of different polymer parameters on the refractive index and amount of different proteins adsorbed.

We are also starting a cooperation with industry to test their polymers on the following aspects.

1) Effect on bloodclotting in general and the effect on different clotting factors in particular, e.g. the activation of clotting factor XII (Hagemanfactor).

2) The adsorptioncharacteristics of different plasma proteins on these polymers.

3) The effect of polymer toxicity on different celcultures and celadhesion.

These aspects are studied in cooperation with the departments of biochemistry and celbiology.

Summarizing:

1) Our institute is specialised in the mechanism of blood coagulation.

2) Ellipsometry offers a good technique to obtain informa-
tion on the interactions of proteins with polymer sur-
faces, such as adsorbed amount and the water content of
the adsorbed protein layer (refractive index), associa-
tion constants and the adsorption and desorption rates.
This information is obtained continuously, in situ,
without using any labels or protein-modifications. So in
the field of biomaterial research of we are interested in
testing biomaterials and further developing of testsys-
tems for biocompatibility.