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Adsorption and Aggregation Properties of Norovirus GI and GII Virus-like Particles Demonstrate Differing Responses to Solution Chemistry

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Abstract

The transport properties (adsorption and aggregation behavior) of virus-like particles (VLPs) of two strains of norovirus (“Norwalk” GI.1 and “Houston” GII.4) were studied in a variety of solution chemistries. GI.1 and GII.4 VLPs were found to be stable against aggregation at pH 4.0–8.0. At pH 9.0, GI.1 VLPs rapidly disintegrated. The attachment efficiencies (α) of GI.1 and GII.4 VLPs to silica increased with increasing ionic strength in NaCl solutions at pH 8.0. The attachment efficiency of GI.1 VLPs decreased as pH was increased above the isoelectric point (pH 5.0), whereas at and below the isoelectric point, the attachment efficiency was erratic. Ca^{2+} and Mg^{2+} dramatically increased the attachment efficiencies of GI.1 and GII.4 VLPs, which may be due to specific interactions with the VLP capsids. Bicarbonate decreased attachment efficiencies for both GI.1 and GII.4 VLPs, whereas phosphate decreased the attachment efficiency of GI.1, while increasing GII.4 attachment efficiency. The observed differences in GI.1 and GII.4 VLP attachment efficiencies in response to solution chemistry may be attributed to differential responses of the unique arrangement of exposed amino acid residues on the capsid surface of each VLP strain.

Introduction

The ability to predict and control the transport of viral pathogens in the aquatic environment is paramount for protecting public health. Enteric viruses such as adenovirus and norovirus have been reported to be present at titers of up to 10^6 and 10^9 PCR-detectable units (PDU) per liter of raw domestic wastewater, respectively (1, 2). Wastewater treatment can eliminate substantial amounts of these viruses, with maximum effluent titers of up to 10^3 (adenovirus) and 10^6 (norovirus) PDU/L, implying that greater than 3 log removal of viruses is common (1, 2). Although a very large percentage (99.9 to 99.999%) of these detected viruses is likely to be noninfectious (3), the small percentage of the discharged virus that remains infectious

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Supporting Information Available

Additional experimental details; TEM images of VLPs (Figure S1); attachment efficiencies of GI.1 and GII.4 in various solution chemistries (Figure S2); and comparison of amino acids in the major capsid proteins of GI.1 and GII.4 noroviruses (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

still may pose a threat to public health. The presence of norovirus in groundwater in documented outbreaks confirms that infectious norovirus from sewage contamination can be transported in the subsurface up to hundreds of meters (4).

Once released into groundwater, viruses can travel long distances and contaminate drinking water sources (5). Viral removal in the subsurface is governed by inactivation and physicochemical removal mechanisms. Inactivation can occur due to unsaturated conditions, temperature changes, adsorption to substrate surfaces (which may also protect against inactivation), and microbial activity. Solution chemistry and pH are generally less important for viral inactivation in the subsurface (6). Physicochemical removal is dependent on the surface-surface interactions governed by solution chemistry and the electrostatic, hydrophobic, and steric interactions between the virus and mineral surface. Predictions of viral transport require an estimation of the attachment efficiency, α , of viral particles to mineral surfaces (6). Published attachment efficiencies for viruses in the subsurface have typically relied on carefully controlled column experiments using bacteriophages, such as MS2, PRD1, ϕ 174, and λ , as well as attenuated poliovirus, as surrogates for viral pathogens (8–11). Recent studies using the quartz crystal microbalance with dissipation (QCM-D) system have allowed sensitive, near real-time determination of attachment efficiency of viral particles (7–9) and other nanoparticles of comparable size (10, 11).

Generally, viral adsorption in the environment is expected to be low at pH values above the isoelectric point (pI) of a given virus, given that most surfaces are negatively charged at environmental pH ranges. Viral adsorption above the pI is expected to be enhanced in high ionic strength conditions, wherein the repulsive charges of the virus and surface are screened by counterions, allowing adsorption. The adsorption of MS2 and poliovirus in columns packed with quartz has followed this behavior broadly (12, 13). However, when ionic strength (IS) is considered as a variable, MS2 has a lower attachment efficiency than would be predicted by electrostatic interactions alone (9, 12–14), possibly due to steric effects caused by hydrophilic loops protruding from the capsid (14). Published attachment efficiencies for viruses often follow qualitatively similar trends with IS and pH, yet, quantitatively, they can be quite inconsistent, likely due to differences in the chemistries of buffers and collector surfaces used (6). Lance and Gerba found that, in addition to expected enhanced adsorption in the presence of divalent cations (Ca^{2+} and Mg^{2+}), the nature of the anion present likewise affected poliovirus adsorption in soil columns, with Cl^- promoting attachment more than NO_3^- , SO_4^{2-} , or H_2PO_4^- salts.

This study evaluates adsorption kinetics of norovirus virus-like particles (VLPs) using the QCM-D system. VLPs are formed by expression of viral capsid proteins in insect cells that spontaneously self-assemble in the formation of VLPs. VLPs are produced in very high concentrations (up to 10^{15} VLPs per mL), facilitating laboratory studies of viral adsorption (using QCM-D), aggregation (using dynamic light scattering, DLS), and electrophoretic mobility, which require up to 10^{12} VLPs per mL. Since there is currently no established permissive cell line for producing norovirus *in vitro*, native virus particles can only be obtained from stool samples from infected individuals, with 10^8 to 10^{11} genome copies per gram, which is insufficient for these experiments (15). While norovirus VLPs are antigenically and morphologically identical to native virions, they lack genomic RNA at the interior of the capsid, which may affect capsid stability and, subsequently, adsorption properties. Norovirus VLPs are considered a surrogate for native virions, with the acknowledgment that future research advances allowing the side-by-side comparison of adsorption properties of VLPs to native virions will further advance our understanding of norovirus transport, with VLP data serving as an important foundation.

The norovirus VLPs used in this study correspond to two strains of norovirus that represent the prototype strain and the current, most prevalent circulating strain (16). The former is the “Norwalk” strain of genogroup I genotype 1 (abbreviated GI.1), first isolated in Norwalk, Ohio in 1972 (17). The latter is the “Houston” strain of GII.4, likewise named for its isolation site. GII.4 strains are responsible for the majority of adult viral gastroenteritis cases worldwide, with new strains emerging frequently (18). The two strains of norovirus cause similar symptoms in infected individuals, are the same size (40 nm in diameter), and have a similar isoelectric point (pI) around pH 5–6 (19). The composition of surface-exposed capsid amino acid residues varies greatly from strain to strain and is responsible for observed differences in biologically specific recognition of carbohydrates and antibodies (20–23). The two strains were chosen to investigate whether GI.1 and GII.4 exhibit a difference in attachment efficiency, which represents nonspecific binding of the VLPs to model mineral surfaces.

The first objective of this study was to systematically evaluate the effect of solution chemistry on the aggregation state and adsorption kinetics (to silica) of GI.1 and GII.4 VLPs. Monovalent and divalent cations were evaluated (Na^+ , Ca^{2+} , and Mg^{2+}) along with a range of anions (Cl^- , HCO_3^- , and HPO_4^{2-}). The second objective was to measure attachment efficiency of GI.1 VLPs as a function of pH to determine whether solution pH can be considered as a major predictor of norovirus VLP transport. We found differences in response between GI.1 and GII.4 both in terms of magnitude and effect of various electrolytes. We also found that GI.1 adsorption dependence on pH is more complicated than can be explained by pI alone, pointing to the possibility that subtle conformational capsid changes dominate adsorption as a function of pH.

Materials and Methods

Preparation of Recombinant Virus-Like Particles (VLPs)

Recombinant GI.1 Norwalk and GII.4 Houston VLPs used in adsorption experiments were prepared using the baculovirus expression system as previously described (24). Final VLP pellets were suspended in their respective storage buffers (Milli-Q water for GI.1 VLPs and 0.5 M NaCl with 0.2 M sodium phosphate adjusted to pH 6 for GII.4 VLPs) and stored at 4 °C. GII.4 VLPs were further purified to remove the phosphate prior to experiments using Vivaspin 2 polyethersulfone centrifugal concentrator columns (Viva Products, Littleton, MA) so that the final phosphate concentration was less than 0.2 μM . The concentration of purified VLPs was determined using the bicinchoninic acid (BCA) protein assay (Thermo Scientific Life Science, Rockford, IL) with bovine serum albumin (BSA) as the protein concentration standard. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements were routinely conducted on purified VLP stocks to ensure correct morphology and size at the time of and following experiment dates. Additional details are given in the Supporting Information.

Solution Chemistry

Solutions for DLS, electrophoretic mobility, and adsorption experiments were prepared using DI water and research grade salts and were filtered through 0.22- μm Millex-GP PES syringe-mounted membranes (Millipore, Cork, Ireland). The pH of solutions was adjusted using small amounts of concentrated NaOH or HCl immediately before experiments were carried out (less than five minutes prior). Additional details are given in the Supporting Information.

Dynamic Light Scattering (DLS) Studies

The size of the VLPs in various solution chemistries was determined using a multidetector DLS unit (ALV-5000, Langen, Germany) with a 532-nm wavelength, solid-state, diode-pumped, frequency-doubled, Nd:vanadate laser (Verdi 2, Coherent, Santa Clara, CA). DLS measurements were conducted at 23 °C and a VLP concentration of 10^{11} particles per mL. Details are given in the Supporting Information.

Electrophoretic Mobility Measurements

The electrophoretic mobility of VLPs was determined at various solution chemistries with a ZetaPALS analyzer (Brookhaven Instruments Corp., Holtsville, NY). Experiments were performed at 25 °C (± 1 °C), immediately after dilution of VLPs to a final concentration of 10^{12} particles per mL in pH-adjusted solutions. Ten measurements were taken of each sample, with at least three samples per condition.

Quartz Crystal Microbalance with Dissipation (QCM-D) Adsorption Studies

The adsorption kinetics of GI.1 and GII.4 VLPs onto a silica surface at various solution chemistries were investigated using the D300 QCM-D (Q-Sense AB, Västra Frölunda, Sweden) and an AT-cut quartz sensor crystal coated with silica (QSX 303, Q-Sense). Steady flow was obtained using a syringe pump in suction mode at a flow rate of 0.2 mL per min, which results in laminar flow in the chamber. The sample solution in the chamber was maintained at 25.1 °C.

After a 30-min stable baseline DI measurement, the electrolyte was adjusted to the desired pH and introduced under flow to establish an electrolyte baseline (10 min). VLPs were then diluted in a fresh pH-adjusted aliquot of electrolyte and introduced to the chamber under flow. VLP adsorption was observed by the shift in oscillating frequency of the sensor. This frequency shift can be thought of as mass per time, according to the Sauerbrey relationship, which holds for a rigid, thin depositing layer (25). Since only the very initial stages of adsorption are observed, the surface remains rigid, as verified by minimal observed dissipation shifts, and thus, the Sauerbrey relationship is valid for this system. At each condition, the first 20 data points during adsorption (1 min) were included to calculate frequency shift, using a linear regression.

Adsorption rates were normalized to the maximum observed adsorption rate to determine the attachment efficiency, α , which represents the fraction of particles that contact the surface and are able to attach at a given condition. The maximum adsorption rate is determined under non-repulsive adsorption, where all particles that approach the surface will attach. Since aggregates of viral particles have a lower adsorption rate due to a lower rate of diffusion toward the surface, particle size was verified for each condition using DLS to ensure that the α reported is independent of the influence of aggregation.

QCM-D adsorption experiments were conducted at a VLP concentration of 1.3×10^{11} particles per mL. Adsorption experiments were carried out three times for each experimental condition for GI.1 VLPs and two times for GII.4 VLPs. Additional details are given in the Supporting Information.

Results

VLP Concentration

GI.1 and GII.4 VLPs had the expected icosahedral structure and size (20 nm radius), according to TEM images (Figure S1 in the Supporting Information). GI.1 stock concentrations were approximately 20 mg protein per mL, whereas the GII.4 stock

concentration had a 10-fold lower yield. This corresponds to stock concentrations of approximately 10^{15} and 10^{14} particles per mL for GI.1 and GII.4, respectively, based on the size (58 kDa) of each capsid protein monomer. The final purification step (for GII.4 only), performed to remove the phosphate buffer required to stabilize VLPs for long-term storage, resulted in a 25–50% recovery of the GII.4 VLPs, yielding a stock concentration for experiments of 10^{13} particles per mL. GII.4 VLPs were stored short-term in 0.1 or 0.5 M NaCl (ambient pH of 5.3), depending on the maximum possible salt concentration to allow for desired experimental IS upon dilution. GII.4 VLPs stored in NaCl were found to be stable for several days and exhibited increased stability of up to several weeks at higher salt concentration (determined by TEM imaging and DLS size verification). In contrast, GI.1 VLPs were stable indefinitely (1 year at the time of final experiments) in DI water.

Norovirus VLP Radii as a Function of pH and Ionic Strength

In high salt solution (100 mM NaCl) at either low (4.0) or high (8.0) pH, GI.1 and GII.4 VLPs had a hydrodynamic radius slightly larger than the radius determined by TEM imaging (20 nm) (Figure 1 and Figure S1 in the Supporting Information). At lower salt concentrations (10 mM NaCl), GI.1 appeared aggregated at pH 4.0 and 8.0 (around 60 and 40 nm, respectively), while GII.4 remained unaggregated at these conditions. At pH 5.2, near their isoelectric point, GII.4 VLPs appeared aggregated at all IS tested (55, 90, and 60 nm for DI water, 10 mM NaCl, and 100 mM NaCl, respectively). For GI.1 at pH 5.2, only DI water caused aggregation (60 nm). GI.1 VLPs did not aggregate at pH 4.0, 5.2, or 8.0 in the presence of high salt solutions (300 and 1000 mM NaCl). At high pH (9.0), the GI.1 VLPs had a very large hydrodynamic radius at all IS.

Norovirus GI.1 VLP Electrophoretic Mobility in Various Buffers

The electrophoretic mobility (EPM) of GI.1 VLPs was measured in the presence of 1 and 10 mM NaCl at various pH (Figure 2). The results confirm the isoelectric point to be between pH 5 and 6, in agreement with published values (19). In 10 mM IS and pH 8, the presence of 1 mM calcium slightly decreased the EPM (absolute value), whereas 0.1 mM phosphate slightly increased the EPM (absolute value), compared to chloride alone.

Norovirus VLP Adsorption Rate Is Governed by Ionic Strength

The attachment efficiencies, α , for GI.1 and GII.4 VLPs at pH 8 at various NaCl concentrations are presented in Figure 3, along with published α for bacteriophages MS2 and λ in NaCl (9, 14). Since the maximum observed adsorption rate was at 100 mM NaCl for the VLPs (regardless of pH or presence of divalent cations, as described in subsequent sections), this condition was considered to be favorable for attachment, and the other conditions were normalized to this rate to calculate α .

The VLP experiments were carried out at pH 8.0 and the phage experiments at pH 5.0, values that, in each case, were 1–2 pH units above the pI in order to allow a comparison of the effect of IS when electrostatic repulsion dominates particle-surface interactions. The published pI values of norovirus VLPs, MS2, and λ range between pH 5–7, 2–4, and 3–4, respectively (9, 14, 19). The attachment efficiency of VLPs and bacteriophages increased with increasing IS from 1 to 100 mM (as NaCl). In 300 mM NaCl, α of the MS2 and GI.1 and GII.4 VLPs decreased slightly from the maximum, observed at 100 mM NaCl. The α of λ is the same at 100 and 300 mM NaCl.

Effect of pH on Norovirus GI.1 VLP Adsorption

The attachment efficiency, α , as a function of pH was investigated for GI.1 VLPs in the presence of 10 and 100 mM NaCl (Figure 4). GI.1 VLPs in 100 mM NaCl had a maximum

α of 1, regardless of pH. In 10 mM NaCl, pH effects were pronounced. In 10 mM NaCl, the GI.1 VLPs had a lower α at pH 8 than at pH 7 (0.08 ± 0.02 and 0.52 ± 0.33 , respectively). At pH 5.2, which is in the range of the pI, the GI.1 VLP α was highly variable in 10 mM NaCl (0.44 ± 0.57). Below this pH, at pH 4.5, the GI.1 VLPs had an α approaching the maximum (0.98 ± 0.20), and, at pH 4.0, the α was again highly variable (0.39 ± 0.38). Experiments were conducted four times at this condition for confirmation.

Effect of Divalent Cations on Norovirus GI.1 and GII.4 VLP Adsorption

Keeping the total ionic strength constant at 10 mM, enhanced adsorption was observed for GI.1 and GII.4 VLPs in the presence of divalent cations. Table 1 summarizes the effects various solutions chemistries have on the α of GI.1 and GII.4 VLPs, with α presented in Figure S2 in the Supporting Information. In the presence of 1 mM Ca^{2+} , GI.1 α was greatly enhanced (by a factor of 13), with an α of 1.0, whereas GII.4 α was enhanced to a lesser extent (by a factor of 3), reaching an α of only 0.1. The presence of 1 mM Mg^{2+} likewise significantly increased GI.1 α (by a factor of 10).

Effect of Buffer Chemistry on Norovirus GI.1 and GII.4 VLP Adsorption

At both 10 and 100 mM total ionic strength, the inclusion of 1 mM NaHCO_3 decreased the α of GI.1 and GII.4 VLPs by a factor of 1/3 and 1/2, respectively (Table 1 and Figure S2). At a total ionic strength of 10 mM and pH 8.0, the inclusion of 0.1 mM Na_2HPO_4 decreased the α of GI.1 and increased that of GII.4 by a factor of 1/4 and 5, respectively.

Discussion

Norovirus VLPs are Stable Against Aggregation over a Wide Range of pH and Ionic Strength

At pH 4.0–8.0, over a range of ionic strengths (NaCl), GI.1 and GII.4 VLPs had hydrodynamic radii slightly larger than the radii determined by TEM but were stable against further aggregation for several hours (data not shown). Exceptions were the solutions of 10 mM NaCl at pH 4 and 1 mM NaCl at pH 8 for GI.1 VLPs, and 10 mM NaCl at pH 5.5 for GII.4 VLPs, in which the VLPs appeared to be slightly aggregated, though the VLPs did not continue to aggregate over time. The hydrodynamic radii were more sensitive to ionic strength than to pH (within the range of pH 4 to 8), with larger VLP sizes measured at lower ionic strength for most pH values. At pH 9.0, the very large hydrodynamic radius of GI.1 VLPs was interpreted as capsid disassembly rather than aggregation. This determination was made because of observations of significantly disrupted capsids and loose proteins in TEM images taken at the same conditions.

Other researchers have shown that the GI.1 VLPs disassemble upon dialysis overnight at a pH greater than 8 (in 20 mM citrate/phosphate buffer) (26) or at pH 8.9 (in 50 mM Tris-HCl) (27) and can subsequently be reassembled to their native form upon lowering the pH. Under our experimental conditions, the GI.1 and GII.4 VLPs did not disassemble at pH 8.0, allowing for experimental determination of α at this pH, which is relevant to wastewater and alkaline groundwater.

For comparison, researchers examining aggregation of four F-specific RNA bacteriophages belonging to the *Leviviridae* family (MS2, GA, Q β , and SP) also used DLS to examine the aggregation state of the viruses in 1 and 100 mM NaNO_3 as a function of pH (pH 1.5 to 7) using an autotitrator setup (28). Above its pI, MS2 particles appeared to have a hydrodynamic radius twice the radius determined by TEM (28), likewise suggesting slight aggregation. MS2 viruses aggregated dramatically near their pI (pH 4) regardless of the IS, going from a hydrodynamic radius of 20 ± 10 nm (pH 5–7) to over 2 μm (pH 2–4). GA and

SP were highly aggregated at all pH and IS. It is notable that GI.1 and GII.4 VLPs do not aggregate significantly below their pI or in high IS solutions, unlike these four bacteriophages. MS2 has protruding hydrophilic polypeptide loops, which have been proposed to enable the steric stabilization responsible for nonelectrostatic behavior of MS2 in adsorption studies (14). Researchers examining rotavirus aggregation (above their pI) likewise have found rotavirus to be stable against aggregation in NaCl solutions up to 600 mM and have suggested that steric stabilization is due to capsid features (7). The extreme stability demonstrated by GI.1 and GII.4 particles near their isoelectric point and in high IS solutions may be due to a capsid feature that likewise results in steric stabilization or may be unique to VLPs, where the lack of RNA could change particle colloidal stability.

Isoelectric Point is Insufficient in Predicting Norovirus VLP Deposition

Both VLP strains followed the similar expected pattern of increasing attachment efficiency, α , with increasing IS at pH 8, due to the compression of the electrical double layers of the negatively charged silica and VLP surfaces in the presence of increasing concentrations of salt (29). Electrophoretic mobility data (Figure 2) demonstrate electric double layer compression of GI.1 VLPs at pH 8, as the VLPs had a less negative electrophoretic mobility in 10 mM NaCl than 1 mM NaCl.

In examining the sensitivities of the VLPs and phages to changes in ionic strength, GI.1, MS2, and λ all had approximately 1 log increase in α as the ionic strength was increased from 10 to 100 mM NaCl (Figure 3). GII.4 showed a steeper response, spanning a 1.5 log difference in α over the same ionic strength range. Differences in response to ionic strength may be due to heterogeneities within the VLP population as well as in molecular scale distribution of surface charge (local patchiness) (30–33). The steeper response of α versus IS of GII.4 compared to GI.1 may indicate that GII.4 VLPs have a less heterogeneous surface charge than GI.1 or that the GII.4 population itself is less heterogeneous than the GI.1 population. At low ionic strengths, differences in population or particle surface charge heterogeneities between the two strains may be more pronounced than at higher ionic strengths, where patchiness is less important due to double layer compression and subsequent masking of heterogeneities (32, 34).

At 300 mM NaCl, the α was slightly lower for both strains than at 100 mM NaCl. Although this phenomenon could be explained by aggregation of the VLPs in higher salt solution, DLS data (Figure 1) demonstrate that the VLPs remain unaggregated at pH 8 at high IS. The slight drop in α at the 300 mM concentration is likely due to hydration forces that are more important at high salt concentrations (35–41).

Solution pH is expected to have an important role in viral attachment. At all pH, the VLP surfaces have positively and negatively charged moieties, including acidic amino acids (aspartic acid, pK_a 3.9, and glutamic acid, pK_a 4.1) and basic amino acids (histidine, pK_a 6.0, lysine, pK_a 10.8, and arginine, pK_a 12.5) (Table S1). The capsids also contain nonionizable monomers of various chemistries (nucleophilic, hydrophobic, aromatic, and amide functionalities) (42). In 100 mM NaCl, regardless of the pH, GI.1 VLPs had an α of 1, indicating that charges are sufficiently screened such that electrostatic interactions are not important (Figure 4). In 10 mM NaCl above the pI, a predicted response was observed. From pH 8 to 7, there was a dramatic increase in α as the VLPs reached a more neutral net charge. At pH 5.2, there was large variability in observed α , though VLP electroneutrality near the pI should theoretically encourage adsorption. This observed variability in α near the pI may be due to small differences in bulk pH that cause dramatic shifts in charge balance. Below the pI, the observed α are quite interesting. At pH 4.5, VLPs had a maximum α , as expected for electrostatic attraction. However, at pH 4.0, again the observed α were quite variable, as seen near the pI.

This high variability at pH 4.0 may be due to the changes in protonation of aspartic and glutamic acid, which have pK_a values near pH 4 (3.9 and 4.1, respectively). Thus, near pH 4, the carboxyl groups on these amino acids dramatically protonate and deprotonate with small shifts in pH. This may affect minor structural features in the capsid, reorienting the charged moieties presented at the surface. The synergistic effects of the combination of all amino acid monomers in the VLP capsid are complex and difficult to interpret, but the erratic GI.1 VLP adsorption behavior near the pK_a values for these monomers is notable. Minor conformational changes of the GI.1 VLPs have been demonstrated for pH changes using a variety of biophysical techniques (including intrinsic and extrinsic fluorescence, high resolution second-derivative UV absorption spectroscopy, circular dichroism (CD), and differential scanning calorimetry), though definitive descriptions of capsid morphology or exposed charged residues at a given pH have not been determined (26). It is possible that pH changes cause subtle reorientation at the capsid surface, which may be responsible for the observed nonmonotonic GI.1 VLP adsorption patterns as a function of pH. In viral transport research, the pI of a given viral pathogen or bacteriophage is often used as the master variable to categorize viruses and predict their transport in aquatic systems. These results suggest that knowledge of the pI of a VLP is insufficient to predict transport as a function of pH, which may have important implications for predictions of viral transport.

Buffer Substitutions Affect GI.1 and GII.4 Deposition Differently

Divalent cations can enhance the adsorption of colloidal particles onto surfaces via two mechanisms: increased shielding of the electric double layer of particles compared to monovalent cations and the formation of bridges between colloidal particles and surfaces due to specific interactions or complexation (7, 8, 29). An increase in α was observed in the presence of Ca^{2+} for both GI.1 and GII.4 VLPs (a 13- and 3-fold increase, respectively), in agreement with classic colloid adsorption and aggregation studies (29, 43). This enhanced adsorption was not due to charge neutralization for GI.1 VLPs, as the EPM was only slightly less negative in the presence of calcium compared to sodium alone (at pH 8 in 10 mM ionic strength) (Figure 2). MS2, PRD1, and rotavirus have all shown enhanced adsorption to silica in the presence of Ca^{2+} , attributed to cation bridging between the viruses and the surfaces (7, 8, 44).

Experimental serendipity highlighted the major role that a common buffer, bicarbonate, plays in norovirus VLP adsorption. In early experiments, bicarbonate was employed as a buffer to maintain pH 8, but regardless of ionic strength, minimal adsorption was observed. Upon elimination of bicarbonate in the system and by instead adjusting the pH quickly with small amounts of NaOH, the expected trend with ionic strength emerged as previously presented. The decrease in α of GI.1 and GII.4 in the presence of bicarbonate may be due to the bicarbonate ions associating with basic amino acids on the surface of the VLP capsids, such as lysine, histidine, and arginine. GI.1 VLPs were slightly more negatively charged in 1 mM $NaHCO_3$ than 1 mM NaCl in the pH 6–8 range, suggesting that bicarbonate ions associate with the capsid. At lower bicarbonate concentrations (0.1 mM $NaHCO_3$), but at the same total ionic strength (1 mM), the GI.1 VLPs had roughly the same electrophoretic mobility as those measured only in NaCl solutions (within the experimental variation), though there was still a noticeable decrease in α in adsorption studies. This demonstrates that electrophoretic mobility measurements alone are not sensitive enough to predict adsorption behavior of virus particles in model transport systems.

It is interesting that the same relative decrease in α was observed for GI.1 and GII.4 VLPs in the presence of 1 mM $NaHCO_3$, regardless of the total ionic strength (a factor of 1/3 and 1/2 for GI.1 and GII.4, respectively). This implies that bicarbonate has a specific interaction with the capsid, since at 100 mM ionic strength all charges should be shielded, and,

therefore, the α should remain equal to 1 regardless of the ions present. The effect of bicarbonate on GI.1 was slightly more pronounced than its effect on GII.4 VLP adsorption, though the effects are of the same order of magnitude, suggesting that the mechanism for the decrease is similar for both VLP strains.

The decrease in α of GI.1 in the presence of 0.1 mM HPO_4^{2-} (by a factor of 1/4) can be explained analogously to the effect observed in HCO_3^- . The surface of GI.1 VLPs was likewise more negative in the presence of 0.1 mM HPO_4^{2-} at 10 mM total IS (Figure 2). GII.4 VLPs, however, demonstrated enhanced adsorption in the presence of HPO_4^{2-} (by a factor of 5, to an α nearing favorable attachment). (Unfortunately, corresponding surface charge data is not available for GII.4 due to stock scarcity.) The opposing trends in adsorption in the presence of phosphate may be due to differences in exposed residues of GI.1 and GII.4 capsids at pH 8.

Taken together, these GI.1 and GII.4 VLP adsorption data (Table 1) suggest that two strains of the same virus may have differing adsorption responses to individual ions in solution. The monomers that make up the GI.1 and GII.4 capsids are not conserved in the surface region (Table S1) (45–47). Differences in exposure and orientation of charged, hydrophobic, and uncharged residues at the surface may account for the individual response to solution chemistry in adsorption.

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Literature Cited

1. da Silva AK, Le Saux J-C, Parnaudeau S, Pommeupuy M, Elimelech M, Le Guyader FS. Evaluation of Removal of Noroviruses during Wastewater Treatment, Using Real-Time Reverse Transcription-PCR: Different Behaviors of Genogroups I and II. *Appl Environ Microbiol.* 2007; 73(24):7891–7897. [PubMed: 17933913]
2. Haramoto E, Katayama H, Oguma K, Ohgaki S. Quantitative analysis of human enteric adenoviruses in aquatic environments. *J Appl Microbiol.* 2007; 103:2153–2159. [PubMed: 18045398]
3. Metcalf T, Melnick J, Estes MK. Environmental Virology: From Detection of Virus in Sewage and Water by Isolation to Identification by Molecular Biology - A Trip of Over 50 Years. *Annu Rev Microbiol.* 1995; 49:461–487. [PubMed: 8561468]
4. Gerba CP. Virus survival and transport in groundwater (Reprinted from *Developments in Industrial Microbiology*, vol 24, pg 247–251, 1983). *J Ind Microbiol Biotechnol.* 1999; 22:535–539.
5. Scandura JE, Sobsey MD. Viral and bacterial contamination of groundwater from on-site sewage treatment systems. *Water Sci Technol.* 1997; 35(11–12):141–146.
6. Schijven JF, Hassanizadeh SM. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. *Crit Rev Environ Sci Technol.* 2000; 30(1):49–127.
7. Gutierrez L, Mylon SE, Nash B, Nguyen TH. Deposition and Aggregation Kinetics of Rotavirus in Divalent Cation Solutions. *Environ Sci Technol.* 2010; 44(12):4552–4557. [PubMed: 20481597]
8. Pham M, Mintz EA, Nguyen TH. Deposition kinetics of bacteriophage MS2 to natural organic matter: Role of divalent cations. *J Colloid Interface Sci.* 2009; 338(1):1–9. [PubMed: 19608192]
9. Yuan BL, Pham M, Nguyen TH. Deposition Kinetics of Bacteriophage MS2 on a Silica Surface Coated with Natural Organic Matter in a Radial Stagnation Point Flow Cell. *Environ Sci Technol.* 2008; 42(20):7628–7633. [PubMed: 18983085]

10. Chen KL, Elimelech M. Aggregation and Deposition Kinetics of Fullerene (C₆₀) Nanoparticles. *Langmuir*. 2006; 22:10994–11001. [PubMed: 17154576]
11. Fatissou J, Domingos RF, Wilkinson KJ, Tufenkji N. Deposition of TiO₂ Nanoparticles onto Silica Measured Using a Quartz Crystal Microbalance with Dissipation Monitoring. *Langmuir*. 2009; 25(11):6062–6069. [PubMed: 19466771]
12. Bales RC, Li SM, Maguire KM, Yahya MT, Gerba CP. MS-2 And Poliovirus Transport In Porous-Media - Hydrophobic Effects And Chemical Perturbations. *Water Resour Res*. 1993; 29:957–963.
13. Redman JA, Grant SB, Olson TM, Hardy ME, Estes MK. Filtration of Recombinant Norwalk Virus Particles and Bacteriophage MS2 in Quartz Sand: Importance of Electrostatic Interactions. *Environ Sci Technol*. 1997; 31:3378–3383.
14. Penrod SL, Olson TM, Grant SB. Deposition kinetics of two viruses in packed beds of quartz granular media. *Langmuir*. 1996; 12(23):5576–5587.
15. Opekun AR, Atmar RL, Gilger MA, Estes MK, Neill FH, Chandrasekaran A, Ugane CL, Graham DY. Magnitude and duration of virus shedding of norwalk virus in a human challenge system. *Gastroenterology*. 2007; 132:A705–A705.
16. Glass RI, Parashar UD, Estes MK. Norovirus Gastroenteritis. *N Engl J Med*. 2009; 361:1776–1785. [PubMed: 19864676]
17. Green, KY.; Chanock, RM.; Kapikian, AZ. *Fields Virology: Chapter 27: Human Caliciviruses*; Lippincott Williams & Wilkins; 2001.
18. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. *Virology*. 2006; 346(2):312–323. [PubMed: 16343580]
19. Goodridge L, Goodridge C, Wu J, Griffiths M, Pawliszyn J. Isoelectric Point Determination of Norovirus Virus-Like Particles by Capillary Isoelectric Focusing with Whole Column Imaging Detection. *Anal Chem*. 2004; 76(1):48–52. [PubMed: 14697031]
20. Chakravarty S, Hutson AM, Estes MK, Prasad BVV. Evolutionary trace residues in noroviruses: Importance in receptor binding, antigenicity, virion assembly, and strain diversity. *J Virol*. 2005; 79:554–568. [PubMed: 15596848]
21. Hutson AM, Atmar RL, Marcus DM, Estes MK. Norwalk virus-like particle hemagglutination by binding to H histo-blood group antigens. *J Virol*. 2003; 77:405–415. [PubMed: 12477845]
22. Le Guyader FS, Loisy F, Atmar RL, Hutson AM, Estes MK, Ruvoen-Clouet N, Pommepuy M, Le Pendu J. Norwalk virus-specific binding to oyster digestive tissues. *Emerg Infect Dis*. 2006; 12(6):931–936. [PubMed: 16707048]
23. Tamura M, Natori K, Kobayashi M, Miyamura T, Takeda N. Genogroup II noroviruses efficiently bind to heparan sulfate proteoglycan associated with the cellular membrane. *J Virol*. 2004; 78:3817–3826. [PubMed: 15047797]
24. Jiang X, Wang M, Graham DY, Estes MK. Expression, Self-Assembly, and Antigenicity of the Norwalk Virus Capsid Protein. *J Virol*. 1992; 66:6527–6532. [PubMed: 1328679]
25. Sauerbrey G. Verwendung Von Schwingquarzen Zur Wagung Dunner Schichten Und Zur Mikrowagung. *Z Phys*. 1959; 155:206–222.
26. Ausar SF, Foubert TR, Hudson MH, Vedvick TS, Middaugh CR. Conformational stability and disassembly of Norwalk virus-like-particles - Effect of pH and temperature. *J Biol Chem*. 2006; 281:19478–19488. [PubMed: 16675449]
27. White LJ, Hardy ME, Estes MK. Biochemical characterization of a smaller form of recombinant Norwalk virus capsids assembled in insect cells. *J Virol*. 1997; 71:8066–8072. [PubMed: 9311906]
28. Langlet J, Gaboriaud F, Duval JFL, Gantzer C. Aggregation and surface properties of F-specific RNA phages: Implication for membrane filtration processes. *Water Res*. 2008; 42:2769–2777. [PubMed: 18329685]
29. Elimelech, M.; Gregory, J.; Jia, X.; Williams, R. *Particle Deposition and Aggregation: Measurement, Modelling, and Simulation*. Butterworth-Heinemann Ltd; Oxford: 1995.
30. Redman JA, Grant SB, Olson TM, Estes MK. Pathogen Filtration, Heterogeneity, and the Potable Reuse of Wastewater. *Environ Sci Technol*. 2001; 35:1798–1805. [PubMed: 11355195]
31. Song LF, Elimelech M. Transient Deposition of Colloidal Particles in Heterogeneous Porous-Media. *J Colloid Interface Sci*. 1994; 167(2):301–313.

32. Song LF, Johnson PR, Elimelech M. Kinetics Of Colloid Deposition Onto Heterogeneously Charged Surfaces In Porous-Media. *Environ Sci Technol.* 1994; 28:1164–1171. [PubMed: 22176246]
33. Tufenkji N, Redman JA, Elimelech M. Interpreting Deposition Patterns of Microbial Particles in Laboratory-Scale Column Experiments. *Environ Sci Technol.* 2003; 37:616–623. [PubMed: 12630480]
34. Chen JY, Klemic JF, Elimelech M. Micropatterning microscopic charge heterogeneity on flat surfaces for studying the interaction between colloidal particles and heterogeneously charged surfaces. *Nano Lett.* 2002; 2(4):393–396.
35. Christenson HK. Non-DLVO Forces Between Surfaces -Solvation, Hydration And Capillary Effects. *J Dispersion Sci Technol.* 1988; 9:171–206.
36. Clasen T, Schmidt WP, Rabie T, Roberts I, Cairncross S. Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. *Br Med J.* 2007; 334(7597):782–785. [PubMed: 17353208]
37. Elimelech M. Indirect Evidence For Hydration Forces In The Deposition Of Polystyrene Latex Colloids On Glass Surfaces. *J Chem Soc Faraday Trans.* 1990; 86:1623–1624.
38. Frens G, Overbeek JTG. Repeptization and the theory of electrostatic colloids. *J Colloid Interface Sci.* 1972; 38:376–387.
39. Healy TW, Homola A, James RO, Hunter RJ. Coagulation Of Amphoteric Latex Colloids - Reversibility And Specific Ion Effects. *Faraday Discuss.* 1978:156–163.
40. Israelachvili JN. Measurements Of Hydration Forces Between Macroscopic Surfaces. *Chem Scr.* 1985; 25:7–14.
41. Lessard RR, Zieminski SA. Bubble Coalescence and Gas Transfer in Aqueous Electrolytic Solutions. *Ind Eng Chem Fundam.* 1971; 10(2):260–269.
42. Nelson, DL.; Cox, MM. *Lehninger Principles of Biochemistry.* 3. Worth Publishers; 2000.
43. Grolmund D, Elimelech M. Aggregation and deposition kinetics of mobile colloidal particles in natural porous media. *Colloids Surf, A.* 2001; (191):179–188.
44. Bales RC, Hinkle SR, Kroeger TW, Stocking K, Gerba CP. Bacteriophage Adsorption During Transport Through Porous-Media - Chemical Perturbations And Reversibility. *Environ Sci Technol.* 1991; 25:2088–2095.
45. Jiang X, Wang M, Wang K, Estes MK. Sequence and genomic organization of Norwalk virus. *Virology.* 1993; 195(1):51–61. [PubMed: 8391187]
46. Larsson MM, Rydell GE, Grahn A, Rodriguez-Diaz J, Akerlind B, Hutson AM, Estes MK, Larson G, Svennson L. Antibody prevalence and titer to norovirus (genogroup II) correlate with secretor (FUT2) but not with ABO phenotype or Lewis (FUT3) genotype. *J Infect Dis.* 2006; 194(10): 1422–1427. [PubMed: 17054072]
47. Allen DJ, Noad R, Samuel D, Gray JJ, Roy P, Iturriza-Gomara M. Characterisation of a GII-4 norovirus variant-specific surface-exposed site involved in antibody binding. *Virol J.* 2009; 6(1): 150. [PubMed: 19781066]

FIGURE 1.

Effect of ionic strength (NaCl) and pH on (a) GI.1 and (b) GII.4 VLP hydrodynamic radius. The pH was adjusted using a small amount of HCl or NaOH, and measurements were taken very quickly after adjustment (total time elapsed is less than 5 min for experiments). VLP concentration was 10^{11} per mL and temperature was fixed at 23 °C. Error bars indicate standard deviations.

FIGURE 2.

Effect of ion composition on GI.1 VLP electrophoretic mobility (EPM) in (a) 1 mM and (b) 10 mM total ionic strength solutions at various pH. At least three experiments were carried out at each condition at a VLP concentration of 10^{12} per mL, with ten measurements per experiment. Temperature was maintained at $25\text{ }^{\circ}\text{C}$ ($\pm 1\text{ }^{\circ}\text{C}$). Error bars indicate standard deviations.

FIGURE 3.

Comparison of sensitivities of attachment efficiencies of GI.1 and GII.4 VLPs, as well as bacteriophages MS2 and λ , to changes in ionic strength (NaCl). MS2 and λ phage attachment efficiency data are reproduced from Yuan et al. (2008) and Penrod et al. (1996), respectively (9, 14). VLP experiments were carried out at pH 8 (roughly 2 pH units above their pI) and phage experiments at pH 5.0 (roughly 1.5 and 1 pH units above the pIs for MS2 and λ , respectively). The VLP concentration was 1.3×10^{11} per mL for each experiment. Phage concentrations ranged from 10^6 to 10^{10} PFU/mL. Error bars indicate standard deviations.

FIGURE 4.

Effect of pH on GI.1 VLP attachment efficiency. VLP concentration was 1.3×10^{11} per mL for each experiment. Experiments were conducted in 10 or 100 mM NaCl at various pH, three times at each condition, except pH 4 at 10 mM NaCl, which was conducted four times. Error bars indicate standard deviations.

TABLE 1

Summary of Sensitivity of GI.1 and GII.4 Attachment Efficiency, α , to Specific Cations and Anions^a

ionic strength (mM)	pH	cation/anion of interest	solution chemistry	fractional increase or decrease in α	
				GI.1	GII.4
10 or 100	8	–	10 or 100 mM NaCl (reference condition)	1	1
10	8	Ca ²⁺	7 mM NaCl + 1 mM CaCl ₂	13	3
10	8	Mg ²⁺	7 mM NaCl + 1 mM MgCl ₂	10	
10	8	HCO ₃ [–]	9 mM NaCl + 1 mM NaHCO ₃	1/3	1/2
100	8	HCO ₃ [–]	99 mM NaCl + 1 mM NaHCO ₃	1/3	1/2
10	8	HPO ₄ ^{2–}	9.7 mM NaCl + 0.1 mM Na ₂ HPO ₄	1/4	5

^aThe table compares the average adsorption rates for experiments in the presence of Ca²⁺, Mg²⁺, HCO₃[–], and HPO₄^{2–}, compared to experiments at the same total IS and pH with NaCl only. The fractional increase or decrease in attachment efficiency in the presence of the substituted ion is indicated, demonstrating that GI.1 and GII.4 attachment efficiencies have different sensitivities to ion substitutions.