A Behavior of Protein Adsorption on Poly(Ethylene Glycol)-Modified Surfaces under Flow Conditions at Relatively Low Concentrations for Microfluidics Systems

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ABSTRACT

As devices, especially biomedical devices, decrease in size, their surface-to-volume ratio increases proportionally. Combined with handling of relatively smaller amount of analytes at low concentrations, the problem of analyte streams depletion due to adsorption to the device surface prior to detector arises. Thus, the basic understanding of an adsorption behavior of biomolecules on the surfaces of these systems is critical for their use in microfluidics as well as bioanalytical devices. Protein adsorption of alkaline phosphatase (AP) and horseradish peroxidase (HRP) onto poly(ethylene glycol) (PEG)-modified microcapillary surfaces has been determined under flow conditions at different flow rates and protein concentrations ranging from 10-300 ng/ml determined. The flow rate affected the protein adsorption on the surfaces at a fixed protein concentration.

Keywords: protein adsorption, flow condition, poly(ethylene glycol) (PEG)-modified surface, effect of flow rate, relatively low concentration (ng/ml)

1 INTRODUCTION

In the last decade microfabrication technology has been used to create new microfluidics systems, bioanalytical devices, and medical instruments.[1,2] The handling of relatively small amounts of analytes in these microsystems, at low concentrations, combined with the fact that the surface-to-volume ratio increases in direct proportion to the device size decreasing, could create potential problems in device utilization. Analytes or target molecules may be completely non-specifically adsorbed on the surfaces of the microdevices before they reach the detector.[3,4] Thus, the basic understanding of the adsorption behavior of biomolecules [5] on the surfaces of these systems is critical for their use in microfluidics as well as bioanalytical devices. It is also essential for the design of biocompatible materials for the use in biomedical and bioanalytical devices.

Protein adsorption is the central event in the biofouling of surfaces. It is not difficult to achieve the adsorption of a protein on the surface, but it is very difficult how to prevent it. The interactions in protein adsorption are mostly noncovalent, i.e. H-bonding, electrostatic, and hydrophobic interactions. Protein adsorption is distinguished by the large size of the protein and by the fact that while the protein adsorbed on the surface can undergo various transformations, both physical and chemical. The transformations often hold changes in the biological activity of the protein like an enzyme activity even though it is apart from the theoretical aspects of the transformations. Even single purified proteins must be suspected of not behaving as they would normally in the environment where they evolved their specific and sometimes unknown functions. Thus it may be argued that to understand the behavior of proteins at interfaces is to understand an important aspect of their normal behavior. [6]

1.1 Enzymes as Model Proteins

The sensitivity of an enzyme activity assay indicates that the presence or absence of particular enzymes can be determined or confirmed confidence and reproducibility at extremely small amounts of specific enzymes as model proteins.[7, 8] Enzymes such as alkaline phosphatase (AP) and horseradish peroxidase (HRP) are sensitive enough to be detected at a very small amount (up to 0.1 ng/ml) of proteins.

We have developed enzyme assays to study protein adsorption to compare and contrast flow and static conditions in microfluidics systems[7,9] because the protein adsorption under flow conditions in microfluidics systems is rare or little.[10] Studies of protein adsorption behavior under flow conditions of two enzymes such as AP and HRP at a fixed concentration and varied flow rate on the PEG-coated surface of a silica capillary are outlined here. We also studied the protein adsorption behavior, which is saturated (“maximum-layered”) on the surface at specific concentrations and flow rates.

1.2 PEG-Modified Surface

We have chosen a PEG-modified surface, which is well known to resist cell adhesion and protein adsorption [11-18]. The PEG-modified surface demonstrates low protein adsorption under static conditions.[13] We have studied protein adsorption behavior with two proteins, AP and HRP as model proteins under flow conditions at low protein concentrations (10-90ng/ml for HRP and 90-300ng/ml for AP) at the range (20-100μL/hr) of flow rates. We show protein adsorption behavior, which although low, was still significant at the low solution concentration (ng/ml) of proteins on the PEG-modified surface and a flow

effect on protein adsorption behavior at a fixed concentration of protein.

2 MATERIALS AND METHODS

The capillary used was fused silica coated with poly(ethylene glycol) (MicrosolveTech, PEG-100, 50μm i.d.). The capillary was cut to the length of 150mm and glued into a Luer Lock needle with an epoxy glue. The capillary was pretreated with the appropriate buffer system, Tris or PBS, for each enzyme at a flow rate of 100μL/hr overnight (approximately 12 hour) prior to use.

Alkaline phosphatase (AP, from calf intestine, >4000 DEA units/mg protein) was obtained from Sigma and horseradish peroxidase (HRP, ImmunoPure®) was obtained from Pierce. AP was diluted with a 50/50 weight percentage mixture of tris(hydroxymethyl)amine hydrochloride (Tris, Sigma) buffer (50mM Tris, 138 mM NaCl, 30mM KCl) and glycerol (Sigma) to make a stock AP solution. The AP stock solution was diluted to make a final working solution with Tris buffer, which contains 5mM $MgCl_2$ and 0.1mM $ZnCl_2$ to preserve AP activity. A HRP stock solution was prepared with a 50/50 weight percent mixture of phosphate-buffered saline (10mM, PBS, Fisher) and glycerin, and diluted with PBS to make a final working solution as required. Water was Milli-Q water (at least 18 M Ohms). All chemicals used were ACS grade or better.

The biochemical assay of the enzyme was based on an enzyme activity of the specific enzyme, which is reacted with a specific substrate at a specific reaction condition. The system was based on a mass balance system. The ratio of the sample activity to the control activity was taken to be proportional to the enzyme remaining in solution. Therefore, the amount of enzyme lost equals to the inlet enzyme concentration multiplied by the fractional decrease in enzyme activity.

A working enzyme solution was prepared immediately from each stock solution prior to use. The syringe was hooked up to a syringe pump (KDS 200), the capillary/Luer Lock needle combination was attached to the syringe, and the syringe pump started at various flow rates at room temperature.

Working enzyme solution was pushed through the capillary into a polypropylene centrifuge tube (VWR) after being drawn into a syringe (10ml, Becton-Dickson). Within 5-10 minutes of the enzyme solution being drawn into the syringe, the first control was taken from the syringe before running the experiment. The enzyme solution was eluted into the tubes, which contained a mixture of 50/50 weight percentage of each buffer and glycerol. An appropriate correction was subsequently made for dilution effects on the apparent enzyme activity. Samples were taken every 20 minutes for the first 2 hours and every hour after that for two additional hours, and then 2 and 4 hour intervals for one additional sample and finally 5 hour interval for two more additional samples. A fresh control was taken from the syringe every hour and every sample interval for additional controls. The enzyme activity assay of the eluants and the controls was calculated for the amount of enzyme lost and amount of protein adsorbed.

We have modified the enzyme activity assay reported previously.[7] The amount of protein lost was calculated by comparing the difference in enzyme activity in the samples and controls. The cumulative protein lost was converted from units of nanograms to nanomoles per square meter. The cumulative eluant volume was converted to time by using a proper flow rate applied.

3 RESULTS AND DISCUSSIONS

3.1 Considerable Protein Adsorption on PEG-Modified Surface

We have demonstrated that protein adsorption behavior on PEG-modified surface under flow condition was evaluated at the relatively low concentration range of 10-300ng/ml, which although low, was still significant. Figure 1 shows that HRP adsorption on PEG surface was evaluated at three different concentrations (10, 30, and 90ng/ml) at a fixed flow rate, 100μL/hr. Figure 2 shows that AP adsorption on the surface was determined at two different concentrations (90 and 300ng/ml). The results have demonstrated that the protein adsorption behavior on the PEG-modified surface is concentration dependent. At higher concentrations (90ng/ml) of HRP, the protein adsorption was much higher than at lower concentrations (10 or 30ng/ml) (see Fig. 1).

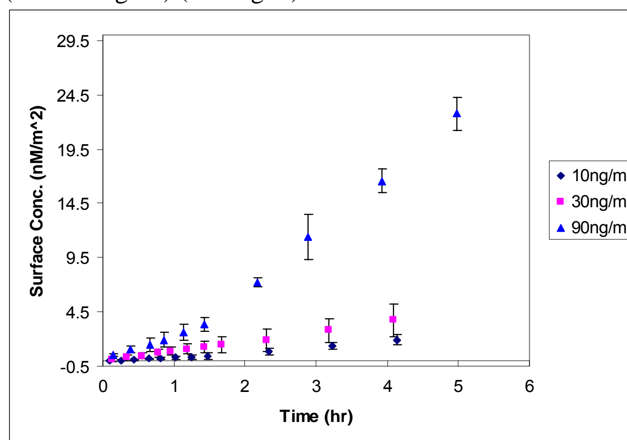

Figure 1. Adsorption of horseradish peroxidase (HRP) on PEG-modified surface at 100uL/hr at varied concentrations.

Horseradish peroxidase (HRP) is a hemo and a glycoprotein. The molecular weight of HRP (44kDa) is much smaller than alkaline phosphatase (140kDa). AP also has a large quantity of saccharides, which is a glycoprotein. The isoelectric points of HRP and AP are 7.2 and 5.6, respectively. Both HRP and AP have spherical shapes. It is believed that those biological, functional, and structural properties may show a different protein adsorption behavior at interfaces. The different biological, structural and

functional properties may cause differences in adsorption behavior.

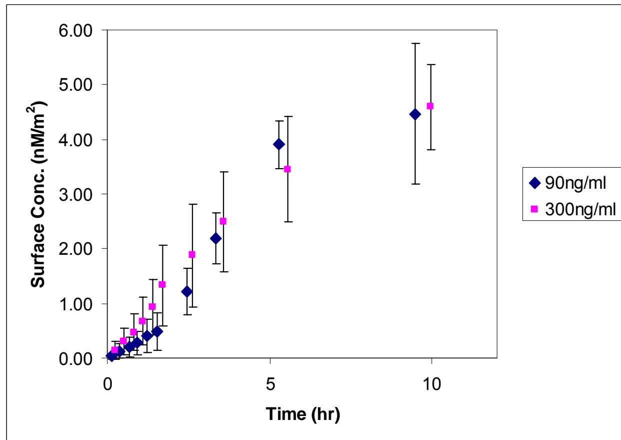

Figure 2. Adsorption of alkaline phosphatase (AP) on PEG-modified surface at 100uL/hr at varied concentrations.

3.2 Flow Effect of Protein Adsorption.

The flow rate affects the protein adsorption on the PEG-modified surface at a fixed protein solution concentration. Figure 3 shows that the flow effect on the

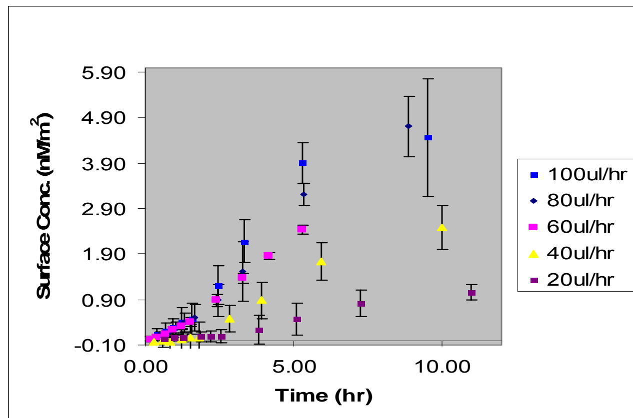

Figure 3. Flow effect of protein adsorption at 90 ng/ml of alkaline phosphatase (AP) at varied flow rates.

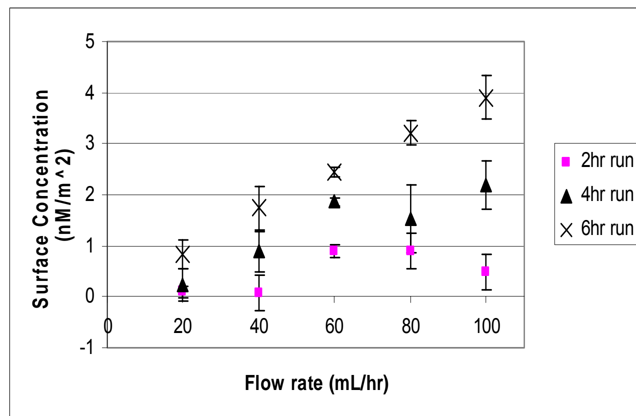

Figure 4. Flow effect of protein adsorption at 90 ng/ml of alkaline phosphatase (AP) at varied flow rates.

protein adsorption behavior was evaluated at varied flow rates (20-100mL/hr) for 90ng/ml solution of AP. The results show that the protein was adsorbed more at higher flow rates (80 and 100mL/hr) than at lower flow rates (20 and 40mL/hr) in Figures 3 and 4.

3.3 Extended Experiments for Simulation Study

We have extended our experimental study to get a saturated (maximum) submonolayer on the PEG-modified surface at a concentration of 300ng/ml at varied flow rates for a simulation (model) study. The result has demonstrated that the protein adsorption is saturated (maximized) at a proper concentration at a proper flow rate for a long term (see Fig. 5). It would be a specific adsorption behavior with specific interaction between specific protein properties and specific properties of the surfaces. It would also be a characteristic property for protein adsorption behavior at the interfaces.

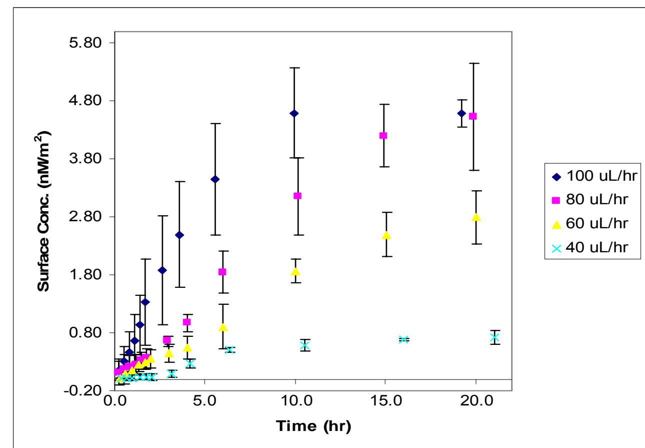

Figure 5. Extended protein adsorption profile at 300 ng/ml of alkaline phosphatase (AP) at varied flow rates.

At higher concentrations of proteins, it would be very difficult to detect the very small amount (submonolayer coverage amount) of protein involved. In the real world of proteins such as blood or urine, the protein adsorption behavior would be a more complex since the protein is not a single protein. It would be a mixture of proteins, which have different affinities to the interface of the surface.[22]

Protein adsorption on a surface of a microfluidic system or bio-analytical medical device is very important. It affects the use of microfluidics systems for of bioanalytical instruments, separation of proteins, and biological samples such as blood, urine, and serum. Furthermore, if we want to shrink instrument sizes, the nonspecific protein adsorption on the surface of the devices is critical to prevent analyte depletion. Therefore, we should understand and determine the interactions between the specific surface of the device and the proteins in the solution that pass through the microfluidics systems and bioanalytical devices.

We are very interested in how the adsorption behavior changes after proteins adhere to the surface and how the

protein interacts with different surfaces and fluid dynamics. Our focus is on whether small amount of proteins adhere to surfaces, which exhibit to be completely protein resistant at high bulk concentrations. Our future work will include the fitting the protein adsorption behavior with fluidic dynamics model to get kinetic coefficients, which are rates of adsorption (K_a) and desorption (K_d), and the density of surface sites (P_{max}).

4 CONCLUSIONS

We have developed assays to evaluate protein adsorption under flow and static conditions at submonolayer coverages on poly(ethylene glycol) (PEG)-modified surfaces, which are well known to resist protein adsorption. Alkaline phosphatase and horseradish peroxidase were used to evaluate protein adsorption behavior at the relatively low concentration range of 10-300 ng/ml, which although low, was still significant. The flow rate was also seen to affect the protein adsorption on the PEG-modified-surfaces at a fixed protein solution concentration. We have extended our experimental study to obtain a saturated (maximum) layer on the PEG-modified surface at a fixed 300 ng/ml, at varied flow rates, in a simulation (model) study for the development of new biocompatible microfluidics systems. This result has demonstrated that the protein adsorption is saturated (maximized) at a specific concentration at a distinct flow rate. Thus, it may be a characteristic property of protein adsorption behavior at an interface.

ACKNOWLEDGMENTS

This work was supported by the MTO office at DARPA through grant number F-30602-01-2-6541, by NSF grant number ECS-0003227 and by NanoScience Technology Center at University of Central University.

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