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Transport Phenomena in Zonal Centrifuge Rotors. XIV. Analytical Characterization in Density Gradient Preparative Centrifuges

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NOTE

Transport Phenomena in Zonal Centrifuge Rotors. XIV. Analytical Characterization in Density Gradient Preparative Centrifuges

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Abstract

The centrifuge has had a long and successful history as a means for isolating particles of biological interest. To achieve separations of higher resolutions, two types of centrifugation have been developed. In the first, particles are separated into discrete zones on the basis of differences in sedimentation rate (rate-zonal centrifugation), and in the second, separation is based on differences in buoyant or banding density (isopycnic-zonal centrifugation). In this paper, methods of a simple analytical characterization in the large-scale isolation using preparative centrifuges in a density gradient solution are demonstrated.

MOLECULAR WEIGHTS OF DNA AND RNA

The very large difference between the buoyant densities of RNA (>1.9 in CsCl) and DNA (>1.7 in CsCl), and between those of DNA and proteins (1.3~1.5 in CsCl) has led to the development of buoyant density-gradient methods for the large-scale isolation of DNA and RNA from tissues, cells, and cell organelles. However, there does not seem to be a universally applicable procedure whereby DNA can be isolated and purified from organism, tissue, or organelle. There are, of course, many excellent methods (*1*), but no single one of them is ideally suited to all situations.

Cs_2SO_4 is the salt most frequently used for buoyant density analyses of DNA–RNA hybrids because DNA, RNA, and the hybrid all band in Cs_2SO_4 gradients (*2*). CsCl can also be used for this purpose although RNA is too dense to band in CsCl gradients. However, neither of these salts is entirely satisfactory since they resolve native and denatured DNA poorly. Birnie (*3*) used NaI gradients for DNA to substitute for CsCl salts and reported that NaI gradients clearly distinguish between DNA, denatured

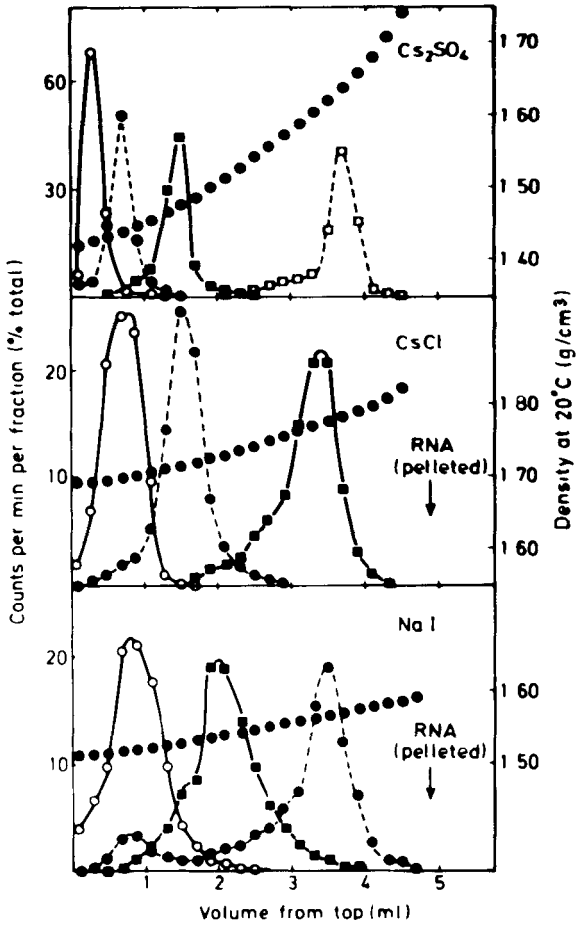

FIG. 1. Isopycnic banding of native DNA, denatured DNA, DNA–RNA hybrid, and RNA from mouse in density gradients formed from Cs_2SO_4, CsCl, and NaI (reproduced from Ref. *4*). (○) Native DNA. (●) denatured DNA. (■) DNA–RNA hybrid, (□) RNA.

DNA, RNA, and DNA–RNA hybrid, and that RNA is soluble in NaI solutions so long as care is taken to remove heavy metal ions from the NaI. His result of separation of native and denatured DNA, RNA, and DNA–RNA hybrid is used here to demonstrate analytical characterization in centrifuges. Materials and methods used in the investigation are in Birnie's article (*3*). Separations using Cs_2SO_4, CsCl, and NaI salts are also summarized by Birnie (*4*) and reproduced here in Fig. 1.

The apparent molecular weight can be obtained from the isopycnic-zonal centrifugal experiments. The formula for the apparent molecular weight in a

density gradient isopycnic banding can be obtained by rearranging a standard deviation σ in a Gaussian concentration distribution at an isopycnic position given by Baldwin (5), which gives

$$M^{(app)} = \frac{RT}{\bar{V}_2(d\rho/dr)\omega^2 r_0 \sigma^2} \quad (1)$$

in which R is the gas constant (8.314×10^7 ergs per degree K per mole), T is the absolute temperature, r_0 is the center of mass of the macromolecular particle from the rotational axis, $\bar{V}_2$ is the partial specific volume of the macromolecules at the center of the mass, $d\rho/dr$ is the slope of the density gradient solution at the center of mass, and σ^2 is the variance in Gaussion distribution.

Birnie used the MSE 10×10 titanium fixed-angle head rotor which has an angle of 35° from the axis of rotation. Distances from the rotational axis to the center of the top and bottom of the centrifugal tube are 3.68 and 7.87 cm, respectively. Therefore, the abscissa in Fig. 1, volume from top (mL), may be converted into distance from the top at the inclined position, which gives 4.099, 4.518, 4.937, 5.356, and 5.775 cm for volumes of 1, 2, 3, 4, and 5 mL, respectively. The centrifugations were performed at 45,000 rpm and 20°C. Hence one has $T = 293$ K and $\omega = (45{,}000 \times 2\pi)/60 = 4{,}712$ s^{-1}, the angular velocity. The quantities appearing in Eq. (1) for the estimation of apparent molecular weights for three separate gradient solutions were estimated from Fig. 1 and are tabulated in Table 1. The partial specific

TABLE 1

Quantities from Fig. 1 for the Calculation of Apparent Molecular Weight Using Eq. (1)

	r_0 (cm)	$\bar{V}_2$(cm^3/g)	$d\rho/dr$ (g/cm^4)	σ^2 (cm^2)	$M^{(app)} \times 10^{-5}$
Cs_2SO_4					
Native DNA	3.80	0.703	0.1193	0.0081	4.25
Denatured DNA	3.97	0.695	0.1200	0.0081	4.09
DNA–RNA hybrid	4.30	0.680	0.1250	0.0144	2.08
RNA	5.20	0.610	0.2153	0.0256	0.63
CsCl					
Native DNA	3.96	0.590	0.057	0.0289	2.85
Denatured DNA	4.31	0.583	0.062	0.0256	2.75
DNA–RNA hybrid	5.11	0.561	0.091	0.0484	1.16
NaI					
Native DNA	4.01	0.660	0.025	0.0361	4.59
Denatured DNA	5.12	0.639	0.050	0.0625	4.40
DNA–RNA hybrid	4.50	0.652	0.025	0.1521	2.39

volumes $\bar{V}_2$ for various DNA, DNA–RNA hybrid, and RNA were obtained from the inversion of the solvated densities of macromolecules at their peak position in the respective density gradient solutions. The apparent molecular weights calculated using Eq. (1) are also listed in Table 1.

We emphasize that the density gradient isopycnic banding method does not possess an advantage over ordinary sedimentation equilibrium in the determination of molecular weight, since heterogenity in the sample causes broadening of the distribution. The method presented here is an estimation of a crude molecular weight from a preparative centrifuge. For an exact estimation, one still has to rely on an analytical ultracentrifuge with a purified sample.

PARTICLE SIZE AND SEDIMENTATION COEFFICIENT OF NUCLEI

The function of the nucleus is in the determination of heredity, and its role in protein synthesis has received intense interest in this organelle. Many biochemists in their studies of nuclei have used rat liver, which is one of the most commonly used materials for biochemical research. The liver is a complex organ. The main functional units are the parenchymal cells. The nonparenchymal cells are diploid. The heterogeneity in type of parenchymal cells and degree of ploidy is a major complication in the interpretation of biochemical experiments involving isolated nuclei.

The results of fractionation of purified rat liver nuclei by low-speed zonal centrifugation using a MSE A-XII rotor to separate them into two main zones (one zone containing diploid and the other tetraploid nuclei) by Johnston et al. (*6*) is used as an example to estimate the size and the sedimentation coefficient of diploid and tetraploid nuclei.

An advantage of the A-type rotor is that it permits continuous direct observation of the position of the particle zones and enables an investigation to be made of their sedimentation behavior. Figure 2 shows the position of the tetraploid and diploid zones as a function of time at a rotor speed of 1200 rpm. [Figure 2 is reproduced from Fig. 14 of Johnston et al. (*6*).]

The sedimentation of a spherical particle in an incompressible density gradient solution, neglecting dispersion between the particle and the density gradient solution, is (*7*)

$$\frac{dV_r}{dt} + \frac{18\mu_m}{\rho_p D_p{}^2} V_r = \frac{\omega^2 r[\rho_p - \rho_m(r)]}{\rho_p} \tag{2}$$

where $V_r = dr/dt$, the sedimentation velocity of the particle, or the rate of solute mobility. Since V_r is very small compared with other quantities (μ_m is the viscosity of the gradient solution, ρ_p and ρ_m are the densities of the

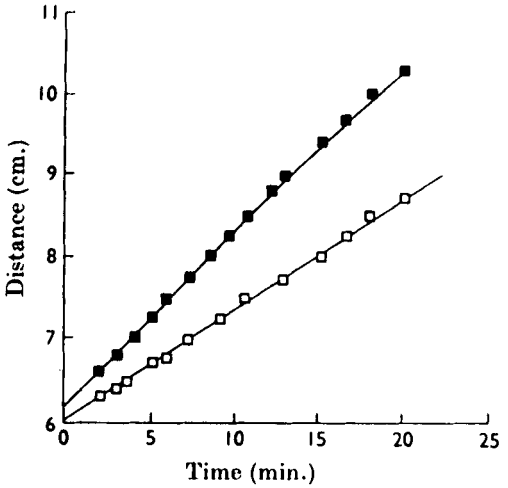

FIG. 2. Distance of nuclei from axis of rotation as a function of time (reproduced from Ref. 6). (□) Diploid, (■) tetraploid.

solvated particle and the gradient solution, D_p is the diameter of the particle or size of the solute, and ω is the angular velocity of the centrifugal field), one may neglect the acceleration term to approximate Eq. (2). Then one has

$$\frac{d \ln r}{dt} = \frac{\omega^2 D_p^2 (\rho_p - \rho_m)}{18 \mu_m} \tag{3}$$

The slope of $d \ln r/dt$ given by Eq. (3) can be evaluated from Fig. 2. The observed sedimentation coefficient of a particle is defined as (*8*):

$$S = \frac{dr/dt}{\omega^2 r} = \frac{1}{\omega^2} \frac{d \ln r}{dt} \tag{4}$$

which is also obtainable from the slope of an $a \ln r$ vs t plot.

The density of the nuclei varies with the density of the gradient medium, presumably because of their permeability to sucrose or other gradient solute solutions. Johnston et al. (*6*) used a gradient of 5–17% (w/w) sucrose at 600 rpm for 40 min and 50–69% (w/w) sucrose at 2500 rpm for 6.5 h to study the density behavior of sedimenting nuclei. They obtained $(\rho_p - \rho_m)$ vs ρ_m data and by extrapolation they concluded that the isopycnic densities for both nuclei are equal and are 1.35 g/cm^3 (i.e., $\rho_p = 1.35$).

Now the data in Table 2 are obtained from Fig. 2.

TABLE 2

	Position from the rotational axis (cm) at the end of centrifugation	Concentration of sucrose gradient at the end position %(w/w)
Diploid nuclei	8.6	25
Tetraploid nuclei	10.45	30

The initial positions of nuclei were at 6.00 and 6.22 cm, and the time of centrifugation was 20 min with 1200 rpm ($\omega = 125.66\ s^{-1}$) centrifugal velocity. The experiments were run at 5°C. The quantities ρ_m and μ_m of sucrose solution at 5°C are listed in Table 3 (*9*).

If the arithmatic average is used as the first approximation to the quantities ρ_m and μ_m in the estimation, one obtains the quantities in Eq. (3) as listed in Table 4.

By rearranging Eq. (3) and substituting the respective values of Table 4 into it, one obtains the diameter of the nuclei

Diploid $D_p = 2.20 \times 10^{-4}$ cm
Tetraploid $D_p = 3.04 \times 10^{-4}$ cm

From Eq. (4) the observed sedimentation coefficients obtained are

Diploid $s = 1.90 \times 10^{5}$ Svedberg units
Tetraploid $s = 2.91 \times 10^{5}$ Svedberg units

If higher order accuracies are required, an exact gradient profile must be incorporated in the estimation (*7*). The calculations become more complicated and more tedious and time consuming. Programmed calculations by electronic computer become essential.

TABLE 3

% (w/w)	ρ_m (g/cm^3)	μ_m (cP)
20	1.0843	3.1354
25	1.1075	4.0425
30	1.1315	5.4216

TABLE 4

	ρ_m	$\rho_p - \rho_m$	$\mu_m \times 10^2$	$d \ln r/dt$
Diploid	1.0959	0.2541	3.5890	3.0×10^{-4}
Tetraploid	1.1079	0.2421	4.2785	4.6×10^{-4}

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