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Protocols

Experimental and computational surface hydrophobicity analysis of a non-enveloped virus and proteins

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ABSTRACT

The physical characteristics of viruses needs to be understood in order to manipulate the interaction of viruses with host cells, as well as to create specific molecular recognition techniques to detect, purify, and remove viruses. Viruses are generally believed to be positively charged at physiological pH, but there are few other defining characteristics. Here, we have experimentally and computationally demonstrated that a non-enveloped virus is more hydrophobic than a panel of model proteins. Reverse-phase and hydrophobic interaction chromatography and ANS fluorescence determined the experimental hydrophobic strength of each entity. Computational surface hydrophobicity was calculated by the solvent exposed surface area of the protein weighted by the hydrophobicity of each amino acid. The results obtained indicate a strong correlation between the computational surface hydrophobicity and experimentally determined hydrophobicity using reverse-phase chromatography and ANS fluorescence. The surface hydrophobicity did not compare strongly to the weighted average of the amino acid sequence hydrophobicity. This demonstrates that our simple method of calculating the surface hydrophobicity gives general hydrophobicity information about proteins and viruses with crystal structures. In the process, this method demonstrated that porcine parvovirus (PPV) is more hydrophobic than the model proteins used in this study. This adds an additional dimension to currently known virus characteristics and can improve our manipulation of viruses for gene therapy targeting, surface adsorption and general understanding of virus interactions.

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1. Introduction

Specific molecular interactions govern the attachment of viruses to cells, initiating the viral infection cycle. The understanding of these specific interactions can lead to the creation of unique therapies for viral diseases. In addition, specific chemical interactions can be harnessed to produce a targeted viral gene therapy vector for gene delivery [1,2], for example, the specific targeting of cancer cells [3]. Virus surface properties can also be exploited to manipulate viruses. Virus removal operations have been designed by absorbing viruses to surfaces [4]. Other uses of virus interactions are in the detection of viruses [5] and in the purification of vaccines or viral gene therapy vectors [6–9]. To produce a general method to adsorb viruses, for any of these applications, the physical properties of viruses that distinguish them from proteins needs to be clearly identified and quantified.

There are limited data on the isoelectric point of viruses [5,10,11]. This is likely due to the difficulty of purifying a virus with a high enough concentration to make the measurement. However, it is generally accepted that viruses are negatively charged at physiological pH. This is shown by the large use of anion exchange chromatography [12–15] and other positively charged moieties [16,17] for the adsorption of viruses.

A lesser-known property of viruses is their higher hydrophobicity as compared to many other proteins. Non-enveloped viruses have been found to bind to hydrophobic surfaces. Examples of this include a significant enhanced adsorption of MS2 bacteriophage to hydrophobic sand, compared to protein-coated silica nanoparticles and rotavirus [18] and the removal of porcine parvovirus by adsorption of small, hydrophobic peptides affixed to a chromatographic support [19,20]. Influenza virus, an enveloped virus, was found to adsorb quickly and with high affinity to a gold surface [21]. Complete xenotropic murine leukemia virus (XMuLV) clearance was achieved under high conductivity with a depth filter, which is large enough for the virus to pass through, suggesting that

Abbreviations: PPV, porcine parvovirus; HEM, bovine hemoglobin; BSA, bovine serum albumin; FIB, fibrinogen; LYS, lysozyme; RNAse, ribonuclease A; IgG, human immunoglobulin G; B19, human B19 parvovirus; MVM, minute virus of mice.

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the hydrophobic interactions strongly contributed to this retrovirus clearance [22]. Bacteriophages MS2 demonstrated a higher removal than ΦX174 by ultrafiltration, which may be attributed that the surface of phage MS2 being more hydrophobic than that of phage ΦX174. This may imply that an increase in hydrophobicity has the potential to assist virus interactions with the membrane material [23]. Viral hydrophobicity was measured using an octyl Sepharose-4 fast flow resin at pH 7.2 and concluded that MS2 and T4 are hydrophobic viruses while ΦX 174 is a hydrophilic virus [24]. Experimentally, hydrophobic interaction chromatography has been used to purify proteins [25], viruses [26] and virus-like particles [27] with minimal theoretical basis. While some work has been conducted on the hydrophobicity of bacteriophages, little work has conclusively determined the hydrophobicity of mammalian viruses and virus-like particles (VLPs).

Other indications of virus hydrophobicity have also been reported. Amino acids and sugars were found to selectively precipitated the human parvovirus B19 [28], PPV [8], and the enveloped Sindbis virus [7], implying that the increased hydrophobicity of the virus as compared to other proteins allowed for the selective precipitation. Polymers created to mimic virus nanoparticles for gene delivery were more efficient when the polymer was more hydrophobic [29]. This may imply that an increase in hydrophobicity has the potential to assist viruses in their mission as gene delivery vehicles.

The hydrophobicity of a protein or a virus is difficult to quantify. The hydrophobic strength of the core of a protein is believed to give the protein structural stability. This is often studied computationally to determine protein folding and stability [30–32]. Protein fragments can be categorized as globular, surface seeking or transmembrane [33]. However, this computational expensive method is only available for small protein segments. Experimental methods that measure surface hydrophobicity include the parameter 1/m* measured by precipitation in solution [34] and a microbial adhesion to hydrocarbons (MATH) assay [35] that has been used to determine the hydrophobicity of MS2 bacteriophage [36] and rotavirus [18]. The hydrophobic/hydrophilic balance of the phages has been indirectly evaluated from adhesion experiments performed on hydrophobic and hydrophilic self-assembled monolayers models [37]. An AFM method that measured the hydrophobic interaction forces between a silicon nitride tip coated with ΦX174, MS2 and Aichi virus and a hydrophobic sand surfaces demonstrated the hydrophobic interaction of the phages [38]. Other experimental measurements of surface hydrophobicity of proteins is the fluorescent probe ANS, that has been shown to measure the surface hydrophobicity of amyloid aggregates [39,40] and the separation of proteins using aqueous two phase system (ATPS) as defined by Log K values [41]. However, without a universal hydrophobicity measurement, it is difficult to compare published results, as has been demonstrated [42].

The hydrophobicity of surfaces can be determined by the oscillation of water molecules in molecular dynamic simulations [43]. Others have noted that to have a more quantitative measure of a hydrophobic surface, the cavity formation of the water structure is needed [44]. For proteins, it is more complicated. The surfaces are very heterogeneous and have complex topographies. It has been shown that the size of the hydrophobic patch on a protein is important in the hydrophobic interaction of proteins [45]. A large hydrophobic patch has more energy to control the water network than a lone hydrophobic amino acid that is surrounded by hydrophilic amino acids.

We desire to quantify the hydrophobicity of viruses as compared to a panel of standard proteins. We compared a computational approach based on the surface accessible surface area calculated by the Eisenberg hydrophobicity scale for protein and virus crystal structures to several different experimental methods to measure hydrophobicity. These methods included hydrophobic interaction chromatography, reverse-phase chromatography and ANS fluorescence. Our variety of methods characterized our model virus, PPV, as hydrophobic compared to the panel of proteins explored. The computational approach verified that other viruses also have a highly hydrophobic surface. This hydrophobicity measurement can be used to better understand virus-cell interactions as well as create improved methods to detect, remove, and purify viruses.

2. Materials and methods

2.1. Materials

The proteins in this study were, bovine serum albumin, BSA, (Sigma, St. Louis, MO), chicken egg white lysozyme, LYS (CalBioChem, Billerica, MA), bovine fibrinogen, FIB (Sigma, St. Louis, MO), bovine hemoglobin, HEM (Sigma, St. Louis, MO), ribonuclease A, RNAse (Sigma, St. Louis, MO) and human immunoglobulin G, IgG (Equitech-Bio, Kerrville, TX). HPLC grade acetonitrile and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO) for C18 chromatography. For the phosphate buffer solution, sodium hypophosphite ($NaH_2PO_4{\cdot}H_2O$) was purchased from VWR (Radnor, PA) and sodium triphosphate (Na_3PO_4) was purchased from Fisher Scientific (Pittsburgh, PA). Ammonium sulfate were purchased from Sigma (St. Louis, MO) for hydrophobic interaction chromatography. Solutions were made with water that was purified with a NanoPure water system (Thermo Scientific, Waltham, MA) to a resistance of >18 MΩ and filtered with a 0.22 μm bottle top filter (Millipore, Billerica, MA) or a 0.2 μm syringe filter (Nalgene, Rochester, NY) prior to use. All proteins were in 10 mM phosphate buffer adjusted to pH 7.2.

2.2. Methods

2.2.1. Virus and conditioned media production and virus titration

Porcine parvovirus (PPV) was produced in porcine kidney (PK-13) cells, as stated elsewhere [46]. Briefly, the virus was produced by infecting PK-13 cells with 10^3 MTT_{50} of PPV and the flasks placed at −20 °C after cell lysis occurred. After returning to room temperature, the cell monolayer was scrapped into the media and centrifuged at 5000 rpm in a Sorvall ST16R Centrifuge (Thermo Scientific, Pittsburgh, PA) at 4 °C for 15 min to remove the cell debris. The supernatant was stored at −80 °C and used directly as the virus lysate for all chromatography experiments. The conditioned media was created by removing the supernatant from PK-13 cells after 3-4 days. This was centrifuged as stated for the virus production and stored at 4 °C.

For the ANS study on PPV, the stock virus was purified. The stock virus was dialyzed using a 1,000,000 Da MWCO cellulose ester membrane from Spectrum Laboratories (Dominguez, CA) to retain only the virus and diffusing out many of the extraneous proteins including BSA (which was interfering with the ANS results). The dialysis was conducted against 50 mM phosphate, 150 mM NaCl at pH 7.2 for 48 h at 20 °C with two buffer exchanges. The dialyzed samples was collected and subjected to fractionation on a desalting column. The desalting column was self-packed with GE sephacryl S-300 HR resin that has an exclusion limit of 2×10^6 Da. In this experiment 1–2 ml of dialyzed sample was added to 10 ml resin column. To elute the virus and proteins of the column an additional 10–12 ml of phosphate buffer was added. The fractioned samples were collected in 1 ml microcentrifuge tubes and the virus titer was confirmed with the cytotoxic MTT assay, described in the next paragraph. The protein content was determined with a microBCA kit (ThermoFisher, Waltham, MA) and the reduction of BSA and

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