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SPRi determination of inter-peptide interaction by using 3D supramolecular co-assembly polyrotaxane film

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ABSTRACT

Accurate measurement of inter-peptide interactions is beneficial for in-depth understanding disease-related protein folding and peptide aggregation, and further for designing and selecting potential peptide drugs to the target antigen. Herein, we demonstrate a 3D polyrotaxane (PRX) surface for detecting peptides interactions by surface plasmon resonance imaging (SPRi). This surface is supramolecular self-assembly monolayer (SAM) structure fabricated by threading α-cyclodextrans (α-CD) through a linear polyethylene glycol (PEG) chain fixed on gold chip surface to form pseudopolyrotaxane, and further capping the pseudopolyrotaxane with bulky terminated group to form PRX film. The hydroxyl groups of α-CD can provide more active sites to increase molecules immobilization density, and PEG chain has unique protein non-fouling feature. We chose Alzheimer's disease marker β-amyloid 40 (Aβ40) as model peptide, and detected the interaction between it and its inhibitors KLVFFK6 by SPRi. As a striking result, the specific adsorption of KLVFFK6 solution at the concentration of 352 μM on Aβ40-PRX was 700 RU, whereas PEG SAM surface gave no significant binding. Interaction between other lower molecular weight peptides was detected *via* PRX surface, and the relatively weak interactions ($K_D = 1.73 \times 10^{-4}$ M) between LPFFD ($Mw = 0.6$ kDa) and amylin20-29 ($Mw = 1.0$ kDa) are successfully detected.

1. Introduction

Inter-peptide interactions mediate a broad range of biological phenomena, such as protein folding and peptide aggregation, and an extensive and in-depth understanding of inter-peptide and peptide–small molecule interactions could be beneficial for designing and selecting potential peptide drugs to the target antigens (Adamczyk et al., 2000; Abdiche et al., 2008).

Surface plasmon resonance imaging (SPRi) biosensor is label-free, highly sensitive and highly throughput technology; it has been widely used in detection of various molecules interactions, such as protein–protein, protein–nucleic acid binding affinities and so on (Anders et al., 2011; Boozer et al., 2006; Bertram and Tanzi 2008). But it is still a challenge for more accurate measurement of interaction between low-molecular-weight analytes such as peptide. A promising approach to help overcome this problem and enhance the sensitivity of SPRi is by increasing the chip binding capability *via* surface modification, such as the commercially available CM5 chip, a kind of gold surface modified by three-dimensional (3D) carboxymethylated dextran. By using CM5 chip, researchers have successfully studied the interactions between estrogen metabolites and estrogen receptor, determined the affinity of netropsin binding on DNA hairpin, and investigated the adsorption behaviors of designed peptides on fibrin (Bost et al., 1985; Chen et al., 2009; Cairo et al., 2002). These works suggest that the high density dextran matrix of CM5 can help detect small molecule–protein and small molecule–DNA interactions. While the analysis of interactions between peptides by SPRi method is still difficult because of the high non-specificity (de-los-Santos-Alvarez et al., 2009). In addition, novel SPRi methods have been proposed and implemented to investigate the binding mechanisms of low-molecular-weight analytes, including nanoparticle and fluorescence label for amplification of SPR signals, while a recurring problem is whether or not the binding reaction kinetics can be affected the label species (Bost et al., 1985; Chen et al., 2009; Cairo et al., 2002; Charles and Gibum 2007; Culha et al., 2003).

In this paper, we demonstrate a strategy for achieving high sensitive SPRi chip feasible in analysis of small analyte such as peptide by using 3D PRX surface combined with α-cyclodextran (α-CD) and poly (ethylene glycol) (PEG). This surface is

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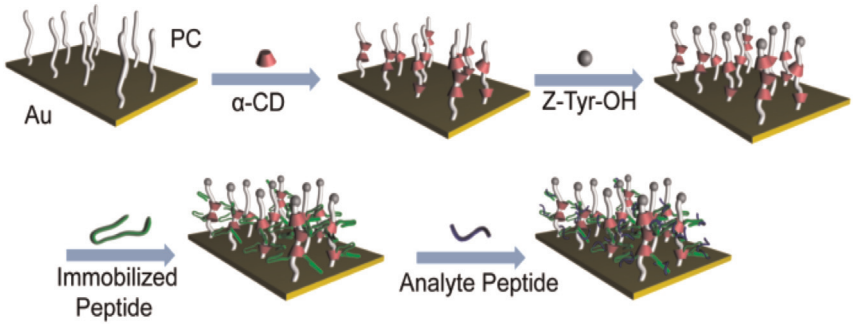

Fig. 1. The scheme of the PRX chip surface fabrication and the detection of inter-peptide interactions by SPRi.

supramolecular co-assembly monolayer (SAM) structure fabricated by threading α-CD through a linear PEG chain self-assembly fixed on gold chip surface to form pseudopolyrotaxane, and further capping the pseudopolyrotaxane with bulky terminated group (Z-Tyr-OH) to form PRX film (Edwards, 2001; Fang et al., 2006). The hydroxyl groups of α-CD can provide more active sites to enhance molecules immobilization, and PEG chain has unique protein non-fouling feature (Gopalakrishnan et al., 2005). In addition, α-CD can freely slide and rotate along the PEG chain, which is beneficial for analyte to access to ligand immobilized on cyclodextran (Harada et al., 1992). Recent work also exhibited the ability to detect target DNA by SPRi using PRX chip surface (Harada, 2001).

Herein we employed the necklace-like PRX surface constructed on SPRi chip as an effective tool to conjugate peptide and measure inter-peptide interactions as shown in Fig. 1. The striking results confirm that the PRX surface is a promising biosensor matrix to detect low-molecular-weight molecules interaction and binding affinity.

2. Experimental section

2.1. Materials

α-CD was purchased from Tokyo Chemical Industry Company, Ltd. HS-PEG-COOH (PC, $Mw \approx 2000$ Da), HS-$(CH_2)_{11}$-O-$(CH_2CH_2O)_6$-COOH (P6, $Mw = 512$ Da), HS-PEG-NH_2 (PN, $Mw \approx 2000$ Da), HS-PEG-OCH_3 (PM, $Mw \approx 2000$ Da) and HS-$(CH_2)_{11}$-$(OCH_2CH_2)_6$-OH ($Mw = 468$ Da) were purchased from Shanghai Yare Biotechnology. Inc. β-amyloid 40 (Aβ40), β-amyloid 42 (Aβ42), KLVFFK6 (KLVFFKKKKKK), K4LVFF (KKKKLVFF), LPFFD, GNNQQNY, 15α (DELERRIRELEARIK), 25Q (polyQ$_{25}$), amylin 20-29 (SNNFGAILSS), GPK4 (GPKGPKGPKGPK), ACTH (SYSMEHFRWGKPVGKKRRPVKVYP), and HTCA (GVHLHRAPLLAHRLAPAEVFHGVR) were purchased from Shanghai Science Peptide Biological Technology Co., Ltd. NaOH, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), succinic anhydride, dimethylaminopyridine (DMAP), Congo red (CR), succinic anhydride, ethanolamine, N-carbobenzyloxy-L-tyrosine (Z-Tyr-OH), polydimethylsiloxane (PDMS), dimethyl sulfoxide (DMSO), N,N-Dimethylformamide (DMF) and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich Co., Ltd. All these reagents were used without further purification. Milli-Q water was obtained by the Millipore ELIX water purification system. All buffers and reagents used were degassed and filtered prior to use in SPRi experiments.

2.2. Nuclear magnetic resonance (NMR) characterization of PRX co-assembly reactions

Four kinds of polyethylene glycol with different terminated groups (PC, PN, PM, and P6, 50 μM) were added into α-CD aqueous solution (128 mM), respectively. The mixture solution was stirred over 12 h at room temperature. White precipitation appeared in the solution. After collected and dried in vacuum oven, the precipitation was dissolved by D_2O for ^{1}H NMR characterization (Bruker AVANCE III, 600 MHz, 30 °C).

2.3. SPRi characterization of in situ PRX co-assembly on gold surface

At firstly, co-assembly capability of α-CD and PEG chain with different terminate groups was *in situ* investigated by SPRi. 1 mM PC, PN, PM, and P6 were printed on SPRi gold chip respectively, and incubated in wet air at −4 °C over 12 h to form uniform self-assembly monolayer. Then the chip was washed by deionized water and ethanol, dried in a stream of N_2. Real-time detection of PRX co-assembly was performed using SPRi apparatus Plexera χ5 V2 (Plexera Bioscience LLC, USA). The entire SPRi chip surface was imaged during the angular scan. The immobilized analyte spots on SPRi chip were selected with 100 μm diameters. For each spot, the reflected intensity was displayed as a function of angle in the plasma resonance curve diagram. Working optical position was automatically calculated according to the plasma curve. Flow rate of auto-injection system was 2 μL s^{-1} in this experiment. Deionized water was the running buffer. After stable baseline was obtained, 600 μL α-CD aqueous solution (128 mM) was injected into flow cell, and was statically maintained for 15 h. When the co-assembly reaction of α-CD binding PEG reached its equilibrium, the flow cell was rinsed with 600 μL deionized water. The baseline was found to be back to original position. The linear correlation between SPRi response and concentration in solution was investigated by injecting a series of α-CD aqueous solutions with different concentrations (16 mM, 32 mM, 48 mM, 64 mM, 80 mM, 96 mM, 112 mM, and 128 mM) into flow cell. 10 mM NaOH aqueous solution was used to remove the α-CD from PEG immobilized on the surface between two α-CD injections.

Then we further *in situ* investigated the procedure of that pseduopolyrotaxane was capped with Z-Tyr-OH and changed into PRX by SPRi. The gold chip was immersed into 1 mM PC solution, and incubated in wet air at −4 °C over 12 h to form uniform self-assembly monolayer. Then the chip was washed by deionized water and ethanol, dried in a stream of N_2. SPRi measurement was carried out as described above. After baseline was obtained, 600 μL 128 mM α-CD aqueous solution was injected into flow cell, without rinse procedure, 1200 μL freshly prepared aqueous mixed solution (containing of 0.1 M NHS, 0.4 M EDC, and 128 mM α-CD) was injected into flow cell continuously. Then another 1200 μL aqueous mixture of 3.2 mM Z-Tyr-OH and 128 mM α-CD was injected to cap with the carboxyl terminus of PC. Finally, the surface was rinsed by water. In order to compare the capping property of Z-Tyr-OH for PC immobilized on gold surface with and without α-CD solution, this experiment was repeated without α-CD in all solution.