INHIBITION OF NUCLEASE ACTIVITY IN BACILLUS SUBTILIS FOLLOWING INFECTION WITH BACTERIOPHAGE SP82G[1]

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Summary

The total nuclease activity of cell extracts of B. subtilis was characterized and shown to decrease following infection with bacteriophage SP82G. This inhibition was maximal at 6 minutes after infection at 37° and 9 minutes after infection at 33°. The bulk (80%) of the inhibition was chloramphenicol sensitive but a small part (20%) was shown to be chloramphenicol insensitive. Extracts from infected cells were shown to have decreased nuclease activity on a variety of substrates.

Transfection with many bacteriophage DNA's (Sp01, SP82G, SPP1) shows a dependency on DNA concentration which suggests that several DNA molecules are required to form an infective center (1-6). Evidence has been presented that the genome of SP82G is "inactivated" during transfection by a process involving an unlinking of genetic markers (7, 8) suggesting that the DNA has been degraded, presumably by a host nuclease. This "intracellular inactivation" can be inhibited by phage infection prior to exposure of competent cells to phage DNA (7) by a process that seems to require protein synthesis (9), thus suggesting a phage induced protein can inhibit the nuclease. In order to investigate these events biochemically, studies have been done to quantitate hose nuclease levels prior to, and following, infection with SP82G.

Our results show that phage infection results in the inhibition of a specific host nuclease activity. Consistent with prior obser-

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vations (9) a portion of this inhibition requires protein synthesis. However, a significant portion of the inhibition of this host nuclease occurs in the absence of protein synthesis. Thus, host nuclease inhibition during infection with SP82G is a complex mechanism involving at least two discreet components. To our knowledge, this is the first time that this type of complex inhibitory process has been seen to occur during bacteriophage infection.

Materials and Methods

Bacterial strains, phage and media. Bacillus subtilis strain SB-1 (his^-, try^-) was used to prepare all cell extracts and was the host for bacteriophage SP82G. Media and techniques for propagation of unlabeled phage have been described (2).

DNA Preparations. [^{3}H]-labeled SP82G DNA (10-15,000 dpm/µg) was prepared by infecting Bacillus subtilis G-1 ($adenine^-$) with SP82G in the presence of ^{3}H-adenine (New England Nuclear; 1 µCi/ml). Bacteriophage were purified by differential centrifugation, banded on a step-wise CsCl gradient and lysed with 3 molar sodium perchlorate. [^{3}H]-DNA from B. subtilis G-1 was prepared by growing cells in the presence of [^{3}H]-adenine and extracting the DNA using the method of Marmur (10). [^{32}P] T_4 DNA and [^{32}P]-silk worm larvae rRNA were gifts from Dr. Donald Nuss.

Preparation of Cell Extracts

Uninfected. Late log phase cells (5 x 10^8 cells/ml) were collected and resuspended (3 ml/g) in a lysozyme (1 mg/ml)-50 mM Tris-HCl (pH 8.0) solution. Cells were frozen at -20°, slowly thawed and refrozen. After a second thawing, extracts were sonicated, centrifuged at 10,000 x g for 20 minutes and stored at -20°.

Infected. Cells were grown to 10^8 cells/ml and then concentrated in warm media to 2.5 x 10^9 cells/ml. SP82G was added at an m.o.i. = 7.5-10 and allowed to adsorb for 1 minute after which the cells were diluted to 10^8 cells/ml and infection allowed to proceed. At the appropriate time after infection, chloramphenicol was added (200 µg/ml) and the suspensions quickly cooled by pouring over ice. Cells were lysed and extracts stored as described above. Assays for viable (i.e., uninfected) bacteria, done before and after the 1 minute phage adsorption period, showed that 95-98% of the cells were successfully infected in the first minute.

Nuclease Assay. The nuclease assay measured the ability of extracts to convert DNA to acid soluble products. Incubation mixtures (1.0 ml) contained 10.0 µg ^{3}H SP82G denatured DNA or 10.0 µg ^{3}H-SP82G native DNA, 50 mM Tris-HCl, pH 8.0 and 10 mM $MgCl_2$. Reactions were started by addition of .01-2.0 mg of crude extract and allowed to proceed for 15 minutes at 37°. Reactions were terminated by addition of 0.5 ml cold 1% bovine serum albumin and 0.5 ml cold 20% trichloroacetic acid. Samples were centrifuged at 2,000 x g for 15 minutes at 4°, and 1 ml of supernatant was added to liquid scintillation counting vials. After neutralization with 1 drop of concentrated NH_4OH, 10 ml of Aquasol (New England Nuclear) were added and the radioactivity determined in a Nuclear Chicago liquid scintillation counter. Efficiencies of counting were determined using the external standard method.

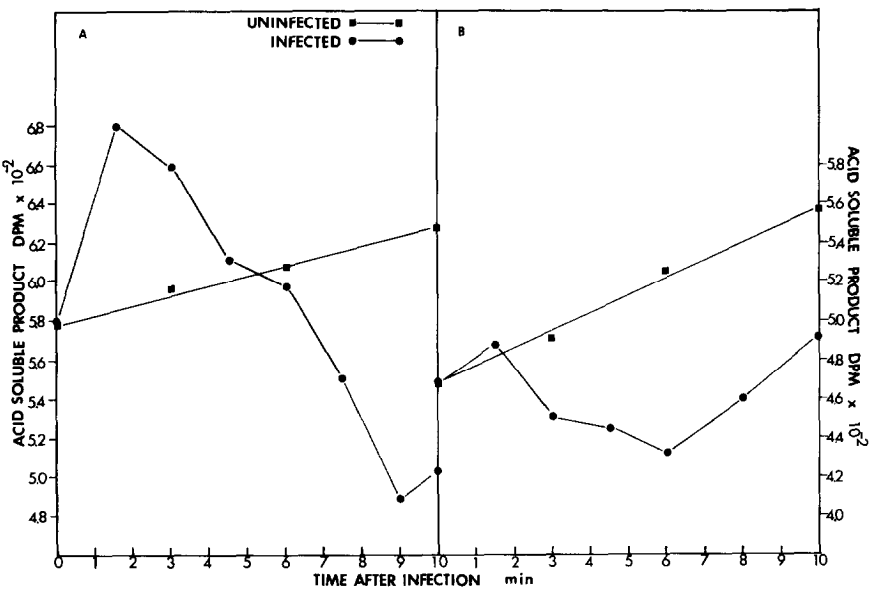

Fig. 1: Effect of infection on nuclease activity. Extracts prepared as described in Materials and Methods. Acid soluble product is shown as a function of time after infection for extracts of infected (●) and uninfected (■) cells. A: Infection at 33°; B: infection at 37°.

Results

Figure 1 shows that a decrease in the rate at which host nucleases degrade SP82G DNA occurs rapidly following infection. The time of onset of this inhibition appears to be temperature dependent, achieving a maximum at 6 minutes after infection at 37° and 9 minutes at 33°. The reason for the increase in nuclease activity at 2 minutes post infection at 33° has not been studied. Similar increases have been reported for nuclease levels in Salmonella typhumurium following P22 infection (11).

To account for the possibility of cell loss due to premature lysis of infected cells, the rate of degradation of SP82G DNA by various extracts was determined on a per mg protein basis (Fig. 2). This clearly shows that at equivalent protein concentrations, the rate of degradation of SP82G DNA by extracts from infected cells is approximately half that

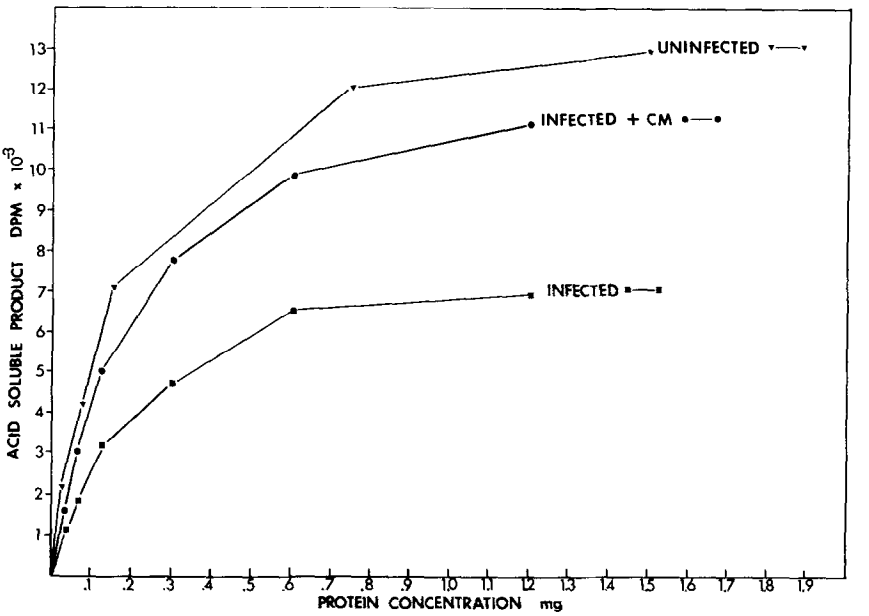

Fig. 2: Quantitation of nuclease activity in cell extracts. Nuclease assays were done as described in Materials and Methods using heat denatured [^{3}H] SP82G DNA as substrate. Acid soluble product formed is shown as a function of mg of protein in extracts from cells infected (■), uninfected (▼) and infected in the presence of CM (●).

of uninfected cells. In addition, it is seen that extracts from cells infected with SP82G in the presence of chloramphenicol degrade SP82G DNA at a rate only slightly less than that of extracts from uninfected cells. Approximately 80% of the inhibition of host cell nuclease levels is prevented when cells are infected in the presence of chloramphenicol. However, even in the presence of chloramphenicol there is a small, but significant, inhibition of the nuclease level in phage infected cells. It is unlikely that this nuclease inhibition is the result of typical protein synthesis since in other studies we have shown that the dose of chloramphenicol used (200 ug/ml) is sufficient to inhibit greater than 98% of amino acid incorporation in infected and uninfected cells (data not shown). In addition, as seen in Fig. 3 the level of this inhibition

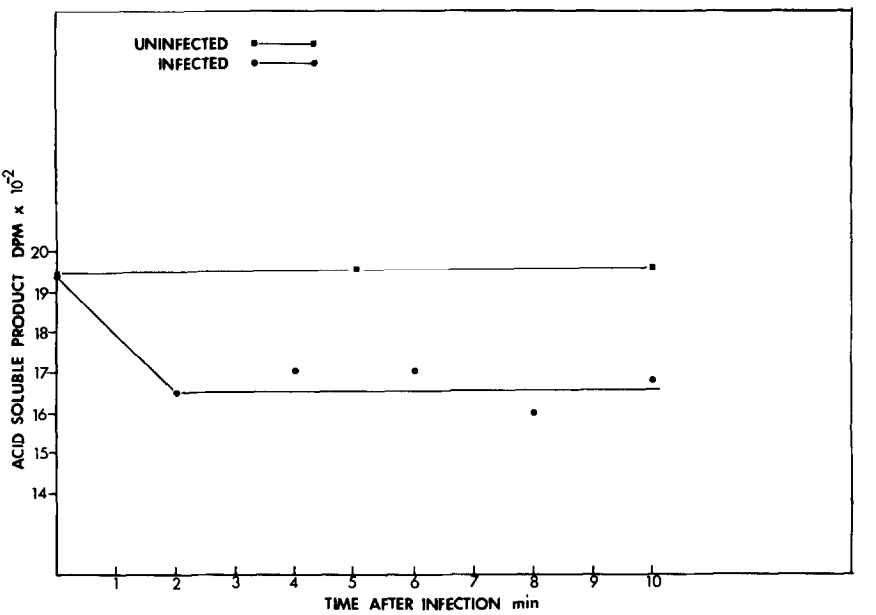

Fig. 3: Effect of infection in presence of chloramphenicol on nuclease activity of cell extracts. Cell extracts prepared as described in Materials and Methods, except that for cells infected in the presence of chloramphenicol, the drug was added to the cells to a final concentration of 200 µg/ml 5 minutes prior to infection. Acid soluble product formed is shown as a function of time after infection for extracts of infected (●) and uninfected (■) cells.

is already maximal at 2 min. post infection. The rapidity of establishment of the CM resistent inhibition seems inconsistent with a requirement for transcription and translation of a phage gene product.

The specificity of the inhibited nuclease(s) was examined by comparing the activities of infected and uninfected cell extracts against a variety of substrates. It was reasoned that if the inhibited enzyme lacked the ability to hydrolyze a given substrate, then the difference between the extracts of infected and uninfected cells would disappear on that substrate. Among the substrates tested were native and denatured SP82G DNA, ultra-violet irradiated SP82G DNA (data not shown), Bacillus subtilis DNA, T4 bacteriophage DNA, and silk-worm rRNA (Fig. 4). Both extracts effectively degraded these substrates to acid soluable products.

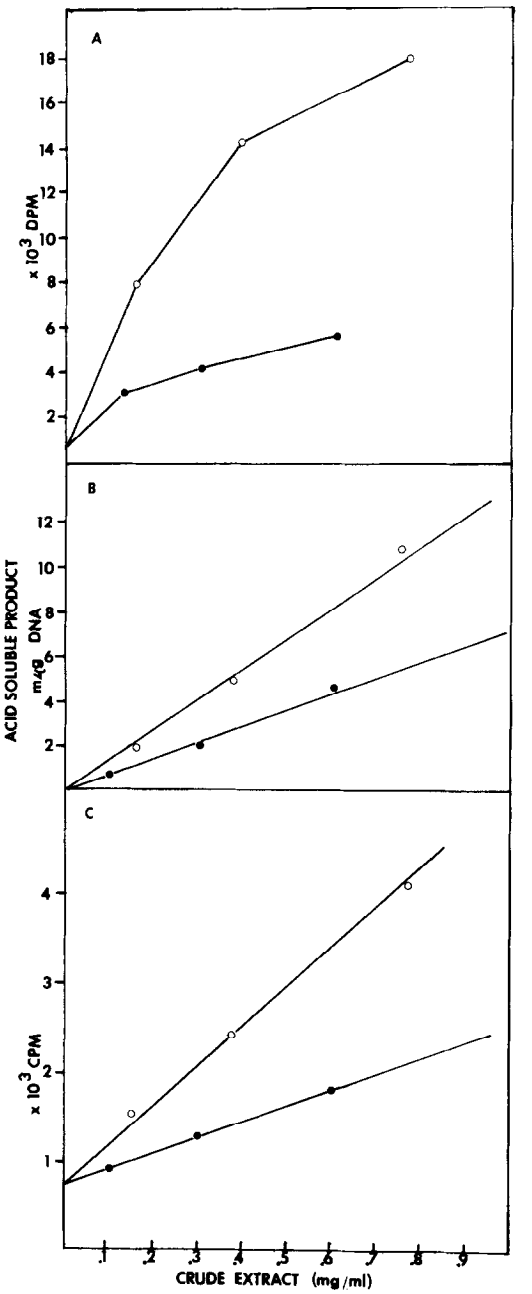

Fig. 4: Comparison of nuclease activity in extracts from infected and uninfected cells using B. subtilis and T4 DNA and silk worm larvae ribosomal RNA as substrate. Total nuclease assays were done as described in Materials and Methods. Acid soluble product formed is shown as a function of mg of protein of extracts from infected (●) and uninfected (O) cells. A: native B. subtilis DNA used as substrate; B: native T4 DNA; C: silkworm larvae ribosomal RNA.

In no case did the difference between extracts from infected and uninfected cells disappear. With Bacillus subtilis DNA, infected extracts were relatively more inhibited than with other substrates.

In conclusion, we have shown that host cell nuclease levels are significantly inhibited at early times after infection with SP82G bacteriophage. At least two steps can be identified in this inhibition; one is an early step (prior to two min) which is resistant to chloramphenicol and the other is a later step (ca. six minutes) which is chloramphenicol sensitive. The inhibited nuclease(s) is active against a wide variety of polynucleotides. In other experiments (J. King, Ph.D. Thesis, UNH, 1974), an intracellular endonuclease which is specifically missing in cells infected with SP82G phage for six minutes at 37°C has been isolated. This endonuclease, in partially purified form, shows the same broad spectrum of activity as that identified by the acid solubilization assay in this paper. This partially purified endonuclease has an absolute requirement for Mg^{2+} or Ca^{2+}, but not for Mn^{2+} and, in this respect, differs from other intracellular exclusively endonucleolytic enzymes of Bacillus subtilis (12, 13, 14). The properties of this endonuclease parallel those hypothesized for the agent responsible for the intracellular inactivation of transfecting DNA in Bacillus subtilis.

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