ISOLATION OF LARGE MOLECULAR WEIGHT DNA FROM AGAROSE GELS FOR FURTHER DIGESTION BY RESTRICTION ENZYMES

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1. Introduction

Specific fragmentation of DNA molecules by restriction enzymes and characterisation of the fragments obtained has developed into a powerful tool for the analysis of viral genomes as well as for cloning of defined DNA sequences. After digestion of DNA with specific nucleases a physical separation of different size classes is usually achieved best by electrophoresis either in polyacrylamid gels for fragments up to about 2×10^6 dalton or in agarose gels for fragments between 2×10^6 and 1×10^8 [1]. The elution of such fragments after electrophoretic separation in polyacrylamide gels and their redigestion with different restriction enzymes has contributed substantially to the physical mapping of viral genomes like SV 40 and Polyoma DNA [2,3]. In contrast, DNA eluted from agarose gels usually resists any further digestion with restriction enzymes, a problem observed in our own as well as in other laboratories. The resistance of 'agarose DNA' to restriction enzymes made it extremely difficult so far to further map large pieces of DNA separated by agarose gel electrophoresis. The inhibition of these specific nucleases was observed whether the DNA was eluted by electrophoresis or by dissolving the agarose at high concentrations of chaotropic ions as iodide followed by precipitation at low ionic strength. Phenol extraction as well as cesium chloride-equilibrium centrifugation of the DNA did not remove the enzyme inhibiting material. We have found a very simple procedure which overcomes this problem: centrifugation of agarose DNA mixtures to equilibrium in potassium iodide gradients (KJ-gradients) yields DNA of high purity and normal sensitivity towards restriction enzymes.

2. Materials and methods

Agarose for gel electrophoresis was purchased from Seakam, MCI-Biochemicals (Rockland, Maine, USA). Potassium iodide was reagent grade from E. Merck (Darmstadt, Germany). Restriction enzyme HindIII was isolated as described by Smith and Wilcox [4]; Sal 1 endonuclease was obtained from streptomyces albus according to Roberts [5]; EcoR1 restriction enzyme [6] was a gift of Dr G. S. Hayward (University of Chicago). The exact digestion conditions for T5 DNA and the mapping of the fragments will be published elsewhere [7]. T5 DNA was prepared as described previously [8].

2.1. *Agarose gel electrophoresis*

DNA electrophoresis was carried out in 0.5% agarose gels [1] at 30 V per gel and 4°C for 18 hr. Between 1 and 3 μg were applied to analytical gels (diameter 1 cm) whereas up to 20 μg could be separated on preparative gels (diameter 3 cm). The DNA bands were stained by immersing the gels in aqueous ethidium bronide solution (0.5 μg/ml). Stained gels were illuminated with ultraviolet light and photographed on Kodak Panatomic x film. In preparative gels only the outside of the DNA bands was stained by short exposure to ethidium bromide in order to avoid any photodynamic effects. From such gels the DNA containing bands were cut and dissolved in saturated KJ solution.

2.2. *Isopycnic centrifugation in potassium iodide gradients*

The DNA and agarose containing KJ solution were adjusted to a final density of 1.5 g/ml and an agarose concentration of 0.1 to 0.2%. This solution is then

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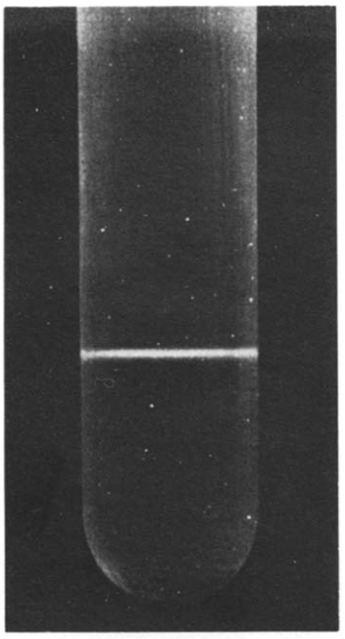

Fig.1. Potassium iodide gradient (initial density 1.45 g/ml) after 40 hr at 40 000 rev/min and 20°C (Spinco rotor SW 56). The gradient contained 25 μg of DNA and 50 μg/ml of ethidium bromide. Up to 45 μg of DNA from 3 preparative agarose gels could be isolated in a single 4.5 ml gradient.

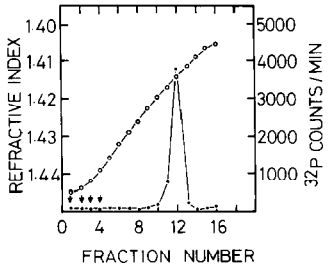

Fig.2. Banding pattern of ^{32}P-labelled T5 DNA (mol. wt 76 × 10^6) and agarose in a potassium iodide density gradient. The density gradient was determined by measuring the refractive index of the gradient fractions. The position of the DNA was determined by monitoring Cherenkov radiation of the different fractions whereas the agarose was detected by its precipitation in 50% ethanol. Arrows indicate the positions of agarose.

centrifuged to equilibrium at about 200 000 *g* for 20 to 40 hr and 20°C. If the KJ gradient contains ethidium bromide (50 μg/ml) the DNA can be directly visualized by ultraviolet illumination (fig.1). In the absence of ethidium bromide the position of the DNA can either be determined by monitoring the radioactivity (fig.2) or by spotting 10 μl samples of the different fractions onto glassfibre paper (Whatman GF/A). After drying the glassfibre paper is immersed in an aqueous ethidium bromide solution (0.5 μg/ml) for several minutes. Spots containing 0.02 μg of DNA or more can clearly be distinguished in ultraviolet light DNA containing fractions were pooled and, if ethidium bromide was present in the gradient, extracted 3 times with *n*-butanol before they were dialyzed against 0.01 M sodium phosphate 0.001 M EDTA, pH 7.9 and stored at 4°C. Such DNA preparations which were obtained in concentrations between 5 and 60 μg per ml depending on the molecular weight of the fragment were directly used for further digestion with restriction enzymes or for transcription studies with *E. coli* RNA polymerase.

3. Results and discussion

The application of potassium iodide gradients for the isolation of DNA from agarose gels appears to be advantageous as compared to a variety of other procedures. Agarose gels dissolve readily in saturated aqueous KJ solutions (G. S. Hayward, personal communication) which subsequently can be adjusted to densities suitable for isopycine centrifugation. For this adjustment the following equation which connects denstiy (ρ; g/ml) with refractive index (n_{20}^D) for KJ solution can be used

$$n_{20}^D = 0.1731 \cdot \rho + 1.1617 \text{ (valid for } 1.3 \leqslant \rho \geqslant 1.7)$$

The high density of agarose in potassium iodide permits it's quantitative removal from DNA (fig.2). The DNA obtained in yields between 90 and 95% is free of any enzyme inhibiting material and is indistinguishable from starting material with respect to its physical properties. Fig.3 shows the electrophoretic pattern of T5 DNA (mol. wt 76 × 10^6) freshly extracted from phage (a) and after reisolation from agarose gels (b). Analysis of alkali denatured DNA on

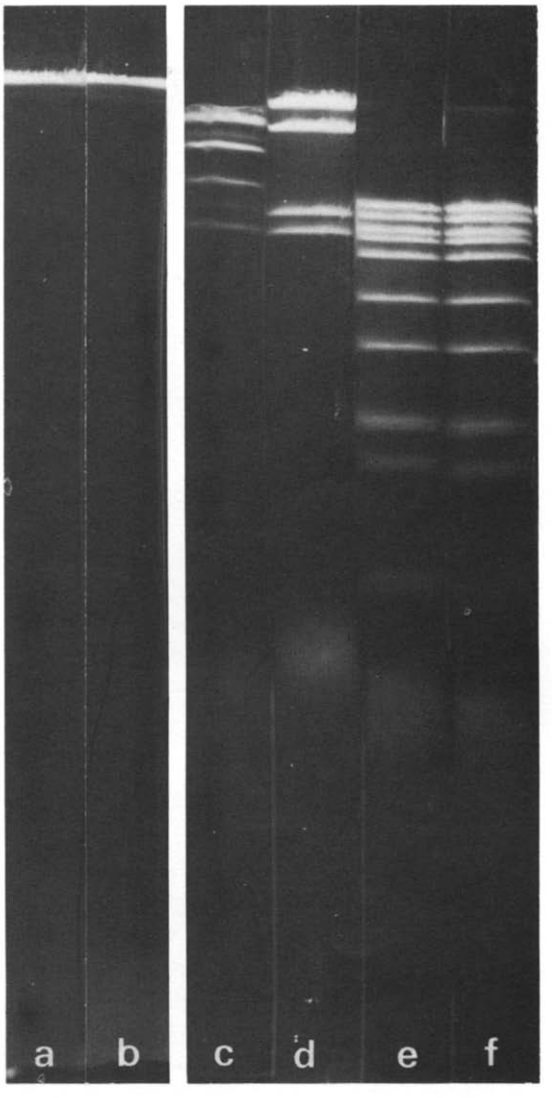

Fig.3. Electrophoresis of T5 DNA. Each gel contains between 1 and 3 μg of DNA. The different gels show the following samples: (a) T5 DNA directly after extraction from phage. (b) Aliquot of the DNA described under (a) after electrophoresis in a 0.5% agarose gel and its reisolation in a potassium iodide density gradient. (c,d,e) show the effect of restriction endonucleases EcoR1, Sal 1 and HindIII respectively on T5sto DNA reisolated from agarose gels. (f) Pattern obtained from freshly extracted T5sto DNA after digestion with HindIII enzyme.

agarose gels yields the same single strand pattern [1] for both DNA's indicating that no single strand breaks are introduced during the isolation procedure (data not shown). Digestions of reisolated DNA with restriction endonucleases EcoR1, Sal 1, HindIII (fig.3 c–e) as well as with Hpa I and Hpa II (data not presented) show that this DNA exhibits the same sensitivity towards these nucleases as normal T5 DNA. Similarly if the reextracted DNA was used as template for *E. coli* RNA polymerase the expected rate of ribonucleotide incorporation was observed (data not shown).

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