Nucleic Acids Research

A general method for isolation of high molecular weight DNA from eukaryotes

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ABSTRACT

A new method for isolation of high molecular weight DNA from eukaryotes is presented. This procedure allows preparation of DNA from a variety of tissues such as calf thymus or human placenta and from cells which were more difficult to lyse until now (e.g. Crypthecodinium cuhnii, a dino-flagellate). The DNA obtained in such a way has an average molecular weight of about 200×10^6 d and contains very few, if any, single strand breaks.

INTRODUCTION

Isolation of large quantities of nick-free, high molecular weight DNA from eukaryotic organisms has heretofore presented considerable technical difficulties. DNA prepared by conventional techniques has been a hetero-geneous population of molecules ranging in molecular weight from 10×10^6 to 20×10^6 d (1, 2). The single strand molecular weight was often around 0.5×10^6 s.

A major improvement was made when Chambon's laboratory (3) introduced the use of proteinase K for DNA isolation. This method is excellent for monolayers of tissue culture cells that can be instantaneously lysed. However, for difficult-to-homogenize tissues, considerable time elapses between cell rupture and the inactivation of nucleases.

We have found that homogenization of tissues in a Waring Blendor in the presence of liquid nitrogen completely disrupts the tissue and allows isolation of "nick"-free DNA. This modification of previous techniques has proved so useful to our laboratory and to numerous others that we wish to describe the method in detail and document the quality of the DNA.

MATERIALS AND METHODS

Agarose for gel electrophoresis was purchased from Seakem (Rockland, Maine). Proteinase K (Merck) and RNase A (Sigma) were commercial enzymes. RNase A was made up to 2 mg/ml in 0.15 M NaCl and heated for 15 minutes at 80°C before use. Sarkosyl was a gift from Ciba-Geigy.

Agarose gel electrophoresis: DNA electrophoresis was carried out in cylindrical 1 x 14 cm 0.5% agarose gels at 30 V and 4°C for 14 hr (4).

For each component, 0.5 µg DNA was applied to the gel. The gels were stained and photographed as previously described (5). The negatives were traced with a Joyce Loebl microdensitometer.

Analytical centrifugation: Sedimentation coefficients of DNA in alkaline and neutral solutions were determined by band sedimentation in a 30 mm cell in the Beckman Model E analytical ultracentrifuge. The average S values and molecular weights of native and denatured DNAs were calculated according to Studier (6). As a test of our conditions we evaluated the sedimentation coefficient of native λ DNA. Our value was within 0.3 S units of the published value (7).

Isolation of DNA: Fresh tissue was immediately frozen in small pieces in liquid nitrogen. A dry metal Waring Blendor was 1/2 filled with liquid nitrogen and the tissue added. The top of the Waring Blendor was covered with 6-8 layers of cheesecloth and its lid was held on with a gloved hand during homogenization. After the tissue was completely powdered, the liquid nitrogen was allowed to evaporate until the powdered tissue was just covered. The powder was then transferred in small aliquots into 2 L of 0.5 M EDTA, 0.5% Sarkosyl, 100 µg/ml proteinase K, pH 8, 50°C in a 4-liter flask. The flask was secured in a New Brunswick gyratory water bath shaker by a masthead knot (8). The solution was swirled at about 200 rev/min during the addition of the powdered tissue and during the subsequent 2-3 hour incubation. The rotary motion aided in solubilizing the tissue and sheared the DNA to about 200×10^6 d. Tissue was added until the solution was very viscous but all the tissue could be solubilized. This resulted in a final DNA concentration of 100-200 µg/ml. Three phenol extractions were carried out with phenol (500 gram), m-Cresol (70 ml) equilibrated with tris buffer pH 8 (9). After equilibration, 8-hydroxyquinoline was added to 0.1%. It was essential that the pH of the phenol phase be around pH 8 to prevent the DNA from being lost in the interphase. The pH of the phenol solution may be measured directly as water saturated phenol behaves as a normal aqueous solution. We poured off the upper DNA solution and found this to be preferable to attempting to pipet the viscous solutions. The final DNA solution was dialyzed into 0.05 M Tris pH 8, 0.01 M EDTA, 0.01 M NaCl. Dialysis was continued until the A_{270} of the supernatant was less than 0.05. Heat-treated RNase A was then added to 100 µg/ml and the RNA was digested for 3 hours at 37°C. During this treatment the solution was swirled as before at 200 rev/min. Two additional phenol extractions were then carried out and the resulting DNA was dialyzed to a dilution of 10^6

against 0.01 M sodium borate, 0.001 M EDTA, pH 9.1.

To achieve the results described here it was necessary that all solutions and glassware be sterilized. All dialysis tubing was boiled in 0.01 M EDTA pH 8 immediately before use and handling of dialysis tubing was done with sterile disposable gloves.

Shearing of DNA: The DNA was sheared by 100 passes through a 10 x 0.04 cm precision bore glass capillary at 30 PSI. The apparatus was similar to the one described by Bowman and Davidson (10) but adapted for about 1.5 L of DNA solution.

RESULTS AND DISCUSSION

We have successfully used the liquid N_2 extraction method for calf thymus and human placenta and the procedure was applied with good results to mouse tissues (F. Polsky, NIH, Bethesda, personal communication) and to protozoan cells (Crypthecodinium cuhnii, a dinoflagellate, R. Steele, Yale University, personal communication). Moreover, the extracted DNA has a high molecular weight. Freshly isolated calf thymus DNA was analyzed by sedimentation velocity. Its large sedimentation coefficient (65.1 S) was calculated from the slope in Figure I a. Using the
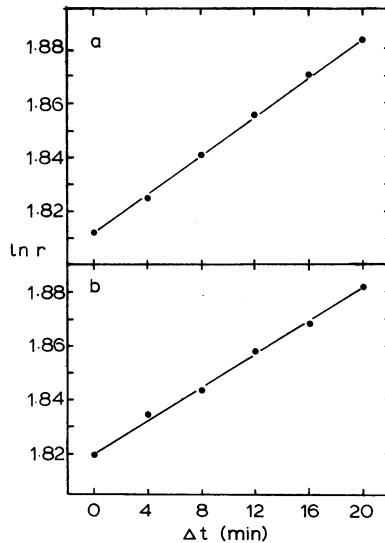

Figure 1. Analytical band sedimentation of bovine DNA
The DNA was isolated from calf thymus tissue using the liquid N_2 method and centrifuged at 31410 rev/min. The motion of the DNA band was converted to ln r and plotted versus Δ t. The sedimentation coefficients of native and alkali denatured DNA were calculated from the slopes of the curves.
(a) native DNA 65.1 S (1.0 M NaCl, 0.01 M TRIS pH 7.8),
(b) alkali denatured DNA 59.3 S (0.9 M NaCl, 0.1 M NaOH).

correlation $S^o_{w,20} = 0.0882 \times M^{0.346}_w$ (6) an average molecular weight of 195×10^6 was obtained. However, an effect of the rotor speed on sedimentation profiles has been noted (11, 12). Our value, therefore, may not represent a true value of molecular weight. To investigate whether single strand breaks were introduced by our extraction method, the DNA was tested by alkali band sedimentation. A value of 74.5 S was found for denatured DNA from human placenta (molecular weight of 75×10^6 d) and similar results were obtained for calf thymus DNA (Figure I b). These data suggest that our high molecular weight DNA preparation contained less than one break per DNA strand.

Because of its large size and relatively high concentration (about 200 µg/ml) the DNA forms a very viscous solution which is difficult to handle. We therefore think that it is important to shear the DNA to a homogeneous size population. Sheared DNA behaves much better in equilibrium density gradients and allows better interpretation of restriction nuclease patterns on agarose gels. Starting with DNA of around 200×10^6 d, shearing at 30 PSI, yielded an homogeneous population of DNA molecules. Figure 2 shows human DNA sheared to size of ca. 30×10^6 d. In order to demonstrate the homogeneity, the sedimentation profile of denatured DNA
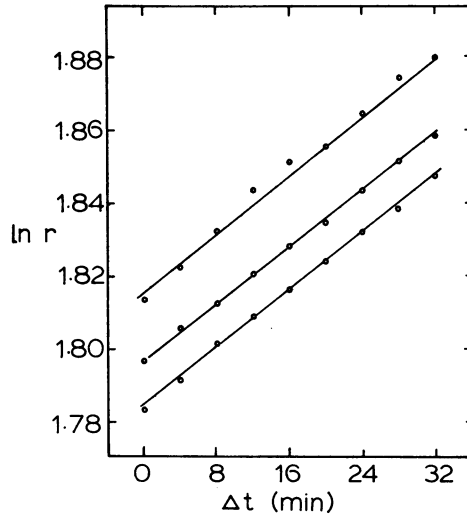
Figure 2. Sedimentation velocity of single strand human placenta DNA
The isolated DNA was sheared at 30 lb/inch^2 at a concentration of 180 µg/ml (100 cycles, 0.01 M $Na_2B_4O_7$, 1 mM EDTA, pH 9.1) and centrifuged at 31410 rev/min (0.9 M NaCl, 0.1 M NaOH). The peak (middle curve) and the points at half height of the leading edge (upper curve) and the trailing edge (lower curve) were taken from the sedimentation profiles and plotted as in Figure 1.

was analyzed. The peak as well as points at half height of the leading and
trailing edge were plotted. Three parallel curves resulted, thus indicating
the homogeneous distribution of the sedimenting band. The $S_{w,20}^{o}$ was 36 S
which corresponded to a molecular weight of 12×10^{6}. The size distribution
of the isolated, sheared DNA was further characterized by agarose gel
electrophoresis. When human placenta or mouse liver DNA was sheared to
ca. 30×10^{6} and run on gels, a sharp band of native DNA was found
(Figure 3) next to a marker band of T 3 DNA (25×10^{6} d). The commercially
available calf thymus DNA, on the contrary, shows a very diffuse distribution.

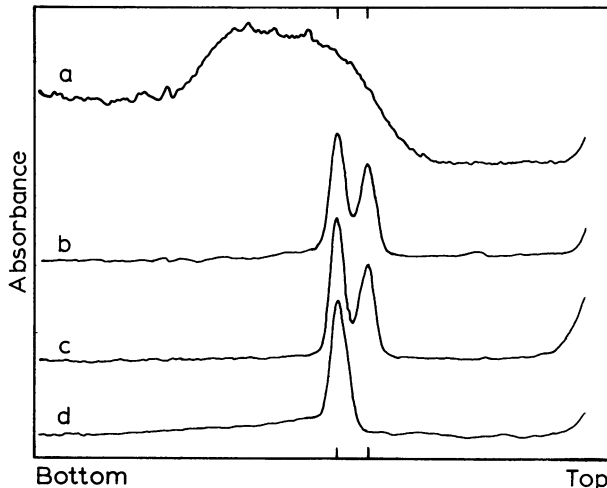Figure 3. Densitometer tracings of agarose gel electrophoresis
　　　　　　Electrophoresis was performed as described in Materials and
　　　　　　Methods. Direction of migration is from right to left. The
　　　　　　tracings show the following samples
　　　　　　(a) commercially available calf thymus DNA
　　　　　　(b) T 3 DNA and human placenta DNA
　　　　　　(c) T 3 DNA and mouse liver DNA
　　　　　　(d) T 3 DNA (25×10^{6} d) (14)
　　　　　　The freshly isolated mammalian DNAs were sheared to about 30×10^{6} d.

　　　　Our method is not limited to the tissues described here or to DNA
isolation. It provides a convenient method for disrupting tissues for
enzyme or RNA extraction and has in fact been used for isolation of nuclei
(Cynthia R. Chuang, University of Texas, Houston, personal communication).
It has not been possible to disrupt yeast by this procedure, probably
because of the limiting particle size one can produce with a conventional
Waring Blendor. Other types of high speed homogenizers may allow the
procedure to be extended to these smaller particles.

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