Note

Isolation of High Molecular Weight Cellular DNA with a Novel Granulated Hydroxylapatite

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We have established a unique method to prepare a novel hydroxylapatite (HA), which is rigidly crystallized (H45) or granulated (H95), having one order of magnitude higher flow rate on column chromatography than Tiselius's HA besides the similarities in other properties.¹⁾ The essential difference in the procedures is that we generate the calcium phosphate, the precursor of HA, at either 45 or 95°C. Saving a great deal of time, labor and cost by using H95, we isolated cellular DNA in a size of about 70 kb, being readily digestible with restriction enzymes, on one hand, and also in practically 100% yield, on the other.

Algal cells (0.5 ml wet cells²⁾) were fixed and suspended in 95% ethanol, and disrupted by being passed only once through a French Pressure Cell at 4°C under 50~80 kg/cm² to result in minimize breakage of cell DNA at the sacrifice of efficiency in cell breakage.³⁾ To the cell materials pelleted by centrifugation were added 5 ml of

PUE—PU (8 M urea in 0.24 M SPB [sodium phosphate buffer, pH 6.9]) plus 20 mM EDTA— and 0.3 ml each of 20% SDS (sodium dodecylsulfate) and 20% Triton X-100. The mixture was left for 30 min at room temperature, and then mixed with 4.6 ml of H95 1×g sediments ($V_s=4.6$ ml), which had been equilibrated with PU. (The V_s -value was two thirds of the total V_s 's of H95 to be used, which had the binding capacity for DNA (400 µg per ml V_s) 5~10 times higher than that sufficient and minimal for adsorbing the DNA to be found in the cell sample.) The resulting slurry was poured onto a H95 column that had been prepared using one third of the H95 ($V_s=2.3$ ml) and equilibrated with PU. The resulting H95 column (column height, 1 cm; tubing attached, 2 cm × 2 mmφ) was washed successively with 35 ml or more

FIG. 1. Agarose Gel Electrophoresis on the *Chlorella* DNAs Obtained by the Single H95 Column Chromatography.

The yield of DNA was about 50% (cell breakage, 50~60%); no DNA remained in the H95 column after the elution. Conditions: 0.4% agarose; buffer, 89 mM Tris-borate buffer, pH 8.3, plus 2.5 mM EDTA; 20 volts for 18 hr; room temperature. Lane 1: λ DNA (20 ng) cleaved by *Hind*III (fragments: 23.6, 9.6, and 6.6 kb); Lanes 2 and 6: intact λ DNA (about 50 kb) (20 ng); Lanes 3 and 4: two *Chlorella* DNA specimens (80 ng) independently isolated and purified by H95 chromatography; Lane 5: the algal DNA (10 ng) obtained from the same cell sample as those in Lanes 3 and 4 but extracted and purified by the conventional phenol—CsCl method.⁴⁾ The main band of the algal DNA (>80% of the DNA having moved) was of 70 ± 5 kb; the major rest was of around 50 kb, 40 kb being the lower limit.

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FIG. 2. Polyacrylamide Gel Electrophoresis on *Chlorella* DNAs Digested with *Hinf*I.

The algal DNA (20 μ g), one of the specimens shown in Fig. 1, was digested with 40 units of *Hinf*I using a buffer containing 10 mM Tris-HCl, 7 mM MgCl₂, 100 mM NaCl, and 7 mM 2-mercaptoethanol at 37°C for 2 hr. Conditions: 12% polyacrylamide; buffer, 40 mM Tris-acetate, pH 8.2, plus 2 mM EDTA; 25 volts for 12 hr; room temperature. Lane 1: pBR322 DNA (0.3 μ g) cleaved by *Hinf*I (fragments: 1631, 516, 506, 396, 344, 298, 220, 154 and 75 bp); Lane 2: *Chlorella* DNA specimen obtained by the single H95-fractionation; Lane 3: *Chlorella* DNA purified by the conventional method.⁴⁾ Because of an excessive amount of the DNA applied to detect clearly the 170 bp, the original sample of which is naturally heterogeneous in base sequence, there can be seen much smear ranging from several hundred to a few thousand base pairs.

each of PU and 0.14M SPB. The DNA was eluted with 20 ml of 0.5M SPB (hydraulic head, 5 cm; flow rate, 1 ml/min) and the DNA-containing fractions were collected. It took only one hour or so to finish the chromatographic procedures (sample charging, washing, and elution), because of the ease in handling and the high flow rate of H95 due to the rigidity and the large size of the component particles.¹⁾

In Figs. 1 and 2 are shown the size and the digestibility with a restriction enzyme of the isolated algal DNA that had been micro-dialyzed briefly⁴⁾ (The results shown here is for *Hinf*I, one of the six restriction enzymes tested; all gave successful cleavages.) It was of about 70 kb and was completely cleaved by *Hinf*I, as revealed by the appearance of the 170 bp band, one of the short repetitive sequences in the algal DNA in the identical manner with the same algal DNA purified by the conventional phenol-CsCl procedures.⁴⁾ The size is sufficient for constructing a genomic DNA library using λ phage vectors. Giant DNAs appear to have been obtained by the column chromatography using Tiselius's HA^{5,6)} or a commercial HA,⁷⁾ but only plasmid DNAs up to about 21 kb have so far been clearly demonstrated.⁸⁾ The chromatography using Tiselius's HA gave us algal DNA that was smaller (15~55 kb) and highly heterogeneous in size, besides being far more time-consuming (about 6 hr for the chromatographic procedures) and inefficient in the elution (yield: 45%). Thus, H95 is really far more advantageous in handling of high molecular weight DNA than Tiselius's HA, and this is the first demonstration for 70 kb DNA being obtainable by HA column chromatography. Since the cell breakage was not very mild, it is a further question whether the DNA size is a limit for the chromatographic procedure or not.

Disrupting the fresh or ethanol-fixed algal cells at 100% cell breakage with a Vibrogen Cell Mill,²⁾ we could also obtain highly purified *Chlorella* DNA rapidly at practically 100% yield ($n=7$; mean, 98.6%; S.D. 3.6%) for the cell samples of 25 mg~3.75 g in dry weight (0.1~15 mg in DNA) even after two cycles of the H95 column chromatography (for the second fractionation we used one third of the amount of H95 in the first one, being equilibrated with 0.1 M SPB). Because of the vigorous disruption of the algal cells, the size of the algal DNA was 0.5~7.5 kb usable for Cot-analyses,⁹⁾ where 100% yield was prerequisite. The DNA specimen contained protein and polysaccharides at 1.8 and 2% of the amount of DNA, respectively, but practically no RNA and no polyphosphates, although those cell constituents were far higher than DNA,¹⁰⁾ as is often the case in plant or microbial cells. We expect that the high molecular weight DNA at high purity in high yield can be attained for the cell species starting from non-walled or wall-deprived cells.

Thus, this method using the novel HA, H95 (H45 being a little less rigid than H95 is considered to give similar results), which is a slight modification of the reported procedures,^{9,11)} is very useful for isolating highly purified cellular DNA in the large size and/or in high yield with great savings in time, labor, and cost (H95 can easily be regenerated with SDS and/or alkali even after use for fractionating cell homogenates). We expect that the method itself as well as the handling of high molecular weight DNA with H95 will be used extensively in various studies involving DNA, not to speak of the currently prevailing recombinant DNA work.

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