Novel antiviral activity of dialdehyde starch

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A significant effort worldwide is being directed toward development of novel biocides against drug-resistant bacterial and viruses because of the significant potential human infection risks in the general population. We report here the discovery of a strong antiviral biocide, dialdheyde starch (DAS). Antiviral tests were carried out against three non-envelop viruses, including two bacterial viruses MS2 and PRD1, and one human virus Poliovirus. Dialdehyde starch aqueous suspensions were effective biocides against these three test viruses in a 1 hr exposure test. The antiviral activity was significantly enhanced in a four-hour exposure test, with maximum seven orders of magnitude reductions against MS2 and PRD1, and four-order reduction against Poliovirus. The antiviral activity of dialdehyde starch was found to be pH dependent, being more active in alkaline and acidic conditions than in neutral conditions.

Dialdehyde starch (DAS) is a polymeric dialdehyde prepared from selective oxidation of starch by periodate ions which cleaves the C2-C3 bond of the anhydroglucose units of the starch polysaccharide chain to form the dialdehyde groups (Fiedorowicz and Para, 2006) as shown in Figure 1.

DAS is quite effective as a crosslinking agent by reaction with other functional groups such as hydroxyl and amino groups. Based on its crosslinking ability, it has been employed in the paper, textile, packaging material and leather industrial applications (Ellis et al. 1998; Kanth et al. 2006). DAS has also been studied for biomedical applications such as the enhancement of the protein absorption and drug delivery carrier (Hersel et al. 2003; Yu et al. 2007). DAS has been reported to show low toxicity toward mammalian cells (Yu et al. 2007) and to exhibit low toxicity to rats by dermal and respiratory routes (Tang et al. 2003).

Unlike the well-studied antimicrobial activity of the small molecular water-soluble dialdehydes, for example glutaraldehyde (McDonnell and Russell, 1999), the study of the antimicrobial behavior of DAS is limited. An early US patent described a method to generate an antibacterial surface by suspending dialdehyde polysaccharide granules
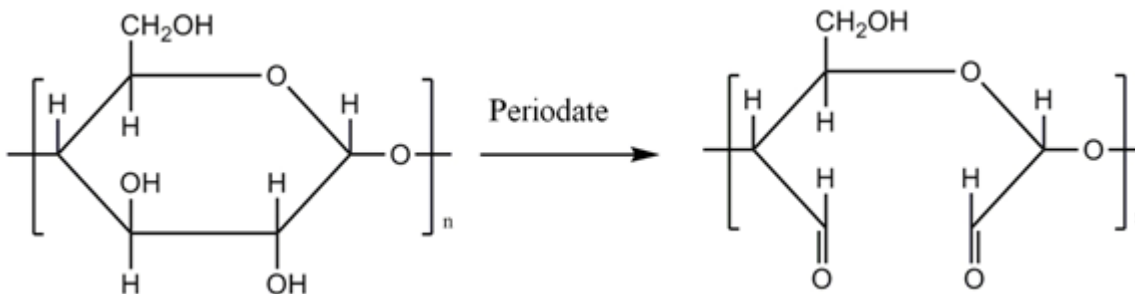
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Figure 1. Periodate oxidation of starch to generate dialdehyde starch.

in the agar solution. Once the agar solution solidified, inhibition of gram-positive/gram-negative bacterial growth was observed on the surface containing dialdehyde cellulose. Inhibition of gram-positive bacterium *S. aureus*, but not gram-negative bacterium *E. coli* was observed on the surface containing DAS (Siragusa, 1977).

With the recent concern about the potential threat from the drug-resistant virus and bacteria, the development of new biocides, which overcome the microbial resistant mechanisms, is necessary and critical. One strategy has been to design a polymeric biocide, for example, N-Alkylated polyethylenimine (Lin et al. 2002; Lin et al. 2003). This class of compound was reported to be active against quaternary ammonium compound (QAC)-resistant *S. aureus*. The immobilized polycations overcame the drug-resistant mechanisms of bacteria such as multi-drug-resistance pumps developed by the bacteria to pump out the normal diffusible biocides (Lewis and Klibanov, 2005). QAC -type biocides have an effect on enveloped but not on non-enveloped viruses; they are frequently used as bactericides (McDonnell and Russell, 1999).

DAS is a reactive polymer with low toxicity, considering its reactive dialdehyde functional groups, similar to glutaraldehyde. The antiviral efficacy of DAS aqueous and granular suspensions at different pH and incubation time against three non-envelop viruses (MS2, PRD1 and Polio) were examined in this study. MS2 is similar to many human viruses in size and in having RNA. PRD1 is a larger virus that has DNA and contains some lipid. Poliovirus is a well-studied example of a human enterovirus. These viruses have been investigated in studies on the inactivation of viruses (McDonnell and Russell, 1999; Lukasik et al. 2000; Katz and Margolin, 2007).

MATERIALS AND METHODS

DAS was purchased from Sigma (P9265) and used without further purification. The as-received DAS was in a granular form. Three grams of DAS was stirred with 97 g of deionized water at 95°C for 2 hrs. The suspension was then cooled to room temperature to obtain a 3% DAS aqueous suspension. The pH values of the DAS suspension before the heat treatment and after the heat treatment were ca. 3.8

and 3.0 respectively. The DAS granular suspension was prepared by simply mixing as-received DAS with deionized water at room temperature (DAS granular suspension).

The phosphate buffer saline (PBS) solutions were prepared with pH equal to 7.4. Other pH values of PBS solutions (from 3.0 to 8.7) were adjusted by HCl/NaOH. The pH values of DAS aqueous suspensions were also adjusted by HCl/NaOH with a PBS buffer.

The bacteriophages used in this study and their hosts were as follows: MS2 (ATCC 15597-B1), *Escherichia coli* C-3000 (ATCC 15597) and PRD1, *Salmonella typhimurium* (ATCC 19585). Their concentrations were approximately 10^9 PFU/ml (Plaque forming units). Human virus Polio virus (1 chat strain) was also selected with approximately 10^7 PFU/ml concentration. All viruses were supplied by the Department of Microbiology and Cell Science, University of Florida.

The antiviral test of DAS granular suspension or aqueous suspension were prepared by adding 0.1 ml virus stock into 9.9 g test medium with the final concentration of DAS fixed at 2.7% weight percentage. The suspensions were stirred for 1 hr and 4 hrs. Samples were taken after 1 hr and 4 hrs for the plate counting. Samples of bacteriophages were serially diluted in 1% tryptic soy broth (TSB broth).
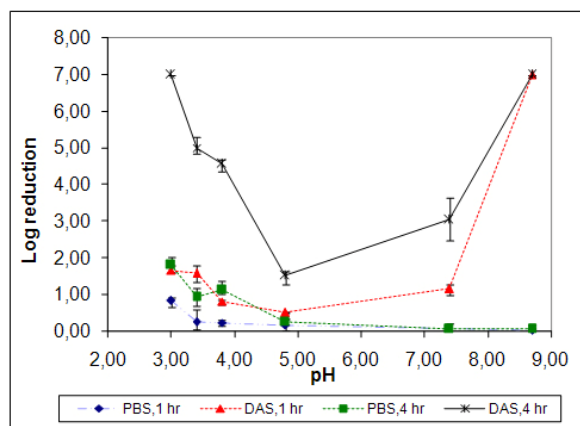

Figure 2. Inhibition of MS2 infectivity by 2.7% DAS aqueous suspension and PBS at different pH and incubation times.
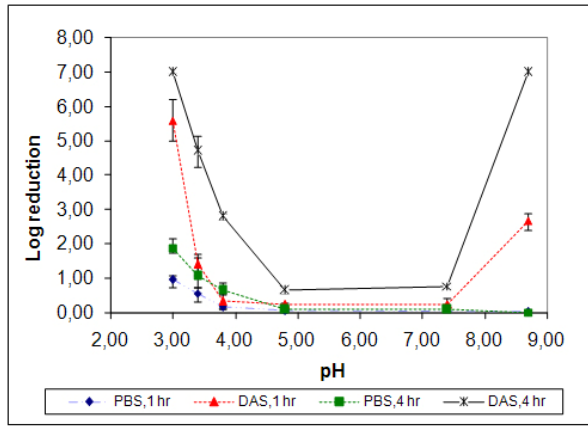
Figure 3. Inhibition of PRD1 infectivity by 2.7% DAS aqueous suspension and PBS at different pH and incubation times.

0.1 ml bacteriophage sample of each dilution was mixed with the appropriate host and assayed using a soft agar overlay. Samples of Polio were diluted in Minimal Essential Medium (MEM) with 2% fetal calf serum (FCS). 0.1 ml Polio sample of each dilution was plated on BGM cells using an agar overlay procedure. More details of culture and plate count of viruses have been described in the literature (Lukasik et al. 2000).

In our study, we found the numbers of virus PFU in PBS control (pH = 7.4) and in as-prepared DAS aqueous suspension at zero incubation time were the same in our experimental condition. The numbers of host bacterial colony forming units in TSB broth with and without residual DAS (based on the zero dilution) were also the same for up to 4 hrs incubation time. It was also found that the incubation time had no effect on the numbers of infectious viruses PFU in PBS control up to 4 hrs. The number of virus PFU in PBS control at the one-hour incubation time was chosen as the baseline to calculate the log reduction

The log reduction, which was defined as: Log reduction = Log N_{c1} - Log N_{tt} was used to determine the antiviral activity of DAS.

Where N_{c1} was the mean value of infectious viral units in the controlled sample at time 1 hr, N_{tt} was the mean value of infectious viral units in the tested sample at time t (t = 1 or 4 hrs). Based on the definition, one log reduction stands for 90% reduction in numbers of infectious virus. The mean log reduction was calculated based on triplicate tests and reported as mean ± standard error in the results.

RESULTS AND DISCUSSION

As-prepared DAS aqueous suspension is acidic with pH 3.0. Antiviral experiments were carried out to understand the virus kill mechanisms of DAS aqueous suspension in different pH values. The pH effect on the antiviral activity was studied by adjusting the pH values of PBS buffer and 2.7% DAS aqueous suspension in the pH range from 3.0 to 8.7 as shown in Figure 2, Figure 3 and Figure 4. For the phosphate buffer saline, weak inactivation of the MS2 and PRD1 were observed in the pH 3.0-3.8. No inactivation was found for MS2 and PRD1 for other pH values. Furthermore, in the selected pH range, inactivation against Polio of the PBS buffer solutions could not be detected. The DAS aqueous suspensions showed a much stronger antiviral activity against chosen viruses in the studied pH range, especially in the acidic and alkaline conditions. The antiviral activity was significantly enhanced with increasing test time. These results strongly suggest that the dominant inactivation mechanism can be attributed to the activity of aldehyde groups instead of the acidity of the DAS aqueous suspension. In the mild condition, for example, at pH = 4.8, the antiviral activity was minimal in the selected pH values for the DAS aqueous suspensions. Sloan et al. (1956) have studied the reaction between DAS aqueous suspension and urea. They found at room temperature, there was no reaction at neutral or slightly acidic pH, but much faster reactions at alkaline pHs (Sloan et al. 1956). The antiviral results presented in our study are consistent with the earlier reports that glutaraldehyde was more active at alkaline than at acidic pHs (McDonnell and Russell, 1999). The pH dependence of DAS activity could be explained by the chemical structure of DAS. It has been reported that the carbonyl groups in DAS are not all free functionalities. Hemiacetal or hemialdal linkages are also formed (Haaksman et al. 2006). However these linkages are fairly easy to break to liberate free carbonyl groups, which can be catalyzed by acid or base (Belarmino et al. 2003).

The antiviral activity of DAS is not only pH-dependent, but also related to its dispersion technology. The antiviral activities of 2.7% DAS aqueous suspensions (DAS-AS) and granular suspensions (DAS-GS) are illustrated in Figure 5. As indicated in Figure 2 and Figure 3, the pH of the PBS buffer itself has inhibition of the infectivity of MS2 and PRD1 in some conditions. This inhibition from pH was subtracted in the reported log reduction shown in Figure 5.
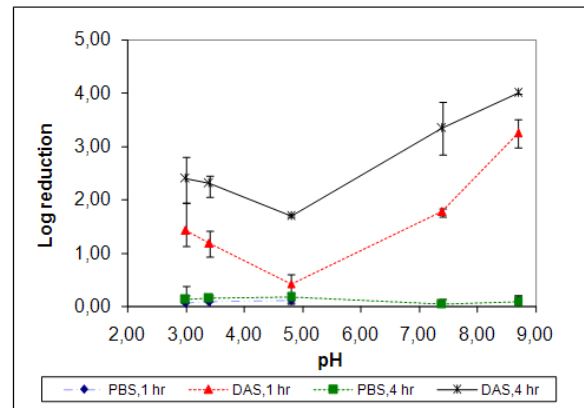

Figure 4. Inhibition of Polio by 2.7% DAS aqueous suspension and PBS at different pH and incubation times.
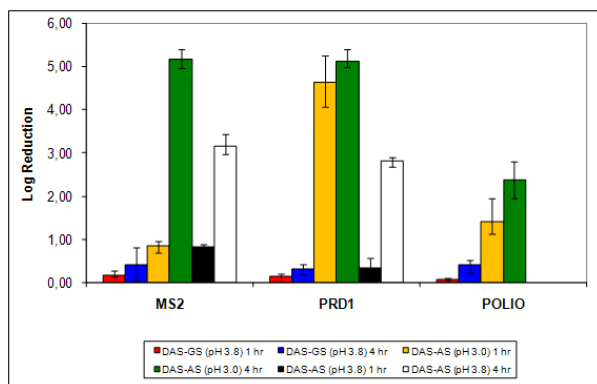
Figure 5. Antiviral test results of 2.7% DAS aqueous and granular suspensions against MS2, PRD1 and POLIO at different incubation times.

The antiviral activities of the DAS granular suspensions (pH 3.8) were very limited for the selected viruses for up to 4 hrs incubation. However, as-prepared DAS aqueous suspensions (pH 3.0) showed a significant antiviral activitie against these three viruses in 1 hr incubation. Furthermore, the antiviral activities were significantly enhanced in four-hour incubation. When the pHs of as-prepared DAS aqueous suspension were adjusted to the same pH value of the DAS granular suspension, DAS aqueous suspensions still demonstrated significant antiviral activities against MS2 and PRD1 in four-hour incubation.

The difference in the antiviral activity between the DAS aqueous suspensions and the DAS granular suspensions may be attributed to the physical and chemical changes of the DAS caused by heating in the water. The molecular weight of the DAS was found to decrease because of the swelling and fragmentation of the DAS granules during heating, but the degradation occurred to a very low extent when the DAS was heated up to 5 hrs at 90ºC with pH below 4 (Veelaert et al. 1997a). The DAS aqueous suspension prepared in our study became homogeneous and transparent with a yellow color upon heating at 95ºC for 2-hrs. After several hours storing at room temperature, gel formation was observed in the DAS aqueous suspension. The weak gel structure could be easily disrupted by simple shaking. Veelaert et al. 1997b, proposed a gelation mechanism including the DAS granules swell first, followed by the disruption of the granules to release the DAS polymeric molecules. Finally, the gel formation by physical entanglements and chemical crosslinks occurs. This gel structure can be destroyed by mechanical force to form the low viscosity suspension (Veelaert et al. 1997b). The stronger antiviral activity of DAS aqueous suspension could be explained by this mechanism, since more DAS polymeric molecules are released from the DAS granule.

In summary, DAS aqueous suspension demonstrated strong antiviral activity against three non-envelop viruses including two bacterial viruses PRD1 and MS2, and another human virus Poliovirus. The antiviral mechanism

of the bioactivity of DAS can be attributed to the reactivity of dialdehyde groups. The reactivity of dialdehyde groups is pH dependent. Studies are currently underway to determine the antiviral mechanistic features of DAS and will be reported in future publications. The investigations of DAS aqueous suspension against other microorganisms such as enveloped virus and gram-negative/gram-positive bacteria as well as the application of DAS onto surface to generate the antimicrobial surface will be presented in other publications.

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