Infection of Mycobacterium tuberculosis with Deoxyribonucleic Acid Extracted from Mycobacteriophage B1

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Received for publication 13 January 1967

Previously, it was reported that deoxyribonucleic acid (DNA) extracted from myobacteriophages could infect host bacteria, without protoplasting or otherwise extensively modifying the cells [T. Tokunaga and M.I. Sellers, J. Exptl. Med. 119:139, 1964; M. I. Sellers and T. Tokunaga, J. Exptl. Med. 123:327, 1966; T. Tokunaga and R. M. Nakamura, Med. Biol. (Tokyo) 71:384, 1965]. Mycobacterial strains employed in these earlier studies were all members of the saprophytic mycobacteria. This paper reports infection of human-type tubercle bacilli with the DNA extracted from B1 phage, isolated originally by K. Takeya and T. Yoshimura (J. Bacteriol. 74:540, 1957).

B1 phage was propagated by use of its host, Mycobacterium smegmatis ATCC 607. The phage lysate was digested with deoxyribonuclease and ribonuclease, concentrated, and purified by differential and sucrose density gradient centrifugation. DNA was extracted by the cold-phenol method and dialyzed against 0.02 M Tris(hydroxymethyl)aminomethane (Tris) buffer supplemented with 0.15 M NaCl and 0.002 M CaCl2. The DNA concentration of the extract determined by use of the diphenylamine method (Z. Dische, Mikrochemie 2:26, 1930) was 616 μg/ml. A 1-μg amount of the DNA could produce 43 plaques on an agar plate when plated with competent host bacteria as an indicator [T. Tokunaga and R. M. Nakamura, Med. Biol. (Tokyo) 72:51, 1966]. The activity of 1,000 μg of the DNA (corresponding to that of 43,000 plaque-forming units) was destroyed completely by the treatment of 1 μg of deoxyribonuclease for 10 min. Neither treatment with antiphage serum, the K value of which was 25.8, nor heating at 60 C for 60 min affected the plaque-forming activity of the DNA, though more than 99.9% of the activity of intact phages was destroyed by either treatment.

M. tuberculosis H37Ra was inoculated on Ogawa’s egg slant and incubated for 2 weeks at 37 C. The bacterial mass was homogenized with a glass homogenizer and suspended in nutrient broth at a concentration of about 2 × 108 per milliliter. An appropriate amount of the B1 was mixed with 0.5 ml of the bacterial suspension and plated quickly onto RVA24 agar plates (W. B. Redmond, Symp. Mycobacteria Exptl. Tuberculosis, Prague, 1965) with 3 ml of 0.6% overlay agar. After 3 to 4 days of incubation at 37 C, clear, round plaques were observed on the bacterial lawn. The diameter of the plaques was about 5 mm, whereas that produced by intact B1 phage was about 12 mm. A 1-μg amount of the DNA could produce about five plaques on the indicator, H37Ra, and deoxyribonuclease treatment at a concentration of 1 μg/ml for 10 min of 100 μg of the DNA before plating completely destroyed the plaque-forming activity.

To obtain an estimation of the time required for the completion of the DNA uptake into a cell, a time course analysis of stabilization of infection against deoxyribonuclease action was carried out as shown in Table 1. It was found that within 70 min of incubation on the plates...
approximately half of the plaque-forming units of the DNA became deoxyribonuclease-insensitive.

Several strains of human tubercle bacilli other than H37Ra, such as H37Rv, AoyamaB, etc., were tested for susceptibility to the B1 DNA, and almost same results were obtained.

Attempts at the genetic transformation of streptomycin resistance with bacterial DNA have been unsuccessful.

This investigation was supported by a grant from the National Tuberculosis Association-American Thoracic Society.