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The Effect of Infection with Bacteriophage on the Electrokinetic Potential of *Rhizobium leguminosarum*

BY J. KLECZKOWSKI AND A. KLECZKOWSKI

Rothamsted Experimental Station, Harpenden, Hertfordshire

SUMMARY: Infection with bacteriophage increased the electrophoretic mobility of pea nodule bacteria (*Rhizobium leguminosarum*) at pH 7, suggesting an alteration of the bacterial surface. This seemed to occur at about the middle of the latent period.

As the electrophoretic mobility of a bacterium in a constant environment depends on properties of the surface of the organism, alterations in mobility have been used as a means of detecting changes in the structure of the surface. Changes have been detected in this way during the germination of spores and during the initial stages of growth of the vegetative form (Douglas & Parker, 1958*b*), at the beginning of growth in a new medium, during transitions between 'smooth' and 'rough' forms (Moyer, 1936), and during treatment with enzymes (Dyar, 1948; Harris, 1953; Douglas & Parker, 1958*a*, *b*) or with bactericidal and related substances (Bradbury & Jordan, 1942; Cohen 1945). The purpose of this work was to find whether, and if so at what stage, infection with bacteriophage alters the bacterial surface in a way reflected in a change of electrophoretic mobility.

METHODS

A strain of pea nodule bacteria (*Rhizobium leguminosarum*) and a corresponding bacteriophage were used. The media and the method of poured plates for obtaining plaques were as previously described (Kleczkowska, 1945). Liquid cultures of the bacteria lysed by the phage, and passed through a Chamberland L3 filter, are referred to as 'stock cultures' of the phage; these were stored at 0°.

Electrophoretic mobility was measured in M/15 phosphate buffer (pH 7) at 0° in the 2 ml. Tiselius cell of a Perkin-Elmer Model 38A electrophoresis apparatus under a gradient of 9·5 V/cm. As visual observation does not distinguish between phage-infected and non-infected bacteria in the same suspension, the optical system of the apparatus could not be used. Instead the mobilities were calculated from results of assays of fluids withdrawn separately from the ascending and descending limbs of the electrophoresis cell after periods of electrophoresis. The mobility of free bacteriophage, which was too dilute to give a visible boundary, was calculated similarly from results of assays. The descending limb and the bottom part of the electrophoresis cell were filled with the fluids to be examined, whereas the ascending limb contained only the buffer. The assays showed the proportions of phage or non-infected

or infected bacteria which had left the descending limb and entered the ascending limb of the Tiselius cell when electrophoresis ended. Assuming that the tested materials were electrophoretically homogeneous, the distances they moved were computed by multiplying 5 cm. (the length of the limbs of the electrophoresis cell) by the proportions. The bacterial suspensions were stable enough for sedimentation under gravity not to affect them appreciably during the time of electrophoresis.

RESULTS

One volume of phage stock culture (containing about 10^9 phage particles/ml. by the plaque count) was added to nine volumes of a 24 hr. liquid bacterial culture (containing about 2×10^8 bacteria/ml. by the colony count) so that about half of the total number of bacteria became infected; the mixture was incubated at 25°. The latent period, i.e. the period between adding phage to the bacteria and the lysis of the first cells, lasts 90 min. and is followed by the first step of the logarithmic increase in the number of infective centres, which also lasts for 90 min. To measure the mobility of the bacteria at any desired stage of interaction with bacteriophage, the progress of the interaction was stopped by cooling to 0°. The bacteria were then washed in cold buffer to remove free bacteriophage, suspended in buffer and subjected to electrophoresis usually for about 30 min. The mobility of infected and of non-infected bacteria was then computed from the results of their assays (by the plaque count and by the colony count, respectively) in the fluids withdrawn from the descending and ascending limbs of the Tiselius cell. The mobility of non-infected bacteria was also measured, using bacterial suspensions to which no phage had been added.

Table 1 shows that in each experiment, when bacteria had been incubated for 60 or 120 min. at 25°, the phage-infected bacteria moved faster than non-infected bacteria (Expts. 6–10). When, however, incubation with phage lasted only 30 min., the mobilities of infected and non-infected bacteria did not differ within the same experiments (Expts. 4 and 5), and comparisons within experiments were much more consistent than comparisons between experiments. Only the difference between the means of mobilities of non-infected and of infected bacteria which had been incubated at 25° for 60 or 120 min., was therefore tested statistically. A statistical test seemed necessary because of the considerable variation between the results of different experiments. The probability of the two means (0·708 and 0·866) differing only by chance is, by the t test, one in more than a thousand, so that the difference can be taken as highly significant.

The electrophoretic mobility of phage-infected bacteria was obtained by measuring the mobility of infective centres; these would include not only infected bacteria, but also any free phage particles that might have been present in spite of the precautions to exclude them. The mobility of free phage was therefore measured and found to be *c.* $-0{\cdot}45 \pm 0{\cdot}02$ μ/sec./V/cm. It is obvious, therefore, that infection increased the mobility of the bacteria, for the infected bacteria moved faster than non-infected bacteria, whereas free

phage moved more slowly than either. There seemed to be only one increase, which occurred between 30 and 60 min. of incubation at 25°, i.e. about the middle of the latent period. The timing must, however, be considered uncertain because of the considerable variation between the results of different experiments.

Table 1. *Electrophoretic mobilities of non-infected and of phage-infected* Rhizobium leguminosarum

Expts. 1–8 were made with bacterial suspensions to which no phage was added. Expts. 4–10 were made with mixtures of bacteria with phage incubated at 25° for indicated periods of time. The mobilities of non-infected bacteria in Expts. 6 and 9 were not estimated because of technical failures.

		Phage-infected bacteria Time of incubation at 25° after adding phage (min.)		
Expt.	Non-infected bacteria	30	60	120
	Mobility (μ/sec./V/cm.) towards anode			
1	0·65	—	—	—
2	0·66	—	—	—
3	0·72	—	—	—
4	0·69	0·69	—	—
5	0·72	0·75	—	—
6	—	—	0·84	—
7	0·75	—	0·88	—
8	0·70	—	0·93	—
9	—	—	—	0·77
10	0·77	—	—	0·91
Mean	0·708 ± 0·01463		0·866 ± 0·02837	

With the system used in this work nothing is known about the progress of phage-host interaction during the latent period. With some coliphages the middle of the latent period corresponds approximately with the end of the eclipse stage, when mature phage particles begin to appear within infected bacteria (Doermann, 1952; Anderson & Doermann, 1952). If it be assumed that the eclipse stage of the Rhizobium bacteriophage also ends at about the middle of the latent period, the increase in the electrokinetic potential of the bacterial surface would approximately coincide with the end of the eclipse stage.

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