Strong Resistance of Phosphorylcholine and Oligo(phosphorylcholine) Self-Assembled Monolayers to Protein Adsorption: Insights into Nonfouling Properties of Zwitterionic Materials

Shengfu Chen, Jie Zheng, Lingyun Liu, Lingyan Li and Shaoyi Jiang
Department of Chemical Engineering, University of Washington, Seattle, WA, 98195

Statement of Purpose: Polyethylene glycol (PEG) is one of the best synthetic non-fouling materials[1]. However, it is now recognized that PEG decomposes in the presence of oxygen and transition metal ions found in most biochemically relevant solutions. It is of great interest to search for alternative nonfouling materials other than PEG[2]. It is generally believed that water plays an important role in surface resistance to protein adsorption. While hydrophilic and neutral PEG forms a hydration layer via hydrogen bonds, zwitterions form a hydration layer via electrostatic interactions. It is expected that zwitterions are capable of binding significant amount of water molecules and therefore are potentially excellent candidates for super-low fouling materials.

In this work[3], we show the strong resistance of zwitterionic phosphorylcholine (PC) and oligo (phosphorylcholine) (OPC) self-assembled monolayers (SAMs) to protein adsorption and examine key factors leading to their nonfouling behavior. PC SAMs with a balanced charge and minimized dipole are excellent candidates as nonfouling materials due to their strong hydration capacity via electrostatic interactions. Despite the existence of negative charge on OPC SAMs and the simple synthesis procedure of OPC thiols, OPC SAMs resist protein adsorption as effectively as or even better than PC SAMs formed from highly purified PC thiols, which indicates that increasing PC repeat units will increase its ability to resist protein adsorption and suppress protein adsorption caused by net charge.

Methods: We synthesized PC and OPC thiols, characterized PC and OPC SAMs on Au(111) using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and ellipsometry, and evaluated protein adsorption using a surface plasmon resonance (SPR) sensor. In addition, we also performed molecular simulation studies of the packing structure and density of PC SAMs on Au(111) to provide molecular-level information for the further interpretation of our experimental results. We also patterned PC and OPC on a surface using a micro-contact printing method to demonstrate its excellent nonfouling property.

Results / Discussion: The adsorption of Fg and BSA on PC and OPC SAMs prepared from the aqueous solution were investigated by an SPR sensor. It is shown in that the adsorbed amounts of Fg and BSA on PC SAMs are 0.03mg/m^2 and 0.01mg/m^2 from PBS buffer (0.15M and pH 7.4) containing 1mg/mL Fg and BSA, Thus, the PC head groups are intrinsically nonfouling. It should be emphasized that the N/P ratio is critical to the nonfouling behavior of a zwitterionic PC surface to achieve charge balance. Comparing our XPS results with those published previously, it can be seen that the N/P ratio from our PC thiol SAM formed at pH 10 is 1.05:1, which is very close to the theoretical value of 1:1 and which has 1% of a ML of Fg. Our simulation results show that the lowest-energy configuration of PC-SAMs has a $(\sqrt{7}\times\sqrt{7})R60°$ lattice structure with a thickness of 14.4 Å. This corresponds to a chain-chain spacing of ~7.63 Å or an area of 50.42 Å^2 per chain, the angle between the vector pointing from P to N and the normal gold surface is about 80^o, indicating that the orientation of PC head groups lies nearly parallel to the gold surface as shown in Figure 1. This was also observed previously for lipids. This appropriate packing is approved by both AFM and ellipsometry results.

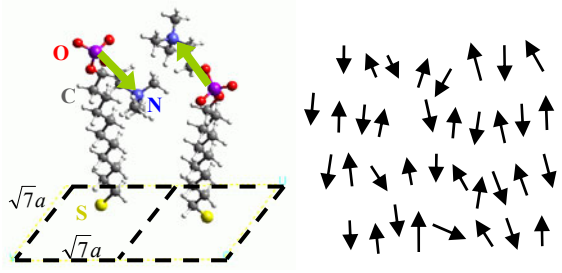

Figure 1. Molecular configuration of PC SAMs (left) and anti-parallel orientation of PC head groups for dipole minimization (right) from molecular simulations.

The adsorbed amounts of Fg, BSA, and Lyz on OPC SAMs are even lower, which are 0.003 mg/m^2, 0.014 mg/m^2, and 0.01 mg/m^2 from PBS buffer (0.15M and pH 7.4) containing 1mg/mL Fg, BSA, Lyz, respectively, although OPC SAMs have the N/P ratios away from 1:1, leading to a negative surface charge. For cell culture on patterned surfaces, after 2-day cell culture, cells remain in their respective bands on both PC and OPN patterned surfaces and exhibit well-defined patterns. No obvious difference was observed in cell behavior between PC and OPC SAMs. Thus, both OPC and PC SAMs can strongly resist protein and cell adsorption.

Conclusions: It is demonstrated in this work that zwitterionic PC SAMs are highly resistant to protein adsorption. PC SAMs have very low protein adsorption when the N/P ratio is close to 1:1 (or balanced charge). OPC SAMs still strongly resist protein adsorption and cell adhesion, even though OPC SAMs have net surface charge. This indicates that increasing PC repeat units will increase its ability to resist protein adsorption and suppress protein adsorption caused by net charge.

References: (1) Ostuni, E.; Chapman, R. G.; Holmlin, R. K.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605.
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(3) Chen, S. F.; Zheng, J.; Li, L. Y.; Jiang, S. *J. Am. Chem. Soc.* **2005**, *127*, 14473.