

ENERGY REQUIREMENT FOR ADSORPTION OF BACTERIOPHAGE ϕ_{u-4} ¹T. L. Thompson and Fred Shafia²

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Received July 9, 1962

Irreversible adsorption of bacteriophages on host bacteria is generally considered to be independent of host's energy yielding mechanisms. Partial inhibition of T_1 adsorption has, however, been reported with various metabolic poisons (Amos and Vollmayer, 1957). This communication deals with the inhibition of adsorption of bacteriophage ϕ_{u-4} on host cells maintained under anaerobic conditions or exposed to metabolic poisons such as 2,4-dinitrophenol, cyanide and carbon monoxide.

METHODS. Bacteriophage ϕ_{u-4} is a minute thermophilic phage less than 10mμ in diameter which is specific for Bacillus stearothermophilus, N.U. strain 10 (Shafia and Thompson, 1962). Ultraviolet inactivated host cells were employed for adsorption studies although the results reported herein have been observed with viable cells. The use of u.v. inactivated cells is advantageous in that adsorption can be followed for several hours without interference from newly released phage. The adsorption rate on u.v. inactivated cells is essentially the same as that on viable cells. Cells were suspended in 0.1 M Tris buffer pH 7.5, diluted to contain approximately 10^7 cells/ml. and irradiated for 7 minutes at a distance of 36 cm. from a G.E. germicidal lamp. Calcium chloride was added to the suspension to give a final concentration of 0.1 M. A phage-cell ratio of approximately 1:100 was employed. Free phage was assayed by the agar layer technique (Adams, 1950).

¹Supported in part by research grant, E-555 (Cl3), from the National Institutes of Allergy and Infectious Diseases, U.S. Public Health Service.

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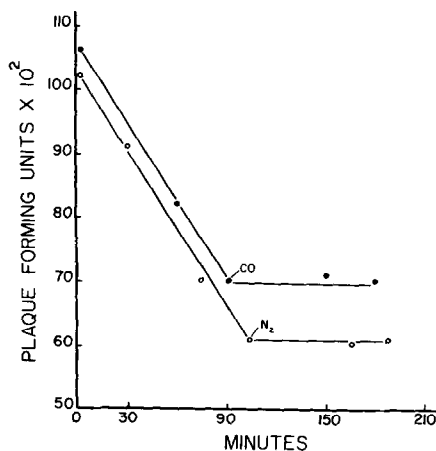


Figure 2. Interruption of normal adsorption of $\phi u-4$ on u.v. inactivated cells by displacement of air with N_2 and CO .

mentioned, however, that only very low concentrations of these compounds could be employed because of their toxicity for the phage.

Table 1.

Adsorption time min.	Irradiated control PFU/ml	Heat killed PFU/ml	NaCN (10^{-3} M) PFU/ml	2,4-DNP (10^{-4} M) PFU/ml
0	112	318	172	166
30	90	312	164	160
90	48	314	170	164
150	19	312	166	168

Table 1. Inhibition of adsorption of $\phi u-4$ on u.v. inactivated cells with sodium cyanide and 2,4-dinitrophenol. PFU represent the number of free phage remaining after each time interval.

From these data, it is evident that the adsorption of phage $\phi u-4$ requires functional terminal respiration, or more specifically, an active energy transfer mechanism. The role of host energy in adsorption of phage $\phi u-4$ is still a matter of speculation. Energy may, however, be required to maintain active receptor sites on the bacterial cell surface. It is also possible that adsorption and penetration may be synonymous. In the latter instance, energy would be required to transport the phage across the cell membrane.

Metabolic inhibitors, NaCN (10^{-3} M) or 2,4-dinitrophenol (10^{-4}), were incubated with the host cells for 20 minutes prior to addition of phage. Carbon monoxide was bubbled through the cell suspension for 60 minutes prior to addition of phage.

RESULTS AND DISCUSSION. As shown in Figure 1, ϕ u-4 fails to adsorb on host cells killed by heating for 10 minutes at 80 C. Ten minutes at this temperature was established as the minimum exposure to give 100 per cent killing.

Adsorption inhibition was readily demonstrated by maintaining the u.v. inactivated host cells under an atmosphere of N_2 . Normal adsorption would occur, however, even after several hours if the N_2 was replaced by air. Conversely, adsorption was inhibited by displacement of air with either N_2 or CO (Figure 2). Carbon monoxide inhibition was not reversed by exposure of the treated cells to intense yellow light.

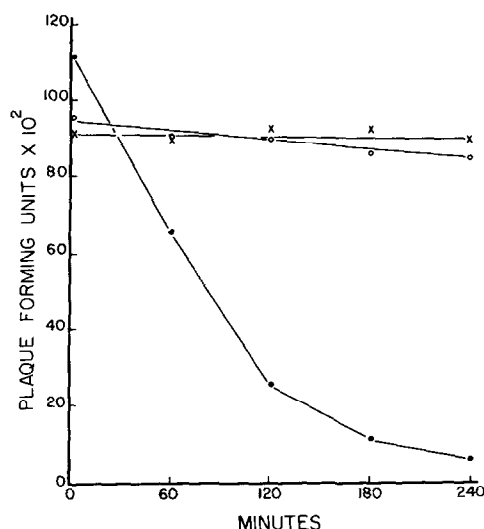


Figure 1. Comparison of adsorption of ϕ u-4 on heat killed and u.v. inactivated host cells. (X) - heat killed cells, (●) - u.v. inactivated cells, (O) - u.v. inactivated cells treated with carbon monoxide.

The combined data from several experiments are shown in Table 1. Cyanide and 2,4-dinitrophenol in the concentrations employed were equally effective in inhibiting phage adsorption. Cyanide inhibition was not reversed by methylene blue or 2,6-dichloroindophenol. It should be

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