Environ. Sci. Technol. **2010,** *44,* 808–812

Detection of Oxidative Damages on Viral Capsid Protein for Evaluating Structural Integrity and Infectivity of Human Norovirus

DAISUKE SANO,[†,‡] ROSA M. PINTÓ,[†] TATSUO OMURA,[§] AND ALBERT BOSCH*[,†]

Enteric Virus Laboratory, Department of Microbiology, School of Biology, University of Barcelona, Av. Diagonal 645, 08028, Barcelona, Spain; Division of Built Environment, Graduate School of Engineering, Hokkaido University, North 13, West 8, Kita-ku, Sapporo, 060-8628, Japan; and Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, Aoba06, Sendai, 980-8579, Japan

Received June 27, 2009. Revised manuscript received November 5, 2009. Accepted November 24, 2009.

The infectivity evaluation of noncultivatable viruses, such as human norovirus, is crucial to address needs for ensuring the safety in usage of water and marine products. In this work, we tested a new approach to evaluate viral particle integrity, in which oxidatively produced carbonyl groups on viral capsid protein were quantitatively detected. As a result, the decrease in the infectivity of human astrovirus, a representative enteric virus, positively correlated with the amount of oxidative damage on viral particles. Furthermore, when human norovirus was treated by 1 ppm free chlorine for 15 min, 49.93% of virions were recovered as oxidatively damaged particles, which represents a 5-fold increase over those treated by 0.5 ppm free chlorine for 15 min. The detection of the carbonylated viral particles could be a powerful tool for the evaluation of the decrease in the infectivity of noncultivatable viruses.

Introduction

Water polluted by pathogenic viruses, essentially nonenveloped enteric viruses, is a global concern because these pathogenic viruses in reservoirs, river water, and seawater pose infectious disease risks via drinking water, recreation water, and marine-cultured products (*1*). Since a large amount of excreted viruses from infected individuals flow into sewage streams (*2, 3*), removal and inactivation of these viruses in wastewater treatment processes are important to prevent viral pollution in water environments (*4, 5*). A variety of disinfection processes using chlorine related oxidants (*6*), ultraviolet (UV) irradiation (*7*), ozonation (*8*), and photocatalysis (*9*) has been proposed and implemented for inactivating pathogenic viruses in water, and mechanisms of viral inactivation with several disinfection processes have been suggested (*10, 11*). However, the presence of noncultivatable viruses, which have no cell cultures and animal models available for assaying their infectivity, makes the situation complicated, because it is impossible to comprehend the effects of these disinfection processes on noncultivatable viruses by the loss of infectivity. An understanding of disinfection effectiveness on these noncultivatable viruses is invaluable to control viral infectious diseases.

One important approach to evaluate the infection risks caused by noncultivatable viruses is the employment of surrogate viruses. For example, feline calicivirus and murine norovirus have been used as surrogates for human norovirus (*12, 13*). However, there are significant differences in the behavior of human norovirus and feline calicivirus (*14*−*16*). Murine norovirus was reported as an appropriate surrogate for human norovirus because of its persistency in various conditions (*17*), but it is still difficult to ascertain how similar this surrogate is to the target noncultivatable viruses. Although the employment of surrogate viruses has greatly contributed to many advances in the field of public health virology, information related to infectivity of noncultivatable viruses per se is the most indispensable to address the problems of environmental contamination by these noncultivatable viruses.

In this work, we propose a new approach to evaluate viral particle integrity, in which cumulative oxidative damages on viral capsid protein are directly detected. Nonenveloped enteric viruses could be injured by exogenous stresses in the natural environment, and the damages on viral capsid protein would lead to an inability of the viruses to recognize cellular receptors to initiate a viral life cycle. In the proposed approach, cumulative oxidative damages on viral particles were detected by labeling with a biotin hydrazide that can form a covalent bond with a carbonyl group (*18*). Residues of some amino acids, including lysine, arginine, threonine, and prorine, can be oxidized by several chemicals, and carbonyl groups are formed on these residues (*19*), and the carbonylation on protein molecules caused by oxidative stresses brings about the loss of protein functions (*20*). A biotin hydrazide could react with a carbonylated capsid protein of a virus particle, and the oxidatively damaged capsid protein could be labeled with biotin. Biotin-modified (= damaged) virus particles are separated from intact virions with avidin-immobilized affinity chromatography. In order to confirm that the accumulation of oxidative damages on viral capsid protein causes the decrease in the virus infectivity, human astrovirus was employed as a representative enteric virus in this study. Then, this approach was applied to human norovirus, an important etiological agent, and this novel technique enabled to quantitatively detect oxidative damages on capsid protein of human norovirus.

Materials and Methods

Viruses and Cell. The preparation and infectious titer assays of human astrovirus serotype 4 were performed by using CaCo-2 cells, as described elsewhere (*21*). Norovirus genogroup II genotype 4 (GII/4), 2006a cluster, from a patient who contracted gastroenteritis during an outbreak on a cruise ship in South Africa, was prepared as described in Supporting Information. The number of genome copies of norovirus GII/4 was measured by a real-time quantitative RT-PCR (qRT-PCR) assay for norovirus GII as described elsewhere (*22*). The detail of the genome quantification for astrovirus by a real-time qRT-PCR is described in Supporting Information. All primers and probes used in the quantification of viral genomes are also indicated in Supporting Information (Table S1).

* Corresponding author phone: +34 93 40 34 620; fax: +34 93 40 34 629; e-mail: abosch@ub.edu.

[†] Enteric Virus Laboratory, Department of Microbiology, School of Biology, University of Barcelona.

[‡] Division of Built Environment, Graduate School of Engineering, Hokkaido University.

[§] Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University.

10.1021/es9018964
Published on Web 12/15/2009

Quantification of Astrovirus Genome Copies by a Real-Time qRT-PCR. A viral genome was extracted with a NucleoSpin RNA virus kit (Macherey−Nagel). A conserved region in ORF1b coding RNA dependent RNA polymerase (RdRp) was used as the target region for the one-step real-time qRT-PCR developed for astrovirus genome copy quantification. Sequences of sense primer (AstVorf1b+), antisense primer (AstVorf1b−) and FAM-modified TaqMan probe (AstVorf1bp), and the details of the real-time qRT-PCR protocol are indicated in Supporting Information.

Oxidation and Biotinylation of Virus Particles. The viruses were treated by free chlorine from sodium hypochlorite (Panreac Quimica Sau). All reagents were prepared as described in the Supporting Information. The concentration of free chlorine was measured with a chlorine and pH test kit (MERCK). A virus stock suspension was diluted in 4 mL of phosphate buffered saline (PBS), and free chlorine concentration was adjusted by a 1% sodium hypochlorite. The virus suspension in PBS was incubated at 4 °C under dark conditions for 5 and 15 min, and then free chlorine in the suspension was neutralized by adding a 1% sodium thiosulfate. As a control, a virus suspension in the absence of free chlorine was also incubated under the same conditions. Virus suspensions after the incubation and the neutralization were stored at −20 °C.

Virus particles were biotinylated as follows. First, 25 μL of 50 mM EZ-Link Biotin Hydrazide (Pierce) in DMSO were added to 1 mL of a virus suspension. Next, the mixture of biotin hydrazide and virus particles was incubated at room temperature for 2 h with moderate mixing. Then, the mixture was purified by Zeba Desalt Spin Column (Thermo Scientific) to remove unreacted biotin hydrazide. Biotinylated virus particles were then stored at −20 °C.

Avidin-Immobilized Affinity Chromatography. All reagents and buffers were prepared as described in Supporting Information. First, the avidin column (Pierce, monomeric) was washed by 8 mL of PBS (0.1 M Na_2HPO_4, 0.15 M NaCl, 0.02% NaN_3, pH7.2). A thawed virus suspension was filtered with a 0.22 μm Milipore Millex-HV filter, and then 2 mL of the filtered virus suspension were loaded into the avidin column. When the entire suspension had entered into the gel, 0.25 mL of PBS was added to force the sample completely into the gel. The avidin column was washed by 48 mL of PBS, and the eluates in the loading and the washing step were combined and stored as an unbound fraction. Then, virus particles bound in the avidin column were eluted by introducing 12 mL of elution buffer (2 mM Biotin in PBS), and strongly bound viral particles were subsequently eluted by introducing 8 mL of glycine buffer (0.1 M glycine, 0.02% NaN_3, pH2.8). The eluate in the elution steps with 1 mM Biotin in PBS and glycine buffer was stored as a bound fraction. After each assay, the gel was regenerated by the washing with 16 mL of PBS and then stored with PBS, including 20 μg/mL RNase A and 0.04% NaN_3 at 4 °C. All buffers and a virus suspension were introduced into the column by natural gravity, and the flow rate was 0.4−0.5 mL/min.

Results

Reductions of Infectious Titers and Genome Copies of Astrovirus by Free Chlorine Treatment. Figure 1 shows the reduction of infectious titers and genome copies of astrovirus type 4 treated with free chlorine. The mean values (triplicates) of the log reduction of the infectious titer by 5- and 15-min treatment with 0.05 ppm free chlorine were 0.43 $\log_{10}$ (SD: 0.23) and 0.67 $\log_{10}$ (SD: 0.35), respectively. Those with 0.5 ppm free chlorine were 1.87 $\log_{10}$ (SD: 0.51) and 2.53 $\log_{10}$ (SD: 0.40), respectively (Figure 1a). Statistically significant ($P < 0.05$) reductions of astrovirus infectivity were detected with a t test by the free chlorine treatments. However, when

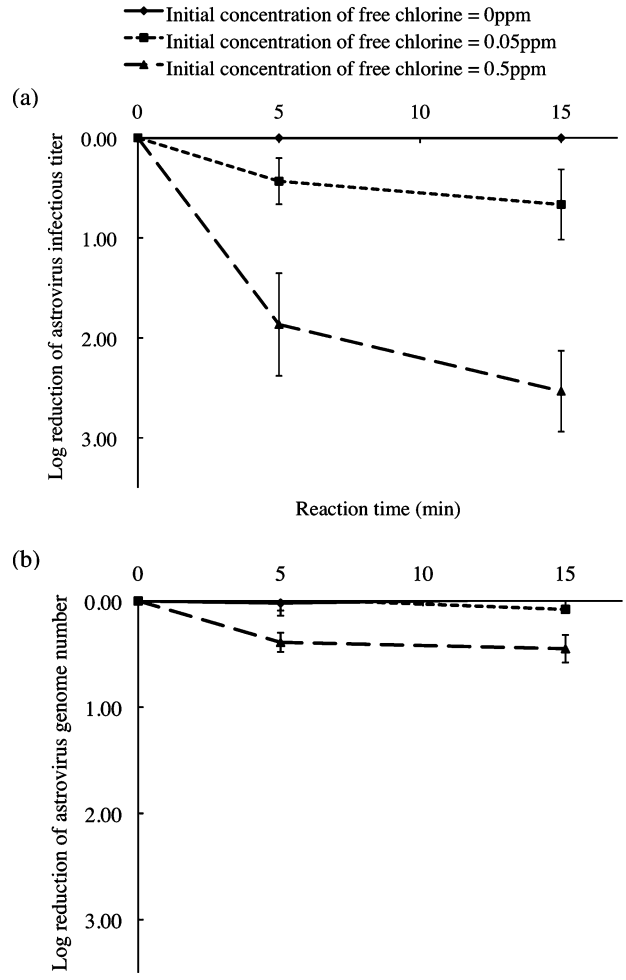

FIGURE 1. Reductions of infectious titer (a) and genome number (b) of astrovirus type 4 by free chlorine treatment (pH 7.2, 4 °C, dark condition). Each data point is an average of triplicates, and each error bar represents standard deviation.

astrovirus particles were treated with 0.05 ppm free chlorine, the mean values (triplicates) of the log reduction of the astrovirus genome copies by 5- and 15-min treatments were −0.02 $\log_{10}$ (SD: 0.11) and 0.08 $\log_{10}$ (SD: 0.03), respectively (Figure 1b). No statistically significant ($P < 0.05$) reductions of the astrovirus genome copies were detected with a t test by the free chlorine treatment at 0.05 ppm. Meanwhile, statistically significant ($P < 0.05$) reductions of the genome copies were observed when astrovirus was treated with 0.5 ppm free chlorine, in which the mean values (triplicates) of log reductions were 0.39 $\log_{10}$ (SD: 0.09) and 0.45 $\log_{10}$ (SD: 0.13) for the 5- and 15-min treatment, respectively (Figure 1b). These results indicate that free chlorine treatments at 0.05 ppm and 0.5 ppm on astrovirus type 4 brought about the infectivity reductions, but the treatment with 0.05 ppm free chlorine for 15 min was not enough to see a significant reduction of the astrovirus genome copies.

Affinity Chromatography of Biotinylated Astrovirus Particles. Figure 2 shows mean values (±data range of duplicates) of the recovery ratio of astrovirus genomes in the bound fraction of the avidin-immobilized affinity chromatography. When biotinylated astrovirus particles without the free chlorine treatment were loaded into the affinity column, 13.0% (±7.2) and 13.5% (±3.2) of virions were bound in the column. Theoretically, a carbonyl group does not exist in intact capsid proteins, but the recovery of about 13% of astrovirus genome in the bound fraction indicates that the intact astrovirus particles had carbonyl groups, which could be originated during the replication of viruses in cells and

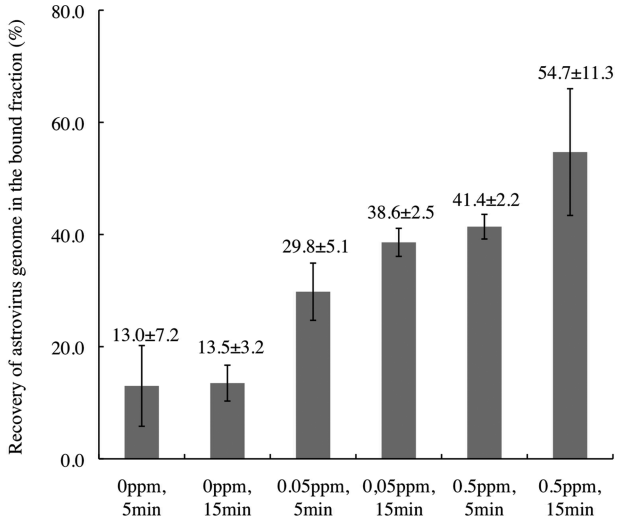

FIGURE 2. Recovery of biotinylated astrovirus type 4 genome in the bound fraction of the avidin-immobilized affinity chromatography. The initial concentration of free chlorine and exposure time were described along the horizontal axis. Each data point is an average of duplicates, and each error bar represents the data range.

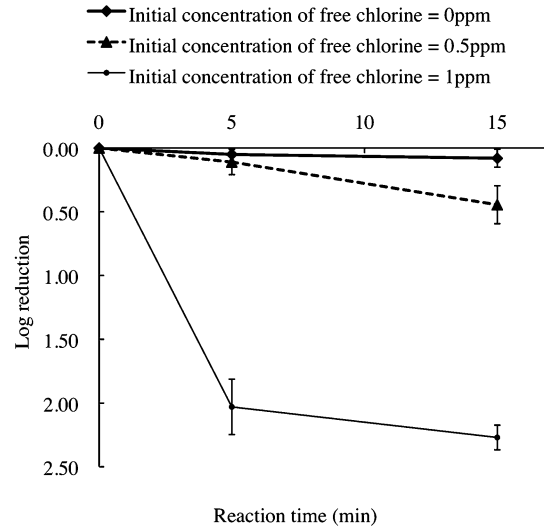

FIGURE 3. Reduction of genome copies of norovirus GII/4 by free chlorine treatment (pH 7.2, 4 °C, dark condition). Each data point is an average of duplicates, and each error bar represents the data range.

the preparation of the virus suspension. Meanwhile, a significant increase in the recovery ratio of bound astrovirus particles was observed when biotinylated virions with the free chlorine treatment were loaded. When astrovirus particles were treated with 0.05 ppm free chlorine for 5 min, 29.8% (±5.1) of astrovirus genomes were recovered in the bound fraction. The recovery ratio of astrovirus genomes in the bound fraction reached 38.6% (±2.5) when astrovirus particles were treated with 0.05 ppm free chlorine for 15 min. These increases in the bound viral particles show that free chlorine-treated astrovirus particles have a larger number of carbonyl groups than intact particles. As shown previously, the free chlorine treatments at 0.05 ppm for 5 and 15 min did not result in a statistically significant ($P < 0.05$) decrease of the astrovirus genome copies (Figure 2), despite the observed inactivation of astrovirus (Figure 1). Oxidative damages on the capsid surface precede damages to viral RNA and the biotinylation assay described here may provide a better measure of viral inactivation than RNA detection.

The recovery ratios of astrovirus genomes in the bound fraction were further increased when astrovirus particles were treated with 0.5 ppm free chlorine. When astrovirus particles were treated with 0.5 ppm free chlorine for 5 min, 41.4% (±2.2) of virions were recovered in the bound fraction, and the recovery ratio rose to 54.7% (±11.3) when astrovirus particles were treated with 0.5 ppm free chlorine for 15 min. The number of bound virions is correlated with the initial concentration of free chlorine and the exposure time, which also supports the speculation that carbonyl groups produced by free chlorine can be accumulated on viral capsid protein, and the amount of oxidative damages can be quantitatively evaluated.

Reductions of the Genome Copies of Norovirus GII/4 by Free Chlorine Treatment. Figure 3 shows log reductions of the norovirus GII/4 genome number by the treatment with free chlorine. No significant reduction of the genome copies was observed in the absence of free chlorine. When norovirus particles were treated with 0.5 ppm free chlorine for 5 and 15 min, the mean values (±data range of duplicates) of the log reduction were 0.11 $\log_{10}$ (±0.10) and 0.45 $\log_{10}$ (±0.15), respectively. Meanwhile, the mean values (±data range in duplicates) of the log reduction of the norovirus genome copies after the treatment with 1.0 ppm free chlorine for 5 and 15 min were 2.03 (±0.22) and 2.27 (±0.10) $\log_{10}$, respectively. Larger log reductions of norovirus genome with the free chlorine treatment at 1.0 ppm was presented previously (*23*), which might be caused by the difference in the length of target region for RT-PCR.

Affinity Chromatography of Biotinylated Norovirus GII/4 Particles. Figure 5 shows the recovery ratio of the norovirus genome extracted from bound virions. The mean values (±data range in duplicates) of the genome recovery ratio of biotinylated norovirus GII/4 without free chlorine treatment in the bound fraction were 1.6% (±1.2) and 1.0% (±1.0) for 5 min- and 15 min-treatment, respectively. These results indicate that norovirus GII/4 particles purified from a gastroenteritis fecal sample have few carbonyl groups on their surfaces and almost no interaction with avidin molecules. There were no significant differences in the mean values between free-chlorine-untreated norovirus particles and oxidized ones after the treatment with 0.5 ppm free chlorine for 5 min (1.0 ± 0.3%). However, the genome recovery ratio of the oxidized norovirus with 0.5 ppm free chlorine for 15 min rose to 10.0% (±2.5), which could be attributed to the formation of carbonyl groups on particle surfaces of norovirus. The mean values of the genome recovery ratio in the bound fraction further increased when norovirus particles were treated with 1.0 ppm free chlorine, in which 21.4% (±3.5) and 49.9% (±2.4) of biotinylated virions were bound in the avidin column after treatment with 1.0 ppm free chlorine for 5 min and 15 min, respectively. These results support that the accumulation of carbonyl groups on norovirus particles by the free chlorine treatment can be detected with the biotin hydrazide treatment and the avidin column separation. This is the first report ever that oxidative damages on particles of a noncultivatable virus can be quantitatively evaluated.

Discussion

One of the important scientific challenges in the field of public health virology has been the evaluation of health risks caused by noncultivatable viruses in natural environments, and several approaches for the viral infectivity evaluation without in vivo or vitro replication of virions have been proposed (*24*). One approach is the employment of RT-PCR to confirm the presence of amplifiable viral genomes, which could be an indication that a viral genome is properly protected by a viral capsid (*25, 26*). A proteinase treatment of virions and an affinity separation of virions against antibody or cell receptors prior to the genome amplification have been

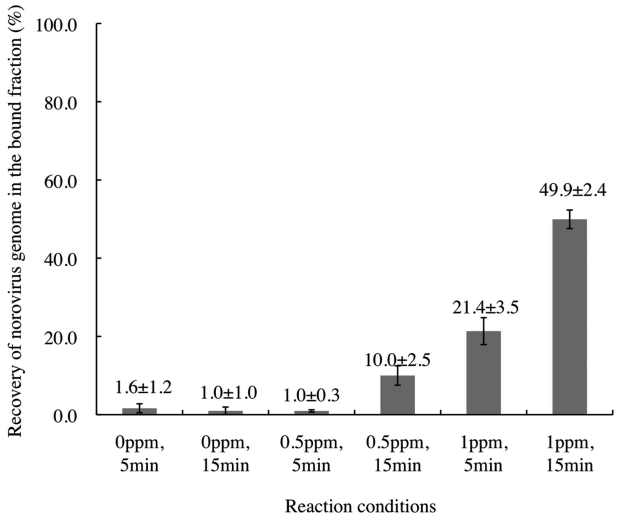

FIGURE 4. Recovery of biotinylated norovirus GII/4 gene in the bound fraction of the avidin-immobilized affinity chromatography. The initial concentration of free chlorine and exposure time were described along the horizontal axis. Each data point is an average of duplicates, and each error bar represents the data range.

attempted to distinguish damaged virions from intact ones (*16, 27*−*30*). These examinations of the viral capsid integrity would be of primarily importance to evaluate the infectivity and the fate of noncultivatable viruses, because capsids from nonenveloped viruses initiate infections of host cells but can also be considered environmental contaminants that interact with natural substances such as colloidal particles in waste stabilization ponds (*31*), soils (*32*), and oyster tissues (*33*).

In this work, a new approach to evaluate the integrity of viral capsid protein by directly detecting oxidized amino acid residues on virion is presented. We employed biotin hydorazide and an avidin-immobilized column to detect oxidized amino acid residues on viral particles, which has been used to evaluate the aging of proteins in cancer-related disorders (*18, 34*) and bacterial proteins (*35*). The oxidative damages on proteins cause the loss of protein functions (*36*), and this study using astrovirus shows that this is also the case for the capsid protein of an enteric virus (Figures 1 and 2). Furthermore, the accumulation of carbonyl groups on norovirus particles was also quantitatively evaluated (Figure 4) and could be highly relevant in the field of public health virology, because it opens the possibility of estimating the decrease in the infectivity of noncultivatable viruses through the accumulation of oxidative damages.

Carbonyl group formation is not the only consequence of oxidation. For example, a dichloramine is formed from a lysine by oxidation with hypochlorite (*37*). Cysteine (*38*), tyrosine (*39*), and methionine (*40*) also form different types of oxidative products from a carbonyl group. These products must be involved in the loss of capsid protein function and virus infectivity, although these other products from a carbonyl group cannot be detected in the proposed approach. However, the latter three amino acid residues (cysteine, tyrosine, and methionine) are relatively minor in capsid proteins of the test viruses, which occupied only 5.74% (31/540) and 5.71% (44/771) of the whole sequence of capsid protein of norovirus GII/4 (accession number: AB496912) and astrovirus type 4 (AB496913), respectively. Given that four target residues of the carbonylation by free chlorine occupied more than 20% in capsid proteins of test viruses, the carbonyl groups should be considered more desirable as a target moiety to evaluate the level of oxidative damages on viral particles.

Free chlorine from sodium hypochlorite, mainly composed of hypochlorite acid and hypochlorite ion, was used in this study, because this is the most common, affordable, and effective disinfectant. Hypochloric acid can cause the formation of carbonyl groups on protein molecules (*20, 41*), and it was proven here that this is also the case in the interaction between nonenveloped viruses and free chlorine from sodium hypochlorite. The further interest in this interaction is the concentration of free chlorine, because an excess oxidation of capsid protein would make viral RNA exposed to outer environment where indigenous RNase exists, which would affect the quantification of viral genome. Furthermore, similar results with this study could be observed when different disinfectants are employed, in which reactive oxygen species (ROS) such as ozone and superoxide created by titanium oxide are included, because ROS oxidize proteins and cause the formation of carbonyl groups as well (*42*). Since ROS can be created by the radiation of photons in water (*43*), the sunlight and ultraviolet light inactivations of viruses could be also attributed to the formation of carbonyl groups on viral capsid protein. Since most noncultivatable enteric viruses are nonenveloped, it would be possible to quantitatively detect the oxidative damages on capsid protein of any enteric viruses. The effects of these viral inactivating factors on capsid protein of other enteric viruses will be investigated with our novel technique in future studies.

In conclusion, it was reported for the first time that oxidative products on viral capsid protein may be quantitatively detected as an indication of virion integrity, which has a significant correlation with viral infectivity. Since viral capsid protein is the first place to contact with any types of oxidants, the direct detection of oxidative lesions by biotinylation and avidin-immobilized affinity chromatography could be a powerful tool for the evaluation of the decrease in infectivity of noncultivatable viruses.

Acknowledgments

This work has been funded by the Healthy Water Project in the European Union (Food-4B-2005-36306) and a Research Fellowship Abroad from the Japanese Society for Promotion of Science (JSPS) for Young Scientists. This work was supported in part by Grant Consolider-Ingenio 2010 CSD2007-00016.

Supporting Information Available

Additional details are provided regarding preparation of reagents, buffers, viral stock suspensions, and primers and probes used in this study (Table S1). This information is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Gerba, C. P.; Rose, J. B.; Haas, C. N.; Crabtree, K. D. Waterborne rotavirus: a risk assessment. *Water Res.* **1996**, *30* (12), 2929–2940.
(2) Abbaszadegan, M.; Stewart, P.; Lechevallier, M. A strategy for detection of viruses in groundwater by PCR. *Appl. Environ. Microbiol.* **1999**, *65* (2), 444–449.
(3) Tu, E. T.-V.; Bull, R. A.; Kim, M.-J.; McIver, C. J.; Heron, L.; Rawlinson, W. D.; White, P. A. Norovirus excretion in an aged-care setting. *J. Clin. Microbiol.* **2008**, *46* (6), 2119–2121, DOI 10.1128/JCM.02198−07.
(4) Ueki, Y.; Sano, D.; Watanabe, T.; Akiyama, K.; Omura, T. Norovirus pathway in water environment estimated by genetic analysis of strains from patients of gastroenteritis, sewage, treated wastewater, river water and oysters. *Water Res.* **2005**, *39* (18), 4271–4280, DOI 10.1016/j.watres.2005.06.035.
(5) Lodder, W. J.; de Roda Husman, A. M. Presence of noroviruses and other enteric viruses in sewage and surface waters in the Netherlands. *Appl. Environ. Microbiol.* **2005**, *71* (3), 1453–1361, DOI 10.1128/AEM.71.3.1453−1461.2005.
(6) Blackmer, F.; Reynolds, K. A.; Gerba, C. P.; Pepper, I. L. Use of integrated cell culture-PCR to evaluate the effectiveness of Poliovirus inactivation by chlorine. *Appl. Environ. Microbiol.* **2000**, *66* (5), 2267–2268.

(7) Thurston-Enriquez, J. A.; Haas, C. N.; Jacangelo, J.; Riley, K.; Gerba, C. P. Inactivation of Feline Calicivirus and Adenovirus type 40 by UV radiation. *Appl. Environ. Microbiol.* **2003**, *69* (1), 577–582, DOI 10.1128/AEM.69.1.577−582.2003.
(8) Bossche, G. V.; Wustmann, U.; Krietemeyer, S. Ozone disinfection dynamics of enteric viruses provide evidence that infectious titer reduction is triggered by alterations to viral colloidal properties. *Microbiol. Res.* **1994**, *149* (4), 351–370.
(9) Watts, R. J.; Kong, S.; Orr, M. P.; Miller, G. C.; Henry, B. E. Photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent. *Water Res.* **1995**, *29* (1), 95–100.
(10) Thurman, R. B.; Gerba, C. P. Molecular mechanisms of viral inactivation by water disinfectants. *Adv. Appl. Microbiol.* **1988**, *33*, 75–105.
(11) Li, J. W.; Xin, Z. T.; Wang, X. W.; Zheng, J. L.; Chao, F. H. Mechanisms of inactivation of Hepatitis A Virus by chlorine. *Appl. Environ. Microbiol.* **2002**, *68* (10), 4951–4955, DOI 10.1128/AEM.68.10.4951−4955.2002.
(12) Urakami, H.; Ikarashi, K.; Okamoto, K.; Abe, Y.; Ikarashi, T.; Kono, T.; Konagaya, Y.; Tanaka, N. Chlorine sensitivity of feline calicivirus, a norovirus surrogate. *Appl. Environ. Microbiol.* **2007**, *73* (17), 5679–5682, DOI 10.1128/AEM.00083−07.
(13) Buckow, R.; Isbarn, S.; Knorr, D.; Heinz, V.; Lehmacher, A. Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Appl. Environ. Micribiol.* **2008**, *74* (4), 1030–1038, DOI 10.1128/AEM.01784−07.
(14) Ueki, Y.; Shoji, M.; Suto, A.; Tanabe, T.; Okimura, Y.; Kikuchi, Y.; Saito, N.; Sano, D.; Omura, T. Persistence of caliciviruses in artificially contaminated oysters during depuration. *Appl. Environ. Microbiol.* **2007**, *73* (17), 5698–5701, DOI 10.1128/AEM.00290−07.
(15) Poschetto, L. F.; Ike, A.; Papp, T.; Mohn, U.; Bohm, R.; Marschang, R. E. Comparison of the sensitivities of noroviruses and feline caliciviruses to chemical disinfection under field-like conditions. *Appl. Environ. Microbiol.* **2007**, *73* (17), 5494–5500, DOI 10.1128/AEM.00482−07.
(16) Lamhoujeb, S.; Fliss, I.; Ngazoa, S. E.; Jean, J. Evaluation of the persistence of infectious human noroviruses on food surfaces by using real-time nucleic acid sequence-based amplification. *Appl. Environ. Micribiol.* **2008**, *74* (11), 3349–3355, DOI 10.1128/AEM.02878−07.
(17) Bae, J.; Schwab, J. Evaluation of murine norovirus, feline calicivirus, poliovirus and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Appl. Environ. Microbiol.* **2008**, *74* (2), 477–484, DOI 10.1128/AEM.02095−06.
(18) Mirzaei, H.; Regnier, F. Affinity chromatographic selection of carbonylated proteins followed by identification of oxidation sites using tandem mass spectrometry. *Anal. Chem.* **2005**, *77* (8), 2386–2392, DOI 10.1021/ac0484373.
(19) Temple, A.; Ten, T.-Y.; Gronert, S. Identification of specific protein carbonylation sites in model oxidations of human serum albumin. *J. Am. Soc. Mass Spectrom.* **2006**, *17* (8), 1172–1180, DOI 10.1016/j.jasms.2006.04.030.
(20) Requena, J. R.; Chao, C. C.; Levine, R. L.; Stadtman, E. R. Glutamic and aminoapidic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins. *PNAS.* **2001**, *98* (1), 69–74, DOI 10.1073/pnas.011526698.
(21) Abad, F. X.; Pintó, R. M.; Villena, C.; Gajardo, R.; Bosch, A. Astrovirus survival in drinking water. *Appl. Environ. Microbiol.* **1997**, *63* (8), 3119–3122.
(22) da Silva, A. K.; Le Saux, J.-C.; Parnaudeau, S.; Pommepuy, M.,; Elimelech, M.; Le Guyader, F. S. 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, DOI 10.1128/AEM.01428.07.
(23) Shin, G.-A.; Sobsey, M. D. Inactivation of norovirus by chlorine disinfection of water. *Water Res.* **2008**, *42* (17), 4562–4568, DOI 10.1016/j.watres.2008.08.001.
(24) Sirikanchana, K.; Shisler, J. L.; Marinas, B. J. Effect of exposure to UV-C irradiation and monochloramine on adenovirus serotype 2 early protein expression and DNA replication. *Appl. Environ. Microbiol.* **2008**, *74* (12), 3774–3782, DOI 10.1128/AEM.02049−07.
(25) Simonet, J.; Gantzer, C. Degradation of the poliovirus 1 genome by chlorine dioxide. *J. Appl. Microbiol.* **2006**, *100* (4), 862–870, DOI 10.1111/j.1365−2672.2005.02850.x.
(26) Simonet, J.; Gantzer, C. Inactivation of poliovirus 1 and F-specific RNA phages and degradation of their genomes by UV irradiation at 254 nanometers. *Appl. Environ. Micribiol.* **2006**, *72* (12), 7671–7677, DOI 10.1128/AEM.01106−06.
(27) Gilpatrick, S. G.; Schwab, K. J.; Estes, M. K.; Atmar, R. L. Development of an immunomagnetic capture reverse transcription-PCR assay for the detection of Norwalk virus. *J. Virol. Methods.* **2000**, *90* (1), 69–78, DOI 10.1016/S0166−0934(00)00220−2.
(28) Myrmel, M.; Rimstad, E.; Wasteson, Y. Immunomagnetic separation of a Norwalk-like virus (genogroup I) in artificially contaminated environmental water samples. *Int. J. Food Microbiol.* **2000**, *62* (1−2), 17–26, DOI 10.1016/S0168−1605(00)00262−2.
(29) Nuanualsuwan, S.; Cliver, D. O. Capsid functions of inactivated human picornaviruses and feline calicivirus. *Appl. Environ. Microbiol.* **2003**, *69* (1), 350–357, DOI 10.1128/AEM.69.1.350−357.2003.
(30) Rodríguez, R. A.; Pepper, I. L.; Gerba, C. P. Application of PCR-based methods to assess the infectivity of enteric viruses in environmental samples. *Appl. Environ. Microbiol.* **2009**, *75* (2), 297–307, DOI 10.1128/AEM.01150−08.
(31) da Silva, A. K.; Le Guyader, F. S.; Le Saux, J.-C.; Pommepuy, M.; Montgomery, M. A.; Elimelech, M. Norovirus removal and particle association in a waste stabilization pond. *Environ. Sci. Technol.* **2008**, *42* (24), 9151–9157, DOI 10.1021/es802787v.
(32) Goyal, S. M.; Gerba, C. P. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. *Appl. Environ. Microbiol.* **1979**, *38* (2), 241–147.
(33) Le Guyader, F. S.; Loisy, F.; Atmar, R. L.; Hutson, A. M.; Estes, M. K.; Ruvoen-Clouet, N.; Pommepuy, M.; Le Pendu, J. Norwalk virus-specific binding to oyster digestive tissues. *Emerg. Infect. Dis.* **2006**, *12* (6), 931–936.
(34) Soreghan, B. A.; Yang, F.; Thomas, S. N.; Hsu, J.; Yang, A. J. High-throughput proteomic-based identification of oxidatively induced protein carbonylation in mouse brain. *Pharm. Res.* **2003**, *20* (11), 1713–1720.
(35) Reverter-Branchat, G.; Cabiscol, E.; Tamarit, J.; Ros, J. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*. *J. Biol. Chem.* **2004**, *279* (30), 31983–31989, DOI 10.1074/jbc.M404849200.
(36) Berlett, B. S.; Stadtman, E. R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.* **1997**, *272* (33), 20313–20316.
(37) Nightingale, Z. D.; Lancha, JR, A. H.; Handelman, S. K.; Dolnikowski, G. G.; Busse, S. C.; Dratz, E. A.; Blumberg, J. B.; Handelman, G. J. Relative reactivity of lysine and other peptide-bound amino acids to oxidation by hypochlorite. *Free Rad. Biol. Med.* **2000**, *29* (5), 425–433.
(38) Sharov, V. S.; Dremina, E. S.; Galeva, N. A.; William, T. D.; Schoneich, C. Quantitative mapping of oxidation-sensitive cysteine residues in SERCA in vivo and in vitro by HPLC-electrospray-tandem MS: selective protein oxidation during biological aging. *Biochem. J.* **2006**, *394*, 605–615.
(39) Winterbourn, C. C.; Kettle, A. J. Biomarkers of myeloperoxidase-derived hypochlorous acid. *Free Rad. Biol. Med.* **2000**, *29* (5), 403–409.
(40) Schfneich, C. Methionine oxidation by reactive oxygen species: reaction mechanisms and relevance to Alzheimer's disease. *Biochim. Biophys. Acta* **2005**, *1703*, 111–119.
(41) Yan, L.-J.; Traber, M. G.; Kobuchi, H.; Matsugo, S.; Tritschler, H. J.; Packer, L. Efficacy of hypochlorous acid scavengers in the prevention of protein carbonyl formation. *Arch. Biochem. Biophys.* **1996**, *327* (2), 330–334.
(42) Stadtman, E. R.; Levine, R. L. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* **2003**, *25*, 207–218, DOI 10.1007/s00726−003−0011−2.
(43) Kohn, T.; Nelson, K. L. Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. *Environ. Sci. Technol.* **2007**, *41* (1), 192–197, DOI 10.1021/es061716i.

ES9018964