

## PHYSICAL CHARACTERIZATION OF THE DNA RELEASED FROM PHAGE PARTICLES BY HEAT INACTIVATION

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### 1. Introduction

A variety of phages including the T-phages [1, 2] P22 [3] and  $\lambda$  [4] lose infectivity when exposed to elevated temperatures (50–60°) particularly in low ionic strength medium or medium containing chelating agents. Heating also releases native DNA molecules from phage particles and for phages T7, SP-8, osaka 1 and  $\alpha$  the DNA was shown to be released as unbroken molecules [4–6]. Since heat inactivation is a simple and rapid method it may offer advantages over those commonly used for extracting DNA from phage particles. This paper reports studies undertaken to investigate the generality of heat inactivation as a method for extracting high molecular weight phage DNA and to extend the physical characterisation of heat released DNA molecules. The results show that for phages T1, T2, T3, T4, T7 and P22 heat inactivation of the particles was accompanied by the release of native DNA which in all cases consisted of unbroken molecules. Furthermore, the heat released DNA molecules from T2 and T7 had intact single strands and a buoyant density identical to that of homologous DNA extracted with phenol.

### 2. Materials and methods

Phages T1<sup>+</sup>, T2H<sup>+</sup>, T3<sup>+</sup>, T4Bo<sub>1</sub> [7] and T7<sup>+</sup> were grown and assayed on *Escherichia coli* strain B. *Salmonella typhimurium* strain LT2 was the host for phage P22<sup>+</sup>. For heat inactivation, phage suspensions were diluted 5–10 fold in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate) and incubated at 60°. Radioactive phages were prepared by infecting bacteria growing with aeration at 37° in TCG medium [8] con-

taining either <sup>3</sup>H-thymidine (10  $\mu$ Ci/ml), added to the culture at the time of infection, or <sup>32</sup>P-orthophosphate (4  $\mu$ Ci/ml) added one hour before infection. Bacteria ( $3 \times 10^8$ /ml) were infected with 2–3 phage/cell for T1, T2, T4 and P22 and 0.25 phage/cell for T3 and T7 and one hour later chloroform was added to complete lysis. Phage particles were concentrated and purified as described previously [9]. Unbroken phage DNA molecules used as reference markers in gradient analysis were gently extracted with phenol and dialysed against 0.05 M NaCl, 0.01 M tris-HCl, pH 8 [9].

For zone sedimentation studies 0.1 ml samples were layered on 5 ml linear gradients of 5–20% sucrose (w/v) dissolved in either 0.1 M NaCl, 0.05 M phosphate, pH 6.8 (neutral sucrose) or 0.9 M NaCl, 0.1 M NaOH, pH 12.1 (alkaline sucrose) and centrifuged at 20° in the SW50 rotor of a Spinco Model L ultracentrifuge. For density gradient analysis 0.2 ml samples were mixed with 3.5 ml CsCl dissolved in 0.05 M NaCl, 0.01 M tris-HCl, pH 8 (density = 1.70 g/ml), overlaid with mineral oil and centrifuged as described for sucrose gradients. Gradient fractions were collected dropwise from the bottom of the centrifuge tube on glass fibre paper and the radioactivity assayed by scintillation counter [1].

### 3. Results and discussion

Radioactive phage suspensions in 2 × SSC were incubated at 60° and at intervals samples were removed and quenched in ice. Samples were assayed for infectivity. The remainder was mixed with differentially-labelled unbroken marker DNA molecules from the same phage type and centrifuged through neutral suc-

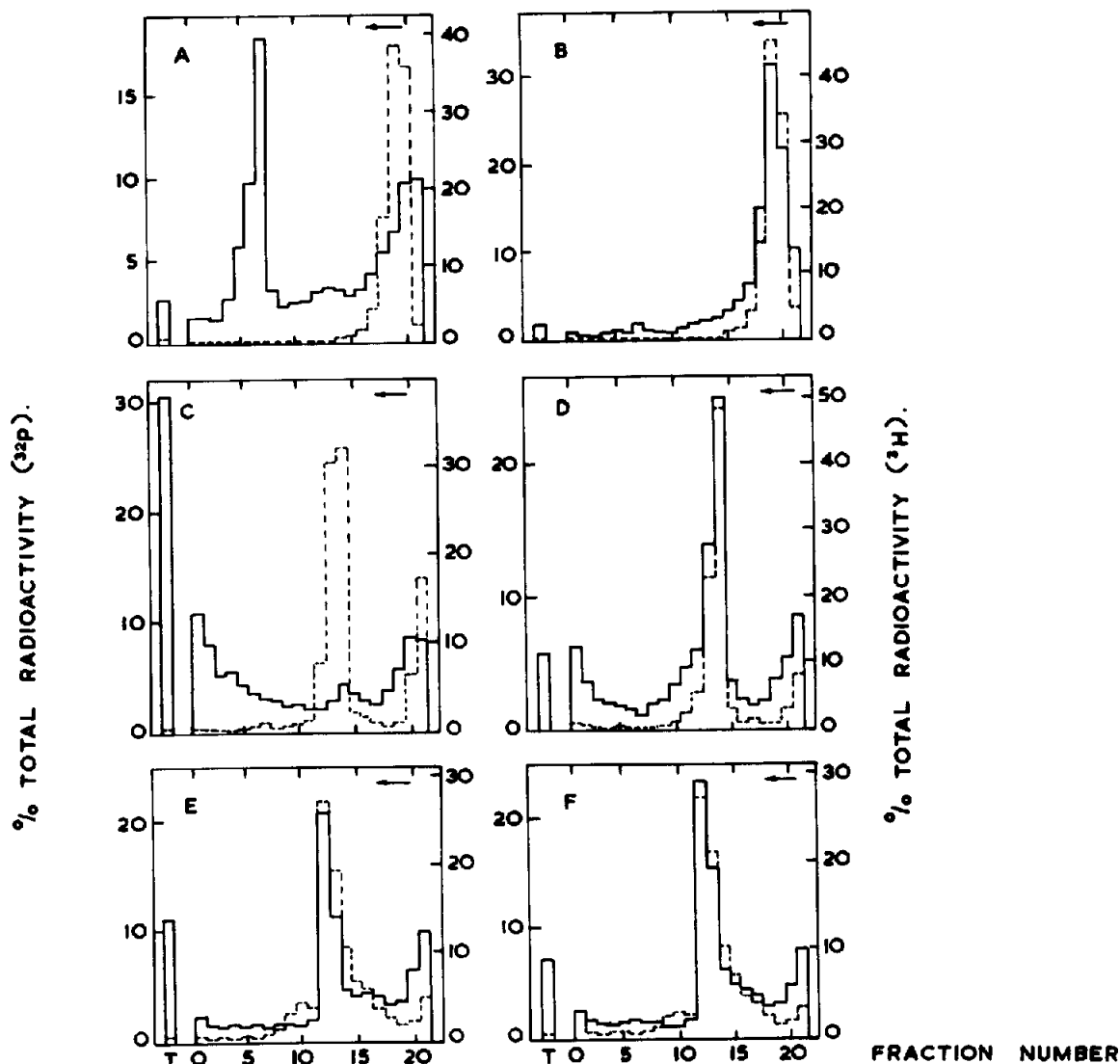


Fig. 1. Zone sedimentation of T7 phage particles before and after heat inactivation.  $^{32}\text{P}$ -labelled phage particles (—) were heat-inactivated for 0 min. (A, C, E) and 20 min. (B, D, F) to give 100% and 0.2% survivors respectively, mixed with  $^3\text{H}$ -labelled phenol extracted T7 DNA (---) and centrifuged as follows: (A, B) neutral sucrose gradients, 24,000 rpm for 0.5 hr; (C, D) neutral sucrose gradients, 41,000 rpm for 1.5 hr; (E, F) alkaline sucrose gradients, 41,000 rpm for 1.5 hr. Arrows indicate direction of sedimentation. Fraction T is the bottom 0.5 cm cut from the centrifuge tube after fractionation.

rose gradients. After brief centrifugation most of the radioactivity of the unheated phage samples had migrated to the position in the gradient expected for infectious phage particles (fig. 1A). (The coincidence of infectivity and radioactivity was confirmed in separate experiments). The  $^{32}\text{P}$  label at the meniscus probably represents DNA from phage particles disrupted during purification. The  $^3\text{H}$ -labelled marker DNA banded near the top of the gradient. Following heat inactivation

most of the  $^{32}\text{P}$  label appeared at the top of the gradient with the marker DNA (fig. 1B). When the centrifugation conditions permitted the marker DNA molecules to migrate part way through the gradient the  $^{32}\text{P}$  label of the unheated phage suspension sedimented to the bottom of the centrifuge tube (fig. 1C) whereas after heat inactivation the  $^{32}\text{P}$  label co-sedimented with the marker DNA (fig. 1D). The rate at which the radioactivity in phage particles was con-

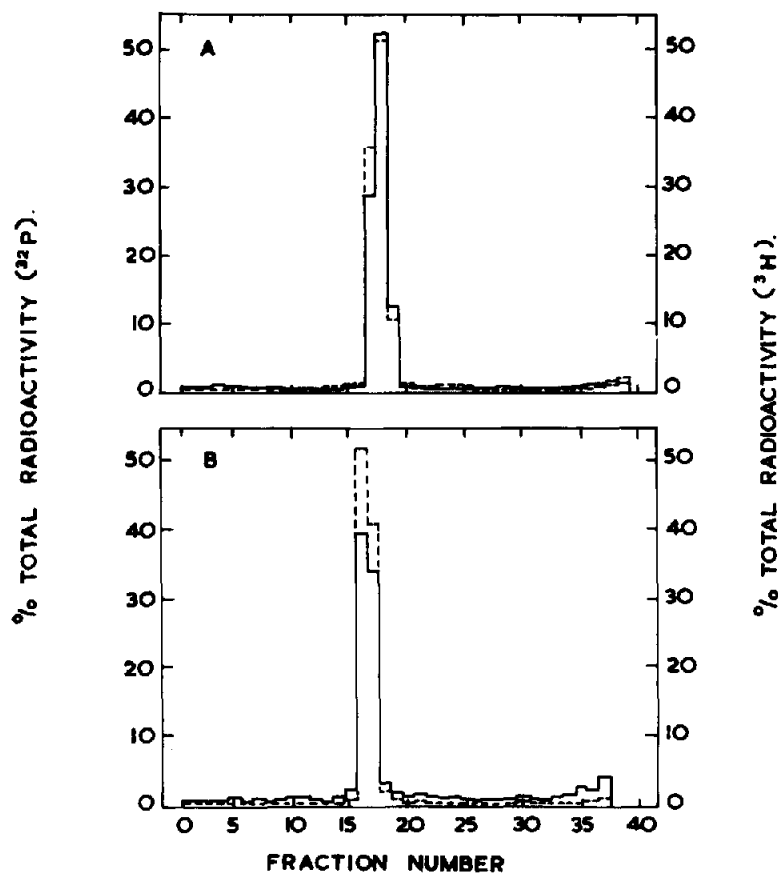


Fig. 2. CsCl density gradient centrifugation of heat inactivated,  $^{32}\text{P}$ -labelled phage particles (—) and phenol extracted,  $^3\text{H}$ -labelled DNA (---). (A) T2 phage particles heat inactivated for 60 min (6% survivors) and T2 DNA. (B) T7 phage particles heat inactivated for 20 min (0.2% survivors) and T7 DNA. Gradients were centrifuged at 40,000 rpm for 60 hr.

verted from the fast to the slow sedimenting form was closely correlated with the rate at which the phages lost infectivity.

These results with T7 show that heat inactivation is associated with the release of DNA from the phage particles and that this DNA has the same sedimentation rate, and therefore molecular weight [10] as unbroken phenol extracted DNA molecules from the same phage type. Essentially identical results were obtained in experiments with phages T1, T2, T3, T4 and P22.

To examine the structure of the single strands,  $^{32}\text{P}$ -labelled T7 phage particles mixed with  $^3\text{H}$ -labelled marker DNA were made 0.1 M with NaOH and sedimented through alkaline sucrose gradients. (This treatment liberates DNA from phage particles and denatures native DNA [5]). Both labels sedimented with

completely overlapping bands which were sharp but with a slight skew on the trailing side (fig. 1E). Thus both DNAs consist predominately of uninterrupted single strands having identical molecular weights [11]. Parallel experiments with heat inactivated phages gave the same result (fig. 1F). Since the sedimentation rates of denatured heat released, alkali released and phenol extracted DNA are identical we conclude that heat inactivation does not induce single strand interruptions. Similar results were obtained with T2. These findings disagree with an earlier report that heat released DNA molecules showed considerable single strand breakage [5].

Heat inactivated  $^{32}\text{P}$ -labelled T2 and T7 phage suspensions were each mixed with their corresponding  $^3\text{H}$ -labelled marker DNA and centrifuged to equi-

librium in CsCl. The density distributions were superimposable showing indistinguishable buoyant densities (fig. 2). With unheated phage particles most of the radioactivity appeared at the top of the gradient as would be expected for complete phage particles.

The results presented in this paper extend earlier reports that the heat inactivation of phage particles causes separation of the DNA from the protein coat [2, 4] and show that the release of the DNA as intact molecules [5, 6] is a widespread property. Additionally we have shown for T2 and T7 that the heat released DNA molecules consist predominantly of unbroken single strands and have the same CsCl buoyant density as phenol extracted DNA. By all three criteria the heat released and phenol extracted DNAs are indistinguishable.

Heat inactivation as a method for extracting highly molecular weight DNA from phage particles offers some advantages over the phenol and NaClO<sub>4</sub> [12] methods. The heat inactivation process is rapid, the longest exposure used, 60 min, released 90% of the DNA from T2 phage particles. Moreover agitation is unnecessary which overcomes the possibility of shear breakage and furthermore the DNA is released into 2 × SSC which is a standard DNA solvent.

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