Selective protein adsorption on stimuli-responsive brushes

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Stimuli-responsive polymer brushes offer promising perspectives for biological and medical applications, including biofouling, chromatography, and regenerative medicine . Understanding and controlling selective protein adsorption will help to delineate their performances for such applications [1].

The aim of this research is to use polymer brushes composed of poly(ethylene oxide) (PEO) and poly(acrylic acid) (PAA) for the tailored adsorption of human serum albumin (HSA), lysozyme (Lys), and fibrinogen (Fb). PEO inhibits protein adsorption. PAA is an anionic polyelectrolyte having variable densities of negative charges depending on pH. Thiol-terminated PAA with M_n = 2000 g/mol and PEO with M_n = 1100, 2000 and 5000 g/mol were used. A gold substrate was modified by these thiolated polymers according to the "grafting to" method. HSA (molar mass of 66 kDa; iep close to 5) was chosen as a common model protein. Lys (14.3 kDa; iep~11) and Fb (340 kDa; iep~6) were additionally chosen based on their iep and structural characteristics with a view to study electrostatic-dependent protein adsorption on mixed PEO/PAA brushes.

The brushes obtained with different PEO:PAA ratios and different PEO chain lengths were characterized by X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM), and water contact angle measurements. Surface characterization of mixed brushes revealed the presence of both polymers in the layers, in variable proportions according to the chosen parameters. Polymer brush formation and protein adsorption were monitored by Quartz Crystal Microbalance. Protein adsorption was studied at different pH values (3.5-9.0) and salt concentrations (10^{-3} - 0.15M). Firstly, protein adsorption was measured from single protein solutions. It was observed that at pH 9.00 and in the ionic strength range 10^{-3}-0.15M, Fb and Lys adsorb on mixed PEO/PAA brushes, while adsorption of HSA was not observed. Next, adsorption was performed from mixtures of two or three proteins. At pH 9.0 and in the ionic strength range 10^{-3}-0.15M, QCM measurements allowed to observe significant protein adsorption. We demonstrated that proteins which adsorbed at pH 9.0 and the ionic strength 10^{-3} and 10^{-2}M could easily be desorbed by rinsing with a sodium chloride solution at pH 9.0 and ionic strength 0.15M. It was observed that the amount of adsorbed and desorbed proteins strongly depend on the molecular weight of PEO and their quantity in the polymer brushes. The supernatant collected upon such rinsing was concentrated and analyzed by gel electrophoresis with silver staining. Silver staining is used for sensitive detection of proteins separated by 1D and 2D SDS PAGE with detection limit from 5-10ng. It was proved that selective adsorption of Lys from the mixture of Lys/HSA/Fb is possible at pH 9.0 and ionic strength 10^{-3}M, while Lys and HSA but not Fb were adsorbed at ionic strength 10^{-2}M and pH 9.0. The results thus demonstrate that by controlling both pH and ionic strength, selective adsorption from protein mixtures can be achieved on PEO/PAA mixed brushes.

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[1] J. Zhang, *Switchable and Responsive Surfaces and Materials for Biomedical Applications*, 2015, Chapter 5, Woodhead Publishing, Cambridge, UK, p.138-140.