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Screening nonspecific interactions of peptides without background interference

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ABSTRACT

The need to discover new peptide sequences to perform particular tasks has lead to a variety of peptide screening methods: phage display, yeast display, bacterial display and resin display. These are effective screening methods because the role of background binding is often insignificant. In the field of nonfouling materials, however, a premium is placed on chemistries that have extremely low levels of nonspecific binding. Due to the presence of background binding, it is not possible to use traditional peptide screening methods to select for nonfouling chemistries. Here, we developed a peptide screening method, as compared to traditional methods, that can successfully evaluate the effectiveness of nonfouling peptide sequences. We have tested the effect of different peptide lengths and chemistries on the adsorption of protein. The order of residues within a single sequence was also adjusted to determine the effect of charge segregation on protein adsorption.

1. Introduction

It has been established that surfaces modified with hydrophilic synthetic polymers, such as poly(ethylene glycol) (PEG), are able to resist types of surface fouling, such as nonspecific protein adsorption and bacterial adhesion/biofilm formation in complex media [1,2]. More recently, synthetic zwitterionic poly(carboxybetaine) and poly(sulfobetaine)) [3–5] as well as synthetic mixed positively and negatively charged polymers [6,7] have been developed and shown to achieve ultralow levels of fouling that have not been achieved before. Despite some progress in the fundamental understanding of molecular-level nonfouling mechanisms and the development of new materials, only a handful of nonfouling materials are currently available. Mixed charge nonfouling polymers are particularly attractive for practical applications because of their simplicity, broad variations, and low-cost [8,9]. Among these, peptides are of particular interest due to their being natural materials. Mimicking the chemistry of zwitterionic polymers, we have also been able to achieve ultralow levels of fouling with peptide-based surfaces [6]. For evaluating nonfouling materials, peptide-based nonfouling polymers offer an indispensible advantage; potentially infinite variability in structure and property, with precise control over length and sequence. In addition to the already rich diversity of naturally occurring amino acids, incorporating synthetically derived unnatural amino acids allows for an even greater number of potential materials [10,11].

Peptide libraries are tools often used to search for new functional chemistry, such as identifying enzyme binding motifs [12], protease cleavage sites [13], mineral binding peptides [14], and antibiotics [15]. To accomplish this, peptide libraries require a display mechanism; commonly phage display, yeast display, bacterial display, or resin display [16–18]. These methods work by evaluating the binding performance of individual peptide sequences to specific materials. The peptide of interest usually makes up only a small portion of the presenting scaffold (i.e. cell, virus, etc.). The presence of background binding from the display mechanism does not interfere because the affinity of the peptide is far greater than the affinity of the background for the material being tested [19].

2. Experimental methods

2.1. Materials

Triisopropylsilane (TIPS), trifluoroacetic acid (TFA), γ-glycidoxypropyltrimethoxysilane (GPTMS), N,N-diisopropylethylamine (DIPEA), glass beads (212–300 μm), fibrinogen from bovine, glass beads (50–70 mesh) and octadecyltriethoxysilane were purchased from Sigma–Aldrich. tetraethylene glycol diamine (PEO_4-Bis Amine) was purchased from Molecular Biosciences. TentaGel MB NH2 resin (capacity: 0.51 mmol/g) was purchased from RAAP Polymere. Alexa Fluor® 488

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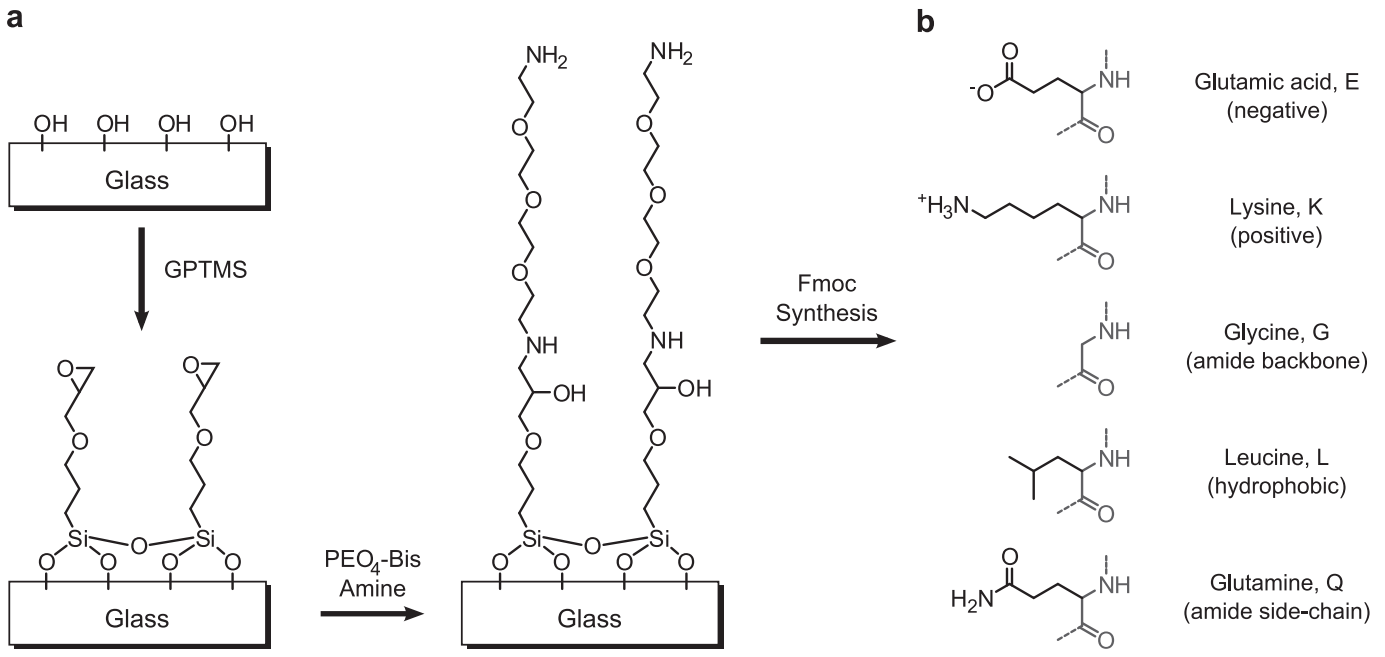

Fig. 1. Steps for surface modification of glass beads. a) GPTMS was first used to introduce epoxide groups to the glass surface. The epoxidized surface was exposed to PEO_4-Bis Amine in order to attach reactive amine groups needed for peptide synthesis. b) Finally, amino acids, with diverse chemical properties, were selected to grow peptides from the glass beads using standard Fmoc peptide synthesis.

carboxylic acid, 2,3,5,6-tetrafluorophenyl ester (Alexa Fluor® 488 5-TFP) was purchased from Fisher Scientific. Hydroxybenzotriazole (HOBt), O-benzotriazole-N,N,N′,N′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and Fmoc protected amino acids were purchased from AAPPTec.

2.2. Glass bead surface modification for peptide synthesis

100 mL of 37% hydrochloric acid was added to 20 g of glass beads and refluxed overnight. The beads were washed several times with water and methanol, then dried under reduced pressure and heating. The glass beads were next treated with a 3:1 volumetric ratio of concentrated sulfuric acid and 30% hydrogen peroxide, and allowed to sit for 30 min. The beads were then rinsed several times with water, then treated with a 5:1:1 volumetric ratio of water to 29% ammonium hydroxide to 30% hydrogen peroxide, and allowed to sit for 30 min. The beads were washed with water several times and then washed several times with methanol. The glass was then dried under reduced pressure and heating. The beads were placed in an oven at 130 °C for 2 h to remove and trace water then allowed to cool to room temperature. For silination, the beads were added to a solution of anhydrous toluene, GPTMS, and DIPEA (94/5/1). The silination occurs at 80 °C for 18 h. After silination, the beads were washed with anhydrous toluene, dichloromethane (DCM), and then dried under reduced pressure and heated at 100 °C for 2 h. The silanized beads were next added to a solution of 5% PEO_4-Bis Amine in anhydrous acetonitrile and heated to 80 °C for 18 h. The beads were then washed with acetonitrile and dried. Unreacted surface epoxide groups were deactivated by incubating in 2% sulfuric acid in water overnight. Then beads were then finally washed with water and methanol, then dried under reduced pressure and heating.

2.3. Peptide synthesis

Solid phase peptide synthesis was performed using a Titan 357 peptide synthesizer (AAPPTec Inc., Louisville, KY). Coupling of Fmoc protected amino acids occurred in 80 mM amino acid, 80 mM HBTU, 80 mM HOBt, 160 mM DIPEA in dimethylformamide (DMF) for 60 min. Piperidine/DMF (20/80) was used to deprotect the Fmoc protected amine from the newly bound amino acid residue. A solution of DMF/DCM (50/50) was used for washing between coupling and deprotection steps. After synthesis was complete, all terminal amine group were acetylated using acetic anhydride/pyridine/DMF (5/5/90). Deprotection of the acid cleavable side-chains occurred as the final step using a solution of TFA/phenol/water/TIPS (88/4/4/4) for 3 h. The beads were then washed with DCM and dried under reduced pressure.

2.4. Protein adsorption and screening

Alexa Fluor 488 labeled fibrinogen was prepared by conjugation with Alexa Fluor® 488 5-TFP. Briefly, 30 mg of fibrinogen was dissolved in 3 mL of 100 mM $NaHCO_3$, pH 9. This solution was added to a vial containing ~1 mg of Alexa Fluor® 488 5-TFP. After 2 h, the labeled conjugates were twice purified using 10 mL Bio-Gel P-6DG Bio-Rad disposable size exclusion columns.

Before adding labeled fibrinogen, peptide coated beads were washed several time with phosphate buffered saline (PBS). The beads were then incubated in a 0.5 mg/mL solution of Alexa Fluor 488 labeled fibrinogen for 60 min. After adsorption, the beads were washing several times with PBS to remove unbound protein and quickly analyzed using a Zeiss LSM 510 confocal microscope. Cross-sectional fluorescent images of the beads were taken (X–Z plane) using 488 nm argon laser for excitation, and detecting emission signal between 500 and 550 nm.

2.5. X-ray photonelectron spectroscopy (XPS) of glass beads

XPS experiments were performed on an S-Probe spectrometer using a monochromatic Al Kα X-ray source ($h\nu = 1486.6$ eV) operated at 10 mA and 15 kV. Survey spectra were acquired with an analyzer pass energy of 80 eV. High-resolution O 1s, N 1s, C 1s and Si 2p spectra were acquired with an analyzer pass energy of 20 eV. All of the XPS data were acquired at a nominal photoelectron takeoff angle of 0°, where the takeoff angle is defined as the angle between the surface normal and the axis of the analyzer lens. Three spots on each sample were examined. The compositional data are averages of the values determined at each analysis spot.

3. Results and discussion

In this work, we screened a large library of peptide sequences to determine nonfouling properties. Since we were looking to determine what peptide sequences have the lowest amount of nonspecific binding, traditional peptide display mechanisms would not work, due to the presence of background binding [20]. Background binding would eliminate the ability to differentiate between nonfouling sequences. For this reason, a new method for screening

Table 1
Summary of XPS-Determined Elemental Composition of bare glass, deactivated epoxy and amine surfaces (atom%). *Theoretical C/N ratio for 100% coupling efficiency of the diamine linker onto the surface.

Sample	Atom%					
	O	N	C	Si	C/N	C/N*
Bare glass	67.5 ± 1.0	–	4.4 ± 1.1	28.1 ± 0.6	–	–
Deactivated epoxy	57.6 ± 2.3	–	21.7 ± 1.6	20.7 ± 0.7	–	–
Amine surface	57.0 ± 2.1	2.0 ± 0.5	18.3 ± 0.6	22.6 ± 1.4	9.1	7.0