SEPARATION OF PHENOL AND DEOXYRIBONUCLEIC ACID BY SEPHADEX GEL FILTRATION*

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(Received April 27th, 1962)

INTRODUCTION

KIRBY has reported on the use of certain salt solutions and phenol in liberating deoxyribonucleic acid (DNA) from mammalian cells[1]. This report has been confirmed and enlarged upon by COLTER *et al.*[2], who showed that DNA preparations isolated by this method are remarkably free of contamination by polypeptides, polysaccharides, and ribonucleic acid (RNA). Most recently, MANDELL AND HERSHEY[3] have demonstrated the utility of phenol for extracting phage DNA of extremely high molecular weight with virtually no shear degradation. Present methods for the removal of phenol from aqueous DNA preparations involve repeated extractions with diethyl ether. The authors suggest the use of Sephadex gel filtration as a method of removing phenol residues from aqueous DNA preparations.

MATERIALS AND METHODS

DNA from two sources was used in these experiments. Labeled DNA was isolated, by mercaptoethanol–tryptic lysis[4], from HeLa S-3 cells grown in culture media containing tritiated thymidine. Purified and highly polymerized salmon sperm DNA was purchased from the California Corporation for Biochemical Research. Sephadex G-25 and G-50 were purchased from Pharmacia Laboratories, Inc. Redistilled phenol was used for extraction purposes, and all chromatographic techniques were carried out at room temperature.

Ultraviolet optical density measurements were carried out in a Beckman DU spectrophotometer. The distribution of tritium activity was followed using a Packard Model 314EX Tri-Carb liquid scintillation counting system.

The presence of deoxyribose in fractions from the Sephadex columns was assessed by the diphenylamine reaction of DISCHE, as described by SCHNEIDER[5] and modified in our laboratory. The diphenylamine reagent consisted of 1 g of diphenylamine recrystallized from hexane, 100 ml of glacial acetic acid, and 2.75 ml of concentrated H_2SO_4. The reagent was prepared immediately before use. To 1 ml of column effluent was added 1 ml of 10% trichloracetic acid and 2 ml of diphenylamine reagent. The mixture was heated for 10 min at 90°, cooled to room temperature, and absorbance at 600 mμ was measured. Preliminary simultaneous deoxyribose and

* Work performed under the auspices of the U.S. Atomic Energy Commission.

phosphorus determinations on salmon sperm DNA samples then permitted quantitative evaluation of the amount of DNA added and recovered in the effluent from the Sephadex columns, expressed as micrograms of DNA phosphorus. Recoveries in each case were essentially quantitative, and phenol did not interfere in the determination.

The Lowry protein determination, as described by CHOU AND GOLDSTEIN[6], was carried out on 1-ml aliquots of samples. The optical density at 750 mμ was determined using a Bausch and Lomb Spectronic 20 spectrophotometer. This method was used to determine the distribution of phenol in column effluents.

EXPERIMENTAL

One hundred mg of salmon sperm DNA were stirred with 100 ml of 0.01 *M* phosphate buffer, pH 7.2, for 48 h at 5°. The resulting suspension was centrifuged at 85,000 × *g*, for 30 min. The supernatant was removed and assayed for deoxyribose, Lowry coloration, and its optical density at 260 mμ. A 20-ml aliquot of this solution was stirred with an equal volume of water-saturated phenol for 30 min at room temperature. The two phases were separated by low-speed centrifugation, and the aqueous layer was removed and assayed for deoxyribose. A 5-ml aliquot was allowed to percolate into a 2 × 30 cm column of Sephadex G-25 which had been equilibrated with 0.01 *M* phosphate buffer, pH 7.2. The column was eluted with 0.01 *M* phosphate buffer, and 4-ml fractions were collected at 5-min intervals. Aliquots of each fraction were analyzed for deoxyribose, phenol, and absorbance at 260 mμ (Fig. 1).

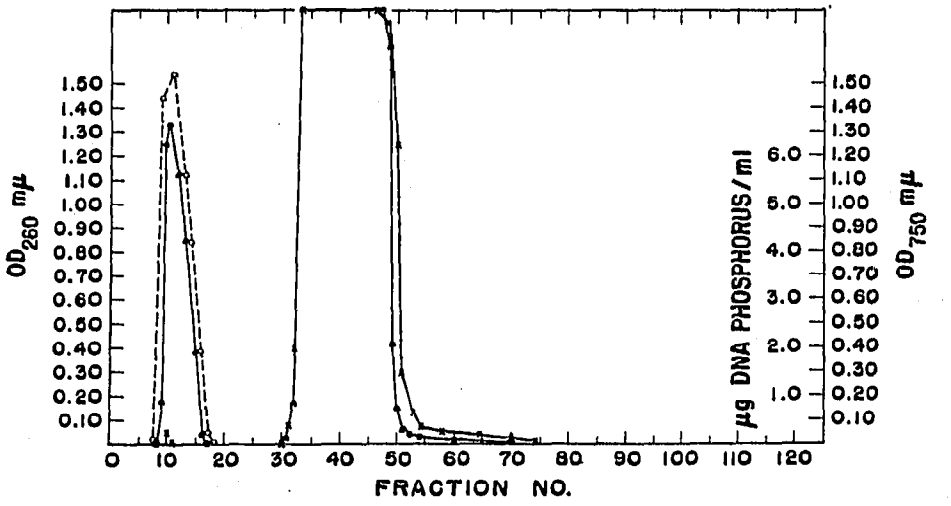

Fig. 1. Gel filtration of an aqueous DNA–phenol mixture. Column, 2.0 × 30 cm; Sephadex G-25; sample volume, 5 ml; eluant, H_2O; fractions collected, 4 ml every 5 min. ●—●—● = O.D. 260 mμ; ×—×—× = O.D. 750 mμ (Lowry determination); O—O—O = μg DNA phosphorus/ml.

The experiment was repeated using a freshly prepared phenol extract of DNA. Two ml of this extract were placed on a 2 × 30 cm column of Sephadex G-25 which had been equilibrated with distilled water. The column was eluted with distilled water, and 5.5-ml fractions were collected at 5-min intervals. Aliquots of each fraction were analyzed for deoxyribose, phenol, and absorbance at 260 mμ (Fig. 2).

The experiment was repeated a third time, the only difference being that the Sephadex column was equilibrated and eluted with 0.015 *M* NaCl. The results obtained were identical to those exemplified in Figs. 1 and 2.

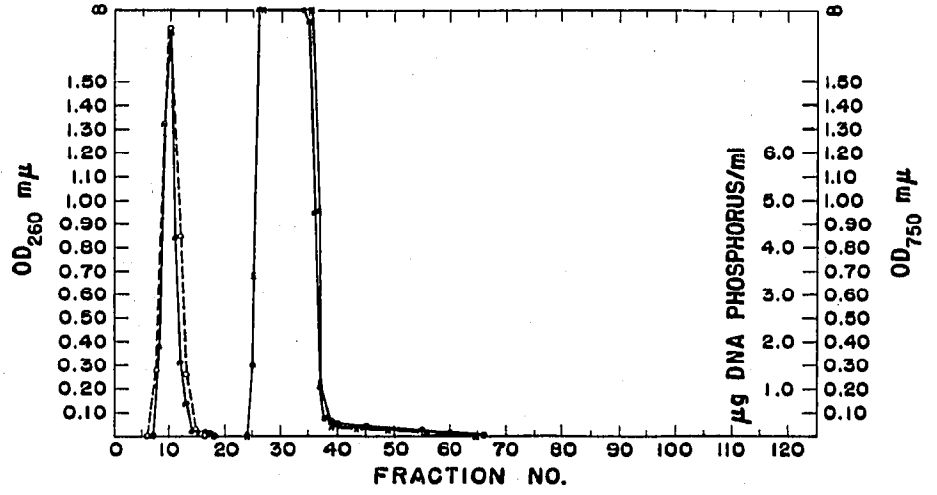

Fig. 2. Gel filtration of an aqueous DNA–phenol mixture. Column, 2.0 × 30 cm; Sephadex G-25; sample volume, 2 ml; eluant, 0.01 *M* phosphate buffer, pH 7.2; fractions collected, 5 ml every 5 min. ●—●—● = O.D. 260 mμ; ×—×—× = O.D. 750 mμ (Lowry determination); ○—○—○ = μg DNA phosphorus/ml.

Finally, an aqueous phenol extract was obtained from HeLa S-3 cell lysate. This extract contained less than 1 μg of DNA per ml and possessed an activity of 16,500 counts/min/0.1 ml, quantity corrected for phenol quenching. A 1-ml aliquot of this extract was percolated into a 1 × 15 cm Sephadex G-50 column which had been equilibrated with distilled water. The column was eluted with distilled water, and 2-ml fractions were collected at 5-min intervals. Aliquots of each fraction were analyzed for optical density at 260 mμ and tritium activity (Fig. 3).

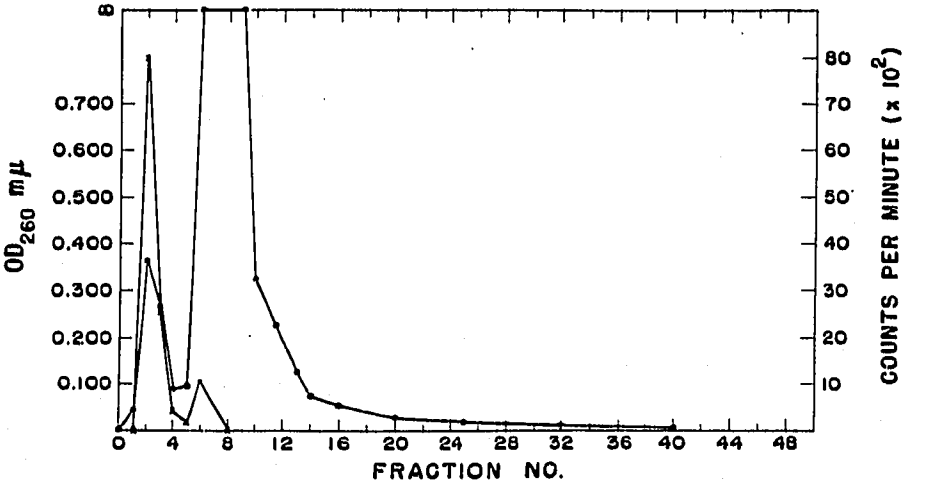

Fig. 3. Gel filtration of an aqueous mixture of phenol and tritium-labeled HeLa S-3 DNA. Column, 1 × 15 cm; Sephadex G-50; sample volume, 1 ml; eluant, H_2O; fractions collected, 2 ml every 5 min. ●—●—● = O.D. 260 mμ; ×—×—× = counts/min.

DISCUSSION

It may be seen that gel filtration on a Sephadex G-25 or G-50 column effects a separation of the components of a phenol–water extract containing DNA. The DNA peak was characterized by its absorbance at 260 mμ and its deoxyribose content. The phenol peak was characterized by its absorbance at 260 mμ and its color formation with the Lowry reagent. Pilot studies carried out on different samples of DNA suggested that purified, protein-free DNA gave a negligible coloration with this reagent.

A separation of DNA and phenol on Sephadex occurred when the column was eluted with water, 0.01 *M* phosphate buffer, or 0.015 *M* NaCl. Duplicate determinations of deoxyribose by the DISCHE method indicated 100% recovery of the applied DNA in the effluent. While it was impossible to carry out parallel assays of the HeLa S-3 DNA eluted from the Sephadex G-50 column, data based on the total counts of each fraction suggest quantitative recovery in this case as well.

The method offers the significant advantage of virtually quantitative recovery of DNA from phenol-extracts of cell lysates in the absence of denaturation by organic solvents and with a minimum of mechanical shear.

SUMMARY

Mixtures of deoxyribonucleic acid (DNA) and aqueous phenol have been separated into their components, phenol and DNA, by Sephadex gel filtration. Recovery of DNA is quantitative. This procedure is suggested as an adjunct to the phenol extraction method for DNA isolation.

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