ISOLATION OF THE NUCLEOID STRUCTURE FROM T_7 BACTERIOPHAGE INFECTED *ESCHERICHIA COLI* B CELLS

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1. Introduction

DNA of *Escherichia coli* forms part of the 'nuclear bodies', which are approximately one micron in diameter and can be observed in the light as well as in the electron microscope (see ref. [1] for review).

After gentle lysing of the cells the folded chromosome can be isolated by sucrose-gradient centrifugation in the presence of high salt concentrations or spermidine. Under these conditions DNA behaves more like a particle than a fiber [2]. Depending upon the lysing conditions, the nucleoid sediments as a 'membrane associated' or as a 'membrane free' particle [3].

The finding that, treatment of the isolated nucleoid from *E. coli* with sodium dodecyl sulfate (SDS) or with pancreatic RNAase, results in complete unfolding of DNA (sedimenting at less than 200 S) [2] and that, DNA from cells which were grown for a few minutes in the presence of rifampicin, was in an unfolded configuration, prompted Pettijohn et al. [4] to conclude that it is mostly metabolically active RNA and protein components which stabilize the folding of DNA.

After infection of *E. coli* with T phages the nuclear body may behave in two differing ways as judged from electron microscopy studies. Using phages T_2, T_4, T_5 and T_6 the nuclear structure of the host is destroyed shortly after infection, while the structure is not disrupted at all after infection with wild-type T_1, T_3 or T_7 [5–7]. The T-even phages and T_5 were classified as 'autonomous virulents', while T_1, T_3 and T_7 were called 'dependent virulents' since their development is apparently dependent upon the nuclear structure of the host [7].

Snustad et al. [8,9] have shown recently that nuclear disruption is not essential for T_4 phage development since burst-size and growth-rate experiments indicate that nuclear disruption-deficient mutants grow as well as the wild-type phage.

It is known that RNA synthesis is shut off shortly after infection with T_7 [10]; in this respect T_7 phage infection is similar to T_4 infection. Since the nucleoid may be stabilized by nascent RNA [4] one would expect that also in the case of T_7 infection nuclear disruption would occur [3]. Since this was not observed in T_7 infection cells using the microscopy technique [7], the sedimentation properties of the nucleoid structure were investigated in sucrose-gradients.

2. Materials and methods

Egg with lysozyme (EC 3.2.1.17 grade I) and Brij 58 (polyoxyethylene 20 cetyl ether) were obtained from Sigma Chemical Co., St. Louis, Mo. Sodium deoxycholate was from E. Merck A. G., Darmstadt, FRG. [2-^{3}H]Glycerol (500 mCi/mmol) and [2-^{14}C] thymidine (59 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, England. All other chemicals used were of reagent grade.

Escherichia coli B and T_7^+L bacteriophage used in this study were donated by Professor F. W. Studier.

The bacteria were grown with aeration at 30°C in M-9 medium supplemented with 0.5% Casamino acids.

Bacterial DNA was labelled with [2-^{14}C]thymidine (0.25 μCi/ml) and the cell membrane with [^{3}H]-glycerol (5 μCi/ml) for two generations.

Culture, 2.5 ml (3 X 10^{8} cells/ml) were quickly cooled and harvested by centrifugation. The remainder of the culture was infected with T_7 (m.o.i. 15) and samples of 2.5 ml were harvested at differing times after infection. The percentage of surviving cells was less than 1%.

Preparations of membrane associated or membrane free nucleoid were obtained by the procedure of Ryder and Smith [3]. The cells were treated with lysozyme for 2 min at 5°C and lysed with a mixture of Brij- 58 and sodium deoxycholate, at 5°C for 10 min for membrane associated nucleoid preparation and at 20°C for 20 min for membrane free nucleoid preparation.

Aliquots of the lysate (0.2 ml) were layered on a 5 ml 10–30% (w/v) sucrose-gradient containing 10 mM Tris (pH 8.1, at 4°C) 1 M NaCl, 1 mM EDTA and 1 mM β-mercapthoethanol. Centrifugation was performed at 16 000 rev./min for 10 min in the SW 50.1 rotor at 2°C for isolation of membrane associated nucleoids and for 25 min for the isolation of membrane free nucleoid. Stonington and Pettijohn [2] introduced a 4000 X g centrifuge step in order to remove unlysed cells, this step, however, was omitted since Ryder and Smith [3] have shown that 50–70% of the chromosomal material is lost by this centrifugation.

Fractions were collected from the bottom of the gradient directly onto glass filters. These were dried, washed in cold 5% TCA and rinsed in ethanol/ether (1:1). In order to measure the radioactivity sedimenting through the gradient, the bottom of the centrifugation tube was always washed after centrifugation and the wash added to fraction 1.

The dried filters were placed in scintillation vials containing toluene based scintillation fluid and radioactivity was determined in the Beckman L 100 scintillation counter. The recovery of radioactivity material was always greater than 85%.

The sedimentation coefficient was estimated directly [11] from the distance sedimented relative to that of T_4 phage (determined by spot-tests on a bacterial lawn).

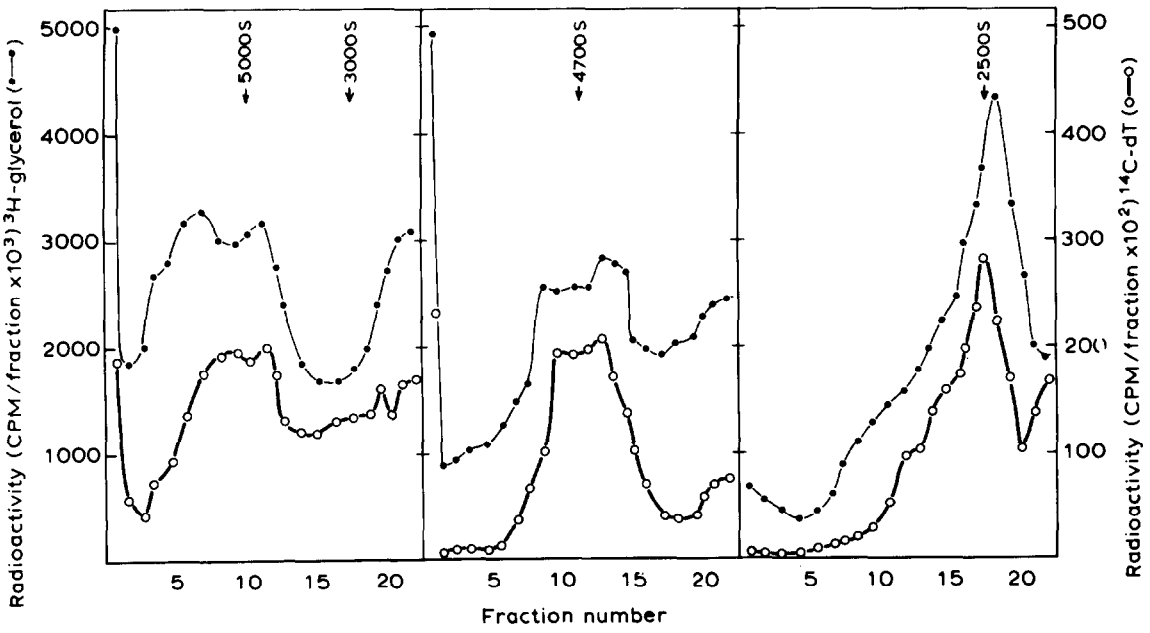

Fig.1. Sucrose-gradient profiles of double-labelled ([^{3}H]-glycerol and [^{14}C]thymidine) membrane associated nucleoids from T_7 infected cells. *E. coli* B cells were grown and labelled as described in Materials and methods. The cells were lysed at 5°C. (a) Uninfected cells, (b) and (c) cells harvested after 7 and 15 min infection, respectively.

3. Results and discussion

Recently, Tutas et al. [12] reported that in T_4 infected cells complete unfolding of the host nuclear structure (the nucleoid) occurs after 5 min infection at 30°C (sedimentation value less than 200 S). In fig.1 it is shown that DNA in T_7 infected cells sediments as a compact structure throughout the whole infection period, the s-value changes from approximately 5000 S for uninfected cells to 2500 S at 15 min after infection. After 7 min infection no change in the sedimentation profile of the nucleoid can be observed (fig.1) although at this time the degradation of the host genome caused by phage coded DNAases has started [13]. Host DNA synthesis is inhibited 6–7 min after infection. Accordingly the nucleoid observed 7 min after infection for the main part host DNA.

During infection host DNA is gradually broken down and the resulting nucleotides are used as precursors for phage DNA synthesis. After 15 min infection most of the genome is degraded [14] and DNA consists mainly of T_7 DNA. As seen from fig.1, most of the DNA from 15 min infected cells sediments at 2500 S. This is most likely the phage nucleoid.

Figure 1 shows that during sucrose-centrifugation approximately 60% of the [^{3}H]glycerol labelled membrane follows DNA when treatment with the detergents occurs at 5°C.

Figure 2 illustrates the sedimentation pattern of the membrane free nuclear structure at differing times after infection. The sedimentation value of the nucleoid decreases with the infection time from 2050–1025 S. This change may be caused by DNA-ases [4]. At 11 min a large fraction of DNA is found on the top of the gradient. Recentrifugation of the top fractions revealed the heterogeneous size-distribution of this DNA since the material had sedimentation values ranging from 200–800 S (data not shown). These sedimentation values are similar to those Serwer [15] found for DNA/protein complexes isolated from T_7 infected cells using other lysing and centrifugation conditions than used in this work.

More than 95% of the membrane (expressed as [^{3}H]glycerol) is found on the top of the gradient (data not shown). This is consistent to what was observed by Ryder and Smith [3] for the membrane when lysis was performed at 20°C.

As mentioned above phage DNA synthesis commences about 7 min after infection, whilst at this time host DNA synthesis is discontinued. Thus [^{3}H] thymidine added 7 min after infection is likely to result in labelled DNA of progeny phage.

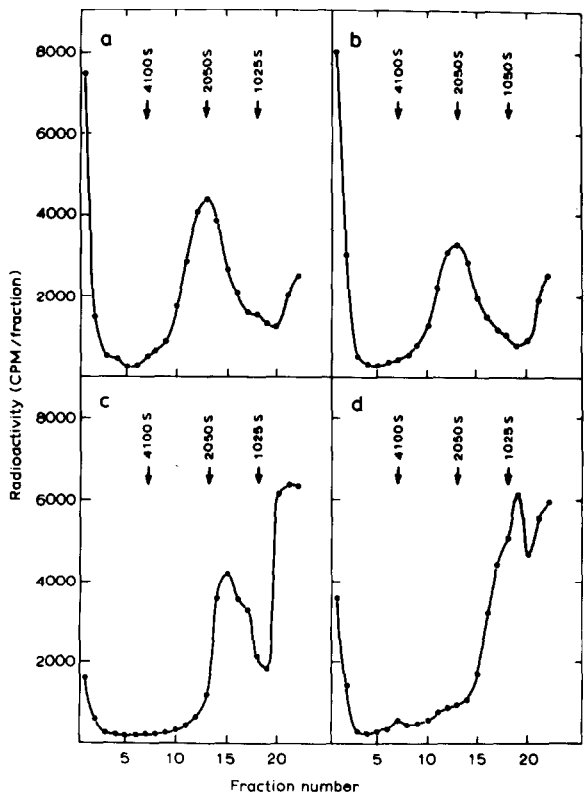

Fig.2. Sucrose-gradient profiles of membrane free nucleoids from T_7 infected cells labelled with 0.25 μCi/ml [^{14}C]-thymidine. The cells were lysed at 20°C as described in Materials and methods and centrifuged for 25 min at 16 000 rev./min on a 10–30% sucrose gradient. (a) Uninfected cells, (b–d), T_7 infected cells harvested at 6.11 min and 17 min after infection.

As seen from fig.3 the replicative forms of phage DNA appears to cosediment with host nucleoid 11 min infection forward. This cosedimentation could either be a coincidence, or progeny phage DNA may be integrated into the nuclear structure of the host [16].

The replication of T_7 DNA proceeds through concatemeric forms of DNA which are three to four times the length of the DNA found in phage particles [17,18].

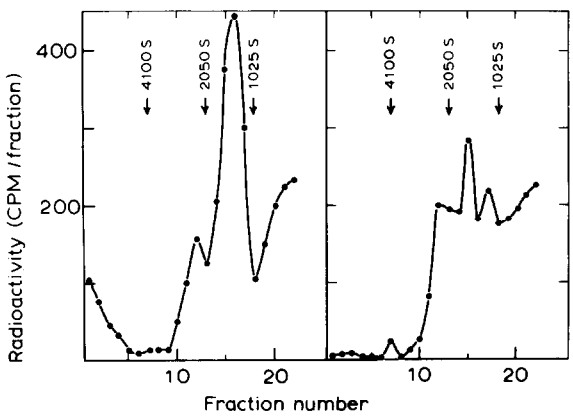

Fig.3. Sedimentation properties of membrane free T_7 nucleoid. *E. coli* B cells were grown and infected as described in the legend to fig.1 except that [^{14}C]-thymidine was added to the culture 7 min after infection. Figure 3(a), cells harvested 11 min after infection. Figure 3(b), cells harvested 17 min after infection. The nucleoid was prepared as described in Materials and methods and centrifuged for 25 min at 16 000 rev./min.

The DNA material sedimenting in the experiment illustrated in fig.3 is likely to represent intermediates between replicating DNA and complexes between bacteriophage T_7 capsids and T_7 DNA [19].

The results obtained in this study show that the nuclear structure observed in *E. coli* during T_7 infection using electron microscopy can be isolated as a nucleoid using the same conditions as previously reported for the *E. coli* nucleotide [2]. It remains to be shown, however, that T_7 development really demands an intact bacterial nucleus and is thus to be classified as a 'dependent-virulent'.

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