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BRIEF NOTE

ADSORPTION SITE OF PHAGE WPK DAMAGED BY ACID EXPOSURE¹CAROLYN M. SABATINO² and SIEGFRIED MAIER,³ Department of Zoology and Microbiology, Ohio University, Athens, OH 45701

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Sabatino and Maier (1980) showed that the low pH used in the adsorption-elution method of virus recovery (Wallis et al. 1972) did not inactivate phage T4 of *Escherichia coli*, but rapidly inactivated phage F116 of *Pseudomonas aeruginosa* PA025 and phage WPK of *E. coli* K12. Since the first step in phage infections is adsorption of phage to host, we tested the hypothesis that acid-inactivation of phage WPK is due to damage of the adsorption site. With this specific damage, acid inactivated phage will not adsorb to host cells and subsequently added active phage WPK will adsorb.

Maintenance of host cultures, preparation and titres of phage stocks, and basic phage titration methods have been

described (Sabatino and Maier 1980). The latent period of phage WPK was determined at a multiplicity of infection (MOI) of 0.04 in 2% nutrient broth (NB) at 37 C using a reciprocating waterbath (170 strokes of 4 cm min⁻¹). Aliquots of filtrates (MF, pore size 0.45 µm) were assayed every 5 min for unadsorbed phage. The latent period lasted 20 min and 97% of input phage was adsorbed within 5 min. In subsequent experiments therefore, we allowed 10-15 min for adsorption which was sufficient time for adsorption of the majority of phage, but not long enough to obscure results by the liberation of new phage progeny.

Host cell densities were derived from previous correlations of *E. coli* K12 optical densities (Bausch and Lomb, Spectronic 20, 420 nm) with viable counts on nutrient agar (24 h, 37 C). However, final MOI's were calculated from respective plate and plaque counts of host and phage dilutions used for a particular experiment. The approximate number of phage

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adsorption sites on the host was determined by increasing the MOI to 1000 and titrating unadsorbed phage. On the average, *E. coli* K12 adsorbed 900 phage WPK/cell.

High titre phage WPK (10^{11} PFU ml⁻¹) was exposed to 0.05 M glycine/HCl buffer (pH 3.2) at 0 C for 5 min (Sabatino and Maier 1980) and neutralized by dilution into 0.1% peptone with 0.02% MgCl₂. Equal volumes of the treated phage and logarithmic phase *E. coli* K12 in 2% NB (MOI \geq 1000) were incubated at 37 C for 15 min with shaking. Control cells were treated similarly, but without phage. Both cell populations were recovered by centrifugation (12,000 \times g, 15 min). Pellets were resuspended to original volumes in 0.1% peptone containing 0.02% MgCl₂. Initial experiments demonstrated the need for MgCl₂ for efficient adsorption. Each cell population was then mixed with active phage WPK (MOI \leq 0.4) and incubated for 10 min at 37 C with shaking. Filtrates from both cell populations were titered for unadsorbed phage.

The number of unadsorbed active phage WPK in the test system compared to unadsorbed active phage in the control was used to indicate if acid-inactivated phage was adsorbed to host cells. A ratio of one would indicate that acid-inactivated phage did not adsorb to host cells, while a ratio much larger than one would signify adsorption of acid-inactivated phage to

host cells.

The mean ratio of 4 experiments was 0.995 ± 0.058 (\pm standard deviation). Therefore, acid-exposure (pH 3.2) damaged at least the ability of phage WPK to adsorb to its host, *E. coli* K12. Electron micrographs showed that phage WPK has a simple short tail (Maier and Preissner 1977) and tailed phage generally adsorb by the tail (Stent 1963). The rapid damage of the relatively simple adsorption structure of phage WPK stands in marked contrast to the acid resistance (Sabatino and Maier 1980) of the more complex adsorption site of phage T4 (Stent 1963). Any other possible acid-induced damage to phage WPK was not investigated.

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