Journal of Colloid and Interface Science 514 (2018) 407–414

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Regular Article

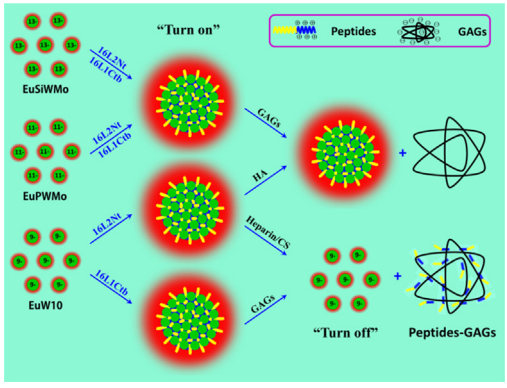
Cell receptor screening for human papillomavirus invasion by using a polyoxometalate-peptide assembly as a probe

Peng-Fan Gao [a], Yu-Xue Liu [a], Lening Zhang [b], Simin Zhang [a], Hong-Wei Li [a,*], Yuqing Wu [a,*], Lixin Wu [a]

[a] State Key Laboratory of Supramolecular Structure and Materials, Institute of Theoretical Chemistry, College of Chemistry, Jilin University, No. 2699 Qianjin Street, Changchun 130012, China
[b] Department of Thoracic Surgery, China-Japan Union Hospital, Jilin University, Changchun 130033, China

GRAPHICAL ABSTRACT

Self-assemblies of Eu-substituted polyoxometalates and different HPV16 peptides have been used to precisely identify cell receptors for viral adhesion prior to cell invasion through competitive recognition.



ARTICLE INFO

Article history:
Received 9 October 2017
Revised 19 December 2017
Accepted 19 December 2017
Available online 20 December 2017

Keywords:
Polyoxometalates (POMs)
Human papillomavirus (HPV)
Cationic peptide
Cell receptor screening

ABSTRACT

The present study constructed a competitive recognition system using cell receptor screening for human papillomavirus (HPV) invasion by using the hybrid-assembly of polyoxometalates (POMs) and cationic peptides as a platform. The fine tuning both of the surface charge of POMs and peptide sequence were precisely performed to develop a luminescence switch of POMs, leading to the establishment of a ternary system to identify which types of glycosaminoglycans (GAGs) are potential cell receptors for HPV infection. In addition, the method was successfully applied to construct a hybrid-assembly with the recombined HPV 16 L1 pentamers from *Escherichia coli* and perform GAGs screening, which validated the system's potential for practical applications. In particular, the intrinsic mechanism for each competitive partner in the system was explained well by using isothermal titration calorimetry (ITC) and time-resolved fluorescence spectra. The present method will be helpful to extend the protocol to other systems by using peptides and POMs with similar properties, and ultimately, we hope it will promote the development of anti-viral agents.

* Corresponding authors.
E-mail addresses: lihongwei@jlu.edu.cn (H.-W. Li), yqwu@jlu.edu.cn (Y. Wu).

https://doi.org/10.1016/j.jcis.2017.12.055
0021-9797/

1. Introduction

High-risk types of human papillomavirus (HPV) attach to and infect epithelial cells; they are the causes of cervical cancer and are extremely harmful to human health [1–4]. The life cycle of HPV starts with its adhesion to host cells *via* specific receptors at the surface. Therefore, it is the first key step to control the viral infection, and exploring the intrinsic mechanism is crucially important for developing anti-viral therapies. As a non-envelope virus, early studies have confirmed the key role of capsid proteins in this process [5–7]; capsid proteins are composed of 72×5 copies of major capsid protein L1 and a somewhat minor capsid protein L2 [8–11]. It has been widely accepted that the viral particle binds to the cell surface essentially *via* the L1 [12–14], while accumulating evidence suggests L2 plays a key role for the entry *via* receptors at the cell surface [12,15–18]. In particular, the cationic peptides of L1 and L2 (Table S1) have been suggested to play a very important role of HPV binding to the surface receptors of cells [19–21]. However, among them, which one plays a more important role in the process is as yet unknown [12–18].

Glycosaminoglycans (GAGs) are a type of very important cell receptors for viral invasion that consist of five types: heparan sulfate/heparin, chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and hyaluronic acid (HA) [22]. Among them, heparin is abundant on most types of cell and has been suggested as a receptor for the initial attachment of many virus including HPV [23–26]. While the specific role of HA [27,28] and CS [29–33] have been demonstrated, other potential cell receptors from the GAGs family that may be involved in viral entry have not yet been identified. Although several studies have proposed the pathways and entry process of HPV into cells and suggested that HA and CS on the cell-surface could be involved in initial recognition and association of viruses with cells [12–14,34], neither of them has been proven to be important. Because the process of viral entry cannot be observed directly through fluorescence or calorimetry methods, there is a need to simplify the complicated interaction between them with a simple model system. Therefore, a convenient and efficient detection method urgently needs to be established.

Polyoxometalates (POMs) have shown extensive bio-applications in the past decades [35–38], especially the europium substituted POMs that are very sensitive to the microenvironment and can interact with cationic peptides of HPV16 capsid proteins. Self-assembled spheres are formed through the electrostatic interactions and hydrogen bonds between them, resulting in significant luminescence enhancement of Eu(III) [39–42]. In addition, the GAGs such as heparin can also interact with the positively charged peptides of HPV with high bind affinity [19]. Therefore, it is possible to construct a binary system with suitable POMs and peptides to screen cell receptors through the competitive interaction between them. In the present study, we have employed europium-substituted POMs as a fluorescence tag and a competitor to study the interaction between the cationic peptides and GAGs to screen for strong binding between them to further confirm which GAGs are more important for viral adhesion prior to cell invasion. The competitive recognition is conveniently and efficiently monitored through fluorescence spectra, establishing a good platform for cell receptor screening.

2. Materials and methods

2.1. Materials and reagents

The cationic peptides from HPV16 capsid proteins (16L1Ctb and 16L2Nt) were purchased from Shanghai A Peptide Co., Ltd. (China), the purities of which are 99.11% as confirmed by HPLC. Their sequences are illustrated in Table S1. 16L1Ctb is the peptide corresponding to the C-terminal residues 492–505 of the HPV16 L1 protein, while HPV16L2Nt is the residues from 1 to 13 at the *N*-terminus of the HPV16 L2 protein. The POMs of $K_{13}[Eu(SiW_{10}MoO_{39})_2]\cdot 28H_2O$ (EuSiWMo), $Na_9[EuW_{10}O_{36}]\cdot 32H_2O$ (EuW10) and $K_{11}[Eu(PW_{11}O_{39})_2]$ (EuPW11) were synthesized and characterized according to a published procedure [43–45]. Chondroitin sulfate (CS) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (China), and its purity is ≥98%. Porcine intestinal mucosa heparin (sodium salt, H4784, ≥180 USP units/mg) was obtained from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). The low-molecular-weight hyaluronic acid (HA) was purchased from Bloomage Freda Blopharm Co., Ltd., and the purity is cosmetic grade. The sodium hydroxide (NaOH, ≥99%) was purchased from Beijing Chemical Reagent Company (China), and 2-(N-morpholino) ethanesulfonic acid monohydrate (MES, ≥99%) was obtained from Aladdin Chemical Co. Ltd. (China). Other chemicals used were from commercial suppliers and were used without further purification before use. Pure water (ρ=18.2 MΩ cm, 25 °C) was obtained from a Millipore Milli-Q water purification system. The stock solutions of peptides were prepared at 1.0 mM and those for GAGs were at 10.0 mg/mL, both in aqueous solution and stored at 4 °C in the dark before use. The recombinant HPV 16 L1 was expressed in *Escherichia coli* and purified using protocols reported previously [46]. The binding assay of EuW10 and HPV L1, and further extraction by GAGs, were performed in the optimized buffer solution of MES-NaOH (10.0 mM, pH 6.0) at 25 °C, based on a previous report [42].

2.2. Luminescence detections

All of the fluorescence spectra were measured on a SHIMADZU (Japan) RF-5301 fluorescence spectrophotometer. The stock solutions of EuSiWMo, EuPW11 and EuW10 were prepared at 2.0 mM in an aqueous solution. The titrations were performed manually, and the corresponding fluorescence spectra were collected after each addition and stirred well. A fixed excitation wavelength at 265 nm was used for all of the photoluminescence detections. Each experiment was repeated three times to obtain more reliable results.

2.3. Time-resolved fluorescence spectra

Life-times were obtained from the luminescent decay curves recorded by using a time-correlated single photon counting technique with an Edinburgh Analytical Instruments-FLS920. To obtain the fluorescence decay curve, the excitation wavelength was fixed at 265 nm, and the intensity at 591 nm was measured. Each experiment was repeated three times to obtain more reliable life-times, and the presented result is one selected as a representative. The sample was prepared in the buffer solution of MES-NaOH (10.0 mM, pH 6.0) at the required concentration.

2.4. Isothermal titration calorimetry (ITC)

A MicroCal ITC200 (GE) was used to measure the ITC curves at several fixed temperatures, and the procedure was modified based on a previous report. All of the experiments were repeated three times, and the displayed curve is a selected representative, not an averaged one. Before titration, each material was prepared in a buffer solution of MES-NaOH (10.0 mM, pH = 6.0) at the required concentration. The titration included the injection of 1.0 μL aliquots of CS/heparin solution and 1.5 μL of HA into a calorimeter cell (V_{cell} = 200 μL) containing peptide solution, using a stirring speed of 1000 rpm and 180 s delay between each injection. The