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Reduced steric hindrance and optimized spatial arrangement of carbohydrate ligands in imprinted monolayers for enhanced protein binding

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ABSTRACT

Imprinted monolayers provide several advantages over bulk imprinting methods. This is especially important for large templates such as proteins. Concanavalin A (Con A)-imprinted binary monolayers consisting of glycolipids with oligo(ethylene glycol) (OEG) spacers and zwitterionic phospholipids (DPPC) were constructed and investigated. The shorter phosphorylcholine (PC) headgroups with an almost flat-on orientation in the binary monolayers gave rise to reduced steric hindrance favorable to the accommodation of Con A with greater ease and facilitated the access of the OEG-linked mannose moieties for enhanced protein binding. Further enhanced binding resulted from optimized spatial rearrangement of the glycolipids at the air–water interface directed by Con A in the subphase to create bivalent binding sites and to minimize steric crowding of neighboring mannose ligands. The combination of the exposed carbohydrate ligands from biologically inert surfaces and the optimized ligand arrangement is the most reasonable solution to enhancement of protein affinity. The bivalent carbohydrate binding sites protruding from the imprinted monolayers were created to be complementary to the Con A binding pockets. This strategy generates tailor-made surfaces with enhanced protein binding and opens the possibility of controlled assembly of intellectual biomaterials and preparation of biosensors.

1. Introduction

Molecular imprinting of biomacromolecules like proteins as the synthetic antibody mimics exhibiting excellent chemical, mechanical, and thermal stability could be substituted for expensive biological antibodies used in isolation, extraction of proteins, biosensors, and the development of biological materials [1–4]. Imprinting of proteins represents one of the most challenging tasks [1]. The benefits of the imprinted monolayers provide several advantages over bulk imprinting methods such as excellent mass transfer of molecules into and out of imprinted sites [3,5]. This is especially important for large templates such as proteins, which can be encapsulated and cannot be removed completely from even thin polymer matrixes [3]. Furthermore, rebinding of the templates is typically fast, and sensing can be further enhanced by the monolayer surfaces that facilitate transduction of binding signals detected in real time [3]. Inspired from the highly dynamic nature of lipid–lipid and lipid–protein interactions in the cell membranes [6], we prepared protein imprintings from binary Langmuir monolayers containing positive-charged lipids or glycolipids at the air–water interface [7–11]. The use of water as solvent provides a biologically friendly environment to proteins although water can reduce hydrogen bonding and electrostatic interactions between the template molecules and the functional monomers [4]. Functionalized lipids at the air–water interface can rearrange to form complementary interactions with proteins in the subphase in the fashions of cooperative and multivalent interactions, followed by horizontal immobilization onto sensor surfaces, and created specific binding sites can be preserved for protein recognition after bound template proteins are removed [7,8,10].

Protein–carbohydrate interactions play an important role in a variety of cellular processes [12,13], and these specific interactions occur between lectins and glycoproteins, glycolipids, and polysaccharides on cell surfaces [12]. The protein–carbohydrate monovalent interactions are of low affinity with the binding constants of 10^3–10^4 M^{-1} [14–16], but interaction strength and specificity are improved for multivalent interactions or several simultaneous binding events with the binding constants of 10^6–10^7 M^{-1} and even higher [14,15,17], which is desirable for protein imprinting in the monolayers at the air–water interface. It has been shown that the surface density and spatial arrangement of carbohydrate ligands play a key role in protein binding [14,17,18]. However, comprehensive understanding of the influence of steric hindrance and spatial arrangement of the ligands on protein binding is still largely lacking.

We recently reported protein imprintings in the binary monolayers composed of double-chained glycolipids directly linked or linked through oligo(ethylene glycol) (OEG) spacers with mannose moieties and corresponding precursor lipids resistant to proteins [10,11], the chemical structures of which are shown Fig. S1 in Appendix A. In this

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work, binary monolayers were composed of synthetic glycolipids, [8-(1,2-di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6-dioxaoctyl]-α-*D*-mannopyranoside (DPEM), and dipalmitoylphosphatidylcholine (DPPC), the chemical structures of which are shown in Fig. 1. The PC-containing phospholipids are also one of the major components of cell membranes. It is well-known that zwitterionic phosphorylcholine (PC) moieties can bind significant amounts of water and possess good biocompatibility to resist protein binding and cell attachment [19,20]. Concanavalin A (Con A, pI 4.5–5.5) [21] exists as a tetramer (104 kDa) at pH > 7.0 [22,23] and is capable of specifically binding mannose and glucose epitopes in the presence of Mn^{2+} and Ca^{2+} ions required for carbohydrate binding activity [22,23] but with more affinity for mannose moieties [24]. The Con A tetramer has four carbohydrate binding sites and presents two binding sites on each face [25]. The orientation of these two binding sites allows Con A to engage in bivalent interactions with the glycolipid monolayers. Herein, the shorter PC headgroups with probable flat-on orientation relative to the OEG spacers of glycolipids in the binary monolayers gave rise to reduced steric hindrance favorable to the accommodation of Con A with greater ease and facilitated the access of the mannose ligands for enhanced protein affinity. Further enhanced binding was attributed to optimized spatial rearrangement of the glycolipids at the air–water interface directed by Con A in the subphase to create bivalent binding sites and to minimize steric crowding of neighboring ligands. The bivalent carbohydrate binding sites protruding from the imprinted monolayers were created to be complementary to the Con A binding pockets. The remaining two binding sites of the bound Con A on the monolayers exposed to solution phase can be available for mannoses, glucoses, and glycoconjugates containing these saccharides such as cells, which would provide a probable means for the construction of Con A gated drug delivery to specific cells.

2. Materials and methods

2.1. Materials

DPEM was synthesized according to the reported routes recently [10,26], and its chemical structure was confirmed by NMR spectra (500 MHz, Bruker DRX-500). L-α-DPPC (~99%) was purchased from Sigma. Their stock solutions were prepared in pretreated chloroform (analytical grade) at a concentration of 1 mM and stored at −20 °C prior to use. The binary mixtures of DPEM and DPPC were prepared volumetrically from their stock solutions. 1-Ocadecanethiol (ODT, 95%) was purchased from Fluka. Triton X-100, ethanol, NaCl, and NaOH were of analytical grade. Con A from *Canavalia ensiformis* (Type V, pI 4.7) was purchased from Sigma. Water used was double-distilled (pH 5.6, resistivity of 18.2 MΩ cm, surface tension of 73.06 mN/m at 22 °C) after a deionized exchange. The solutions of Con A and subphase were prepared from phosphate buffered saline (PBS, 10 mM phosphate, 0.1 mM Mn^{2+}, 0.1 mM Ca^{2+}, and 150 mM NaCl, pH 7.4). U937 and NFS-60 cells were kindly offered by School of Life Science at Nanjing University.

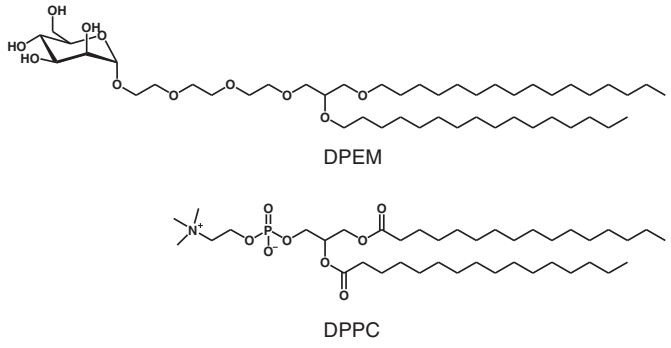

Fig. 1. Chemical structures of DPEM and DPPC.

2.2. Monolayer spreading and isotherm measurements

The surface pressure–molecular area (π–*A*) isotherms were recorded on a Nima 611 Langmuir trough (Nima Technology, England) equipped with a computer control. The maximum available surface area was 30 cm × 10 cm and could be varied continuously by moving two Teflon barriers. A Wilhelmy plate with a small piece of rectangular filter paper was used as the surface pressure sensor with an accuracy of ±0.1 mN/m. Chloroform solutions of DPEM, DPPC, and their mixtures with different molar ratios were spread on the PBS solutions, and then 20 min was allowed for solvent evaporation. Two barriers compressed symmetrically at the same rate of 5 mm/min. The subphase temperature was kept at 22 °C. Each sample was run at least three times to ensure reproducibility.

2.3. Infrared reflection absorption spectroscopy (IRRAS) measurements

In situ IRRAS spectra of the monolayers at the air–water interface were recorded on a Bruker Equinox 55 FTIR spectrometer connected to an XA-511 external reflection attachment (Bruker, Germany) with a shuttle double-trough system and a narrow band mercury-cadmium-telluride (MCT) detector [10,11]. A KRS-5 polarizer was used to generate polarized lights. The IRRAS experiments were carried out at 22 °C. The film-forming molecules were spread from chloroform solution of desired volumes, and then 20 min was allowed for solvent evaporation. The whole attachment system was placed in an air-tight Plexiglass hood to achieve equilibrium of water vapor. After about 4 h, the monolayers were discontinuously compressed to the desired surface pressure of 30 mN/m from about 0 mN/m. After 30 min of relaxation, the two moving barriers were stopped and the monolayer areas were kept constant. Upon protein binding, concentrated Con A solutions were injected into the unstirred subphase underneath the compressed monolayers at a surface pressure of 30 mN/m behind the barriers. The external reflection absorption spectrum of the PBS solution containing Ca^{2+} and Mn^{2+} was used as a reference. The spectra were recorded at an incidence angle of 30° with a resolution of 8 cm^{-1} by coaddition of 1024 scans.

2.4. Surface plasmon resonance (SPR) measurements

Integrated optics SPR sensors (Spreeta, Texas Instruments) [27,28] were employed to study direct binding of soluble proteins from aqueous solution to solid surfaces, so that protein-imprinted binary monolayers could be directly transferred onto the sensor surfaces for biosensing. A Teflon microtrough was homemade with the dimensions of 4 cm × 2 cm × 1 cm [8,9]. The trough walls were undercut by 45° to eliminate the formation of a meniscus presenting a planar interface [7]. The SPR sensor was first cleaned using an aqueous solution of 1% Triton X-100 and 0.1 M NaOH followed by copious double-distilled water. Its sensing gold surface was hydrophobically modified with ODT (2 mM in absolute ethanol) for 20 min followed by rinsing with copious double-distilled water. The ODT-coated SPR sensor was then dried and positioned above the monolayer at the air–water interface. The SPR sensor was initialized in air and calibrated in double-distilled water, and a SPR baseline was obtained in PBS solution. A binary monolayer of DPEM and DPPC was spread until a desired surface pressure of 30 mN/m was reached, and then it was allowed for relaxation for 1 h. In the case of control monolayers (at the solid–water interface), the hydrophobic SPR sensor was slowly lowered into contact with the monolayer using a micromanipulator. Upon contact of the SPR sensor with the monolayer, a step increase of SPR signals from the lipid monolayer was recorded and a new SPR baseline was established for a period of 10 min to ensure the integrity of the transferred monolayer prior to protein injection. Concentrated protein solutions were injected into the subphase to reach a final concentration of 100 μg/mL. The protein binding was allowed