



Antiviral activity of antimony and arsenic oxides

N. Charan, N. Lavanya, B. Praveen, A. Praveen, A. Sridevi and G. Narasimha*

Department of Virology, Sri Venkateswara University, Tirupati, A.P, India

ABSTRACT

The antiviral activity of metals oxides, Arsenic (As_2O_3) and Antimony oxides (Sb_2O_3) were studied. These two metal oxides showed an excellent veridical property on viral strain bacteriophage. With increasing the metaloxide concentration the viral inactivation process also increased. The Antimony oxide at 10 and 12 ppm drastically reduced the viral growth, whereas Arsenic oxide at this concentration completely inhibited the viral multiplication in the host strain is an indication of virucidal nature of metal oxides.

Key words: *Escherichia coli*, Bacteriophage, Arsenic and Antimony oxides, Antiviral activity.

INTRODUCTION

Microorganisms, such as bacteria, fungi, and viruses causes severe infections in human beings. There is need to search for new antimicrobial and antiviral agents from natural and inorganic substances. The antiviral properties on chemical agents iodine and chlorine dioxide against bacteria phage and poliovirus have been reported, and finally concluded that oxidative damage of sulphydryl groups in the protein coat was an important aspect in the killing mechanism [1] The bacteria phage has been recommended in the past as a model phage for chlorine and other disinfectant inactivation studies, however it may be unduly sensitive to iodine. The antiviral mechanism of As_2O_3 and $AgNO_3$ [2] may lead to the development of agents with potent activities against the various viral strains [3]. Information of antiviral property of heavy metals and their oxides is scanty. Hence an attempt was made on this study to know the antiviral effects of metals oxides..

MATERIALS AND METHODS

The bacteriophage isolation is carried out by following steps [4]

Collection of metal oxides:

The metal oxides Arsenic (As_2O_3) and antimony (Sb_2O_3)oxides were obtained from Department of Chemistry, Sri Venkateswara. University, Tirupati, India

Isolation of *E.coli* from sewage:

For isolation of bacterial strain *E.coli*, a main host for virus, one loopful of sewage water was poured on EMB agar medium by quadrant streak method under sterile conditions. And the plate was incubated in incubator at 37 °C for colony development. After incubation, the colonies with metallic shine (unique nature of *E.coli*) was observed. Colonies were transferred to nutrient broth and kept for shaking for preparing *E.coli* suspension.

Enrichment of Bacteriophages

For the enrichment of bacteriophages, 45 ml of sewage water was transferred to conical flask; 5ml of 10X nutrient broth was transferred. This preparation was kept for mechanical shaking for 5-6 hrs at room temperature

Viral inactivation process

Bacterial strains were cultured in TGYE medium with following chemical ingredients g/L (Tryptone; 10, Glucose; 10, Yeast extract; 1, NaCl; 8. Typical phage preparations contain approximately 1×10^7 - 10^{11} cfu/ml. During each experiment, metal oxides were added to concentrations at 2, 4, 8 and 10 and 12 ppm respectively. Bacteriophage suspension was added to each solution and the mixture was vortexed.

Observation of plaque formation

The above enriched sewage sample was filtered through the sterile bacteria retaining filter and filtrate was used as phage source and it was collected in sterile test tube. Actively growing *E.coli* was diluted in sterile nutrient broth and to this phage suspension was added. Now the hard agar medium, being maintained at 70°C in water bath was taken and poured to uniform thickness. Now both the phage suspension and the *E.coli* suspension were mixed in soft agar medium and poured into replicative plates. After solidification is over the plates were incubated at 37°C for 24 to 48 hrs. After incubation, the plates were observed for plaque formation. More number of plaques was observed in 10^{-1} dilution plate when compared to other dilutions inoculated plates. They were identified and marked. Again the plates were kept for incubation, next day size of Plaques was to be increased when compare to previous day. Plaques were confirmed by inoculation.

RESULTS

Observation of plaque formation (after addition of metal ions)

The effect heavy metal oxides on viral strain MS2 bacteriophage were studied and the results listed in table 1. With increasing the metal oxide concentration from 2-12 ppm the plaque formation number decreased on the medium. Various metal concentrations used in this study, the Antimony oxide at 10 and 12 ppm drastically reduced the plaques number, whereas Arsenic oxide at same concentration completely inhibited the viral plaque formation due to complete inactivation of viral growth in *E.coli* bacterial cells/colonies on the medium (table.1). Among two metals oxides tested in this study, As₂O₃ shown more veridical property than the Sb₂O₃. As₂O₃ is more effective in suppressing the cell growth at a lower dose (4-8 ppm), compared to Sb₂O₃. Nearly 50 percentage of viral growth inhibition was observed at 8 to 12 ppm concentration of Sb₂O₃ whereas cent percent inhibition observed in the same concentration of As₂O₃ (Table. 1), than the control (without metal treatment) (Table.2).

Table.1. Antiviral properties Arsenic and Antimony metal oxides

Plate No	Metal oxide conc.(in ppm)	No. of plaques* after metal treatment	
		As ₂ O ₃	Sb ₂ O ₃
1	2	8	14
2	4	5	9
3	8	ND	8
4	10	ND	6
5	12	ND	4

Table.2. Total number of bacteriophages (without metal treatment) on medium (Control)

Plate No	Dilution of the Phage Suspension	No. of Plaques*
6	10^{-1}	66

Note. ND: Not detected

DISCUSSION

The use of phage in the treatment of bacterial infections is an attractive to existing therapies (antibiotics), because unlike broad spectrum antibiotics phage target particular host and are unlike to illicit resistance in untargeted bacterial strains also, unlike chemical therapeutic agents, phages are not susceptible to the onset of bacterial resistance because they have ability to evolve with their host. The bacterial host for the phage isolated in this study was found to be similar to *Citrobacter freudii*, a common enteric bacteria belonging to the family Enterobacteriaceae. *C.freudii* is commonly found in sewage and has been associated with nosocomial

infections in the urinary, respiratory, and biliary tracts of debilitated hospital patients, this organism represents an increased health risk because an important aspect of this organism's physiology is its ability to resist the affects of antibiotics typically prescribed to treat the infections it causes. The mechanism of disinfection by these metal oxides is not completely clear, but could include abrasive properties, as well as oxidative powers. A hypothesis that heavy metal oxides damage virion are stick to them and prevent binding to the host cell is a consideration that need to be explored. In this study, biocidal activities of a series of adduct and graphs of before and after treatment of the metal oxides. Arsenic trioxide compound (As_2O_3) has its ability to suppress cell growth and cell viability.

Metal oxides are potent inhibitors for the bacteriophages at different minimal conditions. number of in vitro studies have contributed to the understanding of possible mechanisms by which arsenic therapy leads to induction of apoptosis, inhibition of growth and angiogenesis, modulation of cellular signaling pathways, perturbation of cellular redox status, and promotion of differentiation[5]Arsenic trioxide inhibits the growth and survival of multiple myeloma cell lines as well as patient cells in a dose and time-dependent manner[6-9]The two primary mechanisms control the oxidant disinfection efficiency by hydroxyl radicals[10]oxidation and disruption of the cell wall and membrane with resulting disintegration of the cell (oxidation ability is due in part to its standard reduction potential[11]diffusion of the disinfectant into the cell or particle where it may inactivate enzymes, damage intracellular components, interfere with protein synthesis[12]. Diffusion of the disinfecting species into the cells is a function of the charge, molecular weight, and half-life of the disinfectant. Hydroxyl radical reacts with most biological molecules at diffusion-controlled rates. Therefore, disinfection by hydroxyl radicals may be limited by mass transfer through the cell wall or cell membrane[13]The Titanium dioxide-mediated poliovirus inactivation, although slow, whereas we reported to be four times faster than *E.coli* form bacteria inactivation, which correlates with the diffusion-controlled oxidation that may be occurring. The lower surface to volume ratio of the viruses may provide greater rates of hydroxyl radical reaction with intracellular biological molecules compared with the larger bacterial cells. The relatively slow diffusion of hydroxyl radicals into viruses, and particularly bacterial cells, may be the cause of its low disinfection rate, and may limit its use as a disinfectant [13]..

CONCLUSION

In this study, we concluded, selected two metal oxides Arsenic and Antimony exhibited antiviral properties on bacteriophage virus. With increasing the metal oxide concentration the viral inactivation process also improved. Compare to Antimony oxide, The Arsenic oxide act as an excellent antiviral agent on Bacteriophage by the indication of decreasing of plaques formation number on the medium.

REFERENCES

- [1] G. M. Byrion and J. Silverstein, *Water Res.*, (1999), 33, 169–179.
- [2] Der-Ren Hwang,Yuan-Chin Tsai,Jin-Ching Lee,Kuo-Kuei Huang,Ren-Kuo Lin,Chia-Hua Ho, Jeng-Min Chiou,Ying-Ting Lin,John T. A. Hsu, and Chau-Ting (2004) *Antimicrobial agents and chemotherapy* , p. 2876–2882.
- [3] P.Roger ,B. Johnson Robert C,Andrew M. Kropinski, Erika J. Lingohr,Carlton L. Gyles. N. Jamalludeen, (2007) *Veterinary Microbiology* 124 47–57
- [4] N. Beaudoin,Danielle R DeCesaro, Debrah LDurkee, and Susan E Barbaro, (2007) *Rivier Academic Journal*, Volume 3, Number 1, Spring.
- [5] W.H. Miller Jr, , Schipper HM, Lee JS, Singer J, Waxman S.(2002). *Cancer Res* ; 62:3893–3903
- [6] W.H.Park, Seol JG, Kim ES, Hyun JM, Jung CW, Lee CC (2006). *Cancer Res*;60: 3065–3071.
- [7] P.Rousselot , Labaume S, Marolleau JP, Larghero J, Noguera MH,Brouet JC(1999). *Cancer Res* ; 59: 1041–1048.
- [8] T.Hayashi, T.Hidemitsu, M.Akiyama Richardson P, Schlossman RL, Chauhan (2002) *Mol Cancer Ther.*; 1: 851–860.
- [9] S.Liu Q, Hilsenbeck, Y.Gazitt (2003). *Blood* ; 101: 4078–4087.
- [10] J.R. Bolton, K.G. Linden (2003), *J. Environ. Eng.* 129; 209–215.
- [11] J.H. Baxendale, J.A. Wilson,(1957). *Trans. Faraday Soc.* 53 ;344–356.
- [12] J.M.Montgomery, (1985) .In. Water Treatment Principles and Design, John Wiley, New York, 1985.
- [13] R.J.Watts, S.Kong,M.P.O,C.M.Glenn,B.H.Henry,(1995). *Water Res.* 29, 95–100.