Antiviral Research 99 (2013) 27–33

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Reduction of porcine parvovirus infectivity in the presence of protecting osmolytes

Maria F. Tafur, K. Saagar Vijayaragavan, Caryn L. Heldt *

Department of Chemical Engineering, Michigan Technological University, 1400 Townsend Dr. Houghton, MI 49931, USA

ARTICLE INFO

Article history:
Received 7 November 2012
Revised 21 April 2013
Accepted 24 April 2013
Available online 3 May 2013

Keywords:
PPV
Glycine
TMAO
Preferential hydration
Protein stability
Self-assembly

ABSTRACT

Osmolytes are natural compounds found in the cells of many organisms that stabilize intracellular proteins against environmental stresses. Protecting osmolytes can promote protein folding, whereas denaturing osmolytes have the opposite effect. A variety of osmolytes were tested for their antiviral activity against porcine parvovirus (PPV). PPV is a non-enveloped, icosahedral, single-strand DNA virus. We have discovered two protecting osmolytes, trimethylamine N-oxide (TMAO) and glycine that reduce the infectivity of PPV by four logs (99.99%). We hypothesize that both osmolytes stabilize viral capsid proteins and prevent them from assembling into viable virus particles. The advantage of the antiviral compounds found is that they can be applied post-infection, which increases their potential to serve as a therapeutic drug.

1. Introduction

Many diseases are caused by pathogenic virus infection. In recent decades, scientists have defined the structure and function of many different viruses. This has aided in the creation of specific antiviral compounds. Compounds that inactivate certain viruses have changed the treatment of many diseases, including respiratory syncytial virus (RSV) (Glatthaar-Saalmüller et al., 2011) and herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2) (Hayashi et al., 2012). The AIDS epidemic has been treated with HIV protease inhibitors that now allow people to live decades with the virus (Antonelli and Turriziani, 2012). However, there is still a need for the continued discovery of antiviral compounds.

Many researchers are now studying natural products as a source of antiviral compounds, since viruses are starting to become resistant to current drugs (Kitazato et al., 2007). In an effort to find natural compounds that have antiviral activity, we screened the antiviral activity of a panel of osmolytes and a salt against the non-enveloped virus porcine parvovirus (PPV). Osmolytes are small organic compounds that are found in the cells of many organisms and they have the ability of stabilize intracellular proteins against environmental stress, such as extreme temperature or high osmotic pressure (Bolen, 2004). A balance between protecting and denaturing osmolytes assist in the delicate equilibrium needed for protein stabilization (Dong et al., 2009). Protecting osmolytes fold proteins by structuring water around themselves and changing the interaction between the water and the protein backbone. Denaturing osmolytes bind directly to the protein backbone, causing the protein to unfold (Street et al., 2006).

Parvoviruses, from the family *Parvoviridae*, are small, non-enveloped, icosahedral, single-stranded DNA viruses that infect vertebrates and arthropods (Halder et al., 2012). PPV infects the intestines of pigs and is the most frequent cause of swine reproductive failure (Boisvert et al., 2010). This virus is often used as a model for the human B19 parvovirus. Although different natural compounds have been studied in recent decades, osmolytes have not been previously shown to have antiviral activity. This study describes the reduction of PPV infectivity in the presence of the protecting osmolytes TMAO and glycine.

2. Materials and methods

2.1. Materials

The osmolytes trimethylamine N-oxide (TMAO) dihydrate, glycine, betaine, D-alanine, D-arginine, sucrose, trehalose dihydrate, urea, and the salt ammonium sulfate were purchased from Sigma–Aldrich (St. Louis, MO) at a minimum purity of ⩾98.0%. Poly-L-lysine, 4′,6-diamidino-2-phenylindole dihydrochloride

Abbreviations: PPV, porcine parvovirus; TMAO, trimethylamine N-oxide; RSV, respiratory syncytial virus; HSV-1, herpes simplex virus type-1; HSV-2, herpes simplex virus type-2; HIV, human immunodeficiency virus; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; IC_{50}, 50% infectious dose.

* Corresponding author. Tel.: +1 906 487 1134; fax: +1 906 487 3213.
E-mail addresses: mftafur@mtu.edu (M.F. Tafur), kskhrupa@mtu.edu (K.S. Vijayaragavan), heldt@mtu.edu (C.L. Heldt).

0166-3542/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2013.04.019

(DAPI), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), agarose type I, low EEO, neutral red solution (0.33%), and sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich (St. Louis, MO). Phosphate-buffered saline (PBS, pH 7.2) and 0.25% trypsin/EDTA for cell propagation were purchased from Life Technologies (Grand Island, NY). 12.1 M hydrochloric acid (HCl) and 3.7% formaldehyde in water were purchased from VWR (Radnor, PA). The monoclonal mouse anti-PPV antibody was purchased from VMRD (Cat no. 3C9D11H11, Pullman, WA) and the polyclonal Alexa fluor 546-conjugated rabbit anti-mouse antibody was purchased from Life Technologies (Cat no. A11060, Grand Island, NY). All solutions were made with NanoPure water (Thermo Scientific, Waltham, MA, resistance >18 MΩ) and filtered with either a 0.2 μm syringe filter (Nalgene, Rochester, NY) or a Millipore 0.2 μm bottle top filter (Billerica, MA) prior to use.

2.2. Cell propagation

Porcine kidney (PK-13) cells were a gift from Dr. Ruben Carbonell at North Carolina State University and were propagated as described previously (Heldt et al., 2006). Briefly, the cells were grown in minimum essential medium (MEM) (Life Technologies, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Oakwood, GA) and 1% penicillin/streptomycin (Life Technologies, Grand Island, NY).

2.3. Virus production and titration

PPV strain NADL-2 was a gift from Dr. Ruben Carbonell at North Carolina State University and were propagated in PK-13 cells, as described previously (Heldt et al., 2006). Briefly, cells were infected with 10^3 MTT_{50} of PPV, and 1.5 h later, 9 ml of supplemented media were added. After 4–6 days, the flasks were placed at −20 °C. The flasks were thawed, and the monolayer was scraped. The scraped cells and media were 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.

PPV was titrated with a colorimetric cell viability assay, the MTT Assay (Heldt et al., 2006). The reduction of the MTT tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) inside the mitochondria of metabolically active cells produces formazan crystals (Mosmann, 1983). Upon dissolving the crystals, the cell viability can be quantified by measuring the absorbance of the solution at 550 nm. This has been shown to be linearly comparable to a $TCID_{50}$ for PPV on PK-13 cells (Heldt et al., 2006). Cells were seeded in 96-well plates, as described earlier (Heldt et al., 2006). The cells were infected with PPV in quadruplicate and 5-fold serial dilutions were made across the plate. After five days, 10 μl/well of 5 mg/ml of MTT in PBS was added. Four hours later, 100 μl/well of solubilizing agent (0.01 M HCl and 10% SDS) were added. Plates were read on a Synergy Mx microplate reader (BioTek, Winooski, VT) at 550 nm between 18–24 h after addition of the solubilizing agent. The 50% infectious dose (MTT_{50}) value was determined to be the virus dilution that yielded 50% of the uninfected cell absorbance. The value was converted to a per milliliter basis and stated as the MTT_{50}/ml titer (Heldt et al., 2006).

2.4. Cytotoxicity assay

Antiviral activity was determined in a similar way to the virus titration described in Section 2.3. After virus was added to the cells, 25 μl of osmolyte or salt at various concentrations were added to the infected cells.

To determine the effect of osmolyte concentration on antiviral activity, 25 μl of either TMAO or glycine with a final concentration ranging from 0.00 to 0.30 M was added to the infected cells. To determine the effect of the time between infection and osmolyte addition on antiviral activity, 0.20 M of either TMAO or glycine was added at various times post-infection. MTT reagent addition was performed after five days, as described in Section 2.3. Calculation of the log reduction is shown in Eq. (1).

$$\text{log reduction} = -\log\left(\frac{\text{virus titer with osmolyte}}{\text{virus titer of control}}\right) \quad (1)$$

2.5. Osmolyte toxicity

Cell viability was assessed with an MTT assay and was used to determine the toxicity of TMAO and glycine to PK-13 cells. Cells were seeded as described in Section 2.3 in 100 μl of media. Osmolytes diluted to a final concentration ranging from 0.00 to 0.60 M in NanoPure water were added to the cells after 24 h at a volume of 25 μl. MTT reagent addition was conducted after five days, as described in Section 2.3. Calculation of the % survival of cells is shown in Eq. (2).

$$\%\ \text{survival} = \left(\frac{\text{absorbance with osmolyte}}{\text{absorbance of control}}\right) \times 100 \quad (2)$$

2.6. Plaque reduction assay

Plaque assays were performed as described previously (Heldt et al., 2006). Briefly, PK-13 cells were seeded into 25 cm^2 tissue culture flasks with a final concentration of 4×10^5 cells per flask and incubated at 37 °C and 5% CO_2 until 70% confluent. Ten-fold serial dilutions of 10^8 MTT_{50}/ml were made in either PBS with 3% FBS or PBS with 3% FBS containing 0.20 M TMAO or 0.20 M glycine. Cells were infected with 200 μl of different sample dilutions. After 1 h of incubation, virus inoculum was removed and infected cells were overlaid with 1:1 of 2% agarose in Nanopure water and 2× supplemented media. Overlay media for osmolytes samples also contained 0.20 M glycine or 0.20 M TMAO. Flasks were stained with 2 ml of 4% neutral red at 3.3 g/L in the overlay media after 4 days of incubation. Plaques were counted 4–8 h after staining.

2.7. Yield reduction assay

Intracellular and extracellular viable virus particles were measured. PK-13 cells were seeded in 6-well plates at the same cell density as the MTT Assay described in Section 2.3. Cells were infected with 2×10^3 MTT_{50} of PPV. After 1 h, the virus inoculum was removed and media added. Osmolytes were added either at this time (Treatment 1) or 5 min prior to sample collection (Treatment 2). Virus supernatant was removed at various times to measure extracellular viable virus particles. To assay intracellular viable virus particles, cells were detached by the addition of trypsin/EDTA and equal volume of media was added to deactivate the trypsin. All samples were frozen at −20 °C for 24 h, thawed at room temperature, and centrifuged at 5000 rpm in a Sorvall ST16R Centrifuge (Thermo Scientific, Pittsburgh, PA) for 15 min at 4 °C. The supernatants were removed and titrated as described in Section 2.3.

2.8. Immunohistochemical detection of virus capsid protein production

Intracellular virus capsid protein production was assessed through immunohistochemistry of PPV-infected cells with and without osmolytes. Glass slides (25 × 75 mm) from VWR (Radnor,

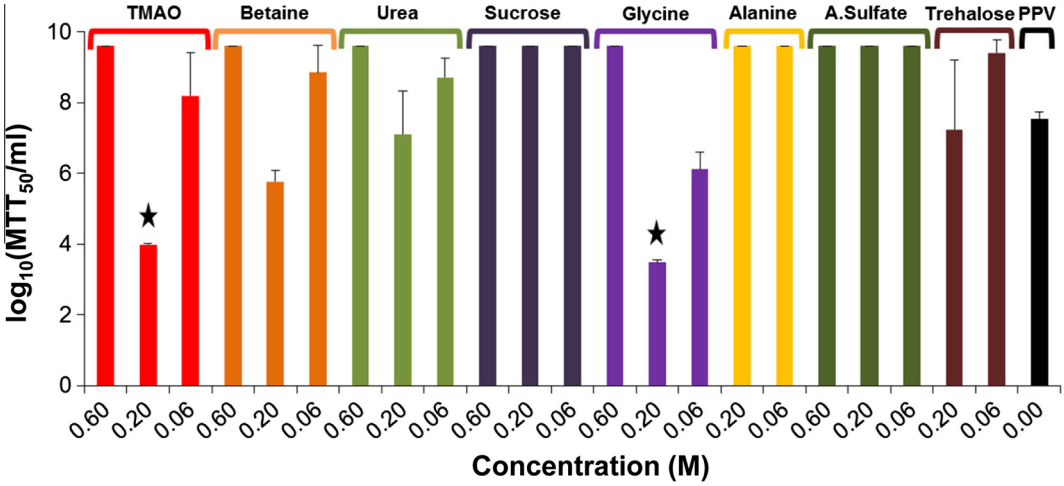

Fig. 1. Antiviral activity of a panel of osmolytes and a salt. 7 $\log_{10}(MTT_{50}/ml)$ of PPV was used to infect cells in media with different osmolyte concentrations for five days. This was followed by the evaluation of virus cytotoxicity with the MTT assay. The stars represent the osmolytes with the greatest antiviral activity. The maximum detection limit of the assay was 9.6 $\log_{10}(MTT_{50}/ml)$. All data points are the average of three separate experiments and the error bars represent the standard deviation.

PA) were soaked in 2 M HCl for 1 h to etch and remove any grease. Then, 200 µl of poly-L-lysine was added on the area of cell growth. After five minutes, the slides were washed with water and dried for 2 h under UV light. Cells were seeded on the slides at a density of 5×10^4 cells/slide with a total volume of 50 µl/slide. Cells were incubated for 6 min at 37 °C, and 5% CO_2. Ten ml of fresh media was added to the petri dish that contained the slides. After 48 h of incubation, the media was removed and cells were washed with PBS. Each slide was infected with 50 µl/slide of either PPV (10^8 MTT_{50}/ml), PPV containing 0.20 M TMAO or glycine (10^8 MTT_{50}/ml), PBS, or media containing 0.20 M TMAO or glycine. After 30 min, the cells were washed twice with PBS to remove any unattached PPV, and 10 ml of fresh media with or without osmolytes were added. The cells were placed at 37 °C, 100% humidity, and 5% CO_2 for different times. The media was removed and the cells were washed once with PBS. The infected cells were fixed with 200 µl of 3.7% formaldehyde for 20 min at room temperature, and then washed twice with PBS. The cells were blocked with 200 µl of 0.3% low-fat milk in PBS. After 1 h, 50 µl of 1:100 v/v anti-PPV antibody were added and incubated for 1 h at 37 °C followed by two PBS washes. Then, 50 µl of Alexa fluor 546-conjugated rabbit anti-mouse IgG (1:500 v/v) were added and incubated for 1 h at 37 °C followed by two PBS washes. Slides were washed again with PBS. To fix the antibodies, 200 µl of 3.7% formaldehyde were added for 20 min at room temperature, and then the cells were washed twice with PBS. Finally, 50 µl of 150 µM DAPI were added for 5 min at room temperature and washed with PBS. Images of the cells were taken with an Olympus IX51 microscope with a DP72 camera (Olympus, Center Valley, PA). Fluorescence per cell was analyzed with ImageJ (NIH). Image study was conducted on 2–3 slides with 10 images per slide. Additional details about the ImageJ analysis can be found in the Supplementary Information.

3. Results

3.1. Virus cytotoxicity in the presence of osmolytes and a salt

Antiviral activity of a group of osmolytes and a salt was screened. These compounds were the protecting osmolytes TMAO, betaine, sucrose, glycine, alanine and trehalose, the denaturing osmolyte urea, and the salt ammonium sulfate. Two to three different concentrations of each osmolyte and salt were tested for their ability to reduce the infectivity of PPV (Fig. 1). Cell viability was measured with the MTT assay. Reduction of cell viability can be caused by either the compound toxicity or the virus cytotoxicity. The MTT assay has been compared to a $TCID_{50}$ (i.e. visual inspection of cell death) for PPV cytotoxicity on PK-13 cells, and shown to be linearly correlated (Heldt et al., 2006).

A value of 9.6 $\log_{10}(MTT_{50}/ml)$ indicates that none of the cells were viable. At all osmolyte concentrations tested, sucrose, alanine

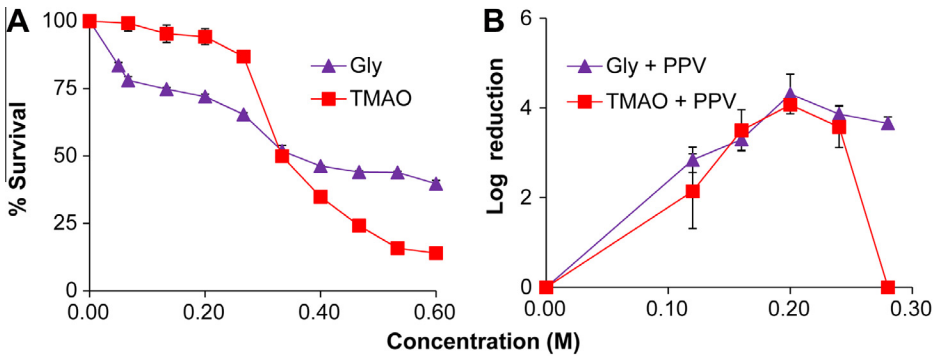

Fig. 2. Osmolyte toxicity and reduction of virus cytotoxicity with different concentrations of TMAO and glycine. (A) Osmolyte toxicity on PK-13 cells, a susceptible host for PPV in the presence of glycine and TMAO, and (B) log reduction of PPV infectivity in the presence of various concentrations of glycine and TMAO. Cells and infected cells were exposed to osmolytes for five days, followed by evaluation of cytotoxicity with the MTT assay. The log reduction is defined in equation 1 and the % survival is defined in equation 2. All data points are the average of three separate experiments and the error bars represent the standard deviation.

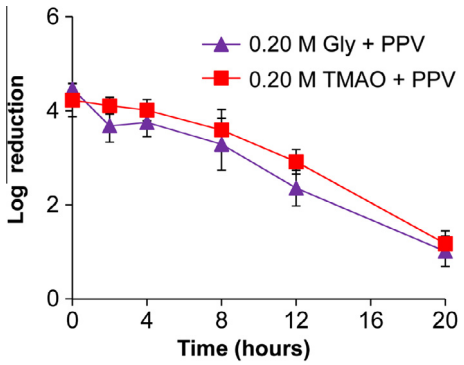

Fig. 3. Reduction of virus cytotoxicity in the presence of osmolytes added post-virus infection. 0.20 M glycine and 0.20 M TMAO were added at different times post-virus infection and after five days, followed by evaluation of virus cytotoxicity with the MTT assay. The log reduction is defined in equation 1. All data points are the average of three separate experiments and the error bars represent the standard deviation.

Table 1
PPV plaque assay.

Sample	Titer (pfu/ml)
PPV	$5.5 \times 10^7 \pm 1.1 \times 10^7$
0.20 M glycine + PPV	ND[a]
0.20 M TMAO + PPV	ND[a]

[a] Not detected.

and ammonium sulfate were found to be toxic to the cells, and therefore no viable cells were detected (Fig. 1). All other compounds tested, except for trehalose, demonstrated compound toxicity at the highest concentrations tested. TMAO, betaine, urea, and glycine were all toxic at 0.60 M. They demonstrated antiviral activitiy at 0.20 M and then returned to near PPV control values at 0.06 M. For most compounds, this return to PPV control values is due to the compound being present at too low of a concentration to have antiviral activity. In the case of betaine and trehalose, the lowest concentration of osmolyte increased the virus titer above the control. It is possible that at low concentrations, the compounds increased and enhanced the virus' ability to infect the cells. This has been seen for other compounds (Tao et al., 2008).

TMAO and glycine at a concentration of 0.20 M showed a four log reduction, which is equal to 99.99% of infectious virus reduction. These two osmolytes had the greatest antiviral activity and were the focus of all subsequent work.

3.2. Osmolyte toxicity

Osmolyte toxicity was measured in PK-13 cells. As Fig. 2A shows, the cells had a high rate of survival at low doses of osmolytes. Increasing the osmolyte concentration resulted in a decrease in cell survival, likely due to hypotonic lysis of the cells. TMAO exhibited a sigmoidal dose response with a calculated 50% inhibitory concentration (IC_{50}) of 0.37 ± 0.01 M. Glycine did not display a clear sigmoidal curve, but an IC_{50} value of 0.42 ± 0.02 M was calculated.

3.3. Reduction of virus cytotoxicity with TMAO and glycine

The antiviral activities of various concentrations of TMAO and glycine were studied. Glycine and TMAO showed the highest log reduction, four logs (99.99%), at 0.20 M (22.29 mg/mL of glycine and 15.01 mg/mL of TMAO) (Fig. 2B). The MTT Assay measures cell viability and this can be caused by compound toxicity or virus infectivity. At high or low osmolyte concentration, cell viability was reduced probably due to compound toxicity or virus infectivity.

Many antiviral compounds work by interrupting virus entry. However, some antiviral compounds work by interrupting replication. To determine if disruption of virus entry was the antiviral mechanism of the osmolytes, the time between virus infection and osmolyte addition was varied. When 0.20 M of TMAO or glycine was added at the initial stages of virus infection, a four log reduction in PPV infectivity was observed for both osmolytes (Fig. 3). As the time between virus infection and osmolyte addition increased, the reduction in infectivity slowly decreased over time and approached one log reduction (90%) at 20 h (Fig. 3). Osmolytes are not likely disrupting virus entry because they were still effective when added 20 h post-virus infection. Treatment of the virus with osmolytes prior to infection did not reduce virus infectivity, shown in Fig. S1. This demonstrates that the osmolytes are likely not disrupting virus attachment or entry.

3.4. Plaque reduction assay

A plaque reduction assay was performed in order to corroborate data from the cytotoxicity assay. A plaque assay was performed for 0.20 M TMAO and 0.20 M glycine and $a > 7$ log reduction was found. No plaques were formed in the presence of 0.20 M TMAO and glycine (Table 1).

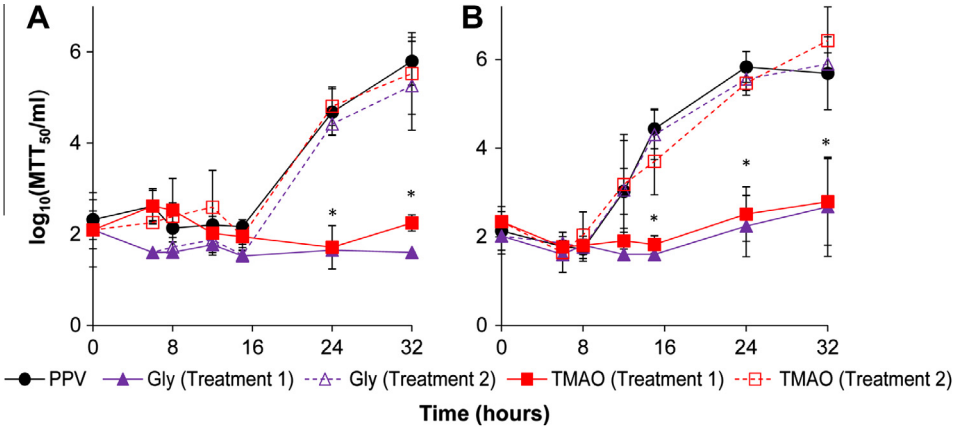

Fig. 4. Impact of osmolytes on infectious virus yield. (A) Extracellular, and (B) intracellular infectious virus yield. Osmolytes were added at the time of infection (Treatment 1) or 5 min before sample collection (Treatment 2). The yield of infectious virus particles was determined with the MTT Assay. The minimum limit of detection of the assay was 1.6 $\log_{10}(MTT_{50}/ml)$. All data points are the average of three separate tests and the error bars represent the standard deviation. Student's *t*-test was used to evaluate the statistical significance between PPV infected cells containing osmolytes for Treatment 1 and PPV infected cells without osmolytes. $^*p$ value of <0.05.

3.5. Impact of osmolytes on infectious virus yield

The presence of intracellular and extracellular viable virus particles was examined. This was studied under two different osmolyte treatments. In Treatment 1, the osmolytes were added at the beginning of the infection cycle and in Treatment 2, they were added five minutes before sample collection. Treatment 2 was done in order to determine if the addition of osmolyte after infection would affect the MTT assay. As shown in Fig. 4, there was no difference between Treatment 2 and the control (i.e., virus infection with no osmolytes addition).

With Treatment 1, there appeared to be little infectious virus extracellularly (Fig. 4A) or intracellularly (Fig. 4B), even after 32 h. Without osmolyte addition, infectious virus began to appear extracellularly after 15 h and intracellularly after 10 h. This is consistent with detection of an increase in DNA at about 8 h post infection (Boisvert et al., 2010). After 32 h, 0.20 M TMAO and glycine had a 3.5 ± 0.5 log and 4.2 ± 0.5 log reduction extracellularly,

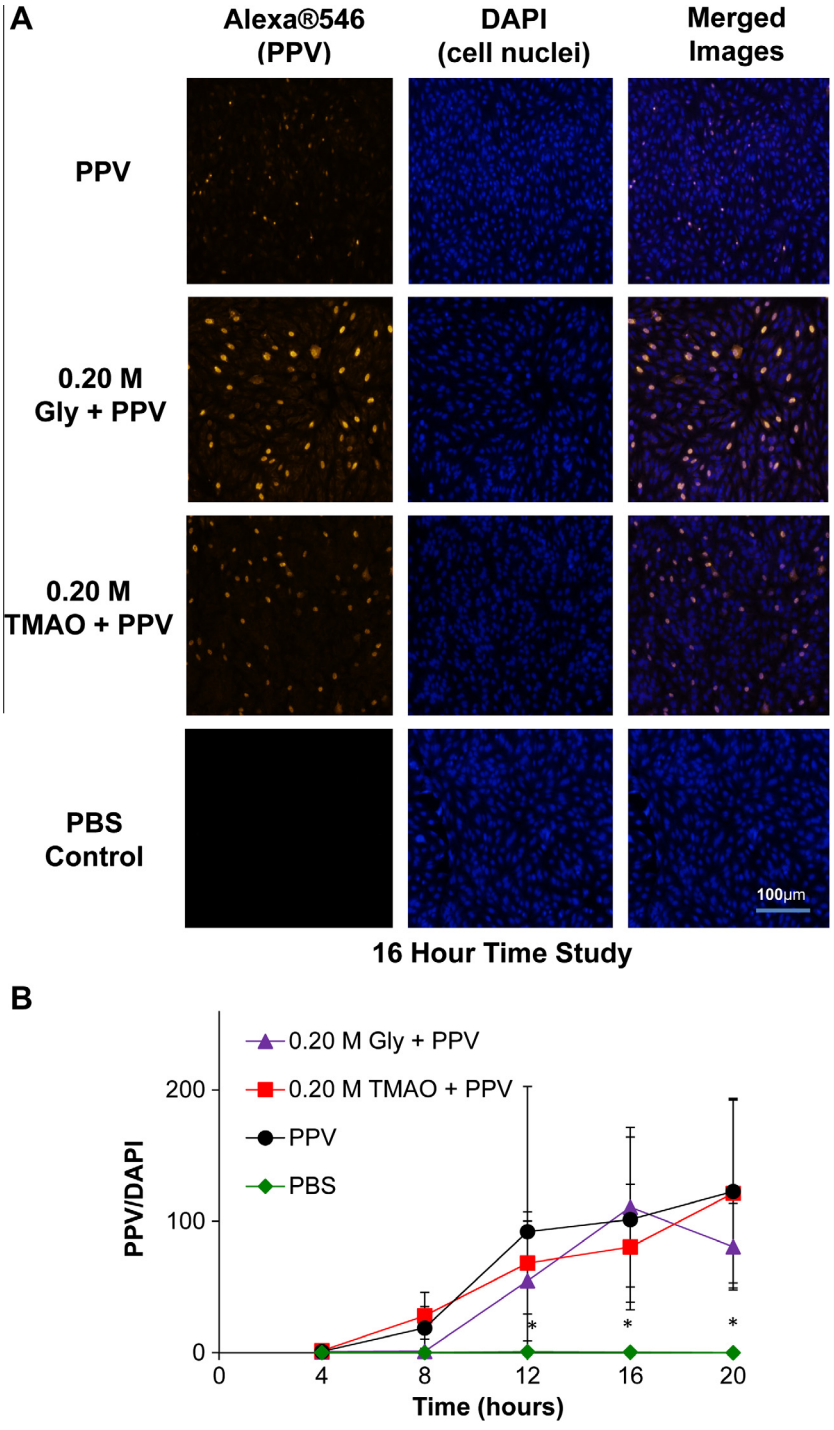

Fig. 5. Impact of osmolytes on virus capsid protein formation. (A) Images of PPV and PBS mock infected cells at 16 h, and (B) the ratio of the fluorescence of PPV capsid proteins to the count of DAPI. All data points are the average of three independent slides with 10 images per slide and the error bars represent the standard deviation. TMAO and glycine were tested in order to determine osmolytes cross-reactivity with antibodies and no virus capsids were found (data not shown). Student's *t*-test was used to evaluate the statistical significance between cells infected with PPV (with or without osmolyte) and the PBS negative control. *p value of <0.05.

respectively, as compared to the PPV control, and a 2.9 ± 0.2 log and a 3.0 ± 0.5 log reduction intracellularly, respectively. This is consistent with $a > 4$ log reduction after five days incubation, as shown in Fig. 3.

3.6. Virus capsid protein production with the addition of osmolytes

It was explored with immunohistochemistry if the virus capsid proteins were being produced within the cells. PPV capsid proteins were found in all samples infected with PPV, including those containing 0.20 M TMAO and 0.20 M glycine (Fig. 5A). PBS without virus was used as a negative control, and did not show any virus capsid protein formation. TMAO and glycine were also tested without virus present and no virus capsid protein formation was detected (data not shown). In Fig. 5B, fluorescence per cell started to increase after 8 h, and this agrees with virus production data (Fig. 4B) and DNA production data (Boisvert et al., 2010), although we did not specifically test if PPV DNA was produced. Similar trends were observed with PPV and osmolytes (Fig. 5B). These results show that viral capsid proteins are produced (Fig. 5B), but viable virus particles are not produced (Fig. 4).

4. Discussion

Antiviral compounds are known to disrupt virus entry, replication, assembly of virus particles, or a combination of these. Antiviral compounds that destroy the enveloped capsid of a virus are required in small quantities (Astani et al., 2011). Other compounds are able to alter the virus-host interactions and are required in higher quantities (Bélec et al., 2000). The advantage of natural compounds that alter the virus-host interactions is that they can be applied post-infection. In this study, two osmolytes, TMAO and glycine, demonstrated this behavior and are therefore promising candidates for therapeutic drugs. TMAO and glycine are both small (molecular weight of 75 g/mol), zwitterionic species. More work will need to be done to determine why these osmolytes, compared to the many that were tested (Fig. 1) demonstrated antiviral activity.

The fact that virus capsid proteins were produced in the presence of osmolytes (Fig. 5) strongly suggests that osmolytes work post-virus infection (Figs. 3 and 4). We hypothesize that osmolytes are disrupting capsid assembly by stabilizing viral capsid proteins and preventing the assembly process. Osmolytes stabilize proteins by causing a preferential hydration around proteins (Bolen, 2004). TMAO has also been shown to preferentially order water molecules around α-chymotrypsin and to stabilize the enzymatic activity of the protein (Attri et al., 2010). This preferential hydration causes proteins to adapt a compact configuration (Street et al., 2010). It is likely that this compact configuration of the VP2 protein in PPV is not able to self-assemble into a virus capsid. The protein capsids of other parvoviruses have been studied and their assembly is likely to be kinetically stable, but not thermodynamically stable (Carreira et al., 2004; Castellanos et al., 2012). This may demonstrate that the osmolytes thermodynamically stabilize the capsid proteins, therefore reducing their propensity to kinetically assemble. The other possibility is that the high osmolyte concentration in the cells reduces the activity of the assembly machinery that guides the DNA inside the virus capsid. However, enzymes are usually stabilized and still have activity in a solution of osmolytes (Attri et al., 2010), therefore, we hypothesize that the osmolytes are disrupting the ability of the capsid proteins to self-assemble.

5. Conclusions

A variety of osmolytes were screened to find antiviral compounds against PPV, a small, nonenveloped, single-stranded DNA virus (Mengeling et al., 2000). We have discovered that two protecting osmolytes, TMAO and glycine, at 0.20 M, reduce the infectivity of PPV by four logs (99.99%). These results are consistent with the literature, since high quantities of antiviral compounds are often required for the inactivation of non-enveloped viruses. Both osmolytes showed antiviral activity after being added 20 h post-infection. In the presence of TMAO or glycine, infected cells produce virus capsid proteins, but not infectious viable virus particles. We propose that the osmolytes TMAO and glycine interfere with the virus capsid formation and are potential candidates for therapeutic drugs.

Acknowledgements

The authors would like to thank Dr. Thomas Werner for his thorough reading of the manuscript. They also thank the Department of Chemical Engineering at Michigan Technological University, the MTU-REF Research Seed Grant, and NSF (CBET-1125585) for funding.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2013.04.019.

References

Antonelli, G., Turriziani, O., 2012. Antiviral therapy: old and current issues. Int. J. Antimicrob. Agents 40 (2), 95–102.
Astani, A., Reichling, J., Schnitzler, P., 2011. Screening for antiviral activities of isolated compounds from essential oils. Evid-Based Complement Alternat. Med. 2011, 253643.
Attri, P., Venkatesu, P., Lee, M.J., 2010. Influence of Osmolytes and Denaturants on the Structure and Enzyme Activity of alpha-Chymotrypsin. J. Phys. Chem. B 114 (3), 1471–1478.
Bélec, L., Tevi-Benissan, C., Bianchi, A., Cotigny, S., Beumont-Mauviel, M., Si-Mohamed, A., Malkin, J.E., 2000. In vitro inactivation of Chlamydia trachomatis and of a panel of DNA (HSV-2, CMV, adenovirus, BK virus) and RNA (RSV, enterovirus) viruses by the spermicide benzalkonium chloride. J. Antimicrob. Chemother. 46 (5), 685–693.
Boisvert, M., Fernandes, S., Tijssen, P., 2010. Multiple pathways involved in porcine parvovirus cellular entry and trafficking toward the nucleus. J. Virol. 84 (15), 7782–7792.
Bolen, D.W., 2004. Effects of naturally occurring osmolytes on protein stability and solubility: issues important in protein crystallization. Methods 34 (3), 312–322.
Carreira, A., Menéndez, M., Reguera, J., Almendral, J.M., Mateu, M.G., 2004. In vitro disassembly of a parvovirus capsid and effect on capsid stability of heterologous peptide insertions in surface loops. J. Biol. Chem. 279 (8), 6517–6525.
Castellanos, M., Pérez, R., Carrillo, P.J.P., de Pablo, P.J., Mateu, M.G., 2012. Mechanical disassembly of single virus particles reveals kinetic intermediates predicted by theory. Biophys. J. 102 (11), 2615–2624.
Dong, X.Y., Liu, J.H., Liu, F.F., Sun, Y., 2009. Self-interaction of native and denatured lysozyme in the presence of osmolytes, L-arginine and guanidine hydrochloride. Biochem. Eng. J. 43 (3), 321–326.
Glatthaar-Saalmüller, B., Rauchhaus, U., Rode, S., Haunschild, J., Saalmüller, A., 2011. Antiviral activity in vitro of two preparations of the herbal medicinal product Sinupret (R) against viruses causing respiratory infections. Phytomedicine 19 (1), 1–7.
Halder, S., Ng, R., Agbandje-McKenna, M., 2012. Parvoviruses: structure and infection. Fut. Virol. 7 (3), 253–278.
Hayashi, K., Iinuma, M., Sasaki, K., Hayashi, T., 2012. In vitro and in vivo evaluation of a novel antiherpetic flavonoid, 4'-phenylflavone, and its synergistic actions with acyclovir. Arch. Virol. 157 (8), 1489–1498.
Heldt, C.L., Hernandez, R., Mudiganti, U., Gurgel, P.V., Brown, D.T., Carbonell, R.G., 2006. A colorimetric assay for viral agents that produce cytopathic effects. J. Virol. Meth. 135 (1), 56–65.
Kitazato, K., Wang, Y., Kobayashi, N., 2007. Viral infectious disease and natural products with antiviral activity. Drug Discover. Ther. 1 (1), 14.
Mengeling, W., Lager, K., Vorwald, A., 2000. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. Anim. Reprod. Sci. 60, 199–210.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65, 55–63.

Street, T.O., Bolen, D.W., Rose, G.D., 2006. A molecular mechanism for osmolyte-induced protein stability. Biophys. J., 217A.

Street, T.O., Krukenberg, K.A., Rosgen, J., Bolen, D.W., Agard, D.A., 2010. Osmolyte-induced conformational changes in the Hsp90 molecular chaperone. Protein Sci. 19 (1), 57–65.

Tao, W., Richards, C., Hamer, K., 2008. Enhancement of HIV infection by cellulose sulfate. AIDS Res. Hum. Retroviruses 24 (7), 925–929.