STUDIES ON THE OSMOTIC SHOCK OF BACTERIOPHAGES

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Investigations of physical properties of viruses have been extensively undertaken since about 1932. A large mass of information has been obtained with the aid of ultrafiltration, analytical ultracentrifugation, electrophoresis, diffusion and viscosity, X-ray diffraction, and electron microscopy. During the last decade it has become evident that the electron microscope is peculiary appealing in its ability to present direct information regarding virus morphology (Anderson, 1953). However, the morphology, of a scale smaller than electron microscopic dimension, has scarcely been made clear.

This paper presents briefly such smaller scale morphology which the writer has been able to obtain of bacteriophage particles by using osmotic shock.

MATERIALS AND METHODS

The T-group of bacteriophages (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) and their host, a strain B of Escherichia coli were used. Adams' routine method for phage experiments was employed throughout this experiments. The saline buffer diluent contained NaCl 0.58 per cent, $MgSO_4$ 0.025 per cent, and was buffered to pH 7.0 with an appropriate mixture of M/15 Na_2HPO_4 and M/15 KH_2PO_4. The phage was diluted to about 10^7 particles per ml in 5 M NaCl solution with osmotic pressures greater than 90 atomospheres and incubated for 30 minutes at room temperature (about 32° C).

0.1 cc of these phage-salt mixtures were diluted rapidly in 10 ccm of water and incubated for 30 minutes. After these shock procedures the sample was appropriately diluted in broth for assay by the agar layer methods. For control the phages in 5 M NaCl solution were diluted 10 fold in 2.5 M NaCl, and incubated for 30 minutes, and diluted 10 fold in 1 M NaCl. After these procedures the sample was also assayed by the same assay methods as for osmotic shock. This control was quite stable.

RESULTS AND DISCUSSION

The susceptibilities of all strains of the T-group phages to osmotic shock are summarized in table 1.

The T-even phages of the T-group were extremely susceptible to osmotic shock. These results are compatible with the view that the envelope of the very susceptible virus particle is somewhat permeable to the solute but much more

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TABLE 1. The Osmotic Shock of the T-group Bacteriophages by 5 M NaCl at about 32° C

Strain	Control titer	Survival titer after osmotic shock	Ratio
T_1	110×10^3	700×10^2	63.6%
T_2	571×10^3	904×10^2	16.0%
T_3	23×10^3	200×10^2	87.0%
T_4	204×10^3	204×10^2	10.0%
T_5	67×10^3	561×10^2	83.8%
T_6	169×10^3	363×10^2	21.4%
T_7	62×10^3	588×10^2	95.0%

permeable to water. Thus, when the particle is placed in solution of high concentration, the soluble molecules enter and water molecules leave until the activities inside and outside are equal. Then, when the particle is returned to water the activities again tend to become equal by slow diffusion of the solute out of the envelope and the much more rapid diffusion of water into it. This unequal rate of diffusion would result in a diffusion pressure on the envelope which, if the pressure is great enough, could develop pores through which the DNA could escape. On the other hand, if it is assumed that the pore of the insusceptible phage is much larger than that of the susceptible one, the envelope of insusceptible phage is much more permeable to the solute. Thus, for this insusceptible phage the unequal rate of diffusion would scarcely result in a diffusion pressure on the envelope.

From table 1, the susceptibilities of the T-group phage for osmotic shock were arranged in the following order.

$$T_4 > T_2 > T_6 \gg T_1 > T_3,\ T_5,\ T_7$$

It may be supposed that biological membranes with smaller pore sizes are more susceptible to osmotic shock because of the difficulty of ion-transport across these membranes. Thus, the order of pore size of protein rich membrane of phage may be expressed by the reversal of the above series. However, even the pores of the most susceptible phage *i.e.* T-even phages may be several times the dimensions of hydrated ions of NaCl salt, because it can be considered that these ions were permeated and equilibrated across the membrane for a minute or two.

These results are reasonable evidence for interpreting that the permeability of dye across membranes is the reverse of the order to osmotic shock (Yamamoto, 1956).

SUMMARY

The susceptibilities of the T-group phages to osmotic shock showed the following order:

$$T_4 > T_2 > T_6 \gg T_1 > T_3,\ T_5,\ T_7$$

It was discussed that biological membranes with smaller pore sizes are more susceptible to osmotic shock because of the difficulty of ion-transport across

these membranes. Thus the order of pore size of the protein rich membrane of phage was expressed by reversal of the above series.

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