INACTIVATION OF BACTERIOPHAGE ØX174 BY SUBLETHAL NITROUS ACID - PRODUCED LESIONS

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Summary

Nitrous acid (HNO_2) treated whole ØX174 bacteriophage particles, but not treated ØX174 DNA, were found to lose viability while being stored at 5°-8°C at pH 8.0. Thus, although the DNA moiety of the virus was the target for HNO_2 inactivation when infectivity was measured immediately after treatment, the protein moiety was implicated in loss of infectivity during storage following HNO_2 treatment.

The viability of HNO_2 treated ØX174 DNA depended on the age of the spheroplast culture used for the bioassay. Survival curves of treated DNA showed slower inactivation on newly prepared spheroplasts than on "old" ones. Some HNO_2 lesions which are normally sublethal are apparently lethal in older spheroplasts.

Introduction

Nitrous acid (HNO_2) has long been used to inactivate and to mutagenize viruses.(1,2,3) It is usually assumed that the primary action for inactivation involves the nucleic acid, in particular, deamination of the bases.(2,4) Recently it has been reported that DNA is the primary target for HNO_2 inactivation for ØX174 bacteriophage.(5) This paper reports inactivation by sublethal lesions apparently caused by HNO_2 action on the coat protein of ØX174 and by HNO_2 action on the DNA.

Materials and Methods

The bacteriophage ØX174 mutant am 3 and the permissive host

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E. coli CR were used.(6,7) The media, phage bioassay, and phage DNA bioassay have been previously described.(5)

The nitrous acid treatment of the virus and the free DNA was performed using acetate-buffered $NaNO_2$ at pH4.4.(3,8) The treatment was halted by diluting an aliquot 1:10 into 0.25 M Tris, 0.012 M EDTA (pH 8.0) at room temperature. Portions of the treated samples were assayed immediately and the remainder stored in a refrigerator (5°-8°C) for further assay. The activation energy for HNO_2 inactivation of ØX174 has been found to be 15 Kcal/mole.(9)

ØX174 DNA was prepared by phenol extraction (at 50°C) from a virus stock of about 10^{13} PFU/ml containing 1 mg bovine serum albumin per ml. The aqueous layer was then exhaustively dialyzed against 0.01 M Tris (pH 8.0), 0.005 M EDTA. The resulting DNA solution was free of phenol and protein as indicated by UV spectroscopy. Bioassay indicated no infective phage particles remained after the deproteinizing treatment.

Results

The survival of HNO_2 treated phage followed one-hit exponential kinetics when assayed immediately after treatment (Fig. 1). When assayed on succeeding days, the survival curve became multi-component in nature. Those viruses treated longest with HNO_2 lost infectivity fastest. Figure 2 shows that the loss of infectivity after a few days storage was exponential with time for all the treated samples. The initial rapid loss of infectivity also increased with increasing HNO_2 treatment. The same results were obtained when the bioassay was done at 31°, 37°, or 42°C or with bacteria from late log phase or stationary phase.

The survival of HNO_2 treated ØX174 DNA during storage was also followed. There were no significant differences in slopes among the

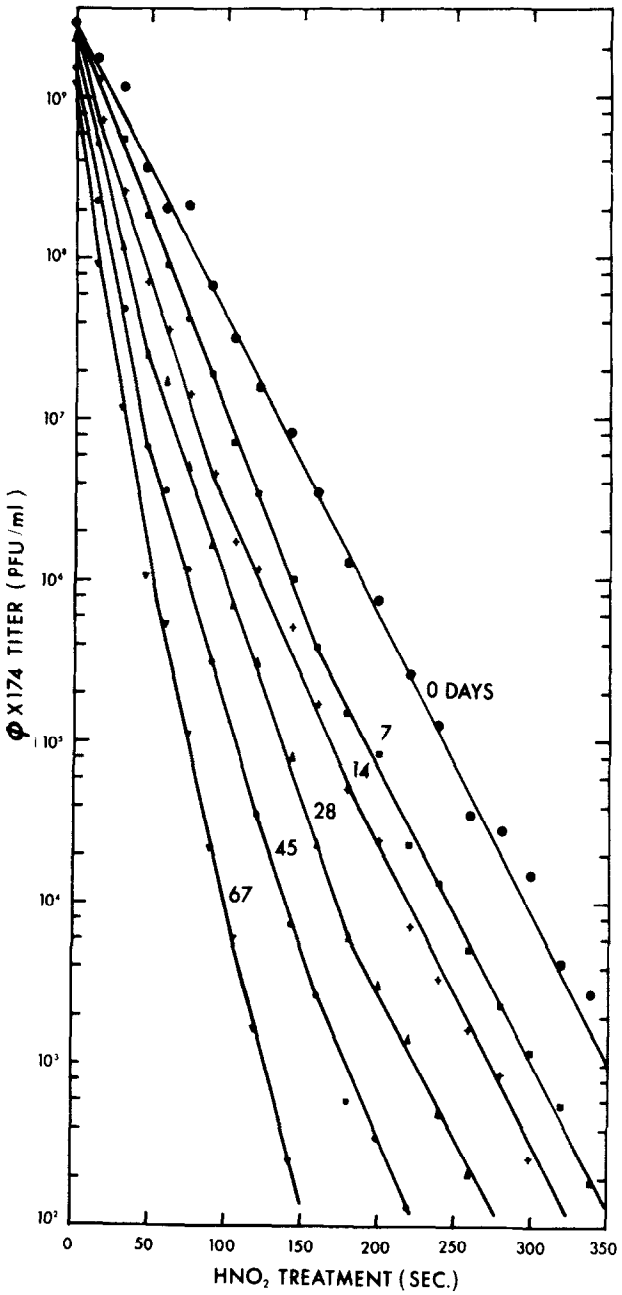

Fig. 1. Survival of ØX174 exposed to acetate buffered 0.5 M $NaNO_2$, (pH 4.4) at 30°C when assayed after different lengths of storage at pH 8.0, 5°-8°C.

survival curves for treated DNA assayed on freshly prepared spheroplasts after storage of the treated DNA up to 37 days.

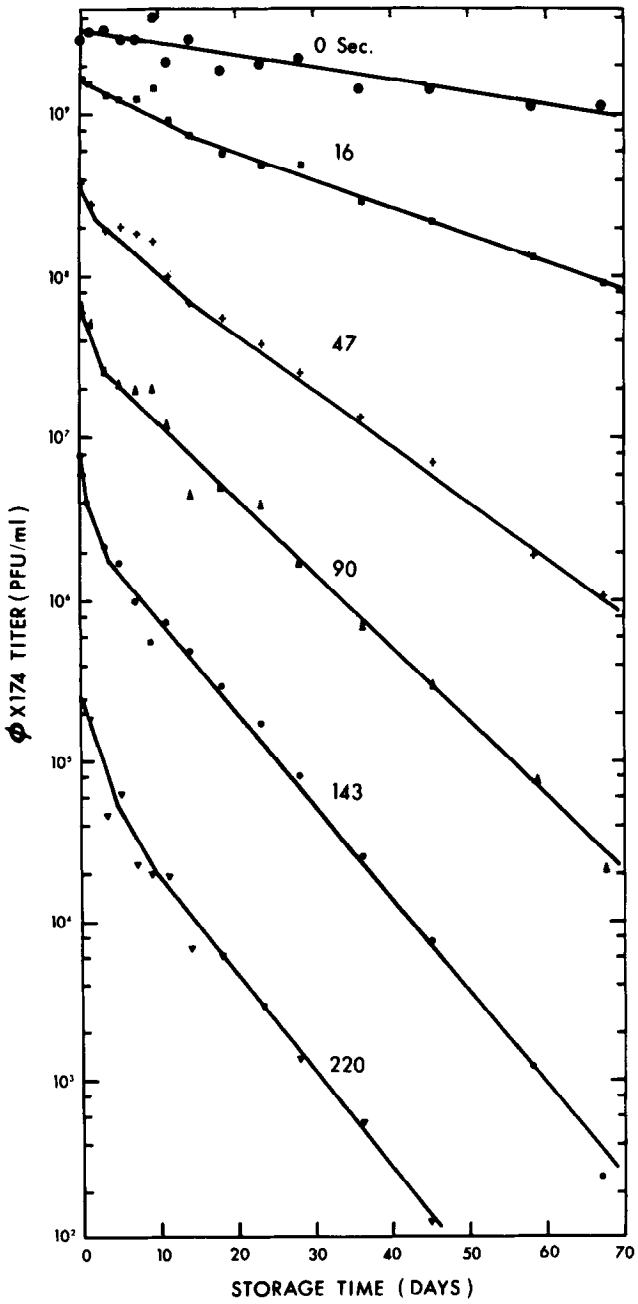

Fig. 2. Survival of ØX174 after storage for samples exposed to different doses of HNO_2. Treatment and storage conditions are same as Fig. 1.

There was, however, a change in survival rate when older spheroplasts were used. Figure 3 shows that the slopes of the survival

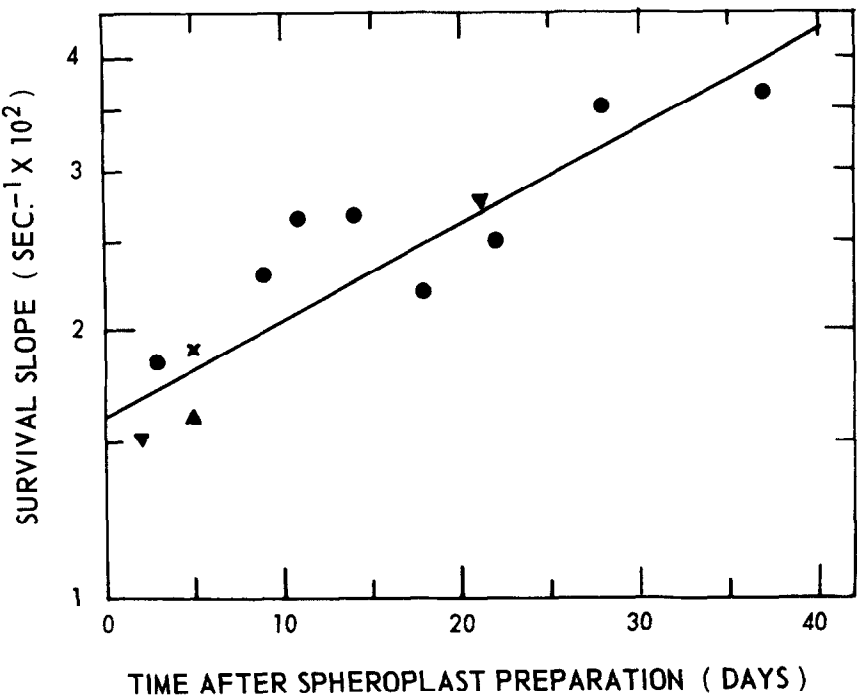

Fig. 3. Slope of survival curves for HNO_2 treated ØX174 DNA for bioassays at different times after preparation of spheroplasts. Time of spheroplast preparation relative to HNO_2 treatment of DNA: 0 -- 3 days before; +, Δ -- 7 days after; ∇ -- 35 days after.

curves increased with time after preparation of the spheroplasts. In no cases were multi-component curves observed.

Discussion

HNO_2 treatment caused loss of plaque-forming ability of ØX174. The loss of infectivity during the HNO_2 treatment is attributable to HNO_2 action on the DNA of the virus.(5) During storage after treatment, additional loss of infectivity occurs for the virus particles. Although the virus particles normally lose infectivity during storage, the treated particles lose infectivity at rates much greater than normal and dependent on time of HNO_2 treatment. Treated DNA, on the other hand, does not lose infectivity during storage. Thus the protein moiety of the virus particle is implicated in this inactivation by sublethal lesions.

Several explanations of our data involving protein are possible. We feel the most likely one is the production of unstable virus particles by direct HNO_2 action on the protein. Deamination of the protein can probably occur by the same mechanism that occurs for the nucleic acid. Protein configuration would be expected to be structurally less stable following deamination. A change of configuration of the protein coat subunits is likely to render the virus noninfective since both attachment to the host bacterium and injection are functions of the coat.

The spheroplast assay of treated DNA indicated that older spheroplasts were less able to permit HNO_2 treated DNA to reproduce. It has been found that HNO_2 treatment of ØX174 produced sublethal lesions of unknown nature, apparently in the DNA, which cause lengthened eclipse and latent periods.(5) Some of the lesions were presumably mutagenic. Freshly prepared spheroplasts allowed replication of DNA containing these sublethal lesions. During storage the spheroplasts lost this ability. The molecular events which effect this change are unknown. This finding indicates, however, that care must be taken in studying infection of spheroplasts using viral nucleic acid, especially nucleic acid treated with inactivating agents.

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