Specific Dissociation of Phage Xp12 by Sodium Citrate

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In general, a low concentration (0·003 M) of citrate ion has little or no effect on phage stability, but concentrations greater than this accelerate phage inactivation (Lark & Adams, 1953). When phage T5, suspended in 0·2 M-potassium solution, is heated at 55°, 90 % is inactivated after incubation for 150 min. When 0·04 M-citrate is added, however, only 20 min. is required (Lark & Adams, 1953). The phage Xp12 from *Xanthomonas oryzae*, possessing an unusual base in its DNA (5-methylcytosine completely replaces cytosine) (Kuo, Huang & Teng, 1968a), was extremely sensitive to low concentrations of citrate. Because of this unusual property the mechanism of the effect of citrate on phage Xp12 was carefully studied; citrate specifically causes the separation of the head from the tail of this phage.

The conditions for growth and maintenance of *Xanthomonas oryzae* strain 507 are described in a previous paper (Kuo *et al.* 1968b). High-titre stocks of Xp12 phage were prepared by inoculating a late log. phase (4×10^8 cells/ml.) culture of bacteria in PS medium at 28°, and this culture was aerated continuously until lysis was complete. Such lysates contained approximately 3×10^{12} p.f.u./ml. The bacterial debris was removed by centrifugation; phage was precipitated with 70 % (w/v) ammonium sulphate resuspended in 0·01 M-tris buffer at pH 7·5 and dialysed against the same buffer overnight. Phage was further purified by both differential and sucrose density gradient centrifugation (Kuo *et al.* 1968b). Finally the phages were dialysed against 0·01 M-tris buffer at pH 7·5.

The sensitivity of Xp12 to sodium citrate was demonstrated by suspending phage particles at a concentration of 2×10^9 p.f.u./ml. in 0·003 M-citrate in 0·01 M-tris buffer at pH 7·5 at room temperature. Less than one phage particle in 10^5 remained viable after as little 1 min. of this treatment (Fig. 1). When inactivated phage suspensions were examined under the electron microscope, it appeared that phage heads and tails were separated. To verify this observation, separated heads and tails were isolated by sucrose gradient centrifugation. Purified phage at a concentration of 3×10^{12} p.f.u./ml. was treated with 0·03 M-sodium citrate. After 10 min. when the phage was assayed, activity was completely lost. One ml. of this inactivated phage was layered on 5 to 50 % linear sucrose gradients made up in tris buffer (0·01 M, pH 7·5) and centrifuged at 24,000 rev./min. in a Spinco SW 25·1 rotor for 4 hr at 4°. Half ml. fractions were collected from the top of the tube by an ISCO model D Density Gradient Fractionator and the extinction read at 280 nm. Three peaks were obtained (Fig. 2a), which were further tested for protein by Lowry's method (Lowry *et al.* 1951) and for DNA by the diphenylamine method (Burton, 1968). Peak 1 contained only DNA, and peaks 2 and 3 only proteins. When samples from peaks 2 and 3 were negatively stained with 2 % uranyl acetate for 5 min. and examined under the electron microscope, peak 2 contained phage tails (Fig. 3a), and peak 3 empty phage heads (Fig. 3b). The tail fraction was very pure because only tails were found; however, in peak 3 there were a few ghosts. For the control, phage without sodium citrate treatment was studied by the same procedure. Only one peak was obtained which possessed a positive DNA and protein fraction (Fig. 2b). This fraction was assayed for phage infectivity; no phage titre was lost. When samples from this peak were also examined under the electron microscope it was found that this peak contained intact phage particles.

After exposure to 0·03 M-sodium citrate, therefore, phage Xp12 separated into three components: DNA, empty heads, and tails. The only intact particles that remained were

phage ghosts. DNA may have been extruded from the phage heads at the site of attachment for tails after removal of tails by sodium citrate. However, the presence of ghosts suggests that DNA could have been extruded from the head through some other site after treatment with sodium citrate.

In order to see whether other chelating agents have a similar effect on Xp12, we treated Xp12 with EDTA, Ethyleneglycol-bis (2-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA) or potassium phosphate with the same procedure. All chelating agents could inactivate Xp12, but the concentrations of different chelating agents required for inactivation of phage was different. When EDTA or potassium phosphate was used, 0·05 M was
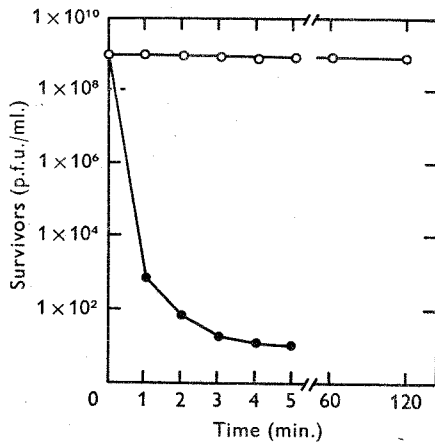

Fig. 1

Fig. 2

Fig. 1. Inactivation of Xp12 phage by sodium citrate. Sodium citrate (0·003 M) was added to a suspension of Xp12 phage (1 × 10⁹ p.f.u./ml.) in 0·01 M-tris buffer at pH 7·5. Samples were withdrawn at 1 min. intervals, then diluted and plated for assay of surviving phage. The mixture was incubated on a shaker at 28°. O—O, without citrate; ●—●, with citrate.
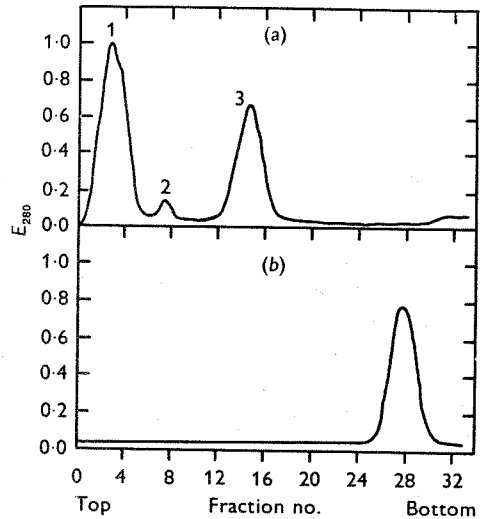
Fig. 2. Sucrose gradient centrifugation pattern of sodium citrate-treated phage Xp12. (*a*) Phages treated with sodium citrate. (*b*) Intact phage.

required to obtain same level of inactivation as with sodium citrate, whereas with EGTA only 0·0008 M was required. EGTA seems more powerful than sodium citrate. Since EGTA is a chelating agent specific to calcium, we suspect that the calcium ion may play an important rôle in the connexion of heads and tails.

The effect of the sodium citrate on the phage Xp12 may be explained on the assumption that, at the connecting site of the head and the tail, there may be a metal-activated protein which is necessary for the connection of the head to the tail. This protein is able to form a complex with various metal cations, including calcium, and is very unstable in the absence of such cations. Sodium citrate reacts with those metal cations, weakening or breaking the bond linking the cation to the protein. The destabilization of this protein causes the separation of heads and tails of phages.
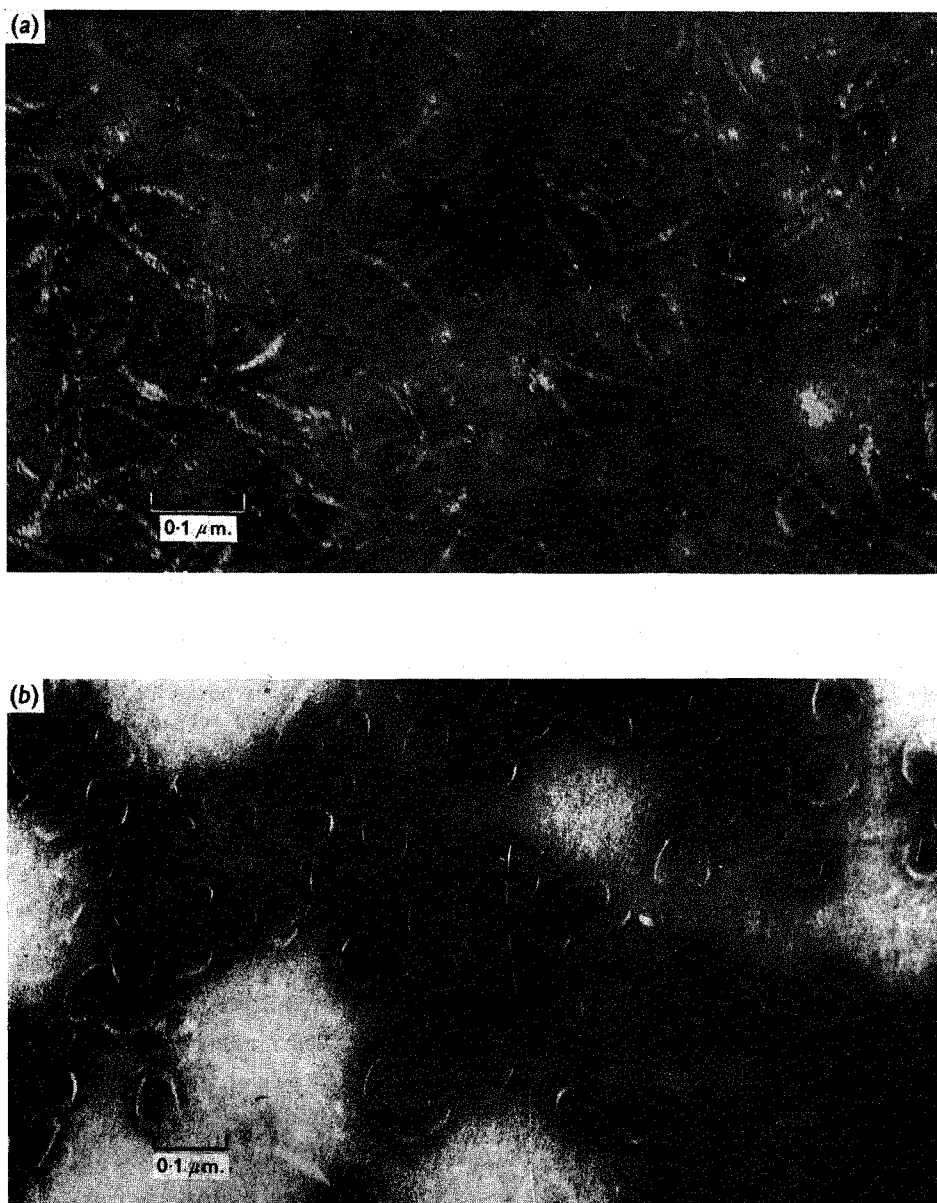
0·1 μm.

0·1 μm.

Fig. 3. Electron micrographs of phage tails (*a*), and heads (*b*).

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