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Short communication

Avidity of influenza virus: Model-based identification of adsorption kinetics from surface plasmon resonance experiments

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ABSTRACT

Affinity chromatography and membrane adsorption are highly promising methods for the downstream processing of cell culture-derived influenza virus. For the optimization of this separation process, it is desirable to quantify the kinetics of virus adsorption. For this reason, the adsorption kinetics of the influenza A virus (Puerto Rico/8/34 (H1N1)) on a surface with the immobilized ligand Euronymus europaeus lectin (EEL) was investigated. The adsorption kinetics was experimentally monitored in a microfluidic flow cell by surface plasmon resonance (SPR) spectroscopy. The boundary layer theory was applied to analyze the convective and diffusive mass transport of the virus particles in the SPR flow cell. A multi-site kinetic adsorption model was found to describe the experimentally recorded adsorption curves adequately. According to the proposed model, under the applied experimental conditions, the number of sites (galactose residuals) binding one single virus particle to the EEL surface is in the range of 300 to 460, which is in average about 4% of the total number of sites available on the virus surface. The avidity of individual virus particles to the EEL surface was estimated to be in the order of magnitude of $10^6\ M^{-1}\ s^{-1}$.

1. Introduction

Affinity chromatography and membrane adsorber are highly promising units of downstream processing in the production of mammalian cell-culture derived influenza vaccines [1]. In order to design and optimize these units, it is necessary to study the virus interaction with the affinity-based membrane surface [2,3]. For this purpose, surface plasmon resonance (SPR) spectroscopy has proven to be a suitable analytical method [4–7]. The viral surface glycoproteins, hemagglutinin (HA), and neuraminidase (NA), contain a vast amount of terminal α-galactose units which enable the use of Euonymus europaeus lectin (EEL) as affinity ligand [6].

In the adsorption experiments performed in a SPR microfluidic flow cell, the microkinetics of virus adsorption is masked by the mass transport phenomena occurring in the flow channel, namely convection and diffusion. In order to extract the real microkinetics from macrokinetic adsorption data, it is necessary to formulate and validate a mathematical model which accounts for axial and radial mass transport effects in combination with virus adsorption on the wall of the SPR microfluidic channel [8–14]. The objective of the present work is to identify a suitable model for the kinetics of adsorption of influenza virus particles with their glycosylated membrane proteins HA and NA containing α-galactose binding sites on EEL-immobilized surfaces.

2. Materials and methods

2.1. Surface preparation

Kinetic adsorption experiments were performed using a BIAcore™ 3000 device (GE Healthcare, Sweden) at 25 °C. The BIAcore sensor chip was equipped with two parallel flow cells. The surface preparation of the sensor chip C1 (GE Healthcare Bio-Sciences AB, Sweden) conformed to the following steps: (i) both flow cells were washed twice by freshly prepared 0.1 M glycine-NaOH, pH 12 containing 0.3% Triton X-100 (1.5 min at 20 μl/min); (ii) 100 mg/ml streptavidin (Biozol, Germany, 16 min at 12 μl/min) in 10 mM acetate pH 5.0 was immobilized on the surfaces of two flow cells by amine coupling kit [15] with running buffer HBS-EP+ (GE Healthcare Bio-Sciences AB, Sweden); (iii) 1 μg/ml EEL (Biozol, Germany, 1 min at 50 μl/min) were injected only through one of the two flow cells by the running buffer (10 mM HEPES, 150 mM NaCl,

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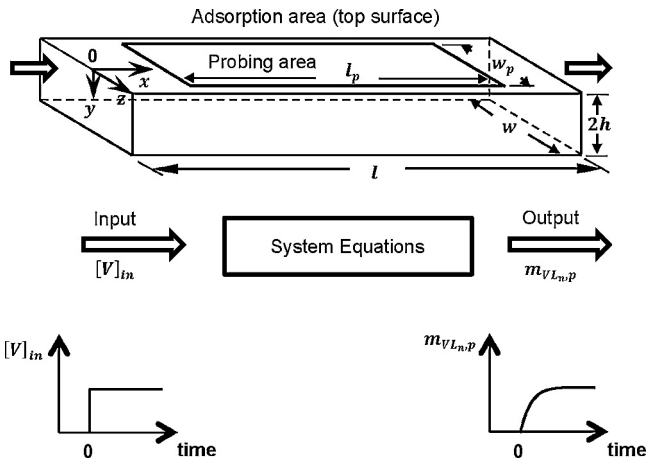

Fig. 1. Sketch of fluid domain of the applied flow cell (above) and illustration of dynamic adsorption experiment in terms of input-output systems behavior (below). The origin of the $x-y$-coordinate system was set on the middle of the top edge of the inlet cross section. The x-direction corresponds to the flow direction, and the virus adsorption takes place on the whole top surface ($y = 0$). The SPR device detects the time dependent amount of adsorbed virus particles at the probing area, $m_{VL_{n,p}}$, while the virus solution flows continuously through the flow channel. The geometrical parameters are $l = 2$ mm, $w = 0.5$ mm, $h = 0.01$ mm, $l_p = 1.8$ mm, and $w_p = 0.2$ mm. Three different inlet virus concentrations were applied, $[V]_{in,1} = 1.015 \times 10^6$ virons/μl, $[V]_{in,2} = 2.03 \times 10^6$ virons/μl, and $[V]_{in,3} = 5.075 \times 10^6$ virons/μl. The flow rate was fixed at $Q = 50$ μl/min.

0.1 mM $CaCl_2$, 0.01 mM $MnCl_2$, pH 7.5). The other flow cell was used as reference cell. The final maximum response of EEL injection to the streptavidin surface was 276.7 RU, i.e., $[L^{max}] \equiv 276.7$ RU.

2.2. Virus injection

The virus adsorption experiments were performed in four main steps: calibration, blank injection, virus injection, and regeneration. In all steps one running buffer (RB) was applied (150 mM NaCl, 50 mM Tris, 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$, 0.005% Tween 20, pH 7.4). The procedure of "double referencing" [16] was employed in order to avoid systematic deviations seen in responses measured on low capacity surfaces. The calibration step included firstly the injection of a small amount of virus and secondly the injection of the same elution buffer as used for regeneration. The blank injection step was performed by RB (6.5 min at 50 μl/min). The virus injection step was carried out at three different concentrations (1.015×10^6 virions/μl, 2.03×10^6 virions/μl, and 5.075×10^6 virions/μl; 6.5 min at 50 μl/min) freshly diluted by RB. The regeneration step consisted of injections of two elution buffers: the first one was 150 mM Lactose in RB (6.5 min at 30 μl/min), and the second one was 2 M NaCl in RB (6.5 min at 30 μl/min). The surface was primed twice by RB between each step in order to remove residual substances and to stabilize the surface.

3. Modeling virus adsorption in SPR flow cell

The flow cell is schematically given in Fig. 1. The fluid in the SPR channel is assumed to be a steady laminar flow with constant total mass density and constant viscosity. Due to the large aspect ratio, a quasi-2D flow can be assumed, i.e., the z-direction is neglected in all model equations. In addition, by applying the classical boundary layer theory [17] to analyze the momentum balances, the entrance region can be neglected, i.e., a fully developed velocity profile can be assumed throughout the SPR flow channel. Assuming that a diffusion boundary layer is established in the SPR flow channel where virus diffusion in flow direction and virus accumulation are negligible, the 2D mass balance of virus particles can be cast into a governing equation for the dimensionless thickness of the diffusional boundary layer, $\delta_D^* = \delta_D/h$:

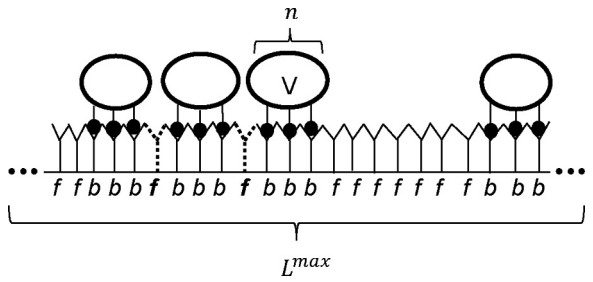

Fig. 2. Multi-site binding of virus particles to ligands represented as linear lattice. Each virus particle (V) occupies n sites on the ligand surface ($n = 3$ shown here). In the model of McGhee and von Hippel, lattice sites are either blocked (b) by a bound virus particle, or free (f). The sign "251658240" means that free sites are not available for the adsorption due to the steric hindrance by two neighboring virus particles. L^{max} stands for the capacity of the surface $L^{max} = 276.7$ RU (see section 2.1).

$$(18(\delta_D^*)^2 - 5(\delta_D^*)^3)R\frac{d\delta_D^*}{dX} + 6(\delta_D^*)^3\frac{\partial R}{\partial X} = 30\left(\frac{l}{h}\right)^2\frac{R}{Pe}, (\delta_D^*(X=0)=0) \quad (1)$$

with the dimensionless virus adsorption rate, $R \equiv (2hr)/(D[V]_{in})$; $X \equiv x/l$; (the value of D, cf. Supplementary data). The mass balance of virus particles on the EEL-immobilized surface, in dimensionless terms sounds:

$$\frac{\partial\theta_{V,s}}{\partial\tau} = R, (\theta_{V,s}(\tau=0,X)=0) \quad (2)$$

where $\theta_{V,s} \equiv [VL_n]/[L^{max}]$ and $\tau \equiv t/(2h[L^{max}]/D[V]_{in})$. The dimensionless BIAcore output quantity is $\Theta_{V,s} \equiv m_{VLn,p}/([L^{max}]\,l_p w_p)$. It can be calculated in discretized form as follows: $\Theta = (l/l_p)\sum_i \theta_{V,s,i}\Delta X$

Each virus particle has a large number of affinity sites on its surface. This results in the multi-site binding situation between a single virus particle and a corresponding cluster of ligands on the EEL-surface. The avidity is described here by applying the probability theory to formulate the distribution of ligand clusters according to a model proposed first by McGhee and von Hippel [18]. According to this adsorption model, each virus particle binds at n sites at a linear lattice (see Fig. 2). The formal stoichiometric equation for the adsorption of a virus particle at a cluster consisting of n ligands is given by:

$$V + L_n \xrightarrow{r} VL_n \quad (3)$$

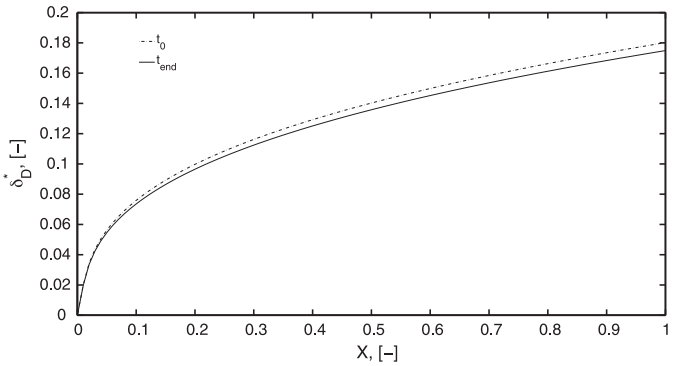

Fig. 3. Profiles of the diffusional boundary layer thickness δ_D^* in X direction at the beginning, t_0, and end, t_{end}, of the adsorption experiment.