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Stability and inactivation of vesicular stomatitis virus, a prototype rhabdovirus

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ABSTRACT

Viruses may remain infectious outside the host cell for considerable time and represent a source of accidental infection if not properly inactivated. In this study, the survival of vesicular stomatitis virus (VSV) in suspension and dried on surfaces was analyzed. In addition, the sensitivity of VSV to disinfectants and physicochemical changes was investigated. VSV showed a notable stability in suspension at 4 °C with virus titers remaining high over several weeks. The presence of serum proteins had a stabilizing effect on virus infectivity, whereas elevated temperatures reduced survival times. VSV dried on polystyrene, glass or stainless steel surfaces remained infectious for at least 6 days at ambient temperature. VSV showed a remarkable resistance to extreme pH in particular in the alkaline range, but could be rapidly inactivated by heating at 55 °C or higher. The virus was highly sensitive to inactivation by commonly used disinfectants such as aldehydes, alcohols, and detergents. The high stability of VSV on surfaces and in suspension may facilitate dissemination of the virus in livestock by contaminated feeding and water troughs, hands, and milking equipment. This knowledge on the sensitivity of VSV to disinfectants will help to set up appropriate hygiene measures.

1. Introduction

The order *Mononegavirales* comprises four related virus families, *Rhabdoviridae*, *Paramyxoviridae*, *Filoviridae*, and *Bornaviridae*, which include several important pathogens of man and animals. The *Mononegavirales* are characterized by a non-segmented, single-stranded, negative-sense RNA genome tightly associated with nucleoproteins and the viral polymerase proteins. All members of the *Mononegavirales* are enveloped by a lipid membrane containing one or more virus-encoded transmembrane glycoproteins.

The *Rhabdoviridae* represent the most diverse family within the *Mononegavirales* (Assenberg et al., 2010) and comprise pathogens of plants (Nucleorhabdovirus and Cytorhabdovirus), fish (Norirhabdovirus), and mammals (Lyssavirus, Vesiculovirus, and Ephemerovirus). The genus Vesiculovirus contains arthropod-born viruses that are transmitted to livestock and humans by insect vectors such as sandflies and mosquitoes. Infection with Chandipura virus has been associated with encephalitis in children (Gurav et al., 2010). The prototype vesicular stomatitis virus (VSV) is endemic in Mexico, Central America, and northern regions of South America with periodic outbreaks in the southwestern United States (Rodriguez, 2002). VSV causes a disease in livestock with symptoms that resemble those induced by foot-and-mouth-disease virus. The virus can be isolated from throat swabs, saliva, and vesicular lesions (Letchworth et al., 1999; Schmitt, 2002). Infection with VSV may also occur through compromised skin. Contaminated feed and water troughs and milking equipment are believed to further spread the virus within a herd. Intimate contact with infected animals may lead to infection of humans with flu-like symptoms (Hanson et al., 1950; Johnson et al., 1966; Patterson et al., 1958).

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VSV has become a favored reagent in cell biology, immunology, and virology. It can be easily propagated to high titers *in vitro* and is open to genetic manipulation (Finke and Conzelmann, 2005). VSV also has attracted a lot of interest because of its eligibility to serve as a viral vector for vaccination and experimental tumor therapy (Lichty et al., 2004). Because of the high sensitivity of VSV to type I interferon, the virus is frequently used for interferon bioassays (Berger Rentsch and Zimmer, 2011; Buckler and Baron, 1966; Hallum et al., 1970; Meager, 2002). Laboratory-acquired infections with VSV have been repeatedly reported (Fields and Hawkins, 1967; Gaidamovich et al., 1966; Hanson et al., 1950; Johnson et al., 1966; Sewell, 1995).

Virus-contaminated surfaces may represent a potential source of unwanted transmission and infection. The higher the "survival" time of the virus outside the cell the higher the risk of exposure to the virus. To assess this risk for a prototype rhabdovirus, we analyzed both long-term stability of VSV in suspension and the survival of VSV on surfaces in dried form. In addition, the effect of temperature, pH value, and protein supplement on VSV infectivity was studied. Since a previous study suggested that VSV is highly resistant to commonly used disinfectants (Wright, 1970a,b), we analyzed the sensitivity of VSV to detergents, aldehydes, and alcohols. Our findings may help to take appropriate hygiene measures for rhabdoviruses and related non-segmented negative-strand RNA viruses.

2. Materials and methods

2.1. Cells

BHK-21 cells were obtained from the German Cell Culture Collection (DSZM, Braunschweig) and grown in Earle's minimal essential medium (EMEM) supplemented with 5% foetal bovine serum (FBS).

2.2. Chemicals

The detergents Triton X-100 (Applichem A4975) and 20% sodium dodecyl sulfate (SDS) solution (Applichem A0675), were of molecular biology grade and purchased from Applichem, Darmstadt, Germany. Ethanol absolute (Merck 1.00983), 2-propanol p.A. (Merck 1.09634), and paraformaldehyde (Merck 104005) were obtained from Merck KGaA, Darmstadt, Germany. Paraformaldehyde was dissolved in phosphate buffered saline (pH 7.2) at 80 °C with stirring until a clear 3% solution was obtained and stored in aliquots at −20 °C. Glutaraldehyde (4%) solution in borate buffer pH 7.6 (Sigma 3802), 1-propanol (Sigma 279544), and methyl cellulose (Sigma M0512) were from Sigma–Aldrich, St. Louis, MO. The glutaraldehyde solution was stored at −20 °C. PD MiniTrap G-25 columns (GE Healthcare 28-9180-07) were from GE Healthcare Life Sciences, Piscataway, NJ.

2.3. Virus and virus titration

VSV*, a recombinant VSV (serotype Indiana) expressing the enhanced green fluorescent protein (eGFP) was previously described (Hoffmann et al., 2010). VSV* was propagated on BHK-21 cells either in the presence or absence of 5% FBS. At 16–20 h post infection, the cell culture supernatant was collected, and cell debris removed by centrifugation at 3000 × *g* for 15 min at 4 °C. The virus was stored frozen in aliquots at −70 °C. Infectious virus titers were determined on confluent BHK-21 cells grown in 96-well microtiter plates. The cells were inoculated in duplicate with 40 μl of serial 10-fold virus dilutions for 90 min at 37 °C. Thereafter, 200 μl of EMEM containing 0.9% methylcellulose were added to each well and the cells incubated at 37 °C. The cells were surveyed 16 h post infection using an inverse fluorescence microscope (Cell Observer, Zeiss, Germany) and infectious virus titres were calculated based on the number of eGFP-positive plaques per well and expressed as focus-forming units per milliliter (ffu/ml). The lower limit of detection of this assay is 12.5 ffu/ml. VSV* stocks titers ranged from 2 to 4×10^9 ffu/ml.

2.4. Inactivation of VSV in suspension

For analyzing the inactivation of VSV* in suspension, stock virus (10 μl) was mixed with FBS (10 μl) and disinfectant (80 μl) and incubated at room temperature (22 °C) for 1 or 5 min. In order to subsequently deplete the cytotoxic disinfectants, the mixture was diluted with 400 μl of MEM cell culture medium with 5% FBS and loaded on small Sephadex G-25 gel filtration columns, which had been equilibrated with MEM medium. According to the manufacturer, Sephadex G-25 is resistant to 0.2 M NaOH, 0.2 M HCl, 24% ethanol, 30% 2-propanol, and 1% SDS. Diluting the samples prior to gel filtration guaranteed that these concentrations were not exceeded. The virus was eluted with 1 ml of MEM containing 5% FBS and titrated on BHK-21 cells. To check whether gel filtration on Sephadex G-25 efficiently depleted any cytotoxic compounds, the same procedure was performed with the highest concentration of the respective disinfectant, in the absence of virus. The medium eluted from the columns was added to confluent BHK-21 cells in 96-well plates, which were then infected with VSV* (multiplicity of infection of 1 ffu/cell). eGFP expression 16 h post infection indicated that >95% of the cells were viable and supported virus replication.

2.5. Survival of VSV on surfaces

VSV* suspended in 10 μl MEM with 5% FBS was added to flat-bottom 24-well culture dishes containing either glass cover slips or stainless steel coupons. Alternatively, the suspension was directly added to the polystyrene bottom of the wells. The virus was dried under a laminar flow for 30 min before the plate was covered with a lid and kept at 22 °C. Subsequently, the dried virus was re-suspended in 250 μl MEM and titrated on BHK-21 cells. Alternatively, BHK-21 cells suspended in MEM + 5% FBS were added to 6 parallel wells (200,000 cells/well) and incubated for 16 h at 37 °C and 5% CO_2. The number of wells containing eGFP-positive cells was determined by inverse fluorescence microscopy. To elucidate the inactivation of VSV* adsorbed to polystyrene surfaces, the virus was dried for 1 h onto the bottom of 24-well culture plates and

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subsequently incubated for 5 min at 22 °C with 250 μl of either SDS (0.025%, 0.05%, or 0.10%), paraformaldehyde (0.10%, 0.25% or 0.50%), or for 1 min with 250 μl of ethanol (10%, 20%, 30%, 40% v/v). After depletion of the disinfectants on Sephadex G-25 columns (see above), the virus was titrated on BHK-21 cells.

2.6. Heat inactivation and pH treatment of VSV

VSV* stock virus was diluted with MEM/5% FBS to give a titre of 2×10^8 ffu/ml. Triplicate samples were incubated for 30 min at different temperatures using a Perkin Elmer 9600 thermal cycler which was run in the incubation mode. The virus were chilled to 4 °C and then titrated on BHK-21 cells.

VSV* (10 μl) was exposed for 30 min at 22 °C to different pH values by incubation with 10 μl FBS and 80 μl of buffer (50 mM phosphate/citrate, 150 mM NaCl, pH 2.0–6.0, or 50 mM Tris/HCl, 150 mM NaCl, pH 7.0–12.0). Before the virus was titrated on BHK-21 cells, the buffers were exchanged against MEM/5% FBS using Sephadex G-25 columns as described above. When VSV* was treated with either 0.05 M NaOH (pH 12.7) or 0.05 M HCl (pH 1.3), pH 7.0, a neutral pH value was restored by adding the corresponding volume of 0.05 M HCl and 0.05 M NaOH, respectively.

3. Results and discussion

3.1. Long-term stability of VSV in suspension

Recombinant VSV (VSV*) expressing the enhanced green fluorescent protein (eGFP) was used throughout this study (Hoffmann et al., 2010). Replication kinetics and titers of the recombinant VSV* did not differ from the parent VSV (serotype Indiana). In addition, ultracentrifugation and SDS polyacrylamide gel electrophoresis indicated that both viruses had the same protein composition (data not shown). VSV* was therefore expected to have the same physicochemical properties as native VSV. VSV* was suspended in minimal essential medium (MEM) with 5% fetal calf serum, incubated for various time periods at either 4 °C, 22 °C, or 37 °C, and finally titrated on BHK-21 cells taking advantage of the eGFP reporter for detection of infected cells by fluorescence microscopy. It turned out that VSV* was remarkably stable at 4 °C in the presence of serum not showing any significant loss in virus titer over 4 weeks (Fig. 1a). Incubation of VSV* at 22 °C resulted in a gradual decrease of infectivity although some infectious virus was still left after 28 days. When VSV* was incubated at 37 °C, infectious titers declined more sharply and dropped below the detection limit (*i.e.* 12.5 ffu/ml) after 21 days of incubation. These findings indicate that long-term stability of VSV is affected by temperature (see Table 1). To study whether protein supplement would stabilize the virus, three different concentrations of VSV* were incubated at 4 °C for up to 28 days in the presence or absence of 5% foetal bovine serum. If VSV* was incubated in the absence of serum, infectivity declined, in particular when low-titer virus suspensions were used (Fig. 1b). This suggests that serum has a stabilizing effect on VSV

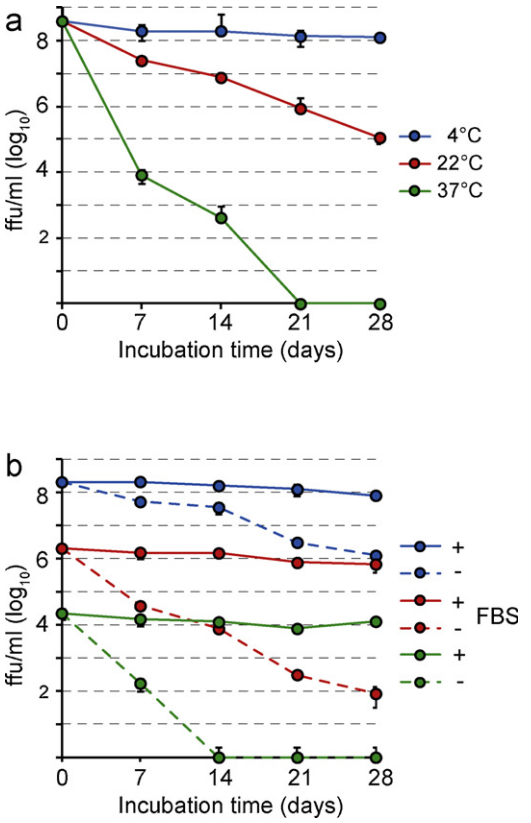

Fig. 1. Stability of VSV in suspension. VSV* was suspended in MEM medium containing 5% FBS and incubated at either 4 °C, 22 °C, or 37 °C for the indicated times. Following incubation, infectious virus titers were determined on BHK-21 cells (a). VSV* was suspended in MEM either containing 5% FBS (continuous lines) or without FBS (interrupted lines) resulting in three different virus concentrations (indicated by blue, red and green lines). The virus was incubated at 4 °C for the indicated times and infectious titers subsequently determined (b). A single long-term experiment with three parallel incubations was performed. Mean values and standard deviations are shown.

Table 1
Life time of VSV as a function of temperature, virus dose, serum supplement, and pH value.[a]

Conditions of incubation	$t_{90\%}$ [b]
Virus suspension + FBS, 4 °C	>28 d
Virus suspension + FBS, 22 °C,	7.8 d
Virus suspension + FBS, 37 °C	2.8 d
Virus suspension (10^8 ffu/ml) + FBS, 4 °C	>28 d
Virus suspension (10^8 ffu/ml) – FBS, 4 °C	12.2 d
Virus suspension (10^6 ffu/ml) + FBS, 4 °C	>28
Virus suspension (10^6 ffu/ml) – FBS, 4 °C	6.4 d
Virus suspension (10^4 ffu/ml) + FBS, 4 °C	>28 d
Virus suspension (10^4 ffu/ml) – FBS, 4 °C	4.3 d
Virus suspension + FBS, 0.05 M NaOH	5.0 min
Virus suspension + FBS, 0.05 M HCl	2.3 min
Virus suspension + FBS, 50 °C	2.2 min
Virus suspension + FBS, 55 °C	25 s

[a] Calculated from Fig. 1a, b, 2a, 3b and 4b, c.
[b] $t_{90\%}$: time required to reduce virus titer by 90% (1 $\log_{10}$).

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infectivity (see Table 1). The high stability of VSV in suspension may facilitate virus spread in livestock husbandry. For example, infected animals release VSV from lesions at throat and mouth into their saliva (Thurmond et al., 1987), which may lead to contamination of water troughs. This may be an important source of infection for other animals drinking from the same trough. On the other hand, VSV is a promising vaccine vector and its stability may favor long-term storage of recombinant live vaccines.

3.2. Stability of VSV* dried on surfaces

In order to elucidate the stability of VSV on surfaces, VSV* suspensions containing 5% serum were added to surfaces composed of either polystyrene, glass, or stainless steel, dried for 30 min under a laminar flow, and incubated for different time periods at room temperature (22 °C, 35% relative humidity). Subsequently, the virus was re-suspended with medium and titrated. Using this approach, infectious virus titers decreased dramatically within the first 24 h (Fig. 2a). Some infectious virus particles could still be recovered after being incubated in dried form for 48 h. The kinetics of virus titer loss slowed down with increasing incubation time. As an alternative approach for assessing VSV "survival", BHK-21 cell suspensions were directly added to virus, which had been dried to either polystyrene, glass or stainless steel surfaces and incubated for different time periods at 22 °C. When VSV* was maintained in dried form for 24 h, infected cells were detected in all six parallel samples (Fig. 2b). The proportion of positive samples decreased from days 1 to 8. At later days, the samples remained negative, indicating that the virus has completely lost infectivity. Infectivity declined on all three materials similarly, suggesting that the material of the surface had no impact on VSV survival. From these findings it can be concluded that the survival time of dried VSV is shorter compared to virus in suspension, probably because humidity is important for the virus to maintain infectivity. The association with organic material (*e.g.* saliva) may prolong the survival time by maintaining a certain level of humidity. In agreement with our findings VSV was found to survive for 3–4 days in infected saliva on pails, mangers, and hay (Hanson, 1952). The ability to survive on surfaces for considerable time may facilitate VSV dissemination by contaminated milking equipment, hands, and feed.

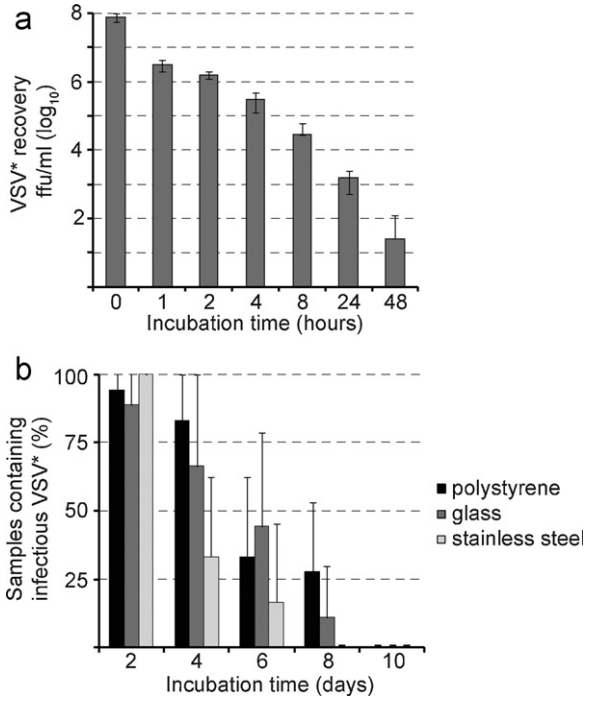

Fig. 2. Stability of VSV dried on surfaces. VSV* suspensions (10 μl) were added to the bottoms of polystyrene 24-well cell culture plates and dried under a laminar flow for 30 min. The dried virus was incubated at 22 °C for the indicated times, suspended in 250 μl MEM with 5% FBS and titrated on BHK-21 cells (a). VSV* (10 μl) was dried to glass cover slips, stainless steel coupons, and polystyrene cell culture plates and incubated up to 8 days at 22 °C (6 parallels per exposure time). BHK-21 cell suspensions were added to the dried virus and incubated for 18 h at 37 °C. Infection of cells was indicated by GFP expression as recorded by fluorescence microscopy (b). Mean values and standard deviations of three experiments are shown.

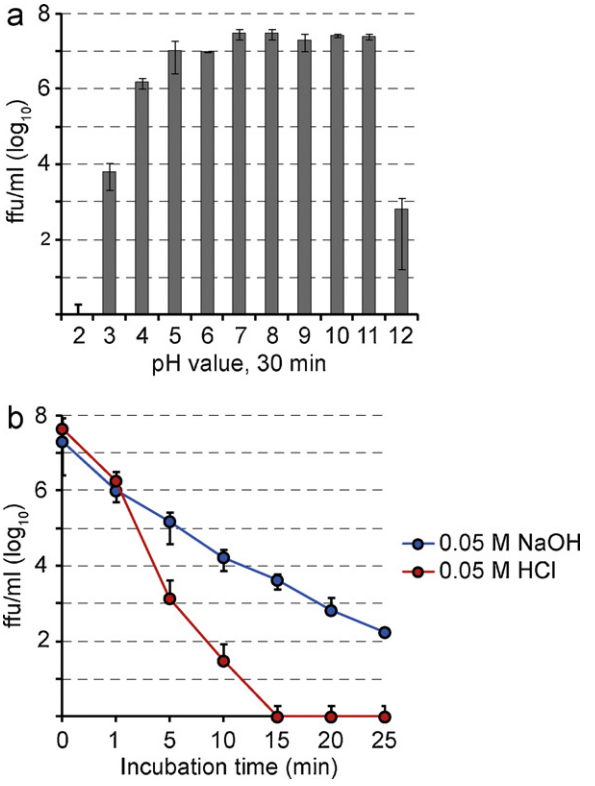

Fig. 3. Effect of proton concentration on VSV infectivity. VSV* was incubated for 30 min at 22 °C with buffers adjusted to the indicated pH values. Subsequently, the buffer was exchanged on Sephadex G-25 columns for MEM cell culture medium and the virus titrated on BHK-21 cells. A representative experiment of two performed is shown. Mean values and standard deviations were calculated from three independent treatments for each pH value (a). The time kinetics of VSV* inactivation by 0.05 M HCl (pH 1.3) and 0.05 M NaOH (pH 12.7) is shown, respectively (b). A representative experiment of two performed is shown. Mean values and standard deviations were calculated from three independent incubations for each time point.

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3.3. Impact of pH value on VSV stability

VSV* was incubated with isotonic buffers adjusted to pH values ranging from 2 to 12 and incubated for 30 min at 22 °C. Thereafter, the buffers were exchanged for cell culture medium taking advantage of small Sephadex G-25 columns. Using this approach, VSV* was found to be fairly stable over a wide pH range (Fig. 3a). In particular basic buffers up to pH 11 did not affect VSV* infectivity at all. Only at pH 12, the virus titer dropped (by 4 $\log_{10}$), however, some infectious virus was still left. Incubation of VSV* with acidic buffers resulted in a pH-dependent reduction of virus infectivity. The lower the pH value the lower the remaining infectivity was. Inactivation of VSV* by a factor of at least 7 $\log_{10}$ was observed when the virus was incubated at pH 2. The time kinetics of inactivation with 0.05 M HCl (pH 1.3) revealed that about 15 min were needed to reduce virus titers below the detection limit (Fig. 3b). In contrast, treatment of VSV* with 0.05 M NaOH (pH 12.7) gradually reduced virus titers with time, although infectious virus was still left after 25 min. The time required to inactivate virus titer by 1 $\log_{10}$ ($t_{90\%}$) were 2.3 min for 0.05 M HCl and 5.0 min for 0.05 M NaOH (Table 1). These results suggest that VSV is more sensitive to acidic than to alkaline conditions. This property may be attributed to the low pH-triggered conformational change of the viral glycoprotein G. The conformational change has been found to be reversible (Roberts et al., 1999), however, the G protein may denature irreversibly if exposed to extreme pH conditions.

3.4. Thermal inactivation of VSV

Inactivation of viruses by heat is a convenient and cheap way of disinfection. In order to determine the sensitivity of VSV* to heat, the virus was suspended in MEM with 5% fetal calf serum, incubated for 30 min at different temperatures, and subsequently titrated on BHK-21 cells. Incubating the virus at 37 °C had no effect on virus titer compared to incubation at 4 °C (Fig. 4a). When the virus was incubated at temperatures up to 46 °C, a moderate decline of infectivity was observed. At 48 °C infectivity dropped drastically (by more than 4 $\log_{10}$ compared to 4 °C), and following incubation at 50 °C infectious virus titers dropped to 25 ffu/ml, which is close to the detection limit. Analysis of time requirements for inactivation of VSV* at 50 °C revealed that 20 min are sufficient to reduce viruses titers by 7 $\log_{10}$ (Fig. 4b). The kinetics of inactivation at 50 °C followed an exponential course (note the logarithmic scale of the axis of ordinate) and took 2.2 min to reduce virus titers by 1 $\log_{10}$ (Table 1). Treatment of VSV at higher temperatures shortened $t_{90\%}$ to 25 s (Table 1). Infectivity was not longer detectable if VSV* was incubated for 4 min at 55 °C (Fig. 4c) or for 1 min at 60 °C (data not shown). These data confirm previously published findings on the thermal inactivation of rabies and other rhabdoviruses (Michalski et al., 1976). Heat treatment of diagnostic samples or blood products may contribute to biosafety as it may inactivate infectious virus without affecting the activity of antibodies or other serum components.

3.5. Chemical disinfection of VSV

Detergents are amphipathic compounds that may affect infectivity of enveloped viruses by solubilizing the viral

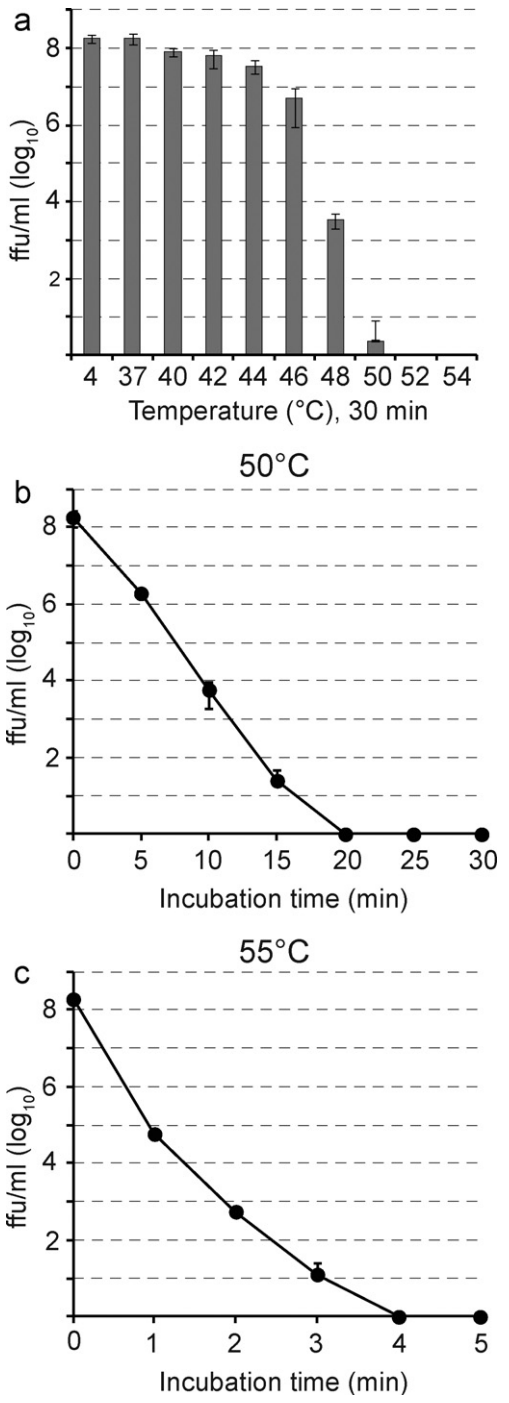

Fig. 4. Thermal stability of VSV. Infectious titers following incubation of VSV* at the indicated temperatures for 30 min (a). Time kinetics of VSV* inactivation at 50 °C (b) and 55 °C (c), respectively. Each experiment was performed two times. Representative experiments (a–c) with three parallel incubations for each temperature is shown. Mean values and standard deviations are indicated.

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membrane along with the integral membrane proteins. The effect of detergents on VSV infectivity was studied with two different compounds Triton X-100, a non-ionic detergent, and sodium dodecyl sulfate (SDS), an anionic denaturing detergent. VSV suspensions containing 10% serum were incubated with the respective detergent for 5 min at 22 °C, purified on Sephadex columns, and titrated on BHK-21 cells. When VSV suspensions were treated with 0.025% Triton X-100, virus titers dropped by 1 $\log_{10}$, while a reduction by 6 $\log_{10}$ was achieved if a 2-fold higher concentration was used (Fig. 5a). In contrast, 0.25% of SDS was required to achieve the same level of inactivation. However, virus dried on polystyrene was already inactivated by 0.05% of SDS. Thus, VSV demonstrates an extraordinary sensitivity to inactivation by detergents with a particular sensitivity to the non-ionic detergent Triton X-100. Since detergents are non-hazardous and cost-effective compounds, they represent attractive disinfectants for the cleaning of contaminated surfaces.

Alcohols such as ethanol, 1-propanol, or 2-propanol are frequently used for disinfection of both surfaces and hands. To study the effect of these alcohols on VSV infectivity, they were directly added to virus suspensions giving final concentrations of 10% up to 70%. Following incubation for 1 min at 22 °C, the mixture was rapidly diluted 1:5 with medium and run on small Sephadex G-25 columns to reduce the alcohol to non-toxic levels. In an alternative approach, the alcohol concentration was reduced to 0.5% by diluting the sample after incubation. The results obtained with the two approaches were essentially the same (Fig. 5b, ethanol, compare black and white bars). However, the approach with the gel filtration columns was more sensitive since virus was not diluted that much.

Treatment of virus with any alcohol at 10% had no major impact on virus infectivity (Fig. 5b). Treatment with 20% (v/v) 1-propanol reduced virus titers by 7 $\log_{10}$, whereas 20% (v/v) of either ethanol or 2-propanol affected virus titers only moderately. However, a reduction factor of at least 7 $\log_{10}$ was achieved when 2-propanol was used at 30% and ethanol at 40% (v/v). These findings indicate that the virucidal activities of the three alcohols differ with 1-propanol being the most effective and ethanol the least effective one (Table 2). Interestingly, 1-propanol has recently been shown to exhibit higher virucidal activity against hepatitis C virus than the two other alcohols (Ciesek et al., 2010). A previous report postulating that alcohols are not effective against VSV (Wright, 1970a,b) was not confirmed. We conclude from our findings that hand disinfectants containing alcohols at $\geq$40% (v/v) or higher will inactivate VSV properly. Ethanol was equally active against VSV* dried on polystyrene (data not shown).

Aldehydes such as formaldehyde and glutaraldehyde inactivate microorganisms by alkylating the amino and sulfhydral groups of proteins and ring nitrogen atoms of purine bases. They are often used for the disinfection of surfaces and medical equipment. In addition, formaldehyde is frequently used for inactivation of viruses in order to prepare safe vaccines. A previous report noted that VSV is relatively resistant to inactivation with formalin (Wright, 1970a), whereas others observed that VSV is sensitive to it (Bachmann et al., 1993). To identify the minimal concentration of formaldehyde required to inactivate VSV, virus suspensions containing 10% serum were incubated with this compound at different concentrations for 5 min at 22 °C. When formaldehyde was used at 0.5%, virus titers were reduced by 7 $\log_{10}$, whereas lower concentrations demonstrated less virucidal activity (Fig. 5c). Glutaraldehyde (pH 7.2) turned out to be more effective than formaldehyde. Virus titers already dropped below the detection limit when incubated with 0.05% of glutaraldehyde. This recommends glutaraldehyde as the preferred aldehyde for inactivation of VSV (Table 2).

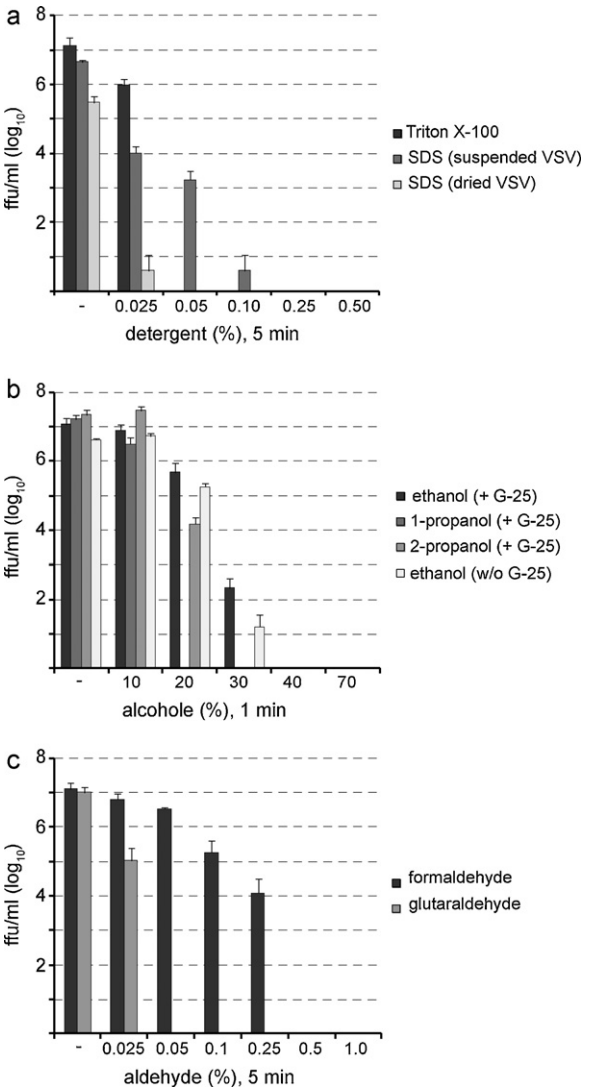

Fig. 5. Inactivation of VSV. VSV* was incubated with either the detergents Triton X-100 and SDS (a), the alcohols ethanol, 1-propanol, and 2-propanol (b), or the aldehydes formaldehyde and glutaraldehyde (c), respectively. The incubations were performed for 5 min (a, c) or 1 min (b) at room temperature (22 °C) followed by depletion of the disinfectants by gel filtration on Sephadex G-25 columns (+G-25) or by dilution (w/o G-25). Virus was titrated on BHK-21 cells. Mean values and standard deviations of three parallel treatments are shown. For each compound, two experiments were performed. One representative experiment is shown (a–c).

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Table 2
Thermal and chemical treatment of VSV leading to reduction of virus titer by $\geq 10^6$ $\log_{10}$.

Treatment at/with	Concentration	Time of treatment (min)	VSV reduction factor ($\log_{10}$)
50 °C	–	30	≥7
55 °C	–	5	≥7
H^+ (high)	pH 2.0	30	≥7
Triton X-100	0.05%	5	≥6
SDS	0.25%	5	≥6
Ethanol	40%	1	≥6
1-Propanol	20%	1	≥6
2-Propanol	30%	1	≥6
Formaldehyde	0.50%	5	≥6
Glutaraldehyde	0.05%	5	≥6

4. Conclusions

The present study suggests that VSV can "survive" outside the host for considerable time, not only in suspension but also in dried form on surfaces. This relatively high tenacity may favor the vector-independent dissemination of the virus. Fortunately, alcohols, aldehydes, and detergents proved to be quite effective in inactivating VSV. Among the compounds tested, 1-propanol, glutaraldehyde, and Triton X-100 turned out to be particularly effective at low concentrations.

Conflict of interest statement

The authors declare that they have no competing interests.

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