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Isoelectric point is an inadequate descriptor of MS2, Phi X 174 and PRD1 phages adhesion on abiotic surfaces

Christelle Dika [a,b], Jérôme F.L. Duval [c,d,*], Gregory Francius [a,b], Aline Perrin [a,b], Christophe Gantzer [a,b,*]

[a] Université de Lorraine, LCPME (Laboratoire de Chimie Physique et Microbiologie pour l'Environnement), UMR 7564, Nancy F-54000, France
[b] CNRS, LCPME, UMR 7564, Nancy F-54000, France
[c] Université de Lorraine, LIEC (Laboratoire Interdisciplinaire des Environnements Continentaux), UMR 7360, Vandoeuvre-lès-Nancy F-54501, France
[d] CNRS, LIEC, UMR 7360, Vandoeuvre-lès-Nancy F-54501, France

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ABSTRACT

MS2, Phi X 174 and PRD1 bacteriophages are commonly used as surrogates to evaluate pathogenic virus behavior in natural aquatic media. The interfacial properties of these model soft bioparticles are herein discussed in connection with their propensities to adhere onto abiotic surfaces that differ in terms of surface charges and hydrophobicities. The phages considered in this work exhibit distinct multilayered surface structures and their electrostatic charges are evaluated from the dependence of their electrophoretic mobilities on electrolyte concentration at neutral pH on the basis of electrokinetic theory for soft (bio)particles. The charges of the viruses probed by electrokinetics vary according to the sequence Phi X 174 ⩽ PRD1 ≪ MS2, where '<' stands for 'less charged than'. The hydrophobic/hydrophilic balances of the phages are further derived from their adhesions onto model hydrophobic and hydrophilic self-assembled mono-layers. The corresponding results lead to the following hydrophobicity sequence Phi X 174 ≪ MS2 < PRD1 where '<' means 'less hydrophobic than'. The respective electrostatic and hydrophobic/hydrophilic features of the phages are further shown to be consistent with their measured adhesions onto polyethersulfone-based membranes with distinct hydrophobicities and charge levels. The methodology clearly demonstrates that the traditionally adopted phage isoelectric point as a relevant physico-chemical descriptor for phage adhesion is not adequate for MS2, Phi X 174 and PRD1 bacteriophages.

1. Introduction

In natural aquatic media, human pathogenic viruses are unable to replicate and they may thus be considered inert bioparticles from a biological point of view. Their surface properties and the composition of the electrolyte medium then become key factors that control their fate and physico-chemical reactivity. This issue led environmental virologists to pay due attention to electrostatic charge and degree of hydrophobicity of phages in order to understand their behavior in defined aquatic systems. The typical diameter of pathogenic enteric viruses such as noroviruses, hepatitis A and E viruses, is 20–30 nm. Fecal bacteriophages like F-specific RNA phages have a similar size and commonly serve as model systems to describe the behavior of pathogenic enteric viruses in water. These viral particles, which are of prime interest in terms of public health [1], further exhibit a complex chemically-stratified structure with a proteic outer layer that encapsulates an internal RNA genome [2]. In view of their outer surface structure (see details below), the MS2, Phi X 174 and PRD1 F-specific RNA phages considered in this work are probably bad models for lipid-enclosed virions because they are so-called nonenveloped viruses, *i.e.* they lack an outer lipid envelop. However, it should be recognized that enveloped viruses may be extremely useful model systems for analyzing *e.g.* virus-host cell interactions because these viruses are known to insert their own proteins and lipids into host-cell derived membrane.

Viruses interact with their close environment according to *e.g.* non-specific electrostatic and hydrophobic forces. The former are long-range forces with characteristic spatial scale the Debye length that is fixed by the medium salinity (~10 nm and ~1 nm for 1 mM and 100 mM salt concentration, respectively). The sign and magnitude of these electrostatic forces are further controlled by

* Corresponding authors. Address: Université de Lorraine, LIEC (Laboratoire Interdisciplinaire des Environnements Continentaux), UMR 7360, Vandoeuvre-lès-Nancy F-54501, France. Fax: +33.3.83.59.62 55 (Jérôme F.L. Duval). Address: Université de Lorraine, LCPME (Laboratoire de Chimie Physique et Microbiologie pour l'Environnement), UMR 7564, Nancy F-54000, France. Fax: +33 3 83 68 23 78 (C. Gantzer).

E-mail addresses: jerome.duval@univ-lorraine.fr (J.F.L. Duval), christophe.gantzer@univ-lorraine.fr (C. Gantzer).

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the density of charges carried by the viruses and by the target surfaces. Hydrophobic interactions take place at shorter separation distances, and become predominant under conditions where electrostatic forces are weakest, *i.e.* at sufficiently large electrolyte concentrations. The nature of these different colloidal interactions highlights the mandatory requirement for acquiring knowledge on both the charge and the hydrophobic/hydrophilic balance pertaining to complex viral particles. In the following, the terms 'hydrophobic interaction' and 'hydrophobic force' are used as they are recurrently found in the literature. However, we draw the attention of the reader on the fact that these terms suggest that there are direct forces between apolar bodies in water, which is not correct. Instead, it is the water (and its high cohesion) that gives rise to the so-called hydrophobic effect. There are phages-environment interactions other than electrostatic and apolar in nature (*e.g.* steric, van der Waals or specific chemical interactions) that likely play a key role in determining the fate of viruses in natural aquatic media. Their measurements and evaluations are however extremely difficult to achieve under liquid conditions with sufficient spatial resolution, especially for nanometric systems like viruses. On the opposite, long-range electrostatic interactions and short-range hydrophobic effects can be satisfactorily addressed *via* the original methodology proposed in this study. We recognize however that adhesion forces addressed here by atomic force microscopy (AFM) and termed 'hydrophobic interactions' for shortening might involve other interactions than those stemming from pure hydrophobic effects. Deciphering the various components of these adhesion forces is a very challenging task from an experimental point of view and it would surely require AFM force measurements with use of virus-coated probes. To the best of our knowledge, no convincing experimental studies have been reported on these aspects yet. Despite the above uncertainties and difficulties, we demonstrate that a consistent interpretation of our results may be given with arguing electrostatic and apolar (or adhesion) interactions only.

The surface charge of viruses is commonly evaluated *via* their zeta-potential determined from electrophoretic mobility measurements [3–5]. While such an approach is still adopted in the literature, it should be realized that the very concept of zeta-potential is physically meaningful for particles that are *stricto sensu* impermeable to water and ions (so-called hard colloids). However, numerous theoretical and experimental studies have now demonstrated that the *a priori* location of a shear plane and the definition of a zeta-potential or a surface charge for complex biosystems such as bacteria [6] and viruses [7] have no physical basis. Instead, these bioparticles are models of soft colloids, *i.e.* colloids that are partly consisting of an interfacial (bio)polymeric structure permeable to water and ions and where charges are three-dimensionally distributed [8]. Viruses further exhibit a complex chemically-stratified structure with the presence of an internal RNA or DNA compartment encapsulated by *e.g.* an outer proteic layer. The electrokinetic properties of these viruses were shown to be dramatically impacted by the three dimensional distribution of charges from the virus center to the peripheral capsid, and by their hydrodynamic permeability [9,10]. The electrophoretic mobility of soft particles such as viruses reaches a non-zero plateau value at large electrolyte concentrations while that of hard colloids tends to zero under similar salinity conditions where particle electrostatic charge is significantly screened by ions in the medium [9,11].

In line with the inexact hard particle representation for viruses, their adhesion capacity (*i.e.* propensity) onto charged abiotic surfaces is often apprehended by the sole consideration of their isoelectric point (IEP) [12], defined as the pH value where electrophoretic mobility is zero. The IEP values of more than 140 viruses have been recently reviewed by Michen and Graule [12] who showed a large discrepancy in IEPs with values ranging between 1.9 and 8.4. Like the zeta-potential, the IEP remains a parameter of limited use for apprehending on a quantitative basis the charge carried by viruses. The first reason is that the classification of adhesion capacity of viruses according to their IEP value is based on experimental data that differ from one study to the other depending on the adopted measurement tool. The second reason is that, despite common acceptation, IEP of soft multilayered particles such as viruses is not only depending on the protolytic properties of the charged groups located at their outer periphery, but also on the complex physico-chemical characteristics of the underlying structure. These include the charge distribution within the capsid and the genome, the thickness of the capsid, the size of the genome, the respective permeability of the capsid and that of the genome viral compartment. These fundamentals of electrokinetics of soft multilayered particles like viruses were first introduced by Langlet et al. [10] and a strong analogy was recently drawn -*via* theory and experiments- with the electrokinetics of other complex soft multilayered interphases like polyelectrolyte multilayers or lipid bilayers supported by charged polymeric cushion [13]. As a consequence, there is no straightforward physical connection between the pH value where mobility of viruses is zero and the very 'surface' charge they carry. Consequently, zeta-potential and IEP parameters should be interpreted very carefully in order to adequately correlate the charge of complex soft multilayered viruses with their adhesion capacity onto charged abiotic surfaces. Instead, analyzing the dependence of virus electrophoretic mobility on ionic strength with the use of adequate formalism where the zeta-potential picture is abandoned, seems to offer a better alternative for defining the searched electrostatic characteristics of viral particles [14]. So far, such a classification of viruses charge on the basis of electrokinetic theory for soft bioparticles is missing.

At sufficiently high ionic strengths, virus electrostatic charges are screened and hydrophobic interactions then significantly favor viral adhesion onto surfaces [15,16]. An evaluation of the magnitude of these interactions requires the measurement of the hydrophobicity degree of the viral interfaces. Most of the methods used for that purpose consist in estimating the affinity of the viruses for a solvent of given polarity (MATS tests) or for a given chosen surface (hydrophobic interactions chromatography, HIC) [17]. These approaches have been however largely criticized in the literature. In particular, Busscher et al. [18] and Ahimou et al. [19] argued that the majority of solvents used in MATS tests as well as other reference surfaces employed in HIC, exhibit a finite charge and may thus electrostatically interact with viruses. Other authors [15] resorted to the use of commercially available membranes to characterize the hydrophobicity of viruses *via* adhesion experiments. These materials are however often ill-defined, and quantitative information on their relative hydrophobicity is rarely provided. Finally, the interpretation of virus adhesion data at large ionic strengths may be impaired by possible virus aggregation [14]. Therefore, it is often impossible to unambiguously classify the adhesion capacity of viruses according to their hydrophilic/hydrophobic balance. Very few classifications of viruses as a function of their hydrophobic character are available in the literature.

Without a clear and accurate knowledge on virus charge and hydrophilic/hydrophobic balance, prediction of virus adhesion to surfaces is impossible or is highly speculative. Therefore the objectives of this study are: (i) to adequately measure the charge and hydrophobicity of different phages currently used in environmental studies, (ii) to address their adhesion onto various membranes of practical interest, and (iii) to analyze the relationship between virus adhesion capacity and virus interfacial properties as determined in (i). The phages selected in this study are classically used as viral surrogates in the environment: MS2, Phi X 174 and PRD1 phages [20–22]. The F-specific RNA bacteriophage MS2 is a member of *Leviviridae* family. This phage consists of a 20–30 nm