Adsorption of Polylysine, Poly(glutamic) acid and their Block Copolymers on Polystyrene and on Carbon Nanotubes

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Introduction

A better understanding of protein behavior at solid-liquid interfaces is important to improve our knowledge of various natural and synthetic processes. A few examples where protein adsorption at a solid-liquid interface may play an important role are found in: blood coagulation, artificial kidney failure, biomedical microdevices, self-assembling microelectromechanical systems (MEMS), protein based microarrays, design and fabrication of biosensors/ biomaterials, filtration systems in bioseparation processes, etc. The structure of adsorbed proteins, its dependence on sorbent and solution properties, and the contribution of protein structural rearrangements to the driving force for adsorption are the most poorly understood aspects of the protein adsorption processes

There are few experimental studies on structure of short peptides at solid surfaces.[1,2] Read and Burkett[1] used circular dichromism (CD) and nuclear magnetic resonance (NMR) to study a 13-residues helical peptide of arginine, alanine, and aspartate adsorbed on anionic and cationic surfaces. They showed that the adsorption is driven by electrostatic complementarity and, as a result, there is a significant helicity loss in the alanine and arginine region of the peptide. Long et al.[2] studied the adsorption of small linear peptides (composed of leucine and lysine residues) of varying periodicities and helix lengths onto porous polystyrene surfaces. They found out that helix length, rather than hydrophobic periodicity, determines the structure of the adsorbed peptides onto the polystyrene surface. They reported that adsorption onto the polystyrene surface induced more helical structure in the shorter peptides and that slight changes in torsion angles were needed for the longer peptides to adsorb onto the polystyrene surface. They concluded that aggregation and formation of the secondary structure in solution precedes adsorption onto the polystyrene surface.

In this paper, we explore the adsorption of several peptides at solid/liquid interfaces. The peptides were designed to either enhance electrostatic or hydrophobic interactions.

Experimental

All the chemicals used in this work were of analytical grade. Before each experiment, buffers were filtered through a Whatman 0.45 μm polysulfone and a Whatman 0.2 μm polyethersulfone membrane filters. The absorbent used was monodisperse polystyrene (PS) latex from Duke Scientific Corp. with a particle average diameter of 102±3 nm (10% solids) or 309±7 nm (1% solids). PS suspensions were dialyzed for 48 hours to remove a proprietary surfactant before they were diluted to the required concentration with phosphate buffer at the desired pHs. . The carbon nanotubes decorated with gold particles were generously provided by Dr. Y. Xing (Chem. Biol. Eng. Dept., UMR).

The block copolymer of L-Phenylalanine and γ-benzyl-D-glutamate (25:75) was prepared as follows. γ-benzyl-D-glutamate-N-carboxyanhydride (BDG-NCA) and L-phenylalanyl-N-carboxyanhydride (L-Phe-NCA) were synthesized according to Daly and Poche[3]. L-Phe-NCA was dissolved in dry benzene (2% solution) and hexylamine used to initiate polymerization (monomer/initiator ratio 40). After one day, BDG-NCA was added to the reaction mixture. After two additional days the reaction was stopped. The polymer was precipitated with ether, washed with ether and chloroform and vacuum dried.

The block copolymer of L-Lysine and L-alanine (50:50) was prepared as follows. ε-benzyloxycarbonyl-L-Lysine-N-carboxyanhydride (Lys-NCA) and L-alanine-N-carboxyanhydride (Ala-NCA) were synthesized according to Ref 3. Lys-NCA was dissolved in dry dioxane (2% solution) and triethylamine used to initiate polymerization (monomer/initiator ratio 100). After two days, Ala-NCA was added to the reaction mixture and after five additional days the reaction was stopped. The polymer was precipitated with ethanol, washed with ethanol and ether and then dried. The benzyloxycarbonyl protecting group was removed using HBr(33% in acetic acid). The polymer was precipitated and washed with ether, dialyzed and then freeze-dried.

Adsorption and dynamic light scattering (DLS) experiments were performed as described in Ref. 4. The thickness of the adsorbed layer was determined by subtracting the diameter of the bare latex nanospheres from the diameter of the spheres after adsorption. SEM and TEM experiments were done in a Hitachi S 570 Scanning Electron Microscopy with Kevex Delta I EDS/WDS system and SESAME automation. Some samples were stained with uranyl acetate.

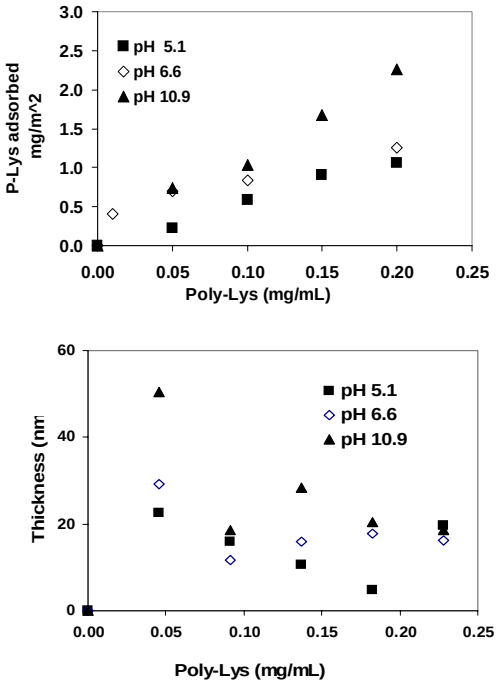

Figure 1. *top: Amount adsorbed. Bottom: thickness of adsorbed layer on PS for poly(lysine).*

Results

The copolymers were analyzed by NMR and acid hydrolysis. The degree of polymerization of (Lys-Ala) obtained by comparing the ratio of initiator and the ε-CH_2 peak of lysine was 38 and the molar ratio for Ala/Lys from acid hydrolysis was 1.43 (degree of polymerization for Ala = 54). This yields a molecular weight of 10,100. A similar analysis of the Phe-BDG yields a molecular weight of 6,800.

A few observations can be made by comparing the thickness of the adsorbed layer and the amount adsorbed. Because of space limitations, we present here a very limited number of data. **Figure 1** shows the observed thickness obtained by DLS and the amount adsorbed for poly(lysine) of a molecular weight of 66,000 Da. The first, an obvious observation is that the thicknesses as well as the amount adsorbed exhibit a maximum at pH 10.9. This pH is close to the isoelectric point of the peptide. The absence of lateral repulsions at isoelectric conditions facilitates the packing of the peptide

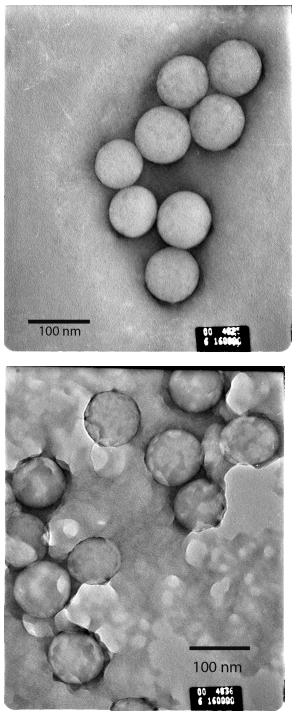

Figure 2. *top: electron micrograph of PS beads (stained with uranyl acetate) before adsorption. bottom: electron micrograph of poly(lysine) adsorbed on PS (stained with uranyl acetate.*

at the surface of the lattice, as expected. It is not clear, however, why at neutral pHs the peptide exhibits a thinner layer. One may expect that at pHs away from isoelectric conditions the peptide will have a tendency to extend into the bulk unless interactions between complementary charges flatten the peptide against the surface. This flattening is obvious at the higher concentrations used by us. Second, the amount adsorbed increases as the bulk concentration increases (as expected) whereas the layer becomes thinner and thinner. The dimensions of the extended peptide are 126 nm by 1.2 nm in diameter whereas the dimensions of the helix are 64 nm in length by 2.4 nm in diameter. The adsorbed layer does not correspond to either one. Experiments performed with higher and lower molecular weights peptides show a similar trend. The data at basic or neutral pH seem to level off at ~ 20 nm. The electron micrographs **(Fig. 2)** indicate that the peptides deposit as amorphous bodies; therefore, the dimensions of the deposits do not correspond to any of the dimensions of the peptides. The point in **Figure 1** at the lowest peptide concentration is, somehow, off. Dynamic light scattering results show that the preparation is highly polydispersed and electron micrographs confirm the formation of very large aggregates. This is consistent with the known fact that when polymers are adsorbed on latex at low concentration they will bridge the lattices together.

Poly(glutamic) acid did not adsorb on polystyrene at any pH (even in the vicinity of its isoelectric point. This is just another evidence of the preponderance of electrostatic interactions in the adsorption process. We have found these same trends in some theoretical studies performed in our group.

We also performed a few experiments with carbon nanotubes "decorated" with gold particles. **Figure 3** shows the results for human IgG. The darker deposits on the tube are gold particles. The increase in the size of the nanotubes is obvious. The protein deposits on both the carbon substrate and the gold nanospheres forming a relatively uniform deposit. The layer is not firmly attached to the tubes since vigorous rinsing seem to wash out most of the deposits.

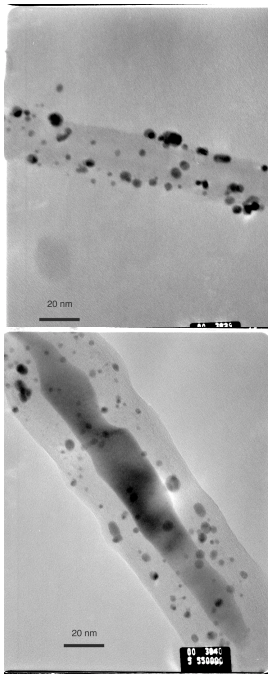

Figure 3*. top: electron micrograph of a carbon nanotube decorated with gold spheres before adsorption. bottom: electron micrograph of IgG adsorbed on the nanotube. .*

References.

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