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Virus disinfection mechanisms: the role of virus composition, structure, and function

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Drinking waters are treated for enteric virus via a number of disinfection techniques including chemical oxidants, irradiation, and heat, however the inactivation mechanisms during disinfection remain elusive. Owing to the fact that a number of significant waterborne virus strains are not readily culturable *in vitro* at this time (e.g. norovirus, hepatitis A), the susceptibility of these viruses to disinfection is largely unknown. An in-depth understanding of the mechanisms involved in virus inactivation would aid in predicting the susceptibility of non-culturable virus strains to disinfection and would foster the development of improved disinfection methods. Recent technological advances in virology research have provided a wealth of information on enteric virus compositions, structures, and biological functions. This knowledge will allow for physical/chemical descriptions of virus inactivation and thus further our understanding of virus disinfection to the most basic mechanistic level.

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Introduction

Obtaining a mechanistic understanding of virus disinfection is a pressing need in environmental engineering owing to the enduring occurrence of waterborne and food-borne virus outbreaks. Many important enteric viruses remain non-culturable to date (e.g. norovirus, hepatitis A); therefore, their susceptibility to disinfection cannot be experimentally tested. Non-culturable virus disinfection kinetics must be either determined with human charge studies or predicted using surrogate viruses that can be cultured *in vitro* but that differ in composition, structure, and function. A framework that enables the accurate prediction of virus inactivation behavior based on a detailed understanding of the processes involved would assist in the development of effective disinfection strategies.

Scientists have long sought to provide mechanistic descriptions of virus inactivation during drinking water disinfection [1]. In the 1960–1980s, researchers employed scintillation spectroscopy and electron microscopy techniques to detect modifications in viral genomes and proteins and typically reported one of two conclusions: 1) inactivation is the result of damage to the virus proteins or 2) inactivation is the result of damage to the genome [2–6]. Although these early studies investigated the molecular mechanisms as much as technologically possible, more recent research has focused less on elucidating mechanisms and more on comparing inactivation kinetics with various virus strains, disinfectants, and water chemistries [7–9]. This is despite the fact that recent technological advances have provided improved tools for probing molecular mechanisms. Collectively, the proposed virus inactivation mechanisms by common water disinfectants vary widely and are often contradictory. For example, the inactivation of poliovirus by chlorine has been attributed to RNA degradation [2] and to capsid protein modifications [10]. At this time, the fundamental questions of what modifications do or do not cause inactivation remain elusive.

Herein, we discuss how the combined knowledge of virus composition, structure and biological function will further our understanding of virus disinfection at the most basic mechanistic level. A physical/chemical description of inactivation is more feasible today than it was ten years ago thanks to advances in genome sequencing, protein mass spectrometry, and structural virology techniques. We focus the majority of our discussion on the disinfection of waterborne enteric viruses [11], in general, and poliovirus, in particular. Enteric viruses are the most relevant to water treatment and poliovirus has been the focus of numerous disinfection studies over the past several decades [12–14]. It should be noted that this discussion could be extrapolated to other settings where disinfection is used to mitigate virus transmission, such as food safety or medical equipment sterilization.

Bottom-up approach to describe virus reactivity: composition only

The reactions that take place between amino acid or nucleotide monomers and common water disinfectants

Table 1

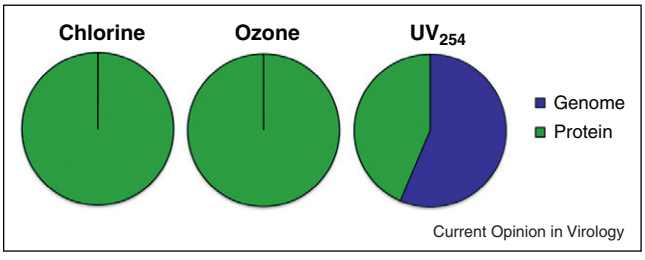
Reported second-order rate constants and photochemical constants for the most reactive amino acid and nucleoside monomers with common disinfectants in aqueous solutions at pH ~ 7

Nucleotides and amino acids	Chlorine	Ozone	UV	
	k (M^{-1} s^{-1})	k (M^{-1} s^{-1})	ε (254 nm) (M^{-1} cm^{-1})	Φ_d
Adenine	6.4[a] [48]	200[c,d] [49,50]	1.2×10^4 [51,52]	4.4×10^{-4}[e] [53]
Cytosine	66[a] [48]	1.4×10^3[c] [49]	3.5×10^3 [51]	5.3×10^{-4}[e] [53]
Guanine	2.1×10^4[a] [48]	5.0×10^4[c] [49]	1.0×10^4 [51]	2.1×10^{-4}[e] [53]
Uracil	5.5×10^3[a] [54]	650 [50]	7.8×10^3 [51]	1.4×10^{-3}[f] 2.6×10^{-3}[g] [53]
Thymine	4.3×10^3[a] [48]	1.6×10^4[c] [49]	6.3×10^3 [51]	9.6×10^{-4}[e] 2.6×10^{-3}[g] [53]
Cysteine	3.0×10^7[b] [55]	$\sim 1 \times 10^9$[d] [56]		
Histidine	1.0×10^5[b] [55]	$\sim 4 \times 10^5$[d] [56]		
Lysine	5.0×10^3[b]			
Methionine	3.8×10^7[b] [55]	$\sim 6 \times 10^6$[d] [56]		
Phenylalanine			140 [57]	0.019 [58]
Tryptophan	1.1×10^4[b] [55]	$\sim 1 \times 10^7$[d] [56]	2.8×10^3 [57]	9.0×10^{-3} [58]
Tyrosine	44[b] [55]	$\sim 4 \times 10^6$[d] [56]	340 [57]	0.022 [58]
Backbone N	≤ 10[b] [55]			
α-amino	1.0×10^5[b] [55]			

[a] Values for AMP, CMP, UMP, and TMP.
[b] Values for pH 7.4.
[c] Values for dAMP, dCMP, dGMP, Dtmp.
[d] Values may include radical pathways.
[e] Quantum yield for nucleotide destruction in air/O_2 saturated solution.
[f] Data for chromophore loss in air/O_2 saturated solution.
[g] Quantum yield of thymine dimer.

such as chlorine, ozone, or UV irradiation are fairly well characterized (Table 1). Consequently, established reaction rate constants for amino acid and nucleotide monomers can be summed based on their known abundance within virus particles to provide predictions of the relative reaction rates of genome and protein targets [15]. When this is done for Poliovirus 1 Mahoney, chemical disinfectants such as chlorine and ozone are much more reactive with viral protein material than with genomic material (Figure 1). On the contrary, UVC radiation affects the genomic material more than the protein material. Unfortunately, such predictions are inaccurate owing to the influence of protein and genome higher-level organization on reaction rates [16–18]. For example, when treated with chlorine dioxide, denatured poliovirus genomes degraded at a different rate than native poliovirus genomes [19•]. Reactions that take place in the genome and proteins during disinfection can form byproducts that further react with amino acids and nucleotides [20]; this makes reactivity predictions even more complicated.

Figure 1



Predicted relative reaction rates for Poliovirus 1 protein and genome components calculated with rate constants and photochemical constants presented in Table 1.

Unlike chemical oxidants, UVC radiation will lead to direct photolysis of photolabile virus components regardless of their solvent accessibility. A genome-size based approach to predict the sensitivity of virus strains to UVC has been proposed [21], although others have reported that genome-size does not always correlate with virus susceptibility to UV disinfection [22,23]. Similar to the genome-size based approach, pyrimidine doublet prevalence in virus genomes was suggested as a framework to predict UVC susceptibility [24•]. Indeed, a plot of the number of potential dimerization sequences in a virus genome versus effective UV dose suggested a correlation. The presence of outlier virus strains, however, indicates that alternative pathways play a role in some UV inactivation mechanisms. Taken together, these discrepancies demonstrate that virus component information (i.e. genome and protein sequences) alone will not allow for an accurate prediction of susceptibility. A prior knowledge of capsid and genome structure should therefore aid in interpreting and predicting virus particle reactivity and in identifying the particle's most susceptible regions.

Coupling structure and composition information aids in our understanding of virus reactivity

X-ray crystal structures have been published for numerous enteric viruses [25,26•,27] and with these reports have come a windfall of valuable information including the location and orientation of capsid protein residues. Cryo-electron microscopy (cryoEM) has expanded our knowledge of virus structures even further, as it allows virologists to study virus particles that are difficult to crystallize due to either complex capsid shapes, inadequate purification, or intermediate, metastable structures (e.g. virus binding or cell entry processes [28,29•]). In fact, recent advances in cryoEM have lead to viral reconstructions at resolutions comparable to those obtained with X-ray crystallography [30,31•]. In addition to ordered capsid protein visualization, cryoEM studies have demonstrated that some viruses have ordered genomes [32] and have described specific interactions between capsid proteins and packaged genomes [33]. It should be noted that resolved near-atomic structures are not yet available for some important enteric viruses such as hepatitis A and human norovirus, although recombinant norovirus-like particles have been reconstructed [34].

Some caution must be taken when using virus structural data to identify the location and solvent accessibility of functional groups. Virus capsids can be fluid in nature and thus functional groups that are normally protected from oxidants in the solvent can be periodically exposed to the capsid surface [35••]. In human rhinovirus, for example, certain regions are static and in agreement with the crystal structure, while other regions are more fluid [36].

Together, composition data and structure data will provide an improved framework to describe the reactivity of virus components with disinfectants. Indeed, a number of studies on nonviral proteins have used structural data to explain the site-specific reactivity of protein components [16,17,37,38]. At this time, however, only a few reports on virus disinfection have mentioned resolved virus structures in the interpretation of results [10,39••,40•]. A study on adenovirus inactivation with chlorine did employ adenovirus structural data to suggest that damaged Met or His residues near a critical motif may contribute to inactivation [39••]. In another study, protein mass spectrometry was used to identify specific residues in MS2's capsid proteins as the virus was inactivated by UV_{254} and singlet oxygen [40•]. Residues on the outside surface of the capsid were modified with $^1O_2$ treatment while residues on the inside surface of the capsid near the viral genome were modified with UV treatment. More research is clearly needed to elucidate the effect of virus structure on the reactivity of virus components.

Knowledge of virus functions is required to understand and predict inactivation mechanisms

Composition and structure information aids in describing where modifications are most likely to occur in a virus particle during disinfection; however, modifications do not always cause inactivation. For example, although ozonation of poliovirus altered viral proteins VP1 and VP2, inactivation was ultimately attributed to genome damage [5]. Solely identifying susceptible virus regions based on composition and structure will be insufficient to describe and predict the effect of a disinfectant on virus infectivity. One must also consider the effect that particular modifications have on fundamental virus functions (e.g. host-cell binding, genome entry, etc.). In order to do this, the fundamental functions and virus components involved in those functions must be well defined.

Virus structural and dynamic information has provided an improved understanding of the biological function of viral domains, including virus–host cell interaction [26•,41•], virus assembly [26•], capsid-RNA interaction sites [33,42•] and RNA release ([42•] and references therein). This new knowledge provides valuable insight into critical structures and biological functions of the virus and thus identifies regions and functions that should be targeted in virus neutralization or inactivation strategies.

The promise of a structure/function-based approach for disinfection is underscored by the fact that the medical field has exploited this method to develop vaccines [41•] and antiviral drugs [43] as well as to better understand the mechanism of previously developed vaccines [41•]. This type of approach has great benefits over the traditional method of screening and isolating fortuitously emerged, non-virulent virus strains or neutralizing antibodies. Namely, it enables the rational design of site-specific antivirals and vaccines that target relevant virus structures common to several strains or species. For example, coronavirus strains were long believed to have host receptors that were too diverse and too prone to mutation to be susceptible to a broad-spectrum antiviral. Recently, however, Yang *et al.* [44••] used function and structure information of all three genetic coronavirus clusters to determine that the main protease has a highly conserved substrate-recognition pocket. Based on this structural information combined with compositional information of conserved amino acids within this region, a protease inhibitor was designed that successfully prevented virus replication of two coronaviruses. Ultimately, the authors suggest that the knowledge of the conserved structure and biochemistry of the protease will lead to the development of a single, broad spectrum antiviral that targets all coronaviruses. A similar approach to water disinfection will lead to the rational design of novel disinfectants.

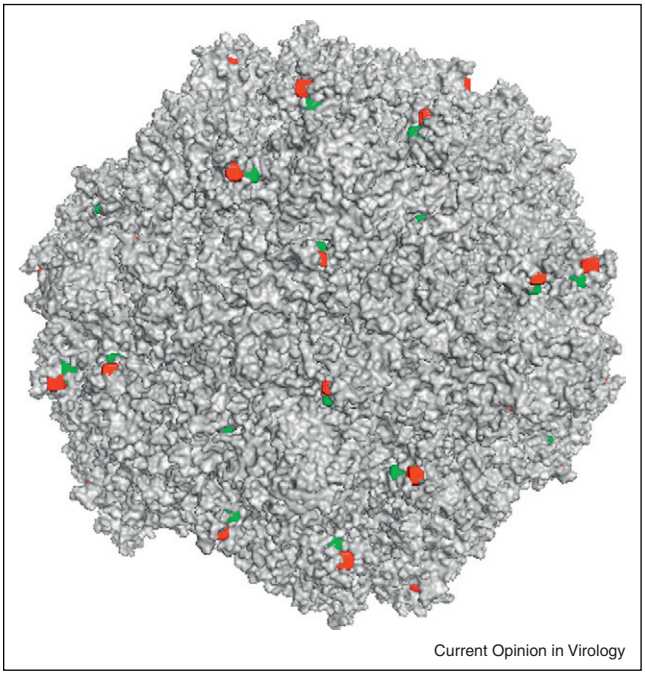
Towards a predictive model for virus susceptibility and disinfection kinetics

An important difference between water disinfection and antiviral drugs, however, is that traditional disinfection does not operate by physically blocking a virus site or particular function via the addition of an external chemical or antibody, but by chemically or structurally altering one or several important sites. We therefore expect that the intrinsic reactivities of the various virus components are of greater importance to disinfection than to drug development. As discussed above, this intrinsic reactivity is dictated by both specific chemical composition and the structure. As such, the structure/function approach used in the medical field would have to be expanded to a composition/structure/function approach in water treatment.

As an example of how structural commonalities and compositional differences may be used to predict susceptibilities to disinfectants, we look at poliovirus 1 Brunhilde and poliovirus 1 Mahoney. The Brunhilde strain was reported to be twice as resistant to chlorination as the Mahoney strain [45,46]. This discrepancy in inactivation kinetics is intriguing as the two strains have nearly identical capsid protein sequences (~98%) and have similarly sized genomes. Inactivation of the Mahoney strain by chlorine was attributed to a loss in ability to attach to the host cell [10], however this has not been examined for the Brunhilde strain. CryoEM analysis has provided a three-dimensional structure of the poliovirus particle bound to its host cell receptor, sPvr [47•], and a number of capsid protein residues were implicated in the virus capsid-host cell interaction. Interestingly, six of these implicated residues differ in the Mahoney and Brunhilde strains, including Mahoney VP2 Met140 (Thr in Brunhilde) and His141 (Tyr in Brunhilde) (Figure 2). Methionine and histidine side chains readily react with chlorine; threonine and tyrosine sidechains, on the contrary, are much less reactive. It is thus possible that the lack of Met and His residues at the binding site is responsible for the greater resistance of the Brunhilde strain to chlorine disinfection.

This is only a hypothetical explanation and will need to be confirmed through experimentation; it does, however, demonstrate the manner in which composition, structure, and function information can assist in deducing virus inactivation mechanisms. It also demonstrates how such knowledge aids in predicting how a new strain will behave based on mutations in the binding site. Suppose, for example, a new poliovirus strain emerges with fewer reactive functional groups in its binding site. The new strain would be expected to be more resistant to disinfection than the well-characterized strain. For fast-mutating viruses like human norovirus, this type of predictive tool would be particularly valuable. Research is needed to assess exactly how similar virus strains should be in structure, composition, and function for such a predictive tool to work.

Figure 2



Poliovirus Mahoney strain capsid. VP2 Met140 is highlighted in green and VP2 His141 is highlighted in red. Structural visualization with PYMOL software (http:/www.pymol.org).

Conclusions and future prospective

To reach a point where a holistic understanding of virus inactivation is in hand, a number of questions will first need to be addressed. Specific questions include: 1) Which virus protein residues are involved with fundamental functions and how do these vary amongst different strains and species; 2) What specific chemical modifications take place in the genome and capsid during disinfection and what effects do these modifications have on virus structure and function; 3) How similar are disinfectant-induced modifications amongst various enteric viruses? Answering these questions is a lofty goal given the numerous virus-disinfectant pairs that will need to be examined.

In conclusion, the study of virus fate in water treatment is entering an exciting new phase thanks to the advent of tools that provide insight into virus structure and function. As a result, virus inactivation predictive tools and the design of highly efficient disinfectants may be a reality in the not-so-distant future.

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