A PROCEDURE FOR THE EXTRACTION OF DNA AND PROTEIN GHOSTS FROM BACTERIOPHAGE LAMBDA

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Bacteriophage DNA is commonly prepared by denaturing the viral coat protein with such agents as phenol, guanidine, hydrochloride, urea, sodium perchlorate, or by using extremes of pH. Of these methods, phenol extraction has been the most widely used (Gierer and Schramm, 1956). For those phages which are subject to osmotic shock, rapid dilution from a concentrated salt solution will release the DNA (Anderson, et al., 1953) apparently without doing serious damage to the coat protein. In addition, at least a few phages release their DNA when subjected to crenation in concentrated sucrose (Anderson, et al., 1953), and it has been reported that pyrophosphates can be used to separate the ghost and DNA of the coliphage T2 (Van Vunakis and Herriot, 1962). This communication describes a procedure for obtaining either the DNA or protein ghosts of the bacteriophage lambda, a virus which is subject neither to osmotic shock nor to crenation in sucrose. It is readily adaptable to microliter samples of low concentration and

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results in essentially complete recovery of either DNA, the protein ghosts, or both simultaneously without apparent degradation or denaturation.

It was found that when a suspension of lambda (in a buffer containing 0.05 M NaCl and 0.01 M tris (hydroxymethyl) aminomethane at pH 7.3) was mixed and incubated with an equal volume of 10 M LiCl at 46° C for a few minutes before diluting with several volumes of buffer, a complete separation of phage protein and DNA takes place in such a manner that either component can be recovered.

That the separation occurs in the LiCl and not as a result of osmotic shock on dilution was demonstrated in a sedimentation velocity experiment in which the phage and LiCl were mixed directly in an ultracentrifuge cell and examined without dilution. Two sedimenting boundaries were observed using the schlieren optical system of the Spinco Model E analytical ultracentrifuge, the faster boundary having a sedimentation coefficient approximately corresponding to that of lambda ghosts (after correction for the estimated viscosity of the medium) and the slower one exhibiting the characteristic DNA spike.

If the incubation of the LiCl suspension of lambda is allowed to take place at lower temperatures, at higher LiCl concentrations, or for considerably longer times, a slow denaturation of the protein takes place, eventually leaving the DNA alone in solution. Thus, for example, undegraded DNA free of native protein was prepared by exhaustive dialysis at 0° C against 5 M LiCl buffered with 0.05 M tris, pH 7.5, followed by removal of the LiCl by dialysis against

a dilute buffer. Both solutions contained 0.001 M EDTA to guard against degradation by the trace amounts of deoxyribonuclease that seemed to be present in all the phage preparations used. Although pancreatic deoxyribonuclease was found to be inactive in LiCl concentrations above one or two molar, enzymatic activity was restored by dialyzing out the LiCl.

PREPARATION OF GHOSTS

For routine recovery of the phage protein the virus, at a concentration of about five milligrams per milliliter, was incubated for 10 minutes in 5 M LiCl at 46° C, then diluted ten-fold with a pH 7.5 tris buffer containing 0.001 M $MgCl_2$ and 10 mg/ml of deoxyribonuclease. The diluted solution was allowed to stand in a 37° C water bath for half an hour to allow the enzymatic degradation to take place and then centrifuged at 75,000 x g for three hours in a Spinco Model L preparative ultracentrifuge. The ghosts, after three washings in small quantities of dilute buffer, were estimated to have less than 1% nucleic acid contamination as measured by the ultraviolet absorbencies at 260 and 280 millimicrons.

For purposes of comparison, ghosts were isolated from lysates of ultraviolet-induced E. coli K12 (λ) by differential centrifugation and further purified by density gradient sedimentation in sucrose (Martin and Ames, 1961). These ghosts, which are probably the result of imperfect or incomplete phage synthesis, were compared with the LiCl produced ghosts. The two preparations had essentially identical amino acid profiles (as measured on a Spinco

amino acid analyzer after a 24-hour hydrolysis in 6 M HCl at 110° C), optical rotatory dispersion curves in the ultraviolet, and sedimentation coefficients. At a concentration of 0.58 mg/ml the "native" ghosts gave an $S_{20,w}$ of 146 svedbergs and a specific rotation of 5300 $(deg/dm)(gm/ml)^{-1}$ at 233 mu, while a 0.65 mg/ml solution of the LiCl-derived ghosts gave values of 146 and 5100, respectively. (Concentrations were estimated from refractive index measurements using bovine serum albumen as a standard.) From these comparisons it would seem that the LiCl-derived ghosts consist of undenatured protein that is structurally and chemically similar to or identical with the native lambda protein.

PREPARATION OF PROTEIN-FREE DNA

To obtain the phage DNA free of all traces of the phage protein, the following procedure was followed. After the 46° C incubation in LiCl, the solution was diluted with three volumes of tris buffer containing EDTA, then dialyzed against the same buffer to remove the LiCl. The dialyzed solution was brought to a density of 1.71 gm/ml with CsCl and spun in the SW-39 rotor of the Spinco preparative ultracentrifuge for 24 hours at 33,000 rpm, thus separating the DNA and protein in a density gradient (Meselson, et al., 1957). The density was chosen so that the DNA banded near the center of the tube (Rolfe, cited in Kaiser and Hogness, 1960) while the protein floated to the top, allowing its easy removal.

It is, of course, important that the LiCl-released DNA be undenatured and undegraded if the method is to be a useful preparative technique. For this reason a

sample of lambda DNA, released at 46° C in 5 M LiCl, was studied in the analytical ultracentrifuge under similar conditions of temperature, rotor speed, buffer and solute concentration as those employed in the characterization of phenol-extracted DNA (Hershey, et al., 1963). The observed rate of sedimentation was the same as that reported by Hershey, to within the accuracy of the method employed.

APPLICATION TO OTHER VIRAL SYSTEMS

Concentrated LiCl could undoubtedly be used in the extraction of DNA from most of the other phages because of its ability to denature protein under certain conditions (Mandelkern and Roberts, 1961) and in fact has been used in this laboratory for the extraction of T2 and T4 DNA as well as that of lambda. In general, it has those advantages outlined by Freifelder for sodium perchlorate extraction (Freifelder, 1965), especially in that it avoids the use of ultraviolet-opaque reagents and allows easy inactivation of deoxyribonuclease. The mechanism whereby it is able to release lambda DNA without serious disruption of the protein is unknown, but preliminary experiments indicate that T2 may be similarly affected.

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