STRUCTURE OF BACILLUS SUBTILIS PHAGE SPO2 AND ITS DNA: SIMILARITY OF BACILLUS SUBTILIS PHAGES SPO2, ø105 AND SPP1

LuBelle Boice[1], F. A. Eiserling[2] and W. R. Romig[2]

[1]Department of Zoology and [2]Department of Bacteriology
University of California, Los Angeles, California 90024

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Several properties of B. subtilis phage SPO2 are described and compared with two other phages, SPP1 and ø105. All three phages are similar in morphology and size; each has a hexagonal-shaped head, which is probably a regular icosahedron, and a long flexible tail without a sheath. SPO2 and ø105 have complex structures at the base of the tail which consist of six subunits surrounding a central core. The diameter of the head of SPO2 is 495 Å and the length of the tail is 1770 Å. Phages SPO2 and ø105 are antigenically related. The DNA from SPO2 and SPP1 phage particles is very similar in molecular weight (25-26 x 10^6 daltons). The DNA from phages SPO2, SPP1 and ø105 is infectious in a transfection system. The DNA from SPO2 phage has cohesive ends.

INTRODUCTION

Bacteriophage SPO2 of Bacillus subtilis was isolated in 1963 from soil in Chicago, Illinois, by Shunzo Okubo (personal communication). It has been found to contain DNA which is infectious in a transfection system (Okubo and Romig, 1965).

The purpose of this paper is to describe the morphology and size of phage SPO2, and the molecular weight and structure of its DNA. Some properties of two other phages of B. subtilis which appear to be very similar to SPO2 are described and compared to SPO2: phage SPP1 (Riva and Polsinelli, 1968a and 1968b, and Riva et al., 1968) and phage ø105 (Bernard E. Reilly, personal communication).

MATERIALS AND METHODS

Strains and Media Phage SPO2 c_1 (Okubo and Romig, 1965) was used in all the SPO2 experiments described in this paper. Phage SPO2 c_1 was propagated on B. subtilis 168B (Brodetsky and Romig, 1965). B. subtilis 168(ø105) was obtained from Bernard E. Reilly. Phage ø105 was obtained by induction of 168(ø105) with Mitomycin C (Nutritional Biochemical Corp.) by a method which will be described in a later paper. Robert J. Huskey provided stocks of λ^{++} and $\lambda b_2 b_5 c$. Lysates of SPO2

were prepared and assayed on NY media (Okubo and Romig, 1966). Lysates of ϕ105 were prepared and assayed on TY media (Romig, 1962). Tris buffer for SP02 consisted of 0.1M Tris (Trizma base, Sigma Chemical Co., neutralized with HCl to pH 7.2) containing $MgSO_4$ at a final concentration of 5×10^{-3}M (Shunzo Okubo, personal communication).

Purification of phage for electron microscopy and preparation of antisera Phages SP02 and ϕ105 were concentrated by differential centrifugation. Phage pellets were resuspended in NY broth. The phage were purified by centrifugation in a density gradient of CsCl (99.9% grade, Research Inorganic Chemical Co.) dissolved in NY broth. The CsCl was removed from SP02 by dialysis against Tris buffer, and from ϕ105 by dialysis against the minimal medium of Spizizen (1958) without glucose.

Electron microscopy Phages were applied to the surface of a thin carbon film supported on a carbon stabilized Parlodion (Mallinckrodt) net where they were rinsed with 1% uranyl acetate at pH 5 to give them optical contrast. Photographs were taken in a Hitachi HU11A electron microscope.

Determination of the molecular weight of SP02 DNA The length of the DNA molecule from phage SP02 was determined by the Kleinschmidt method as adapted by Lucien Caro (Caro, 1965).

Preparation of phage antisera Purified SP02 and ϕ105 were mixed separately with Freund's Adjuvant (Difco) for us by Eli E. Sercarz and each was injected into a rabbit; sera were removed one month later. Before immunization the serum of each rabbit was tested for neutralizing activity against SP02 and ϕ105; neither serum had detectable ability to neutralize the phages. The neutralizing capacity of the sera, reported as the K value, were determined by standard methods (Adams, 1959).

RESULTS

Morphology and size of SP02 phage particles A schematic representation of the structure of SP02 is shown in Figure 1. The dimensions of SP02 were determined by measuring fifteen phage particles with the Scherr-Tumico optical comparator. Twenty-two extended sheaths of T4D on the same electron microscope plates were used as a standard. The length of the extended sheath of T4D was assumed to be 950 Å (Kellenberger et al., 1965).

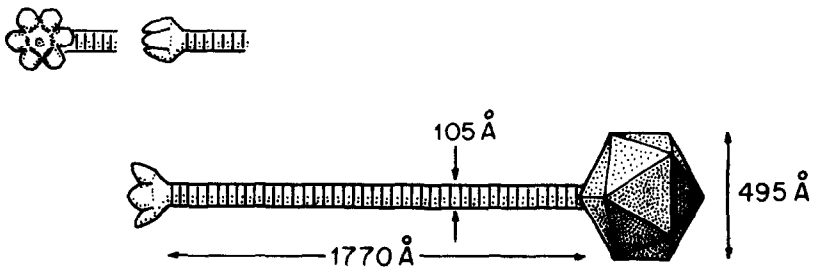

Fig. 1 A schematic drawing of phage SP02 showing different configurations of the tail base.

An independent measurement of the dimensions of SP02 was made by Larry D. Farrell with a Bausch and Lomb measuring magnifier on electron micrograph prints. Magnification was determined from photographs of a standard diffraction grating (Fullam). His results are in good agreement with those shown in Figure 1.

Complete heads of phage SP02 usually have a hexagonal outline. The complete head (containing DNA) of SP02 resembles a regular icosahedron, as shown in Figure 1. However, individual facets have not yet been resolved. The internal volume of the head is calculated to be 3.1×10^7 Å^3, assuming a capsid of 495 Å diameter and 50 Å in thickness. By comparison, the internal volume of phage λ is 4.3×10^7 Å^3, based on measurement of ten phage particles on the same electron microscope plates as the SP02 particles, and assuming a capsid of 540 Å diameter and a thickness of 50 Å. This value for the diameter of λ is less than that of 650 Å reported by Eiserling and Boy de la Tour (1965), but it falls within the range of values found for the diameter of λ (Eiserling, unpublished).

Phage SP02 has a flexible tail with 42±4 striations and no sheath, judging by examination of seventeen particles. The structure at the end of the phage tail is complex, and it assumes several configurations. Six subunits surround a central core which has a hole; the subunits sometimes appear closed, but at other times are spread open as the petals of a flower (Figure 1). A longitudinal channel is seen in the tail of ghosts of SP02, similar to that in phage λ (Eiserling and Boy de la Tour, 1965, and Kaiser, 1966) and in phage SPP1 (Riva et al., 1968).

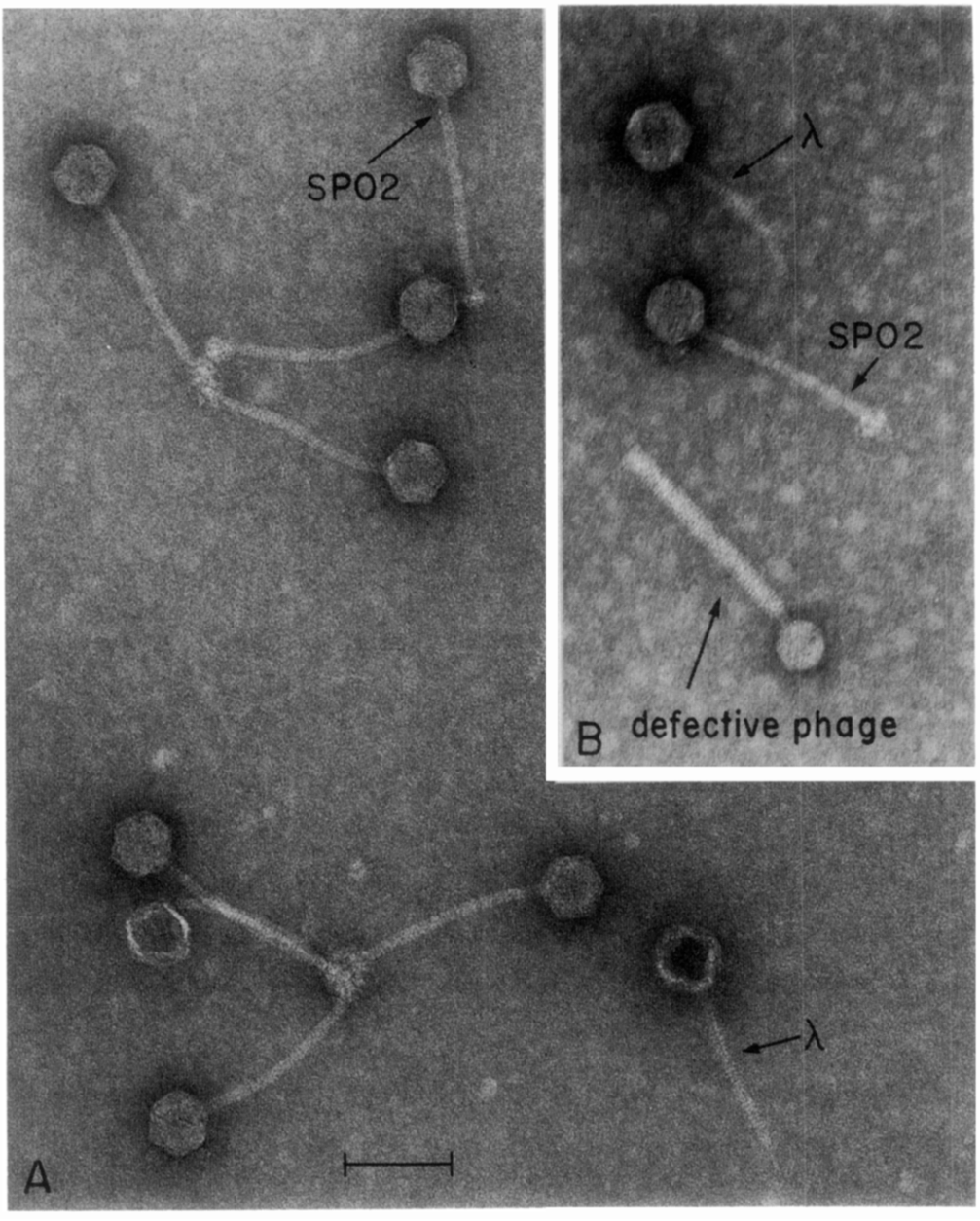

Fig. 2 Electron micrographs of (a) a λ phage and a SP02 phage and two aggregates of SP02 phages, and (b) a λ phage and a SP02 phage and a defective B. subtilis phage (Okamoto et. al., 1968). The bar represents 1000 Å.

Morphology of phage Ø105 The structure of Ø105 appears to be indistinguishable from SP02. Occasional preparations of phage Ø105 contained particles without any

structure at the tip of the tail, and it is possible that the joint between the tail base and the tail is quite fragile. All preparations of Ø105 also contained complete heads lacking any tail structure. Whether these heads are phage precursors, breakdown products of intact phage, or have a more subtle origin is not known.

Buoyant densities of SP02 and Ø105 phage particles Two types of λ phage having different buoyant densities, λ^{++} = 1.508 gm/cm^3 in CsCl (Kellenberger et al., 1960) and $\lambda b_2 b_5 c$ = 1.484 gm/cm^3 (Kellenberger et al., 1961), were mixed with SP02 and Ø105 phage particles in NB medium containing CsCl and centrifuged to equilibrium in a Spinco Model L centrifuge at 34,000 rpm for twenty hours. Phage SP02 has a buoyant density of 1.495 gm/cm^3; Ø105 has a buoyant density of 1.484 gm/cm^3.

Antigenic relationship of SP02 and Ø105 Phages SP02 and Ø105 are serologically related:

	K against SP02	K against Ø105
antiserum against SP02	238	55
antiserum against Ø105	2.3	338

Molecular weight and structure of SP02 DNA The average contour length of fifteen linear SP02 DNA molecules is 13.2±.6 microns, which corresponds to an approximate molecular weight of 26 x 10^6 daltons. Several circular molecules and a double linear molecule were photographed. The observation of circular DNA molecules is evidence that the DNA molecules released from phage particles have cohesive ends.

DISCUSSION

We have found that SP02 and Ø105 are morphologically indistinguishable. The electron micrograph prints shown by Riva et al.,1968 for SPP1 indicate that its head and tail structure are very similar to SP02 and Ø105. The size given for the tail of SPP1 is shorter (1400 Å) than what we obtained for SP02 (1700 Å). The absence of a tail base on SPP1 has not been explained; the phage may either be formed without a base, or its base may have been lost during purification, as we have observed in some experiments with Ø105.

Another criterion for the similarity of phages is serological relationship (Luria, 1945, and Delbrück, 1946). We have found that SP02 and Ø105 are serologically related; so far SPP1 has not been tested.

The molecular weight of SPP1 DNA is reported by Riva and Polsinelli (1968a) to be 25 x 10^6. This is very close to our value for SP02: 26 x 10^6 daltons. The DNA of all three phages (SP02, SPP1, and ∅105) is infectious in a transfection system (Okubo and Romig, 1965, Riva and Polsinelli, 1968a, and Bernard E. Reilly, personal communication).

A final similarity of SP02 and ∅105 is that both are temperate phages, as will be discussed in another paper. Our evidence suggests that they may represent a related family of temperate B. subtilis phages which may also include phage SPP1. This relationship is formally analagous to that among the lambdoid phages of Escherichia coli.

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