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Thickness and morphology of polyelectrolyte coatings on silica surfaces before and after protein exposure studied by atomic force microscopy

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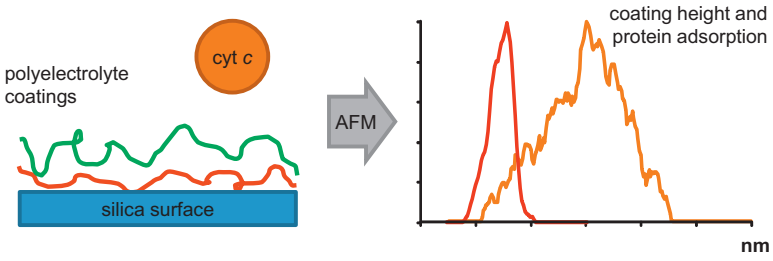
HIGHLIGHTS

- Atomic force microscopy is used to characterize polyelectrolyte coatings.
- Coating procedure leads to nm-thick layers on a silica surface.
- Polyelectrolyte coatings effectively prevent protein adsorption.
- AFM provides the high resolution to investigate these thin films.
- AFM results support earlier findings obtained with capillary electrophoresis.

GRAPHICAL ABSTRACT



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ABSTRACT

Analyte–wall interaction is a significant problem in capillary electrophoresis (CE) as it may compromise separation efficiencies and migration time repeatability. In CE, self-assembled polyelectrolyte multilayer films of Polybrene (PB) and dextran sulfate (DS) or poly(vinylsulfonic acid) (PVS) have been used to coat the capillary inner wall and thereby prevent analyte adsorption. In this study, atomic force microscopy (AFM) was employed to investigate the layer thickness and surface morphology of monolayer (PB), bilayer, (PB-DS and PB-PVS), and trilayer (PB-DS-PB and PB-PVS-PB) coatings on glass surfaces. AFM nanoshaving experiments providing height distributions demonstrated that the coating procedures led to average layer thicknesses between 1 nm (PB) and 5 nm (PB-DS-PB), suggesting the individual polyelectrolytes adhere flat on the silica surface. Investigation of the surface morphology of the different coatings by AFM revealed that the PB coating does not completely cover the silica surface, whereas full coverage was observed for the trilayer coatings. The DS-containing coatings appeared on average 1 nm thicker than the corresponding PVS-containing coatings, which could be attributed to the molecular structure of the anionic polymers applied. Upon exposure to the basic protein cytochrome *c*, AFM measurements showed an increase of the layer thickness for bare (3.1 nm) and PB-DS-coated (4.6 nm) silica, indicating substantial protein adsorption. In contrast, a very small or no increase of the layer thickness was observed for the PB and PB-DS-PB coatings, demonstrating their effectiveness against protein adsorption. The AFM results are consistent with earlier obtained CE data obtained for proteins using the same polyelectrolyte coatings.

Abbreviations: AFM, atomic force microscopy; cyt *c*, cytochrome *c*; PB, polybrene; DS, dextran sulfate; PVS, poly (vinylsulfonic acid); CE, capillary electrophoresis; SMIL, successive multiple ionic polymer layers; EOF, electroosmotic flow; FWHM, full-width-half-maximum.

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1. Introduction

Capillary electrophoresis (CE) is a powerful analytical technique, capable of separating a variety of substances ranging from macromolecular biological compounds to inorganic ions. CE characteristics include narrow analyte peaks and high resolution. However, when using bare fused-silica capillaries, separation efficiencies and migration time repeatability may be compromised due to analyte–wall interactions. In order to avoid analyte adsorption to the inner surface of the capillary and to improve CE performance, chemical modification of the silica surface has shown to be an effective option. Over the last years various capillary coatings have been proposed and their usefulness in, for example, protein and DNA analysis has been shown [1–3].

A flexible, and convenient, coating approach used in CE is the application of self-assembled polyelectrolyte multilayer films. This concept was introduced by Decher et al. [4] and applied in CE for the first time by Katayama et al. [5]. Referring to the way of production these multilayers were coined "successive multiple ionic polymer layered (SMIL) coatings". SMIL coatings are made by simply flushing the capillary with aqueous solutions of polyelectrolytes. The coating procedure is relatively fast and can be easily repeated. Consequently, SMIL coatings have found widespread use in CE for a number of applications [2,3].

Over the last years, our group has demonstrated the usefulness of noncovalent polyelectrolyte coatings of polybrene-poly(vinylsulfonic acid) (PB-PVS) and polybrene-dextran sulfate-polybrene (PB-DS-PB) for highly efficient CE analysis. These coatings were applied for metabolic profiling [6–8], peptide analysis [9,10] and protein characterization [11–13], providing highly reproducible and efficient separations and good compatibility with various detection schemes, like mass spectrometry [7,13] and fluorescence [11]. The performance of these noncovalent polyelectrolyte coatings was thoroughly studied by monitoring analyte migration time repeatabilities and peak widths and shapes. The effectiveness of these coatings against protein adsorption was also investigated *in situ* by evanescent-wave cavity ring-down spectroscopy employing a basic protein as probe [14]. Physical characterization of the layered coatings in terms of thicknesses and surface morphology before and after protein exposure was not investigated before. Still, knowledge on the constitution of SMIL coatings can be essential to understand the (degree of) surface coverage and orientation of the polymers and, thus, properly appreciate their capacity to prevent adsorption and provide a stable electroosmotic flow (EOF).

Various techniques are available for the probing of surfaces and their morphology on a molecular scale [15]. Among these, atomic force microscopy (AFM) has become an important tool due to its good lateral and vertical resolution – down to sub-nm dimensions – and low sample pretreatment requirements. AFM has shown useful for the assessment of the morphology of bare [16,17] and coated [18–22] silica surfaces as met in CE. Capillary coatings probed by AFM include micelles [18,20], polyacrylamides [19,21], and hydrogels [22], with observed layer thicknesses ranging from sub-nm [18] up to several hundreds of nm [22].

In the present study, we used AFM to examine the thickness and morphology of noncovalent polyelectrolyte coatings frequently used by our group in CE of biomolecules. The coatings were applied to silica surfaces (glass substrates) to allow AFM. The studied coatings were a monolayer of PB, bilayers of PB-DS and PB-PVS, and trilayers of PB-DS-PB and PB-PVS-PB. In order to mimic CE conditions, the respective coatings were exposed to an aqueous solution of potassium phosphate (pH 7.4) while being examined by AFM. Average layer thicknesses of the different coatings were determined using tip-induced displacement of the layers (*i.e.* nanoshaving) and surface morphologies were assessed. Potential protein adsorption to the various surfaces was probed by exposure to the basic protein cytochrome *c* and subsequent AFM analysis. Results obtained with AFM were correlated to data previously obtained during CE analysis of cytochrome *c* using different coated capillaries.

2. Experimental

2.1. Chemicals

Potassium hydroxide, sodium hydroxide, polybrene (hexadimethrine bromide, PB), poly(vinylsulfonic acid) (PVS) and dextran sulfate (DS) sodium salt were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate was obtained from Merck (Darmstadt, Germany). A 10 mM phosphate buffer (pH 7.4) was prepared by dissolving 0.136 g potassium dihydrogen phosphate in 100 mL deionized water and adjusting the pH with potassium hydroxide. Solutions of 10% (w/v) PB, 1% (v/v) PVS and 3% (w/v) DS were prepared in deionized water. The solutions were filtered over a 0.45 μm filter type HA (Millipore, Molsheim, France) prior to use. For the protein adsorption measurements, a stock solution of 80 μM (1 mg mL^{-1}) cytochrome *c* (cyt *c*, Sigma–Aldrich) was prepared in deionized water, and diluted in 10 mM potassium phosphate buffer to a final cyt *c* concentration of 16 μM.

2.2. Substrate coating

Microscope glasses (Menzel Gläser, Braunschweig, Germany) were used as substrates and first thoroughly cleaned by washing the surface with ethanol and deionized water, respectively. Subsequently, the microscope glasses were immersed in 1 M NaOH at room temperature for 15 min to activate the surface silanol groups. Finally, the substrate was rinsed with deionized water and dried using a flow of nitrogen gas and then the substrate was ready for coating. A single layer of PB was produced by covering the substrate surface with the 10% PB solution and incubated for 10 min at room temperature. Subsequently, the substrate was thoroughly washed with deionized water in order to remove nonbound polymer. For the bilayer and trilayer coatings the coating procedure was repeated using the respective polyelectrolyte solutions in order to obtain the desired coatings, *i.e.* PB-DS, PB-PVS, PB-DS-PB, and PB-PVS-PB. Fig. 1 shows the molecular structures of the coating agents. The various surfaces were also exposed to cyt *c* by coverage with a 16 μM solution followed by a 15 min incubation and subsequent flush with water.

2.3. Atomic force microscopy

AFM experiments were performed using a JPK NanowizardII AFM instrument (JPK Instruments, Berlin, Germany). AFM graphs of the differently treated glass substrates were recorded in phosphate buffer solution (pH 7.4) using the contact mode. A CSC 37 cantilever (Micromash, Estonia) was applied with a nominal spring constant of 1.2 N m^{-1}. Using the JPK SPM software, the exact cantilever spring constant was determined and the force applied to the sample was adjusted manually to approximately 50 pN. AFM pictures were recorded at line-scan rates of 1 Hz and the grid size was 512 × 512 pixels. The different coatings and adsorbed protein were shaved from the glass surface by applying the maximal amount of force (approximately 250 nN) at a line-scan rate of 20 Hz. Three randomly chosen sections of the surface were investigated ($n = 3$). Per section, an area of 1 × 1 μm was shaved with high force to create a piece of bare silica surface in order to allow establishment of a zero point (0 nm) for height measurements. Subsequently, an adjacent area of 1 × 1 μm was scanned and the