

Protein resistance properties of short monodisperse PEG derivatives

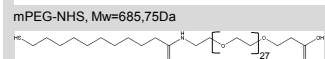
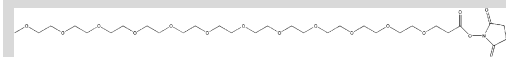
Mattias Rudh and Erik Agner
Polypure AS, Gaustadalleen 21, N-0349 Oslo, Norway

Can low molecular PEG derivatives block protein binding? Recent studies [1] have shown that the adsorption resistant properties of polyethylene glycol (PEG) exist even when a PEG with a comparably short number of repetitive ethylene glycol units is used (Mw~650Da). In this study we investigated the resistance towards non specific adsorption, of a short PEG chain. Two types of interactions were investigated, BSA-antiBSA and BSA-goat serum. The data shows that PEGylation with a short PEG chain had an effect on the protein-antibody interaction but not on the adsorption of serum components to the protein.

PEG synthesis and purification

PEG is a polymeric material and hence polydisperse in nature. PEG polydispersity is the reason behind the difficulties in preparing homogenous bifunctional derivatives. We have developed a purification technique that enables the fractionation of polydisperse PEG into single oligomers. Our purification technology is based on **Sample Displacement Chromatography**. The synthesis of advanced derivatives is thereby facilitated, and products can be made in very high purity.

In order to obtain molecules that could either be immobilized on a surface or covalently linked to proteins, two different PEG derivatives were synthesized. A NHS functionalized short chain length PEG was made in order to link it to proteins and an alkane thiol terminated acid was made in order for SAM formation.

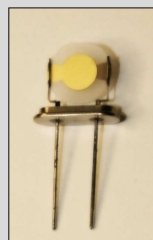


QCM analysis

The performance of the PEG molecules was evaluated with Quartz Crystal Microbalance (QCM), the Attana 100 system [2]. The real time QCM principle is based upon the linear relation between oscillation frequency and adsorbed mass, first presented by Sauerbrey [3]. In the present work the raw data have been converted into mass by using the relation.

$$\frac{\Delta m}{A} = \frac{\Delta \rho_q v_q}{2f_0^2}$$

Where A is the active electrode area, ρ_q is density of the quartz, v_q velocity of sound in quartz, f_0 the fundamental resonance frequency, Δm is the adsorbed mass.



10MHz Quartz Crystal from Attana.

Materials and methods

Gold coated quartz crystals (Attana AB) were cleaned by repeated treatment with Piranha solution (highly concentrated H₂SO₄ and H₂O₂), followed by sonication in toluene and acetonitrile. After drying with nitrogen, the gold crystals were either used immediately or treated with 100uM aqueous solutions of SH-PEG-acid for 20 hours to form a self assembled monolayer (SAM). NHS activation of the SH-PEG-acid was done prior to mounting the quartz crystal in the instrument.

The mPEG-NHS was dissolved in MeOH and mixed with buffer just prior to injection into the instrument. Throughout the entire experiment PBS buffer pH 7.4 (Sigma) was used as running buffer of 50µl/min. BSA, goat serum and polyclonal rabbit anti albumin were purchased from Sigma, divided and diluted in PBS buffer and kept frozen at -20°C. Upon each experiment, one aliquot was thawed, diluted and used within 3 days.

In all experiments the injection volume is set to 43 µl.

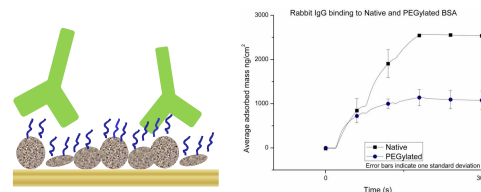
References

- [1] Desito, G et al. Chemistry&Biology 14, 1152-1162, October 2007. DOI 10.1016/j.chembiol.2007.08.015
- [2] www.attana.com
- [3] G. Sauerbrey, Z. Phys. 155, 206 (1959).

Results

PEGylation effect on antibody interaction

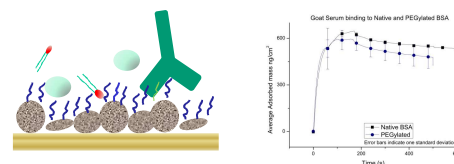
After a stabilization period of approximately 1 hour in the QCM instrument, 10 mg/ml concentration of BSA was repeatedly injected in order to completely cover the surface. In subsequent steps the BSA layer was treated with double injections of freshly prepared mPEG-NHS (20 mg/ml). In order to evaluate the PEGylation effect of the short PEG chain, rabbit anti albumin polyclonal antibody was injected following the binding event. The BSA-antibody complex was desorbed by repetitive washes of 150mM HCl and 100 mM NaOH. The procedure was cycled over many times and the results clearly show that less antibody binds to the PEGylated BSA, compared to the native BSA.



PEGylation experiment. The quartz crystal was first covered with BSA, subsequent treatment with NHS-PEG showed a significant reduction in antibody binding. Binding curve showing the antibody-BSA binding.

PEGylation effect on serum interaction

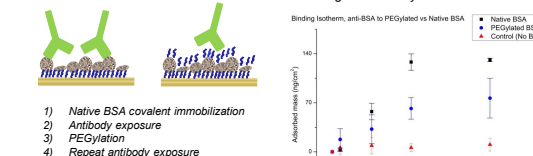
A similar experiment was also done with goat serum. Proteins, lipids and sugars present in the serum provide multiple types of interactions with the PEGylated BSA. BSA was immobilized on the sensor surface, treated with mPEG-NHS and subsequently with 1:200 dilution of goat serum. Components in the serum provide other types of interactions with the BSA (hydrophobic tail of lipids) that is not blocked by the PEGylation.



PEGylation experiment. BSA was immobilized on the sensor surface, treatment with NHS-PEG showed a minor reduction in serum binding.

Binding isotherm

BSA was coupled to a prefucionalized NHS-ester terminated PEG SAM. Once immobilization was completed, remaining NHS ester was quenched by NH₂-PEG. In a control experiment the NHS ester was immediately quenched with NH₂-PEG. The BSA was exposed to different concentrations of polyclonal rabbit anti albumin, followed by repetitive BSA regeneration washes of 150 mM HCl and 100mM NaOH. After collecting sufficient data, the immobilized BSA was PEGylated by triplicate injections of freshly prepared mPEG-NHS (20mg/ml). The PEGylated BSA was again exposed to different concentrations of antibody, followed by repetitive regeneration washes with 150 mM HCl and 100mM NaOH. The results show a reduced binding to the PEGylated BSA.



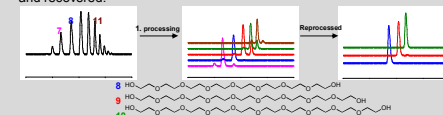
- 1) Native BSA covalent immobilization
- 2) Antibody exposure
- 3) PEGylation
- 4) Repeat antibody exposure

Conclusions

In this work we have investigated the effect of PEGylation of BSA with a short chain PEG. The results show that there is a reduction in binding for the polyclonal antibody, while some of the components in goat serum still can bind to the PEGylated BSA.

Sample displacement chromatography

The chromatographic technique relies exclusively on competition effects towards binding sites on the stationary phase. Several samples are applied on the column top. A high sample loading ensures that strong binding components displace weaker binders, which in turn are transported further through the column system by the flow of a carrier solution. At the end of the separation, the entire column is saturated with products, having different composition throughout the column. The sample components are finally extracted from the segmented column and recovered.



Standard configuration, 1 litre column system



1. Separation step, serial connection; sample displacement
2. Recovery step, parallel connection; MeOH extraction



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