Immunosensor for Fibrinogen in Human plasma

A study was initiated to investigate the use of surface plasmon resonance (SPR), Autolab ESPRIT, for the detection of fibrinogen. Fibrinogen is a plasma glycoprotein, essential for blood clotting, synthesized and secreted by hepatic parenchymal cells, also known as Coagulation Factor I. We used fibrinogen (human plasma) from Calbiochem, Cat no. 341576. To detect fibrinogen, a polyclonal IgG Rabbit anti Human anti-Fibrinogen antibody (Calbiochem, cat 341552) was covalently attached to Mercapto-undecanoic acid modified gold surfaces.

During the injection of Fibrinogen in buffer, an acceleration of the interaction process was observed and the signal continued to increase until the incubation time was finished. After the injection was finished, some of the bound material desorbed as expected, resulting in a signal decrease. The fibrinogen is lyophilized from 18.968 ml of 20mM sodium citrate HCl pH 7.4. The interaction of fibrinogen could be measured in $5*10^4$ dilution.

The sensor

The design of the surface layer, which is in direct contact with the sample, is crucial. This is especially true when complex materials, like plasma samples have to be analyzed. In these samples, the analyte (i.e. the ligand) is typically present in a lower concentration compared to other molecules. Non-specific adsorption of these latter components may overwhelm or even prevent the specific interaction signal. Hence, the design and preparation of surface coatings that suppress or avoid non-specific protein adsorption is important in biosensor design to ensure specific recognition of the analyte only. The suitability of the self assembled monolayer (SAM), Mercaptoundecanoic acid (MUA), has been studied.

Experiment

Preparation of the SPR sensor
The SPR sensor disks were extensively cleaned in a freshly prepared piranha (H_2SO_4/H_2O_2: 70% sulfuric acid : 30% hydrogen peroxide) solution. After 1 hour, the disks were thoroughly rinsed with water, dried in a stream of nitrogen gas and immediately incubated overnight in a 1mM solution of MUA in ethanol. After SAM formation, the disks were subsequently washed with ethanol and water, and dried with nitrogen gas.

Immobilization of Anti-Fibrinogen
MUA-modified surfaces were activated using a 1:1 mixture of 400mM EDC (Dimethylaminopropyl-N'EthylcarbodiimideN-3- Hydrochloride) and 100mM NHS (N-Hydroxy Succinimide) in water. In channel 1 the NHS-esters produced reacted with amine functions present in the 10µgml^{-1} antibody in 10mM NaAc pH4.7 (sodium acetate) solution. Different ligand sample concentrations 50, 100 and 400µg/ml have been used fore immobilization. After coupling, remaining activated groups were deactivated with a 1M ethanolamine solution at pH8.5.

An example of antibody immobilization is presented in Fig. 1, showing a typical SPR graph for the immobilization of anti-fibrinogen on a MUA surface. Injection for activation, antibody attachment and subsequent blocking of remaining activated groups, are shown.
In this case, the immobilization level obtained was 1.2ngmm^{-2} (≅ 145m°). This can be calculated from the obtained angle shift as 120m° is equivalent to an amount of attached protein of 1ngmm^{-2}.
The amount of antibody bound to the MUA surfaces, increases with increasing amount of antibody concentration.

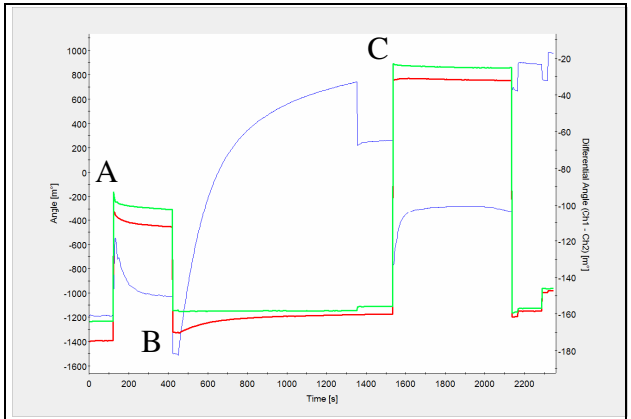

Figure 1. Immobilization of the anti-fibrinogen antibody on a MUA surface, with subsequent injections of EDC–NHS (A), Anti-fibrinogen (B) and ethanolamine (C). The antibody immobilized gives rise to a response of 145m°, which is equivalent to an amount of 1.2ngmm^{-2}.

SPR experiments
SPR measurements were made by recording the angle shift as a function of time. Fibrinogen samples were diluted in 10mM HEPES pH7.4, 150mM NaCl, 3mM EDTA, 0.005% Surfactant Tween P20 running buffer. Sample injections of 50µl of diluted human plasma were used to measure the interaction. Regeneration of material bound to the sensor

surface was accomplished by injections of 50µl of a 10mM glycine solution pH2.5. The experiment was done under temperature control at 23.0 C°.
An example of the interaction is shown in Fig. 2. The plasma showed a lot of non-specific interaction onto the MUA surface. Sample concentrations between the 3nM to 25nM gave good responses. Outside this range, the non-specific binding overwhelmed the specific interaction.

To be able to measure lower sample concentrations, higher immobilization rates of rabbit anti human anti-fibrinogen antibody were used, but also in those cases, the overwhelming power of the non-specific adsorption did not change on the lower range as well as on the higher range.

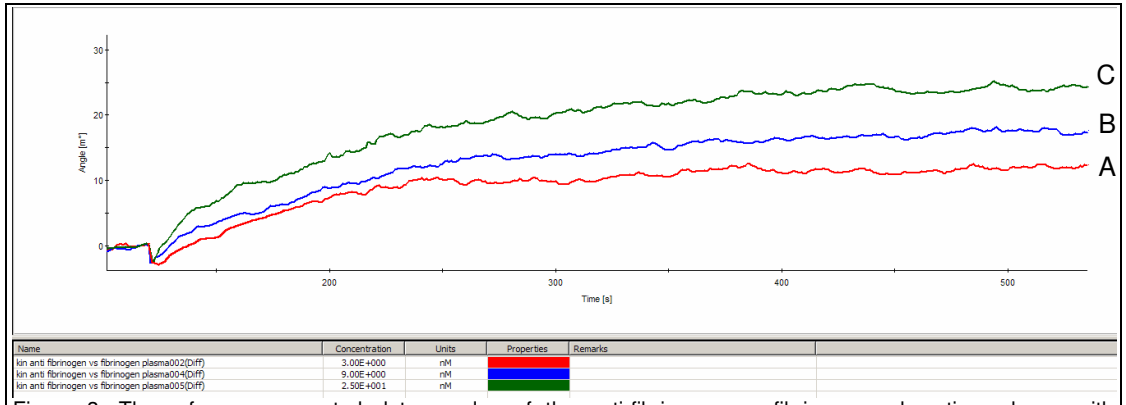

Figure 3. The reference corrected data overlay of the anti-fibrinogen vs fibrinogen adsorption, shown with subsequent fibrinogen plasma samples (A), 3nM (B), 9nM (C), 25nM.

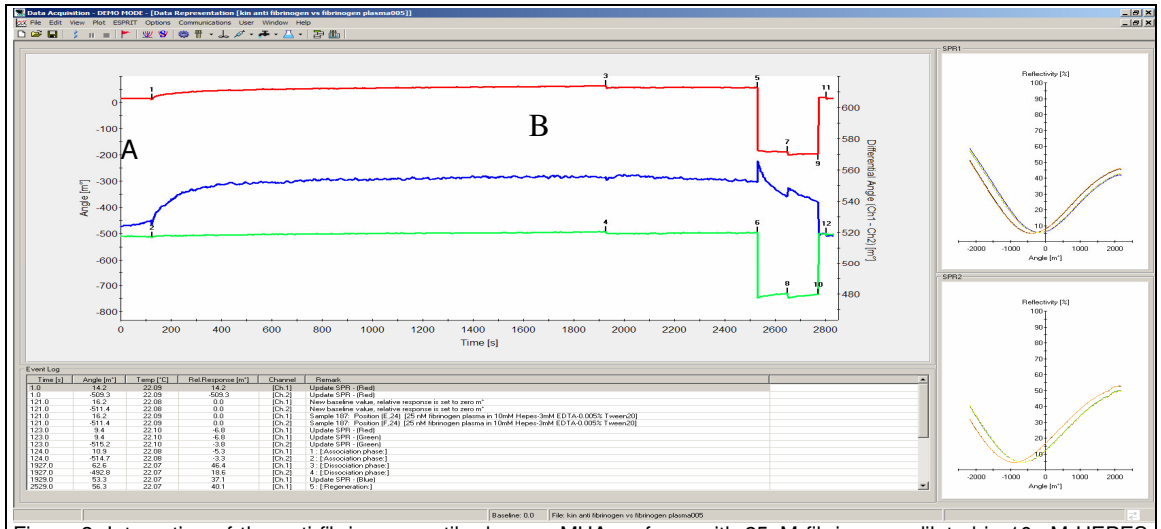

Figure 2. Interaction of the anti-fibrinogen antibody on a MUA surface, with 25nM fibrinogen, diluted in 10mM HEPES pH7.4, 150mM NaCl, 3.5mM EDTA, 0.005% Surfactant Tween P20. The subsequent injection are, running buffer as baseline, association with fibrinogen (A), dissociation with running buffer, (B) twice a regeneration with 10mM Glycine HCL pH2.5, and the end is a wash with running buffer. The top line shows data from channel 1, lowest line shows the data of channel 2 and the middle line shows the differential between channel 1 and 2, the so-called corrected measurement data line.

Figure 3 shows an overlay of sample concentrations, which worked best in this study.

Conclusion

The best results in terms of plasma adsorption and surface regenerability for the tested samples are obtained with a limited range of concentrations, between 3nM to 25nM fibrinogen.
This is due to the very high non-specific adsorption levels on the MUA surface. Future research needs to focus obtaining higher immobilization levels on alternative modified surfaces that are more suitable to quantify proteins with ‘sticky’ properties.