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Effect of Cadmium on the Infection of Lactobacillus lactis by Bacteriophage LL-H

By T. ALATOSAVA, T. JUVONEN, AND R.-L. HUHTINEN
Departments of Genetics and Biochemistry, University of Oulu, SF 90570 Oulu 57, Finland
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SUMMARY

The infectivity of Lactobacillus lactis bacteriophage LL-H was shown to be calcium-dependent. Of 10 different divalent cations screened, cadmium specifically decreased the infectivity of LL-H in the presence of calcium. At 1 to 2 mM, CdCl₂ resulted in a decrease of the burst size of about 2.5- to 4-fold. Cd²⁺ was shown to reduce specifically the level of total phage DNA synthesis, resulting in a reduced progeny phage yield. Moreover, Cd²⁺ had the most profound irreversible effect on progeny phage production between 20 and 60 min after LL-H infection. This paralleled the beginning of phage DNA synthesis. Possible modes of action of Cd²⁺ on phage DNA replication are discussed.

It is well documented, especially among bacteriophages active against Bacillus or Lactobacillus and other lactic acid-producing bacterial strains, that a Ca²⁺-dependent step(s) is frequently involved in the infection cycle (Potter & Nelson, 1953; Sozzi, 1972; Watanabe & Takesue, 1972; Steensma & Blok, 1979; Landry & Zsigay, 1980). This holds true also in the case of the Lactobacillus lactis bacteriophage LL-H. Of 10 different divalent cations screened, only Ca²⁺ and Mg²⁺ were capable of significantly promoting the infectivity of LL-H. Moreover, among these cations, cadmium was unique in being able to decrease the infectivity of LL-H in the presence of Ca²⁺. The basis of this special effect of Cd²⁺ on LL-H multiplication is investigated in this communication.

Procedures for the growth of L. lactis and phage LL-H have been reported previously (Alatossava & Pyhtilä, 1980). All plating experiments were carried out by the agar overlay technique described by Adams (1959). Soft and hard agar contained the same concentrations of divalent cations and chloramphenicol (Boehringer Mannheim) used as supplements in MRS medium (Difco). Standard procedures were used for one-step growth curve and burst size experiments.

To reveal the roles of various elements on phage LL-H infectivity, the following cations were considered: Na⁺, K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Sr²⁺, Mn²⁺, Zn²⁺, Cu²⁺, Fe²⁺, Hg²⁺, Cd²⁺, and in addition spermine. Of these, only Ca²⁺ and Mg²⁺ were able to increase the efficiency of plating of LL-H. The optimal concentrations for Ca²⁺ and Mg²⁺ were between 20 and 40 mM. Supplementing MRS medium with 20 mM-CaCl₂ did not affect the rate of growth or DNA and protein syntheses of the host, L. lactis (results not shown). Of the cations studied, cadmium was the only one able to decrease the infectivity of LL-H in the presence of Ca²⁺.

To determine the Cd²⁺-sensitive step or steps in the lytic cycle of LL-H, the phage adsorption kinetics was first studied by determining the proportion of free phage in samples using Nuclepore membrane filters (pore size 0.4 μm). These filtration experiments showed that Cd²⁺ did not inhibit phage infection at this step nor did LL-H adsorb to the Nuclepore filter. Accordingly, the Cd²⁺-sensitive step(s) must occur after the phage had adsorbed.

Analysis of phage LL-H DNA replication showed this step to be sensitive to the action of
Cd\(^{2+}\). The kinetics of DNA synthesis in LL-H-infected bacterial cultures was followed by the incorporation of \(\left[{^{14}}C\right]\)thymidine into 10\% trichloroacetic acid (TCA)-insoluble material. *L. lactis* cells growing exponentially at 37 \(^\circ\)C in 200 ml/MRS medium supplemented with 20 mm-CaCl\(_2\) were collected by centrifugation (at \(A_{600} = 0.3\)). The bacterial pellet was suspended in 10 ml of fresh, warmed MRS medium supplemented with 20 mm-CaCl\(_2\) and 7 \(\mu\)Ci [2-\(\text{14}C\)]thymidine (61.5 mCi/mmol; Amersham International). For bacterial controls, three 1 ml aliquots of this suspension were diluted 20-fold with fresh, preincubated MRS medium supplemented with 20 mm-CaCl\(_2\) (Ca control), with 20 mm-CaCl\(_2\) plus 1 mm-CdCl\(_2\) (Cd control) or with 20 mm-CaCl\(_2\) plus 3 \(\mu\)g/ml chloramphenicol (CAL control). The rest of the cell suspension was infected with phage LL-H at an m.o.i. of about 1. Immediately after infection, a 1 ml aliquot was withdrawn and further supplemented with CdCl\(_2\) to 2 mm to test the effect of cadmium on the phage DNA injection process. After an adsorption period of 15 min, each infected 1 ml aliquot was diluted 20-fold with fresh preincubated MRS medium supplemented with 20 mm-CaCl\(_2\), with 20 mm-CaCl\(_2\) plus 1 mm-CdCl\(_2\) or with 20 mm-CaCl\(_2\) plus 3 \(\mu\)g/ml chloramphenicol. The 1 ml infected aliquot supplemented by 2 mm-CdCl\(_2\) during adsorption was also diluted 20-fold with MRS medium containing 20 mm-CaCl\(_2\) plus 1 mm-CdCl\(_2\).

The inhibition of host DNA synthesis was equally severe in medium supplemented with 1 mm-CdCl\(_2\) or with 3 \(\mu\)g/ml chloramphenicol (Fig. 1a). DNA synthesis in all phage-infected systems (Cd\(^{2+}\), CAL and Ca\(^{2+}\) control system) proceeded at a considerable rate for 40 to 90 min, after which the rate of synthesis slowed down both in Cd\(^{2+}\) and control (no inhibitors present) systems and finally reached a limit after 150 min. At late times of infection in Cd\(^{2+}\)-containing and control systems, the amount of TCA-insoluble radioactive material decreased slightly, perhaps because of nuclease activity. On the other hand, in the presence of chloramphenicol (CAL system), DNA synthesis continued after 150 min at a high rate and by 4 h after infection equalled the level of the control culture. Furthermore, the presence of 2 mm-CdCl\(_2\) during the 15 min adsorption period had no effect either on the kinetics of DNA synthesis or on the amount of DNA synthesized. Hence the phage DNA injection process was not blocked by Cd\(^{2+}\).

The kinetics of DNA synthesis in LL-H-infected *L. lactis* cells (Ca control system) are practically identical to those reported for *Lactobacillus lactis* phage LL55 (Sarimo & Aaltonen, 1978). This may be expected, since restriction endonuclease analysis of phage DNA and phage neutralization tests using LL-H and LL55 antisera have shown phages LL55 and LL-H to be genetically closely related but not identical (unpublished observations).

The kinetics of DNA synthesis in LL-H-infected bacterial cultures correlated with the kinetics of progeny phage production, with a delay of about 30 min during the early rise period (Fig. 1b). Accordingly, the burst size of LL-H was proportional to the level of phage DNA replication and, furthermore, this showed itself to be the stage at which Cd\(^{2+}\) affects phage LL-H multiplication. On the other hand, chloramphenicol did not reduce the burst size of LL-H, but rather prolonged the rise period as a consequence of the reduced rate of phage DNA replication.

The burst size of LL-H showed itself to be dependent on the amount of progeny phage DNA molecules but not on the rate at which these were synthesized. The modes of action of Cd\(^{2+}\) and chloramphenicol differed in this respect. But there was one more difference between the effects of these inhibitors. Unlike chloramphenicol, the effect of Cd\(^{2+}\) on phage multiplication was irreversible, as shown in Fig. 2. In this experiment each inhibitor had an effect only up to the first 120 min of infection, and progeny phage determinations were made at 150 min and 20 h. At the concentrations used, the inhibitory effect of both substances was apparent after the first 20 min of infection. However, progeny phage production was restored only in the chloramphenicol-treated cultures [in fact the phage titres of these cultures were a little higher than in the control (zero min) culture]. When 2 mm-CdCl\(_2\) was used, the progeny phage production was irreversibly reduced to a level about fourfold lower than the control or chloramphenicol cultures. The most pronounced drop in phage titres affected by Cd\(^{2+}\) treatment was observed at a period of 20 to 60 min after infection, at the time of the beginning of phage DNA replication.

A question arising from the present results concerns the mode of action of Cd\(^{2+}\) in the host cell. The cell membrane plays an essential role in the replication of host chromosome,
Fig. 1. Effect of cadmium on phage LL-H DNA synthesis and phage production. (a) Effects of cadmium (Cd++) and chloramphenicol (CAL) on DNA synthesis in phage LL-H-infected (-----) and uninfected (---) cultures of Lactobacillus lactis. An m.o.i. of about 1 was used. The pH of the MRS medium varied between 5.8 and 6.1 and the temperature was 37°C. MRS medium was supplemented with 20 mM-CaCl₂ (■), with 20 mM-CaCl₂ plus 1 mM-CdCl₂ (○), or with 20 mM-CaCl₂ plus 3 µg/ml CAL (▲). During the 15 min adsorption period, MRS medium was supplemented with 20 mM-CaCl₂ plus 2 mM-CdCl₂ instead of 20 mM-CaCl₂ (△). From each 20 ml culture, 4.5 ml samples were withdrawn at 40 min, 1.5 h, 2.5 h and 4.5 h after phage infection, and supplemented with 40 µg/ml CAL to stop further growth. Samples were placed in ice before disruption of the cells by sonication (two to five times 3 min at 20 kHz; MSE 100-W Ultrasonic Disintegrator). To each sonicated sample (5 ml), 5 ml ice-cold 20% TCA was added. The acid-insoluble material was centrifuged (8000 g for 15 min) after standing overnight at 4°C. The pellet was washed with ice-cold 10% TCA and dissolved in 0.4 ml of 0.1 M-NaOH-1% SDS and further mixed with 0.7 ml Bio-Solv BBS-3 (Beckman Instruments) to dissolve the sample in 10 ml scintillation fluid. The fluid was prepared by dissolving 4.25 g PPO (Packard) and 285 mg POPOP (New England Nuclear) in 1 litre toluene, to which 550 ml Triton X-100 (Koch-Light) was then added. Radioactivity was counted in a Ultrabeta 1210 Liquid Scintillation Counter (LKB Wallac). (b) Effects of cadmium and chloramphenicol on progeny phage production. Symbols and conditions for phage infection are as for (a). Infected cells were disrupted by chloroform treatment before plating. The dashed line illustrates extracellular phage appearance in the control (MRS medium supplemented with 20 mM-CaCl₂) phage infection.
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Fig. 2. Effects of cadmium and chloramphenicol present during the first 120 min of phage LL-H infection on progeny phage production. *L. lactis* culture in MRS medium supplemented with 20 mM-CaCl₂ was infected with LL-H at an m.o.i. of about 1. The infected culture was immediately divided into three samples; one was supplemented with 2 mM-CdCl₂ (■) and another with 30 μg/ml CAL (■) (the third was the control). After 15 min adsorption, each culture was dilated 10²-fold with preincubated MRS medium containing the same supplements as before dilution. After periods of 20, 60 and 120 min from the beginning of infection, a sample was withdrawn from each culture and diluted 10⁵-fold with preincubated MRS medium supplemented with 20 mM-CaCl₂ to dilute the inhibitors to a sub-inhibitory concentration. After incubation for 150 min and 20 h at 37 °C, a portion of each sample was treated with chloroform before plating for progeny titre determination.

bacteriophages and plasmids (e.g. Siegel & Schaechter, 1973; Timmis & Pühler, 1979). Cd²⁺ may affect the phage DNA replication machinery indirectly (from outside the cell through the membrane) or directly (within the cell). Utilization of the manganese transport system for accumulation of Cd²⁺ into *Staphylococcus aureus* cells has been reported by Weiss et al. (1978). In addition, other host- or phage-specific components acting during phage DNA replication may also be possible targets for Cd²⁺ within infected cells: possibilities include the phage DNA itself, enzymes for nucleic acid metabolism and regulator proteins.

Although Cd²⁺ and Ca²⁺ showed opposite effects on LL-H infectivity, the basis and site of the effect of Ca²⁺ is of course not necessarily the same as for Cd²⁺. Hence, the characterization of Ca²⁺-dependent step(s) in the lytic cycle of LL-H merits further study.

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REFERENCES


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