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Tobacco mosaic virus adsorption on self-assembled and Langmuir–Blodgett monolayers studied by TIRF and SFM

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Abstract

The adsorption of tobacco mosaic virus (TMV) on self-assembled and Langmuir–Blodgett monolayers was investigated using total internal reflection fluorescence (TIRF) spectroscopy and scanning force microscopy (SFM). Substrates were chosen to probe electrostatic, hydrophobic and surface fluidity effects on TMV adsorption. Positively charged and hydrophobic surfaces demonstrated similar initial rates of TMV adsorption; however, their respective surface TMV coverages differed greatly. Likewise, positively charged surfaces which differed primarily in surface fluidity exhibited similar adsorption rates for TMV, but different TMV surface coverages. In contrast, virus adsorption to negatively charged and zwitterionic substrates was negligible. To elucidate these differences in adsorption behavior, SFM was used to image the distribution and the aggregation state of adsorbed TMV.

Keywords

Virus adsorption; Tobacco mosaic virus; Langmuir–Blodgett film; Total internal reflection fluorescence; Scanning force microscopy

1. Introduction

The adsorption and mobility of viruses on model surfaces are of interest for studies of virus–host interactions, viral detection, filtration and purification [1–4]. Virus adsorption onto surfaces which mimic certain properties of the cell membrane can provide a simplified picture of the initial stages of the virus–host interaction. Viruses, due to their symmetric geometry, may also serve as model systems for studying interactions between bio-colloids and surfaces. The adsorption behavior of tobacco mosaic virus (TMV) on several different self-assembled and Langmuir–Blodgett (LB) monolayers was investigated here to gain insight into the fundamental interactions influencing non-enveloped virus adsorption. While TMV is commonly deposited on surfaces as a size standard in electron and scanning force microscopies (SFM) [5,6], relatively few studies have investigated the adsorption kinetics of this virus, and then, only in a qualitative manner [1,7,8]. In this study the adsorption kinetics of TMV on model substrates are determined quantitatively using total internal reflection fluorescence (TIRF) spectroscopy while the distribution and the aggregation state of adsorbed virus are determined using SFM. The results indicate that the adsorption of TMV is dominated by electrostatic interactions between the surface and the virus while hydrophobic interactions contribute to a lesser degree; this is in agreement with studies of TMV adsorption to phospholipid vesicles [1,7]. The distribution of adsorbed TMV particles

on the various substrates and their respective aggregation states suggest that charged and hydrated surfaces allow for more efficient packing of TMV, while hydrophobic surfaces lead to a partial disruption of TMV rod-like structure.

2. Materials and methods

2.1. TMV

TMV is an 18-nm wide and 300-nm long rod-like particle, composed of 2130 identical 17.5 kDa protein subunits which symmetrically spiral around a single strand of RNA [9]. The subunits interact with each other primarily through hydrophobic interactions; in addition each subunit forms three salt bridges with three phosphate groups on the RNA [9]. The intact virus has a diffusion coefficient of $4.4 \times 10^{-8} \text{ cm}^2/\text{s}$, an isoelectric point near pH 3.3 and possesses between 200 and 1000 elementary negative charges at pH 7 [9–11]. Solutions of purified tobacco mosaic virus (American Type Culture Collection) were made in 10 mM PBS, pH 7.3.

2.2. Surface preparation and characterization

Octadecyltrichlorosilane (OTS) and LB films were prepared on fused silica as previously described [12,13]. Silica slides were aminated using *N*-(2-aminoethyl)(3-aminopropyl)trimethoxysilane (EDA) according to the method of Stenger et al. [14] and stored under nitrogen until use. Single-layer LB films were prepared on hydrophobic OTS-slides using three phospholipids (Avanti Polar Lipids): dipalmitoylphosphatidylserine (DPPS), dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylethylphosphocholine (Et-DPPC) which were doped with 1 mol% of the dye 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) (Molecular Probes). LB transfer ratios were typically near unity.

All surfaces were characterized using the sessile drop and captive air bubble water contact angle techniques. Monolayer stability and packing density were analyzed using SFM operating under PBS solutions. Fluorescence of the DiO molecules in the monolayers was used as an indicator of film stability during the TIRF experiments and in fluorescence microscopy analysis. EDA-modified surfaces were also characterized using X-ray photoelectron spectroscopy (XPS) (Hewlett–Packard 5950B).

2.3. TMV adsorption experiments

The adsorption kinetics of TMV were followed using total internal reflection fluorescence (TIRF) spectroscopy. The operation and calibration of the TIRF instrument were performed as previously described [15]. Fluorescence of adsorbed TMV particles was excited at 295 nm and their intrinsic fluorescence emission was monitored at 330 nm. The 0.025 mg/ml TMV solution in PBS was flowed through the TIRF cell at solution flow rates of 0.2 ml/min or 1 ml/min while the fluorescence was continuously recorded [15].

2.4. Fluorescence and scanning force microscopy (SFM)

An inverted microscope (Nikon Diaphot 200) with a 100 $\times$, N.A. 1.25 oil immersion objective lens was used to image domains in the DiO-doped LB monolayers. SFM was operated in both the contact and tapping modes in ambient conditions and under PBS. Two SFM instruments, a Nanoscope II (Digital Instruments) and an Explorer 2000 (Topometrix) were used. 180- μm long V-shaped cantilevers (Ultralever, Microlever; Park Scientific) with probe radii of 10–40 nm and nominal force constants of 0.05–0.24 N/m (manufacturer's specifications) and resonance frequencies between 20–40 kHz (experimentally verified) were used in SFM imaging. TMV was adsorbed on substrates for SFM analysis by placing a 90- μl drop of PBS on the substrate, then adding 10 μl of 0.25 mg/ml TMV solution to the existing drop. After 15–60 min the drop was gently rinsed away with 1 ml of PBS and the

sample dried with filtered nitrogen. In situ SFM imaging was performed by omitting the drying step and imaging the samples under PBS.

3. Results and discussion

3.1. Surface modification

Fig. 1 shows the three phospholipids' π -A isotherms and the transfer conditions. Water contact angles of modified surfaces are given in Table 1. The contact angles of the OTS and EDA surfaces are in good agreement with literature values [14,16]. The contact angles attained for the phospholipid headgroups, however, are rather high, a possible result from monolayer rearrangement in the presence of the captive air bubble [13]. The higher contact angle for cationic Et-DPPC monolayers versus the DPPS and DPPC monolayers is attributed to a less dense packing due to steric hindrance and charge repulsion. The monolayer packing densities were further investigated with SFM. An SFM image of a DPPC monolayer oriented with the polar heads facing the solution (Fig. 2) displayed a lattice with a 1.2 nm periodicity, which is about twice the expected distance between the headgroups of a tightly packed double-chain phospholipid monolayer [17]. Other regions of the DPPC monolayer showed similar lattice spacing which was independent of scan direction. This large head-to-head distance suggests DPPC might be in a liquid expanded phase. However, fluorescence microscopy analysis of a DPPC LB monolayer showed many dark domains, about 1 μ m in diameter, separated by narrow DiO (fluorescent) regions (data not shown). The immiscibility of the DiO in these dark regions suggest that they consist of tightly packed, condensed DPPC phase. Hence, we attribute the larger DPPC lattice to an SFM tip-induced artifact due to the pressure exerted on the sample during the imaging. Imaging with reduced loading forces, however, yielded no periodic lattice in the images. The Et-DPPC and DPPS monolayers did not show periodicity in SFM images, a manifestation of a packing regime less dense than DPPC. Fluorescence microscopy images of transferred Et-DPPC displayed a uniform DiO fluorescence, with no distinct domains visible at high magnification (data not shown). Overall, the monolayers were found to be free of gross defects. XPS survey spectra performed within 24 h after preparation of the EDA surfaces confirmed the presence of all the expected elements. A high-resolution XPS spectrum showed two peaks (399 eV and 401 eV) in the N1s region, the latter of which corresponds to protonated amine groups (data not shown).

3.2. TMV adsorption kinetics

The silica, DPPS and DPPC surfaces all showed negligible TMV adsorption (Fig. 3). In contrast, OTS, EDA and Et-DPPC surfaces all demonstrated much faster TMV adsorption. The initial lag in these three adsorption kinetics is a manifestation of the low TMV diffusivity which significantly increases the time needed for the development of a concentration profile near the interface. Once the concentration profile had been established, the adsorption rate on these three substrates became similar, suggesting that the adsorption process was transport-limited. After about 15 min adsorption time, the adsorption on OTS reached a steady state at about 0.6 mg/m². On EDA and Et-DPPC surfaces the adsorption continued to rise steadily past 40 min adsorption time (see Fig. 3, inset). The steady state kinetics on OTS suggested either an adsorption equilibrium or a blockage of the TMV binding sites on OTS. On EDA surfaces using a faster flow rate the TMV adsorption kinetics reached a steady state at 2.1 mg/m² after 90 min (see Fig. 3, inset). To the contrary, a plateau in the adsorption kinetics was not attained on the Et-DPPC substrates up to 160 min. The respective adsorption was 3.4 mg/m², an amount that, based on the estimated maximum surface coverage of 12.3 mg/m² for TMV close-packed on a surface, was sufficient to cover 28% of the surface. The larger TMV adsorption on Et-DPPC vs. EDA may arise from differences in both the surface chemistry and mobility. While both substrates

carry a positive charge, their actual surface potentials were unknown. As indicated below, the SFM imaging of adsorbed TMV on Et-DPPC surfaces was not successful. One explanation is that the fluid Et-DPPC monolayer carries a mobile adsorbed TMV layer which is easily disrupted under the influence of the SFM tip.

3.3. SFM imaging of adsorbed TMV

SFM analysis of TMV adsorbed on mica, OTS and EDA surfaces (Fig. 4) showed different packing arrangements, conformation and/or aggregation state of the virus. Fig. 4A shows TMV aggregates on negatively charged mica. Note that this image has been recorded in a dry state as in situ SFM imaging could not show TMV adsorbed to mica or silica. The aggregation of adsorbed TMV is thought to result from the weaker interactions between TMV and the substrate which are affected during drying, sweeping the TMV particles into tight clusters by the receding water droplet. On OTS (Fig. 4B, imaged under PBS) the conformation of TMV is distorted and the rods are of varying lengths, suggesting that they have disaggregated. Indeed, many small particles, 12–18 nm tall, are found on the OTS surface between the TMV rods. Given the major role hydrophobic interactions play in the self-assembly of the TMV coat proteins [9], it is likely that hydrophobic OTS disrupts the packing of TMV. By disassembling into smaller aggregates of coat protein, relatively few virus particles could interact with a large surface area, blocking further TMV adsorption and thus explaining a low adsorption steady-state plateau (Fig. 3).

On EDA (Fig. 4C,D, imaged in air), numerous intact TMV rods, end-to-end aggregates and overlapping TMV particles are seen randomly deposited on the substrate, a manifestation of the high affinity and low mobility of TMV on this surface. The low surface coverage image (Fig. 4C) also shows a smaller number of round features indicating that some TMV rods might have partially disaggregated. Once SFM tip-induced broadening is accounted for, a 15% TMV surface coverage is calculated from Fig. 4D which equates to about 1.8 mg/m². Higher surface coverage may be hindered by steric restrictions and/or electrostatic surface charge compensation imposed on the remaining binding sites by the adsorbed TMV. It must be noted that surface coverages determined from SFM cannot be compared directly with those determined from TIRF since different transport mechanisms delivered the virus to the substrates (i.e. static vs. flowing solutions). Nonetheless, information on the geometry and conformation of adsorbed TMV attained using SFM appears to be useful in explaining the adsorption kinetics attained with TIRF.

Desorption experiments in PBS suggest that TMV is irreversibly bound to the substrates once adsorbed (data not shown). The large TMV–substrate interaction area requires many interactions to be broken simultaneously for the virus to desorb. Previous studies demonstrate that high salt concentrations are required to desorb TMV [8].

In a recent study we demonstrated that LB films with greater mobility adsorbed less human growth hormone than surfaces of similar chemistry but decreased mobility [13]. We attributed this effect to an unfavorable loss of surface conformational entropy upon protein adsorption. In the present study, the opposite appears true, that a more fluid surface in fact binds more of the virus. These results, however, are not contradictory. The losses in surface conformational entropy upon TMV adsorption may simply be insufficient to overcome the favorable gains in interaction energy. In order to interact with a maximum number of binding sites on the surface, either the substrate or the virus must be flexible. In fact, on immobile heterogeneous surfaces it has been shown that the rigid TMV rods actually bend to maximize the number of favorable interactions [18].

4. Conclusions

The adsorption behavior of the rod-shaped tobacco mosaic virus on self-assembled and LB monolayers has been investigated to gain an understanding of the fundamental interactions influencing virus adsorption on planar substrates. Quantitative virus adsorption kinetics have been determined on unlabeled TMV using TIRF spectroscopy. The kinetics indicate that both electrostatic and hydrophobic interactions influence TMV adsorption; however, as shown by SFM, hydrophobic surfaces lead to viral disassembly. In addition, TIRF and SFM results suggest that the lateral mobility of LB films may allow for post-adsorption reorientations to occur which increase the surface capacity of these films for TMV.

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Fig. 1. Surface pressure–area isotherms for DPPS, DPPC and Et-DPPC monolayers doped with 1% DiO.

Fig. 2. Contact SFM image ($100 \times 100 \text{ nm}^2$, imaged under PBS) of DPPC demonstrating lattice periodicity of DPPC headgroups.

Fig. 3. Adsorption kinetics from 0.025 mg/ml TMV solutions flowing at the rates of 0.2 ml/min (large graph) and 1 ml/min (inset). The axes on the inset graph have the same units as the large graph.

Fig. 4. SFM images of TMV packing, aggregation and conformation. (A) Contact SFM image of TMV tightly aggregated on mica after 15 min adsorption ($1 \times 1 \mu\text{m}^2$, imaged in air). (B) Tapping SFM image of TMV on OTS after 15 min adsorption ($0.4 \times 0.4 \mu\text{m}^2$, imaged in PBS). (C, D) Contact SFM images of TMV randomly oriented on EDA-silica after 15 min (C) and 1 h (D) adsorption times ($3 \times 3 \mu\text{m}^2$, imaged in air).

Table 1

Water contact angles on modified surfaces

Type of surface	Surface property	Sessile drop contact angle	Captive bubble contact angle
Silica	Negative charge	0°	0°
EDA	Positive charge	30°	35°
OTS	Hydrophobic	107°	114°
DPPS	Negative charge	–	65°
DPPC	Zwitterionic	–	65°
Et-DPPC	Positive charge	–	75°