EXTRACTION AND SEPARATION OF NUCLEIC ACIDS FROM CESIUM CHLORIDE HOMOGENATES OF WHOLE CELLS

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Present methods for nucleic acid extraction from cells either degrade the nucleic acids (NA's) or, leaving them in a highly polymerized form, do not accomplish a quantitative extraction--especially with small quantities. This paper describes a method, useful for small samples, which adapts equilibrium density gradient centrifugation (Meselson et al. 1957) to separate the undegraded NA and nucleoproteins (NP) directly from other constituents.

In a cesium chloride (CsCl) gradient, the NA, proteins (PR), NP, polysaccharides (PS) and lipoproteins (LP) separate at equilibrium by virtue of their different buoyant densities. By selection of a gradient of appropriate mean density one can centrifuge the NA, NP and PS into a pellet from a homogenate of cells ground directly in saturated CsCl. In the CsCl, enzymatic degradation of the NA is presumably minimized. The method has been applied successfully to both animal and plant material.

CsCl as obtained from American Chemical and Potash Co., Trona, Calif., had a sharp absorption peak at 270 mμ, which interfered with determination of the ultraviolet absorption (UV) spectra of the NA and NP. The contaminants were removed as follows: CsCl, crystallized from 5 M solution at 0°C by adding 5x's its volume of 95% alcohol, was filtered and dried. The crystals were then melted in a porcelain crucible over an open flame; the impurities turn brown and become flocculent in the melt. After cooling, the melt is dissolved in water, shaken with freshly ignited, acid washed charcoal, filtered and dried.

For these experiments, 0.05 M phosphate buffer pH 6.4 containing 0.01 M $MgSO_4$ was saturated with the purified CsCl.

Since the capacity of each tube in the SW39L rotor used in the Spinco Model L centrifuge is 5.0 ml, the maximum volume of tissue desirable for the density gradient is 1 ml. The tissue was homogenized at 0°C in 1 ml saturated CsCl. The refractive index of the homogenate was estimated in an Abbé refractometer. From this value, the amounts of CsCl and buffer to be added for a final volume of 4.5 ml of the desired mean density could be calculated. When a sample of mean density 1.40 g cm^{-3} is run for 24 hours at 86,000 x g, a gradient ranging from 1.30 to 1.50 g cm^{-3} is usually obtained. The buoyant density of PR in CsCl is 1.3 g cm^{-3}, of deoxyribonucleoprotein (DNP), 1.5 g cm^{-3}, that of deoxyribonucleic acid (DNA) and ribonucleoprotein (RNP), 1.7 g cm^{-3} and that of ribonucleic acid (RNA) approximately 2.0 g cm^{-3} (Weigle et al. 1959; Davern et al. 1960). Thus, the floating layer will contain PR and LP; the pellet NA, NP and PS.

The pellet, the CsCl gradient and a perchloric acid extract of the floating layer were analyzed for NA and PR by conventional methods: UV, ribose, nitrogen and phosphorus determinations (details in Levenbook et al. 1958). For the PS, the anthrone method for glucose determination was used (Seifter et al. 1950). DNA was estimated by the microbiological assay of Hoff-Jorgensen (Miller 1958).

For analyses other than UV spectrophotometry, the CsCl was removed by micro dialysis as follows: the 0.2 ml volumes of the gradient fractions were placed in 6 x 50 mm tubes, the tubes were covered with squares of dialysis tubing held in place with rubber bands and inverted so that their tops were immersed in a beaker containing one liter of 0.01 M $MgSO_4$. The tubes were shaken several times during the dialysis period (18 hrs at 0°C).

An exploratory trial with rat liver indicated the applicability of the method. The extraction was not quantitative due to incomplete homogenization, with whole cells present in the floating layer. The pellet from 1 ml of rat liver (buffer-perfused, starved for 20 hrs) contained 0.58 mg RNA, 0.03 mg DNA and from the PS, 1.3 mg glucose. The N/P ratio was 3.4, expected for a nucleoprotein of 40% NA to 60% PR by weight.

A similar experiment was carried out with the apical meristems from a Nicotiana langsdorfii x glauca hybrid. After the initial homogenization the buffer required for the gradient was added and the cell walls were separated by a preliminary centrifugation (800 x g, 10 minutes). The remainder of the CsCl was added to the supernatant fraction. The pellet from an initial sample of 1 gm contained 0.16 mg RNA, 0.01 mg DNA and 0.10 mg glucose (PS). In this case the N/P ratio was 1.82 indicating very little protein.

Egg cells present special difficulties for ordinary extraction procedures due to the presence of massive amounts of yolk. From the sea urchin (Strongylocentrotus purpuratus), 1 ml of packed, unfertilized eggs yielded a pellet containing 5.3 mg RNA and 0.09 mg DNA. With large yolky eggs (i.e., from the frog), the volumes involved in the method are too small for the number of eggs required to yield a measurable amount of nucleic acid. Recent experiments have shown that larger volumes can be accommodated by substituting an angle head rotor for the swinging bucket, the method being otherwise as described.

The original purpose in devising these methods was to fractionate the diverse NA's of Drosophila melanogaster as part of a study of the genetic control of the constitution of the RNA's in this species (Schultz 1956). From 1 g of flies, a pellet was obtained containing 0.33 mg RNA, 0.01 mg DNA and 0.40 mg glucose (PS). The N/P ratio was 1.64, which is close to expectation for pure NA.

Detailed studies have been made on the unfertilized Drosophila egg in which comparisons could be made with analyses by other methods. Since a separation of the RNA from the DNA was desired, a slightly different gradient, whose mean density was 1.51 g cm^{-3} was established. In such a gradient, the RNA and RNP were expected in the pellet and the DNP as a band at a density 1.54 g cm^{-3}. The pellet from 17,200 eggs contained 0.245 µg RNA per egg; this is a higher yield than the 0.190 µg RNA per egg obtained from a hot 10% perchloric acid extract. The N/P ratio was 3.4, indicating NP. The base compositions of the RNA of the pellets obtained by the new method agree

closely with RNA obtained by sodium chloride extraction (Table 1). The pellet also contained a small amount of DNA.

Table 1

Type of extraction	Moles of base per 100 moles phosphorus			
	A	G	C	U
NaCl extract (Levenbook et al. 1958)	29.8	23.1	18.5	28.5
CsCl pellet	28.8	23.1	19.5	28.5

A large amount of E_{260} absorbing material in the gradient could be removed by dialysis. This presumably is the pool of low molecular weight NA components (nucleosides, etc.) already described (Travaglini et al. 1958). After dialysis, the material at both densities 1.46 g cm^{-3} and 1.54 g cm^{-3} was found to contain DNA.

Where homogenization was complete, there was no NA found in the floating protein layer.

The RNP's of the different materials appear to respond differently to the cesium gradient; in some cases, RNA was dissociated from its protein, in others it was still combined with an appreciable amount of protein.

On the basis of the present experience, the method would appear to have a wide application where a quantitative recovery of highly polymerized (native?) NA is required. It is clear that further fractionation, some of which has already been accomplished, can easily be carried out by resuspending the pellet in a new gradient. The method has obvious advantages over other procedures for quantitative recovery of NA from small samples since it does not require alcohol precipitation. It does provide a source of material with potential use in tests of biological activities of the different NA's.

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References

Davern, C. I. and Meselson, M. J. Mol. Biol. 2:153 (1960).
Levenbook, L., Travaglini, E. C. and Schultz, J. Exp. Cell Res. 15:43 (1958).
Meselson, M., Stahl, F. W. and Vinograd, J. Proc. Nat. Acad. Sci. Wash. 43: 581 (1957).
Miller, H. K. Methods of Biochem. Anal. (Interscience Publ. Inc.) 6:31 (1958).
Schultz, J. Cold Spring Harbor Quant. Biol. XXI:307 (1956).
Seifter, S., Dayton, S., Novic, B. and Muntwyler, E. Arch. Biochem. 25: 191 (1950).
Travaglini, E. C., Levenbook, L. and Schultz, J. Exp. Cell Res. 15:62 (1958).
Weigle, J., Meselson, M. and Paigen, K. Brookhaven Symp. in Biol. 12:125 (1959)