Electronmicroscopy of the anti-bacterial agent produced by *Escherichia coli* 15

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SUMMARY

Some properties of the phage ψ of *Escherichia coli* 15, as revealed by electronmicroscopy of an u.v.-induced lysate, are described. The differences between this defective phage and a bacteriocine and the type of interaction between this phage and *E. coli* 15 are discussed.

INTRODUCTION

When it was first observed that the strain 15 of *Escherichia coli*, after u.v.-irradiation and further incubation, would eventually lyse and release an anti-bacterial agent, this agent was considered to be a 'colicine' (Mukai, 1960; Ryan, Fried & Mukai, 1955). The possibility that, in addition, a defective phage was produced, was left open. But, since in an u.v.-induced lysate of this strain all anti-bacterial activity can be spun down by applying centrifugal forces of only 20,000 *g* for 2 hr (Mukai, 1960) and since those colicinogenic strains, which do lyse after u.v.-irradiation, were found to be also lysogenic (Kellenberger & Kellenberger, 1956), we concluded (Mennigmann, 1964*b*) that the anti-bacterial agent must be a large particle, probably a phage. In this communication we wish to verify these conclusions by presenting some electronmicroscopical pictures of such lysates, which show a defective phage to be present. Thus, regarding the antibacterial agent produced by *E. coli* 15, we rather favour the opposite of the above picture: the anti-bacterial agent is a defective phage and the possibility of the additional presence of a colicine has to be left open.

METHODS

The bacterial strain *Escherichia coli* 15 T—obtained through the courtesy of Professor O. Maaløe—was grown in a minimal medium (Maaløe & Hanawalt, 1961) enriched with 1.5% Difco casamino acids and 2×10^{-5} M thymine. A logarithmically growing culture ($OD_{578} = 0.15/\text{cm.}$) was u.v.-irradiated (layer of about 1 mm., 250 ergs/mm.² from an Osram germicidal lamp HNS 12 ofr) and further incubated. At the time of maximum lysis the culture was shaken with 1/10 volume chloroform for 15 sec. and the bacterial debris centrifuged at low speed. From the supernatant specimens were prepared by the phosphotungstate negative staining method and examined under the electronmicroscope 'Siemens Elmiscop I'.

RESULTS

The pictures revealed a phage about which the following can be said. It consists (Pl. 1, fig. 1) of a polyhedric head (about 600 Å in diameter) and a tail (overall length of about 1100 Å, and about 170 Å in diameter). In some cases, the head seems to be empty (Pl. 1, fig. 2). The tail is complex: an outer sheath (Pl. 1, figs 2-4) covers an inner core (Pl. 1, figs. 2, 4). To this is attached a base plate with at least three spikes (Pl. 1, figs. 1, 3 and 5). No fibres could be detected. The sheath appears 'striated' (Pl. 1, figs. 1-4) and is contractile (Pl. 1, figs. 2 and 4). The part of the tail between the head and the sheath is of smaller diameter than the sheath, but bigger than the core and is somewhat reminiscent of the 'neck' of the T-even phages. Thus, this new phage is not much different from *Escherichia coli* phages of the T-even series, except that its head is somewhat smaller. We have named this phage ψ (because the Greek letter psi is—with sufficient imagination—reminiscent of an inner core plus base plate plus three spikes; see Pl. 1, fig. 5).

A surprising feature of the lysate, prepared as described above, was the relatively large number of tails without heads as compared with the number of morphologically 'intact' phages. Even though this treatment does very little, if any, harm to *Escherichia coli* phages of the T-series, it was thought possible that some kind of destruction might have occurred when shaking the crude lysate with chloroform. But omitting this purification step did not result in fewer tails as compared with the number of 'intact' phages. Thus, it can be concluded either that the phage is so fragile that it falls apart when the bacterial cell lyses, or that the 'assembly machinery' does not work properly, or that the cells predominantly produce tails. In favour of the latter possibility is the fact that one does not find as many heads as one can see tails (assuming that the heads are not preferentially obscured by the large amount of cell debris still present after low-speed centrifugation).

In addition to the phages or phage-related structures there are at least two more types of particles with a defined shape, which have not been found in T4- or λ -lysates as obtained from other strains of *Escherichia coli* and whose nature so far is unknown: round cyst-like particles much smaller than the phage head and four-lobed structures (Pl. 1, fig. 6).

DISCUSSION

It was found that by u.v.-irradiation (Mukai, 1960; Ryan *et al.* 1955) and by thymine deprivation (Luzzati & Chevallier, 1964; Mennigmann, 1964*a*; Sicard & Devoret, 1962) the bacterial strain *Escherichia coli* 15 can be induced to lyse, and that it concomitantly produces an anti-bacterial agent—presumably a colicine—active against the same bacterial strain (Mukai, 1960; Ryan *et al.* 1955). From the sedimentability at 20,000 *g* (Mukai, 1960) it was concluded (Mennigmann, 1964*b*) that the agent must be larger than other known true colicines. Because it was found (Kellenberger & Kellenberger, 1956) that, whenever a colicinogenic strain lyses after u.v.-irradiation, phages are also produced, we assumed this also to be the case (Mennigmann, 1964*b*) with the presumably colicinogenic strain *E. coli* 15 (Mukai, 1960; Ryan *et al.* 1955). This assumption was verified by the finding reported in this communication at least to the extent that phages (and parts of them) are produced. There is no evidence for the presence, in addition to the particulate agent, of an ordinary colicine. The nature of the other constituents of the

lysate (Pl. 1, fig. 6) will have to be elucidated. Thus, since the lysate can be freed from the anti-bacterial activity by the same centrifugal forces which effectively sediment phages, we conclude that the active agent in lysates from *E. coli* 15 is the defective phage found in the electronmicroscopical pictures. The observation (Mukai, 1960) that the anti-bacterial agent is sensitive to the action of commercial trypsin was thought to be in favour of the agent being a bacteriocine rather than a bacteriophage (Mennigmann, 1964*b*). This argument no longer seems to be valid, since in the meantime Northrop (1964) has reported, that some phages are also sensitive to this enzyme. Thus, the only remaining observation, which was said (Mennigmann, 1964*b*) to argue against the agent being a bacteriophage, is its non-transmissibility. Since this argument only holds true if one excludes defective phages, it is only a very vague one—as one can see from the findings reported in this communication.

In connexion with the fact that *Escherichia coli* 15 is lysogenic for a defective phage, which after induction produces mostly parts of phages, it is interesting to note that the same has been reported for other strains of *E. coli* (Arber & Kellenberger, 1958) and for a strain of *Proteus mirabilis* (Taubeneck, 1963). In the case of virulent phages a number of mutants have been found, which on certain strains of *E. coli* produce complete phages, while on other strains they only produce parts of phages (Epstein *et al.* 1963; Kellenberger & Boy de la Tour, 1963).

Finally, knowing now that the presumed colicine (Ryan *et al.* 1955; Mukai, 1960) in fact seems to be a phage (or part of it), it might be interesting to study more closely (a) the observed relationship (Mukai, 1960) between the acquired sensitivity to the other T-phages of *Escherichia coli* and the concomitantly acquired resistance to the phage ψ , and (b) the type of interaction between this phage and the sensitive strain of *E. coli* 15, which leads to a reversible inhibition of cell division (Mennigmann, 1964*b*). The latter might be of particular interest, since from what has been reported so far it seems to be equally difficult to classify this interaction as 'resistance' (=no adsorption), 'restriction' (=destruction of the injected DNA), or 'immunity' (=prevention of the phenotypic expression of the lytic functions).

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EXPLANATION OF PLATE

Constituents of a crude lysate of *Escherichia coli* 15 T⁻ after u.v.-induction (× 300,000).

Fig. 1. Morphologically 'intact' phage particle.

Fig. 2. Phage particle with an empty head and contracted sheath, thus making visible part of the inner core.

Fig. 3. Phage tail with base plate and spikes. This type of particle is more prevalent than the other phage-related structures.

Fig. 4. Phage tail with contracted sheath, thus making visible part of the inner core.

Fig. 5. Part of the inner core and base plate with spikes.

Fig. 6. Cyst-like particles and four-lobed structures frequently seen in the lysates.

Note added in proof. While this communication was in the press, two other papers have been published which also show that *Escherichia coli* 15 harbours a defective prophage (Endo *et al.* 1965; Sandoval, Reilly & Tandler, 1965).