Sedimentation Equilibrium of DNA Samples
Heterogeneous in Density

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Synopsis

The problem of determining the molecular weight of DNA samples by sedimentation equilibrium in a buoyant-density gradient is considered for the case of DNA samples with density heterogeneity. By determining apparent molecular weights in two or more buoyant mediums, quantitative measure of the amount of density heterogeneity can be determined. This method may be employed to determine both the true molecular weight and the extent of base composition heterogeneity.

INTRODUCTION

For samples that are homogeneous in buoyant density it is now possible to determine the molecular weight of DNA in the 1 to 100 megadalton range by sedimentation equilibrium in a buoyant-density gradient. The buoyant density of DNA depends on the base composition and samples that are heterogeneous in base composition are correspondingly heterogeneous in buoyant density. It has long been recognized that density heterogeneity will broaden the equilibrium concentration distribution and therefore lower the apparent molecular weight.

A simple test for density heterogeneity would be valuable for the characterization of a new DNA and for an assessment of the validity of a molecular weight measured by density-gradient sedimentation equilibrium. Such a test is possible by performing molecular weight studies in two or more different salts, since the resolution of density differences depends on various properties of the banding medium. By this same method it is possible in principle to analytically determine both a sample's base composition heterogeneity and molecular weight. Characterization of the base composition heterogeneity is itself a subject of recent interest.

It will also be experimentally demonstrated here that the same techniques used to increase or decrease the effects of density heterogeneity may be used to increase or decrease the resolution of different components within a sample.
Sueoka has considered the problem of any general distribution of densities where the molecular weight distribution of the entire sample describes the molecular weight distribution of any single density component. This assumption should be good for the highly fragmented DNA's usually isolated from bacteria and higher organisms. For this case

$$\langle \delta_T^2 \rangle = \langle \delta_M^2 \rangle + \langle \delta_d^2 \rangle$$  \hspace{1cm} (1)$$

where $T$ refers to the total variance; $M$, the spreading from diffusion; and $d$, the spreading due to density differences. The total variance is calculated from experimental data using the following equation

$$\langle \delta_T^2 \rangle = \frac{\int_{-\infty}^{+\infty} \delta^2 C(\delta) \, d\delta}{\int_{-\infty}^{+\infty} C(\delta) \, d\delta}$$

where $\delta$ is the distance from band center and $C(\delta)$ is the concentration at that point.

The variance due to density heterogeneity can be related to the variance of the base composition heterogeneity by linear transformations. Two DNAs having the buoyant density difference $(\rho_{s,o} - \bar{\rho}_{s,o})$ will be separated by the distance $\delta_d$:

$$\delta_d = \frac{(\rho_{s,o} - \bar{\rho}_{s,o})}{\left( \frac{dp}{dr} \right)_{buoyancy}} = (\rho_{s,o} - \bar{\rho}_{s,o}) \frac{\beta_B}{\omega^2 r}$$

If the plot of buoyant density versus base composition (GC fraction, $f$) is linear with a slope of $a$; and if $\bar{\rho}_{s,o}$ is the mean density of the sample, then:

$$(\rho_{s,o} - \bar{\rho}_{s,o}) = a(\Delta f)$$

$$\delta_d = \frac{a\beta_B}{\omega^2 r} (\Delta f)$$

where $\Delta f$ represents the difference in GC fraction from that of the mean of the distribution. With the above transformations Equation (1) may be written as

$$\langle \delta_T^2 \rangle = \frac{\beta_{eff} \rho_{s,o} RT}{M_2(1 + \Gamma') \omega^4 r_0^2} + \frac{a^2 \beta_B^2}{\omega^4 r_0^2} \langle (\Delta f)^2 \rangle$$  \hspace{1cm} (2)$$

or

$$\frac{1}{M_{app}} \equiv \langle \delta_T^2 \rangle \frac{(1 + \Gamma')}{\beta_{eff}} \left( \omega^4 r_0^2 \right) = \frac{1}{M_2} + \left( \frac{a^2 \beta_B^2}{\beta_{eff} RT} \right) \left( \frac{1}{\rho_{s,o}} \right) \left( \frac{1}{1 + \Gamma'} \right) \left( \frac{1}{\rho_{s,o}} \right) \langle (\Delta f)^2 \rangle$$  \hspace{1cm} (3)$$
where the definition of the equilibrium band width$^{1,2,3,6,10}$

$$\langle \delta M^2 \rangle = \frac{1}{M_3} \frac{\rho_{s,0} R T}{\omega^2 r_0^2} \frac{\beta_{\text{eff}}}{(1 + \Gamma')}$$

has been used,

and $r_0$ is the distance of band center from the center of rotation  
$\omega$ is the speed in radians/sec  
$\rho_{s,0}$ is the buoyant density of the DNA  
$M_3$ is the molecular weight of dry cesium DNA  
$(1 + \Gamma')/\beta_{\text{eff}}$ is a buoyancy factor.

The buoyancy factor $(1 + \Gamma')/\beta_{\text{eff}}$ has been experimentally determined for DNA in several cesium salts.$^{2,3}$ The quantity $a\beta_B$ determines the distance by which two DNAs differing in base composition will be separated in a density gradient. For CsCl this quantity may be evaluated from the data of Schildkraut, Marmur, and Doty.$^4$ For a new salt it is only necessary to compare the distance between two DNAs in the new salt solution relative the distance in CsCl. Besides simplicity this method has the virtue of permitting the accurate determination of relative values of $a\beta_B$. This device is used in this work.

Inspection of Equation (2) reveals the well-known conclusion that the resolution of density differences is independent of speed, or for the present purpose, that the apparent molecular weight, $M_{\text{app}}$, in the presence of density heterogeneity is speed-independent.$^5,6,7$ However, the apparent molecular weight does depend on several properties of the salt, Equation (3), since the quantity $a\beta_B^2(1 + \Gamma')/\beta_{\text{eff}}\rho_{s,0}$ is a composite of several terms each unique for a given medium.

**EXPERIMENTAL**

Centrifugation was performed at 25°C on a Beckman Model E ultracentrifuge equipped with a photoelectric scanner using double-sector centerpieces. Harshaw optical grade CsCl and Cs$_2$SO$_4$ dissolved in 0.01M tris pH 7.2 and a concentrated stock of Harshaw optical grade cesium formate, pH 6.95, diluted with doubly distilled water, were used as the buoyant mediums.

DNA was prepared from coliphage $\lambda$cIS57 and *lysoedicticus* phage D3 by phenol extraction. Calf thymus DNA (Calbiochem) was purified by phenol extraction.

The $\lambda$ DNA was mechanically sheared by passing it through a capillary and identified as halves by band centrifugation in CsCl.$^{11}$ The cohesive ends of the $\lambda$ halves were repaired by DNA polymerase to prevent aggregation of the halves.$^{12}$ This material was repurified by phenol extraction.

The $\lambda$ halves were chosen as a model system possessing density heterogeneity since both the molecular weight$^{13}$ and density heterogeneity$^{14}$ in this system have been well characterized.
RESULTS

To obtain the quantity \((a\beta_a)\) for cesium formate, the distance between the \(\lambda\) halves, Figure 1, in this salt was measured relative to the separation in CsCl (Fig. 1). For CsCl the value of \(a\beta_a\) was determined from the data of Schildkraut et al. These quantities are reported in Table I.

![Optical Density vs Distance](image)

Fig. 1. Equilibrium concentration distributions of \(\lambda \text{e}1857\) DNA mechanically sheared to halves. All traces at the same magnification. Trace A is the distribution in Cs formate at 35,000 rpm, B is in CsCl at 35,000 rpm, and C is in CsSO4 at 30,000 rpm.

| Salt            | \((\beta a) \times 10^{-3}\) | \(R T \rho_a \beta_{\text{eff}} \times 10^{-4}\) | \(M_{\text{app}} \times 10^{-6}\), daltons | \(|\langle \Delta f \rangle\rangle|_{1/2}\) |
|-----------------|-----------------------------|---------------------------------------------|-------------------------------------------|----------------|
| Cs formate      | 2.17                        | 4.254                                      | 1.17                                      | 0.0435         |
| Cs chloride     | 1.17                        | 2.544                                      | 1.88                                      | 0.0436         |
| Cs2 sulfate     | 0.218                       | 0.246                                      | 7.08                                      | 0.0614         |

* CsCl is taken as the standard, and the other salts are calibrated to it as explained in the text. The value of \((a\beta_a)\) for Cs2SO4 is for a 49% GC DNA. The value of \((a\beta_a)\) varies by a factor of two in Cs2SO4 over the range 25% to 70% GC. The apparent molecular weight is defined by Equation (3) for CsDNA.

In Cs2SO4 the buoyant density is not linearly dependent on the base composition and \((a\beta_a)\) for this salt will depend on the base composition. For D3 DNA 54% GC and calf thymus DNA 39% GC the ratio of the distances between the bands in cesium chloride and cesium sulfate was found to be 5.8. Although these bands were incompletely resolved, this value is consistent with Szybalski's data which was used to estimate the value of the ratio for a DNA with the base composition of \(\lambda\) DNA. This value of \((a\beta_a)\) for Cs2SO4 solutions of 5.35 was employed in the ensuing calculations (Table I).

The resolution of \(\lambda\) DNA halves increase from sulfate to chloride to formate. This is predictable from the data in Table I and Equation (2).

The apparent molecular weights were calculated from Equation (3) and the variance of each distribution. For Cs2SO4 a small virial correction was
employed. In CsCl and cesium formate the variance of the distribution is almost entirely due to density heterogeneity (Fig. 1), and no virial effect was observed. The base composition heterogeneity (Table I) was calculated from these apparent molecular weights using Equation (3).

**DISCUSSION**

The apparent molecular weight of the λ DNA halves depends on the salt used, and in general this method may be employed qualitatively to recognize the existence of density heterogeneity. The base composition heterogeneity observed in cesium formate and chloride is in quantitative agreement with that expected for two DNAs having the density differences of the λ DNA halves. In cesium sulfate the observed density heterogeneity is in poor agreement with the results in chloride and formate (Table I) and with the expected result.

The poor agreement between the sulfate and chloride is probably the result of two problems. First, for λ DNA halves the molecular weight and buoyant density are correlated, this violates the original assumption of Equation (1). Second, the GC difference between the λ halves is large; while in cesium sulfate the quantity $a_{B}$ is not constant over such a GC range, varying by about 25%. The linear transformation from density differences to base composition differences will therefore be in error. A better high-gradient salt than CsSO₄ is needed.

The most general derivation of the effects of density heterogeneity should include a correlation between the molecular weight and density distributions as well as corrections for the nonlinearity in $a_{B}$. This is presently impractical. The case of λ DNA halves is a very stringent test of Equation (3), which is successful in CsCl and Cs formate solutions. It is possible that Equation (3) would adequately describe the equilibrium concentration distribution of simpler cases, such as fragmented bacterial DNAs even in CsSO₄, although this has not been tested because a test system as well defined as the λ DNA halves is not available.

The resolution obtained in different salts is noteworthy. In salts having steep gradients, bands are narrower and density separations are smaller. The first factor enhances the resolution of density differences, the second factor decreases the resolution. In steep gradients such as in CsSO₄ the second effect is the dominant one. In cesium sulfate the gradient is steep, and therefore resolution is poor, making it a good solvent to band low-molecular-weight heterogeneous DNAs. In cesium formate the density gradient is weak and the resolution is excellent, approaching that observed for the λ DNA halves in the Hg⁺⁺-cesium sulfate system.

It is anticipated that with the use of additional cesium salts which have a linear dependence of buoyant density upon GC composition, these methods will provide accurate means of evaluating and extrapolating out the effects of base composition heterogeneity upon molecular weight determinations in density-gradient sedimentation equilibrium.
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References


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