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Reversible Inactivation and Desiccation Tolerance of Silicified Viruses

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Long-distance host-independent virus dispersal is poorly understood, especially for viruses found in isolated ecosystems. To demonstrate a possible dispersal mechanism, we show that bacteriophage T4, archaeal virus *Sulfolobus* spindle-shaped virus Kamchatka, and vaccinia virus are reversibly inactivated by mineralization in silica under conditions similar to volcanic hot springs. In contrast, bacteriophage PRD1 is not silicified. Moreover, silicification provides viruses with remarkable desiccation resistance, which could allow extensive aerial dispersal.

The mechanisms and extent of virus dispersal are often unclear. Given the importance of viruses in maintaining microbial diversity and recycling nutrients on a global scale (1) and causing disease (2), understanding virus distribution is essential. However, it is not clear whether virus species are cosmopolitan (3) or display regional endemism (4–8). Interestingly, local hot spring virus dispersal can result from aerosolization by fumaroles (8), indicating at least one possible host-independent dispersal mechanism.

Stratospheric winds are capable of carrying bacteria and fungi from the Sahara Desert as far as the Caribbean Sea (9, 10). However, a critically limiting factor for wind-borne virus spread is the ability of the virus to resist drying; most viruses are highly sensitive to desiccation (for examples, see references 11 to 13). However, if viruses could be reversibly coated in a protective coat in addition to their capsid, they could potentially spread very widely. Silica coating is a particularly attractive possibility, since in hot spring environments, viruses can be coated with silica (14, 15). However, the effect of silicification on virus infectivity was not known. Therefore, we tested both enveloped and unenveloped viruses for their susceptibility and response to silicification. Viruses tested included bacteriophage T4 (16), bacteriophage PRD1 (17), the archaeal virus *Sulfolobus* spindle-shaped virus Kamchatka (SSV-K) (18), and vaccinia virus (VACV) (19).

Bacteriophage T4, PRD1, SSV-K, and VACV were propagated in host cell cultures using *Escherichia coli* B, *Salmonella enterica* serovar Typhimurium LT2, *Sulfolobus solfataricus* strain GΘ, and murine BSC-1 cells, respectively. After growth, cell debris was removed. The resulting viruses were mixed with freshly prepared pH 7.0 to 7.1 sodium metasilicate solution in either 10 mM sodium bicarbonate–5 mM magnesium chloride for bacteriophage T4, PRD1, and SSV-K or Dulbecco's phosphate-buffered saline for VACV to final silica concentrations of 0, 5, and 10 mM (0, 300, and 600 ppm). Solutions were placed in dialysis tubing in a reservoir of the same buffer and silica concentration. The bathing solution was replaced daily. Samples were withdrawn immediately and on days 1, 3, 8, and 10. The virus titer was determined in triplicate by plaque assay. On day 10, aliquots were diluted 1:10 with a 0-ppm silica solution. Plaque assays were performed with these diluted samples on days 12, 14, 16, and 20. On day 10, aliquots were also removed for desiccation tests. Initial drying (except for VACV) was performed with a vacuum concentrator at 4°C and 13 mbar for 4 h. Samples were then desiccated at a pressure of 250 to 300 mbar for 10, 30, and 90 days. Vaccinia virus was air-dried in a biosafety cabinet. Desiccated virus samples were rehydrated with a 0-ppm silica solution. Titers were determined at 1 h and at 10 days after rehydration.

Treatment of viruses in silica solutions had a variable effect on virus infectivity (Fig. 1). Treatment of bacteriophage T4 with silica at 600 ppm (10 mM) caused a loss of infectivity of up to three orders of magnitude (Fig. 1). Effects were less in 300-ppm silica solutions. In contrast, bacteriophage PRD1 was insensitive to silica treatment. The archaeal fusellovirus SSV-K, which is indigenous to high-silica hot spring environments, had an intermediate degree of silica-induced inactivation (Fig. 1). Vaccinia virus responded similarly to bacteriophage T4 to silica treatment (Fig. 1). In summary, bacteriophage T4, the archaeal virus SSV-K, and the animal virus VACV can be inactivated at silica concentrations similar to those found in terrestrial hot springs (20–22). Based on previous silicification studies with bacteria, archaea (23, 24), and viruses (14, 15), infectivity loss on silicification is not unexpected. However, even in supersaturated silica solutions (600 ppm), different viruses were not equally affected (Fig. 1). These data strongly suggest that virus surface characteristics significantly impact silica deposition and thereby their susceptibility to inactivation. Bacteriophage T4, PRD1, and SSV-K have protein capsids (16–18) but have quite different inactivation profiles (Fig. 1). Inactivation of the enveloped virus VACV by silica exposure was similar in magnitude to that of bacteriophage T4, but more rapid (Fig. 1). SSV-K, which is endemic to high silica environments, may be resistant to silicification.

Viruses inactivated by silicification could be reactivated merely by lowering the external silica concentration to below saturation. Following 10 days of silica exposure, both bacteriophage T4 and SSV-K regained infectivity to at least 10% of the initial titer (Fig. 1). Similarly, silicified VACV recovered slightly over 5% of its original infectivity. However, when the 600-ppm silica treatment is compared to the control, VACV demonstrated a nearly 400-fold

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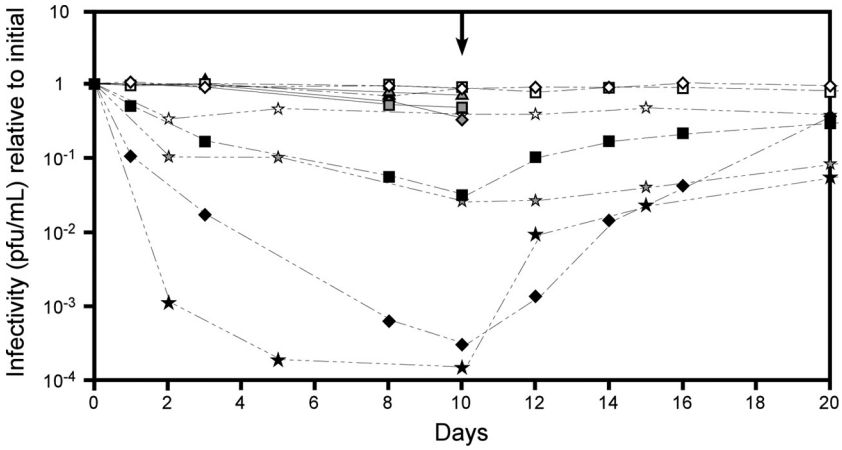

FIG 1 Reversible inactivation of viruses by silica treatment. Effect of silicification on infectivity of bacteriophage T4 (diamonds), SSV-K (squares), PRD1 (triangles), and VACV (stars), normalized to initial infectivity. Black symbols, 600-ppm (10 mM) silica solution; gray symbols, 300-ppm (5 mM) silica solution; white symbols, control (0-ppm silica solution). The vertical black arrow indicates the transfer to a low external concentration of silica. All plaque assays were performed in triplicate with triplicate biological replicates, except for VACV, which had only a single biological replicate. Error bars are obscured by data point symbols.

increase in titer compared to that after 10 days of silica exposure. Beyond showing that the effect of silicification on infectivity is at least partially reversible, these results support the hypothesis that the effect on infectivity was due to the silica coating rather than a physical or chemical damage, which would have led to an irreversible loss of infectivity.

Silicified bacteriophage T4 and the archaeal virus SSV-K have greatly enhanced resistance to desiccation compared to unsilicified virus under conditions similar to stratospheric pressures and dryness. Silicified bacteriophage T4 had detectable infectivity after up to 30 days of desiccation (Fig. 2), whereas unsilicified viruses lost more than seven orders of magnitude of infectivity. SSV-K was similarly protected by silicification, but to a lesser extent than bacteriophage T4 (Fig. 2). SSV-K, however, has a lower starting titer than that of bacteriophage T4, limiting the ability to compare their desiccation resistance levels at later times. Desiccation protection was not absolute, however, as bacteriophage T4 lost more than seven orders of magnitude of titer after 90 days of desiccation. Only VACV—well-known for its innate desiccation resistance—had any infectivity after desiccation. The infectivity of unsilicified VACV dropped three orders of magnitude after desiccation (1.4×10^8 PFU/ml to 2.1×10^5 PFU/ml), consistent with previous data (25), while the silicified VACV dropped four orders of magnitude (1.4×10^8 PFU/ml to 1.6×10^4 PFU/ml). The additional loss of infectivity for the silicified VACV may be the result of damage during silicification. These desiccation results indicate that, for at least some viruses, silicification may provide protection from the effects of drying, thus allowing the viruses to persist for days to weeks under stratospheric pressure and humidity, which may in turn allow global dispersal (10). These data potentially explain some of the conflicting results of virus distribution (3–7). This is particularly true for silicified hot spring viruses that could be aerosolized by fumarole outgassing or dispersed by volcanic activity (6, 8). Responses of silicified viruses to other conditions remain to be tested.

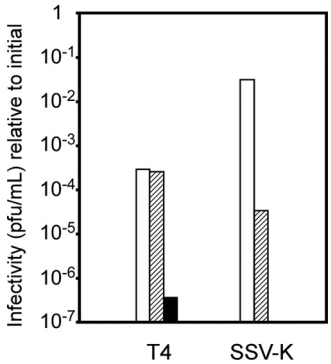

FIG 2 Silicified viruses are resistant to desiccation. Effect of silicification on infectivity of silicifiable viruses after desiccation (VACV data shown in text), normalized to the initial infectivity. White bars, infectivity after 10 days of silicification; crosshatched bars, infectivity after 10 days of desiccation and 10 days of rehydration; black bars, infectivity after 30 days of desiccation and 10 days of rehydration.

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