# A NOVEL METHOD FOR THE RELEASE OF BACTERIOPHAGE DNA

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Ideally a procedure for the isolation of undamaged DNA from bacteriophage should have the following characteristics: (i) it should be rapid, (ii) the extracting material should have no optical absorption at 260 mμ, (iii) nucleases should be inactivated, (iv) no adverse chemical effects should result, (v) the method should be applicable to semimicro samples, e.g., 10 μliters, and to samples of low concentration, i.e., 1-10 μg/ml, and (vi) it should work for all phages. Of the methods in current use, phenol extraction (Mandell and Hershey, 1960) requires additional procedures to remove the high $OD_{260}$ left by residual phenol and does not satisfy criteria (iii) and (v); release by alkali or heating (Davison and Freifelder, 1962) fails for items (iii) and (vi) and possibly for (iv). This report describes a new technique which seems to satisfy these six criteria and which is probably also useful for extraction of nucleic acids from animal and plant viruses and bacteria.

DNA has been extracted from coliphages T4, T7 and λ, and Pseudomonas aeroginosa phage B3 by treatment with neutralized 5 M $NaClO_4$ containing $5 \times 10^{-3}$ M EDTA. The procedure consists simply of adding a phage suspension to a concentrated $NaClO_4$ solution, mixing, and waiting until DNA release occurs. Release can be stopped at any time after addition of the phage by dilution with sodium phosphate, pH 7.8, to a perchlorate concentration of 1 molar. The rate of release has been measured by determining in the analytic ultracentrifuge the relative amounts of material sedimenting in both the phage and DNA boundaries

after various times of exposure to high perchlorate concentrations. It was found that the rate of release varies with the phage species and is dependent on the perchlorate concentration. At 5 M the process is complete in 10 sec for B3, 20 sec for λ and T7, and 1 min for T4. The concentration dependence of the rate of release for phage T7 is shown in Table I. It should be noticed that for both 2.3 and 3.7 M the kinetics are peculiarly biphasic. This enables the phage population to be fractionated in a way not yet understood.

TABLE I

Percentage of DNA Released from Phage T7
As a Function of Time for Various Concentrations of $NaClO_4$

| Concentration | Time, sec | % Release |
|---|---|---|
| 5.0 M | 5 | 50 |
| " | 10 | 90 |
| " | 20 | 100 |
| 3.7 M | 10,20 | 65 |
| " | 30 | 70 |
| " | 40 | 80 |
| " | 50 | 100 |
| 2.3 M | 20 | 40 |
| " | 50,60,90,100,150, 270,600,900 | 50 |
| " | 30 min | 100 |

DNA isolated by perchlorate extraction from T7 and T4 is intact by the criterion of sedimentation velocity homogeneity (Davison and Freifelder, 1962). For λ and B3, but not for T4 or T7, an unexpected phenomenon occurs - that is, the DNA is released stepwise - first, as a rapidly sedimenting form and then (only if the high concentration of perchlorate is maintained) as the form with the usual sedimentation

coefficient. This will be described in detail in a subsequent publication. For each of these phages it has also been possible to extract the DNA in the denatured state simply by raising the perchlorate concentration and adding HCHO. This result was not unexpected in view of the denaturing effect on DNA of very high concentrations of $NaClO_4$ (Hamaguchi and Geiduschek, 1962) and of HCHO (Freifelder and Davison, 1963).

If T7 phage is mixed with 10 μg/ml of pancreatic DNAase in $10^{-3}$ M $MgSO_4$ - $10^{-2}$ M $PO_4$, pH 7.8, and the DNA is released by incubating for 10 min either at pH 7.8, 70°C or at pH 11.5, 25°C, enough DNAase survives the extraction procedure that the DNA is severely damaged. In a typical experiment the $S_{20,w}$ was 20 instead of the normal value of 29. Treatment of a similar sample with 5 M $NaClO_4$ yielded DNA with no indication of nuclease damage. Whether the DNA-ase is irreversibly inactivated or if the result is a consequence of the insolubility of $Mg(ClO_4)_2$ is not yet clear.

In preliminary experiments, transforming DNA has been extracted with 2 M $NaClO_4$ from B. subtilis phage SP50 (Trautner, personal communication).

The mechanism of action of $NaClO_4$ is not known. It is not simply osmotic shock because (i) NaCl at equivalent concentrations has no effect, (ii) the procedure works for T4 $O^r$, the osmotic shock-resistant form of T4, and (iii) the effect is independent of dilution rate and in fact does not even require dilution, i.e., if the phage is added to 2.3 M $NaClO_4$ and the undiluted solution centrifuged, the sedimentation boundary is that of DNA.

Experiments in progress include detailed studies of the concentration dependence and kinetics of release, the use of perchlorate as a denaturing agent for DNA and RNA and as an extracting agent of DNA in bacteria and animal cells, and electron micrographic studies of perchlorate-released DNA.

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