Effect of Iron-Doped Apatite Nanoparticles on a Eukaryotic Host-Virus System

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Abstract

Bacteriophage (phage) are bacteria-specific viruses that can provide alternative antibiotic treatment.[1-7] Previous investigations have found specific apatite nanoparticles (ANPs) exhibit an unprecedented effect on phage infections *in vitro*. Through the addition of iron-doped apatite nanoparticles (IDANPs) to phage in solution, bacterial death zones (plaques) increase up to 128 % higher than phage-alone controls.[8] These results are therefore of great interest for antibacterial applications. The ANPs described have been synthesized under many conditions and tested *in vitro* with gram-negative and gram-positive strains of bacteria. Results indicate apatite nanoparticles specifically synthesized at 25°C to 45°C and doped with 20 to 30% iron provide the greatest increase in phage infections in both gram types. IDANPs used resemble hydroxyapatite, a mineral that is well known to be biocompatible and most analogous to the inorganic constituent of mammalian bone and teeth,[9] thereby allowing such particles to enter a physiological system without significant risk of immune system rejection. While the phenomenon of IDANP enhancement of phage infection has been replicated many times in the laboratory, the specific mechanisms involved remain elusive. In order to further understand the phenomenon of increasing phage infections seen in a prokaryotic system, the present authors have replicated the experiments in a eukaryotic system. Prokaryotic IDANP experiments have been replicated with Chlorella NC64A and its virus, PBCV-1. Specimens were received from the University of Nebraska where both have been extensively studied and published.[10-15] Results indicate that IDANPs synthesized at 25°C and doped with 30% iron do not increase infection of Chlorella NC64A by PBCV-1 (Figure 1 and 2), indicating that characteristics inherent to prokaryotic systems may allow for the effect of IDANPs on viral infection. Presently, IDANPs synthesized under various conditions, including alternative synthesis temperatures, iron-doping inclusion percentages, citrate amounts, as well as those synthesized with alternative doping agents are being studied in the Chlorella system. Subsequent results of further investigations will allow for a complete comparison of one eukaryotic system with that of the prokaryotic systems previously studied.

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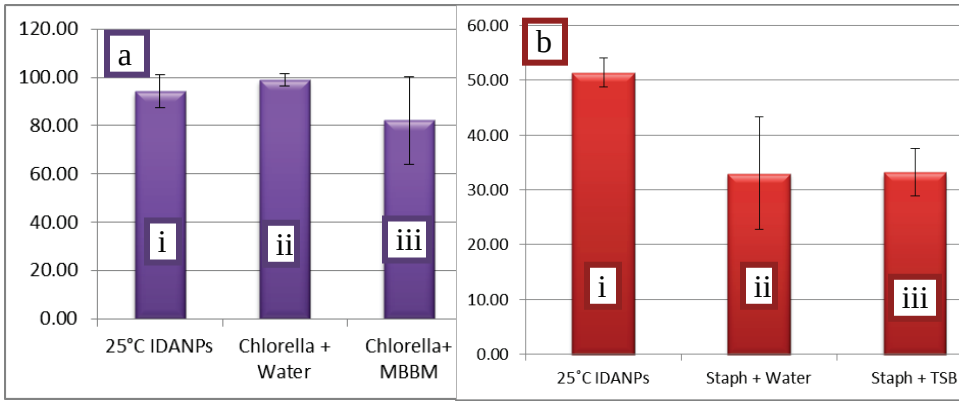

Figure 1. Excel data of results obtained during two different viral infection experiments. Numbers on the left axis indicate the average number of holes found on five agar plates. Negative controls in all cases contained no holes in the agar. Error bars were determined by a single standard deviation as calculated from all data obtained in each category. Results show that while a statistically significant increase in phage infection occurs in a prokaryotic system when IDANPs are added, there appears to be no significant increase in viral infection in the Chlorella NC64A system when IDANPs are added. **a.** Chlorella NC64A infections by PBCV-1 virus in the case that: i. IDANPs synthesized at 25ºC with 30% iron incorporation were added to Tryptic Soy Top Agar, virus, and cells prior to plating, ii. sterile deionized water was added to Tryptic Soy Top Agar, virus, and cells prior to plating, or iii. cell media was added to Tryptic Soy Top Agar, virus, and cells prior to plating. **b.** *Staphylococcus aureus* infections by phage in the case that: i. IDANPs synthesized at 25ºC with 30% iron incorporation were added to Tryptic Soy Top Agar, phage, and cells prior to plating, ii. sterile deionized water was added to Tryptic Soy Top Agar, phage, and cells prior to plating, or iii. cell media was added to Tryptic Soy Top Agar, phage, and cells prior to plating.

Figure 2. Bio Rad Molecular Imager Gel Doc XR+ with Image Lab Software was used to obtain images of agar plates following a single viral infection experiment. Holes in the agar indicate sites of viral infection and cell lysis of Chlorella cells by virus PBCV-1. Results indicate viral infections do not increase at statistically significant amounts when IDANPs are added to virus and cells. **a.** Chlorella NC64A cell lawn incorporated with PBCV-1 virus, Tryptic Soy Top Agar, and cell media. **b.** Chlorella NC64A cell lawn incorporated with PBCV-1 virus, Tryptic Soy Top Agar, and IDANPs synthesized at 25ºC with 30% iron incorporation and dispersed with citrate. **c.** Chlorella NC64A cell lawn incorporated with PBCV-1 virus, Tryptic Soy Top Agar, and sterile deionized water.