Electron Microscopy of *Streptococcus lactis* Phage Plaque Margins

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**SUMMARY**

Ultrathin sections of plaques produced by *Streptococcus lactis* phages O712 and m13 were examined by transmission electron microscopy. The clear central area of the plaque was found to contain hardly any cellular material but the turbid margin contained abundant plasma membranes and some partially lysed cells whose appearance suggests a novel mechanism for the termination of plaque growth.

Several plausible mechanisms for the termination of plaque growth have been proposed, relating it to cessation of bacterial growth due to exhaustion of nutrients and accumulation of inhibitory products of metabolism, particularly acids. None of them is completely satisfying or established on firm evidence, however, and it is commonly observed that bacterial lawns may increase in density after plaques have achieved their final diameter, suggesting that some more-specific influence is responsible. To explore this problem further we have examined the margins of plaques of a number of phages of Gram-positive bacteria, using ultramicrotomy and electron microscopy. Qanber-Agha (1976) found abundant residual plasma membranes at the margin of plaques of *Staphylococcus aureus* phage 42D. In this paper we describe similar membranous residues at the margins of plaques of *Streptococcus lactis* phages O712 and m13.

This work is of special interest for the following reasons. Oram (1971) found that the receptor substance for phage m13 is located in the plasma membrane of the host bacterium; this observation remains unique but unchallenged at the present time. Plasma membranes extracted by Oram after mechanical rupture of the cells would neutralize phage m13 *in vitro*. Moussavi-Jahed (1979) confirmed this and showed that membranes extracted after removal of the cell wall by the lysozyme and salt method (Metcalf & Deibel, 1969) would neutralize with even greater efficiency. An exhaustive search was therefore made for virions adsorbed to residual plasma membranes at plaque margins, indicating intactness of the phage receptor after exposure of the membrane to the products of phage infection.

*S. lactis* phages O712 and m13 and homologous host strains 712 and ML3 were obtained from the National Collection of Dairy Organisms, NIRD, Shinfield, Reading, U.K. They were propagated in broth and assayed by the double-layer agar plaque technique using the GT media of Douglas *et al.* (1974). Plaques were cut out of the soft agar layer and prefixed for 90 min at 4 °C in 2.5% glutaraldehyde in 0.1 M-cacodylate buffer followed by fixation in OsO₄ (1% in cacodylate) for 1 h at room temperature. They were then stained with uranyl acetate (0.25% in acetate buffer) for 1 h at room temperature, dehydrated through ascending ethanol concentrations, transferred to acetone and embedded in low-viscosity epoxy resin (Spurr, 1969). Tangential sections were cut on an LKB Ultratome and examined in a Jeol 100C electron microscope at 80 kV.

Plaques of phages m13 and O712 usually comprise four concentric zones, although the relative widths of the zones vary according to the growth conditions, and one or more zones may be so narrow as to be invisible to the unaided eye in some cases. The plaque proper (zone A) is very clear and was found to contain hardly any particulate matter at all except that, on rare occasions, a seemingly intact cell was observed. This may have been a resistant mutant or a mutant unable to replicate for various reasons. The turbid margin (zone C), which may account for three-quarters of the diameter of the plaque, is of uniform turbidity
Fig. 1. Electron micrographs of sections of *Streptococcus lactis* phage plaque margins. (a) Phage m13; zone C. (b) Phage m13: zone D, showing the beginning of equatorial lysis, especially in the uppermost cell (arrowed). (c) Phage m13; zone C/D. Arrow indicates inverted distal hemisphere of one of a pair of daughter cells. (d) Phage m13; zone C/D, showing expulsion of swollen protoplast from equatorially ruptured cell (arrowed) whose detached hemisphere was not seen.
Fig. 2. Electron micrographs of sections of *Streptococcus lactis* phage plaque margins. (a) Phage O712; zone B/C, showing extrusion of bubbles of cytoplasm (arrowed). (b) Phage m13; zone B/C, showing residual intercellular annulus (arrowed A) and inverted distal hemisphere (arrowed B). (c) Phage O712; zone B, showing swollen protoplasts (arrowed A) and phage-saturated cell (arrowed B). (d) Phage m13; zone B, showing protoplast with partially digested membrane (arrowed A) and phage-saturated cells (arrowed B).
except for its inner edge (zone B), where there is a very narrow band of enhanced turbidity, and its outer edge (zone D) where it fades into the unaffected lawn. The turbidity of zone C is due to abundant residual plasma membranes (Fig. 1a) of cells digested from without by lysin diffusing from the plaque proper. These membranes are vesicular in form, some vesicles having a diameter much greater than that of *S. lactis* cells and others very much smaller. Examination of some partially lysed cells shows how they may arise. Diffusing lysin attacks the cell wall equatorially (Fig. 1b). The distal hemisphere (with reference to the pair of cells indicated) separates, sometimes inverting itself (Fig. 1c and Fig. 2b) and the protoplast expels itself by osmotic expansion (Fig. 1d). Alternatively, an equatorial crack develops and membrane-bound ‘bubbles’ of dilute cytoplasm are extruded (Fig. 2a); this occurs with both infected and uninfected cells. The annulus of residual wall between two daughter cells (seen in section as two opposed ‘v’ shapes) is lysin-resistant and often seen when little else of the cell remains (Fig. 2b).

The inner edge of the turbid margin zone B is of considerable interest since it contains, as well as membranes, numerous phage-saturated but otherwise seemingly intact cells (Fig. 2c, d) responsible for its enhanced turbidity. Their intactness is surprising since, if not lysed-from-without by the massive infection, they should have been digested by surplus lysin from other lysed cells nearby. Stent (1963), discussing the phenomenon of ‘lysis inhibition’, suggested that “superinfection temporarily antagonizes the lytic action of phage lysozyme, and postpones the moment of final disruption of the cell by its internal pressure.” Receptors are clearly less abundant on the m13 cell surface; we have calculated geometrically that a saturated cell of 712 (as in Fig. 2c) may bear upward of 2000 adsorbed virions. These, it will be noted, are mainly ‘ghosts’. Cells of ML3 on the other hand do not show such close packing of adsorbed m13; Fig. 2(d) shows the maximum packing density recorded, and the irregular appearance is typical.

The absence of membranes from the plaque proper suggests that membranolytic enzymes are active in plaque formation. Observed by light microscopy, individual infected cells can be seen to lyse in a few seconds at the end of the latent period and we have envisaged a mechanism whereby such an enzyme weakens the membrane, allowing it to rupture suddenly so that preformed internal lysin gains instant access to the peptidoglycan of the wall. The absence of free virions from most of our preparations is not surprising because it is well known that virions are easily washed out of soft agar gels. Therefore, they would have been removed during the preparative treatment unless adsorbed to large particulate debris. Virions adsorbed to cell wall fragments are abundantly seen in zone B but we attach the greatest significance to the almost complete absence of virions convincingly adsorbed to residual membrane to which, especially at the inner edge of the turbid margin, they must have been intimately exposed. We do not doubt that the m13 receptor is located in the plasma membrane. On the other hand, it would seem logical strategy for nascent phage to destroy redundant receptors to prevent inactivation by adsorption to debris. A separate receptor-destroying enzyme is probably involved. A mechanism for the termination of plaque growth can now be envisaged as follows. After the first few cycles of lysis the small but growing plaque will contain progeny virions and surplus lytic enzymes, both of which will diffuse radially. Being much smaller than virions, the enzyme molecules will diffuse faster and establish, ahead of the virions, a zone in which the host has been rendered resistant by alteration of wall, membrane or receptor, or any combination of these. Growth of the plaque proper, by infection and lysis-from-within, will then cease, but growth, especially of the turbid zone as noted by Douglas *et al.* (1974), may continue by enzyme action alone.

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REFERENCES


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