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# Mass spectrometric mapping of fibrinogen conformations at poly(ethylene terephthalate) interfaces ☆

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**Abstract**

We have characterized the adsorption of bovine fibrinogen onto the biomedical polymer polyethylene terephthalate (PET) by performing mass spectrometric mapping with a lysine-reactive biotin label. After digestion with trypsin, MALDI-TOF mass spectrometry was used to detect peptides from biotinylated bovine fibrinogen, with the goal of identifying lysines that were more accessible for reaction with the chemical label after adsorption. Peptides within domains that are believed to contribute to heparin binding, leukocyte activation, and platelet adhesion were found to be biotin labeled only after bovine fibrinogen adsorbed to the PET surface. Additionally, the accessibility of lysine residues throughout the entire molecule was observed to increase as the concentration of the adsorbing bovine fibrinogen solution decreased, suggesting that the proximity of biologically active motifs to hydrophilic residues leads to their exposure. The surface area per adsorbed bovine fibrinogen molecule was quantified on PET using optical waveguide lightmode spectroscopy (OWLS), which revealed higher surface densities for bovine fibrinogen adsorbed from higher concentration solutions. By measuring changes in both the identity and conformation of proteins that adsorb from complex mixtures such as blood or plasma, this technique may have applications in fundamental studies of protein adsorption and may allow for more accurate predictions of the biocompatibility of materials.
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*Keywords:* Protein adsorption; Fibrinogen; Biocompatibility; Polyethylene terephthalate

## 1. Introduction

Upon exposure to blood, implanted materials adsorb a thin layer of proteins onto their surfaces [1–3]. The rate of adsorption and the chemical properties of the surface influence protein conformations and surface coverage as the adsorbed proteins denature and unfold at this solid/liquid interface. Some adsorbed proteins expose previously hidden binding domains that participate in inflammatory, thrombotic, and immunologic responses [4–8]. The post-adsorptive conformations of fibrinogen have been investigated on surfaces of varying surface energy [9,10] and these transformations expose sites responsible for platelet activation and aggregation [11–14]. Here, we introduce mass spectrometric mapping to probe local conformational changes within bovine fibrinogen (bFg) following adsorption to the biomedical polymer polyethylene terephthalate (PET).

Fibrinogen is a 340 kDa dimeric glycoprotein composed of A$\alpha$, B$\beta$, and $\gamma$ chains held together by 29 disulfide bonds. The structure is trinodular, composed of one inner and two outer globular domains linked by regions of coiled-coils [15]. The inner domain (E domain) contains the amino termini of the A$\alpha$, B$\beta$, and $\gamma$ chains [16], while the two outer domains (D domains) consist of the carboxyl termini of the B$\beta$, and $\gamma$ chains [17]. Extending from the D domains, beginning near A$\alpha$224 in bFg, are the mobile and highly flexible $\alpha$C domains that associate non-covalently with the E domain [18]. Fibrinogen possesses several cell adhesion and protein interaction domains in all three of its major

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chains, including RGD integrin binding motifs in the A$\alpha$ chain [19], a heparin [20] and cadherin [21] binding domain in the B$\beta$ chain, and a dodecapeptide essential for platelet aggregation in the $\gamma$ chain [22].

When proteins are reacted with a chemical label prior to enzymatic digestion, labeled peptides with increased masses can be identified by mass spectrometry. Since the rate of reaction of protein functional groups is dependent on their degree of solution exposure [23], mass spectrometry can be used to map changes in protein conformation by varying the experimental conditions and tracking the accessibility of specific residues to chemical modification. Mass spectrometric mapping has been previously utilized to discern protein–protein interactions and protein conformations in solution [24–26]. Arginine, lysine, tryptophan, and tyrosine are the most common targets of amino acid modification [24,26,27]. Since lysines occur frequently throughout proteins, are charged and have a high degree of solution exposure, amine-specific *N*-hydroxysuccinimide (NHS) ester labels have been used in various applications, including isotope-tagging for relative absolute quantification (ITRAQ) [28,29]. The availability of water-soluble derivatives of NHS-esters provides an advantage over most other reagents used for protein labeling, in that the reaction can be performed under physiologic conditions [30].

Techniques such as antibody labeling [11], atomic force microscopy (AFM) [9,31–35], circular dichroism [36], sum frequency generation (SFG) [37], total internal reflectance fluorescence (TIRF) [34], and Fourier transform infrared spectroscopy (FTIR) [35,38,39] have demonstrated structural changes in fibrinogen after adsorption onto surfaces. Each of these techniques has advantages for elucidating protein structural changes but generally provides information at the domain-level. Antibody binding can be used to correlate the exposure of cell interaction motifs to post-adsorptive changes in fibrinogen conformation [11]. While antibody binding verifies the exposure of previously inaccessible domains of fibrinogen, it is dependent on the availability of specific antibodies and can only reveal changes in protein conformation in areas of the protein complementary to these antibodies. AFM has provided detailed information on the overall post-adsorptive structure of fibrinogen by revealing the dimensions of the fibrinogen D and E domains on various surfaces. However, AFM is not well suited for discerning transitions in dense monolayers of protein. Detailed studies on the global changes in fibrinogen secondary structure after adsorption have been performed using SFG, TIRF, and FTIR, also yielding information at the domain-level. We believe that mass spectrometric mapping may contribute to the study of adsorbed proteins by providing residue-specific information on surface-dependent conformational changes under physiologically relevant experimental conditions.

We have developed a proteomics-based strategy to analyze the adsorption of bFg onto PET, which supplements our earlier proteomic studies on the adsorption of serum and plasma proteins on biomaterials [40]. The conformational changes that occur upon fibrinogen adsorption were examined by comparing the mass spectra of bFg that was biotin labeled in solution to bFg that was labeled after adsorption to the surfaces of PET particles. We hypothesized that there was a tendency for sections of bFg to be exposed to solution during the adsorption process, and that peptides at these locations would be biotinylated at higher rates. We targeted primary amines with a chemical label due to their reactive properties and presence at 107 sites throughout the bFg molecule. Labeled residues could be analyzed with respect to their location in three-dimensional space due to the availability of the nearly complete bFg crystal structure. The reactions were performed under physiological conditions with an NHS-ester label. These residue-specific mass spectrometric mapping experiments correlated well with optical waveguide lightmode spectroscopy (OWLS) results, both of which demonstrated concentration-dependent changes in fibrinogen spreading on PET surfaces. Our data support previous findings that large-scale changes in fibrinogen conformation occur upon adsorption from dilute solutions, evidenced by enhanced exposure of hydrophilic lysine groups throughout the protein. Furthermore, post-adsorptive increases in lysine exposure were detected for several residues localized within biologically relevant fibrinogen motifs. Thus this method may be useful in localizing adsorption-induced conformational changes to specific sites within proteins with a residue-level resolution.

## 2. Materials and methods

### 2.1. Spin coating

Unless otherwise noted, all reagents were purchased from Sigma. Glass OWLS chips coated with a $Si/Ti/O_2$ waveguide layer ($48 \times 16 \times 0.5\,mm^3$, MicroVacuum Ltd.) and silicon wafers ($48 \times 16 \times 0.5\,mm^3$) were cleaned by immersion in piranha solution (3:1 $H_2SO_4$ and $H_2O_2$) for 5 min followed by rinsing in DI water. PET (0.05 mm thick, McMaster Carr, Chicago, IL) sheets were dissolved in chloroform with 5% (w/v) trifluoroacetic acid (TFA) to make 0.8% solutions of PET. The PET solution (100 $\mu$L) was applied to the surfaces of either the wafers or OWLS chips and spun at 4500 RPM for 50 s with a model KW-4A spin coater (CHEMAT Technology). PET layer thicknesses on the silicon wafers were confirmed with a variable angle Stokes ellipsometer (Gaertner Scientific Corporation). X-ray photoelectron spectroscopy (XPS, Kratos Analytical Axis 165 spectrometer, University of Missouri Rolla) was performed to verify the composition of the layers.

### 2.2. OWLS analysis

PET coated waveguide chips were placed inside the flow chamber of a MicroVacuum OWLS 110 optical waveguide lightmode spectrometer. The adsorption of bFg from 1, 0.5 and 0.1 mg/mL solutions in phosphate buffered saline (PBS; 0.2 g/L KCl, 0.2 g/L $KH_2PO_4$, 8 g/L NaCl, 1.15 g/L anhydrous $Na_2HPO_4$, pH 7.4) was monitored with a time step of 30 s. PBS was flowed over the surface of the chips for at least 1 h, or until a baseline with a transverse magnetic mode refractive index variation less than $1 \times 10^{-6}$ was obtained. Solutions of bFg (0.1, 0.5, or 1 mg/mL) were allowed to flow over the surface for 4 h at 0.15 mL/min. The surface was then washed with PBS at 0.15 mL/min until a steady baseline was again

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