Effect of Isotopic Label on Buoyant Density Determinations of Viral DNA in the Preparative Ultracentrifuge

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The buoyant density of herpes simplex virus DNA may be significantly increased by isotopic labeling, particularly with ^{14}C . The apparent increase in buoyant density may be readily calculated from the specific activity of the DNA and is easily demonstrated by isopycnic centrifugation in both the preparative and analytical centrifuge.

The determination of the base composition of viral DNA is often included in studies designed to compare or classify viruses having common morphological or biological properties (2). In cases where (i) DNA yields are too low or technical limitations preclude chemical analysis, or (ii) an analytical ultracentrifuge is unavailable, the determinations are often based on buoyant density determinations of isotopically labeled DNA in CsCl solutions. Characteristically these determinations involve isopycnic banding, along with appropriate density standards, in preparative ultracentrifuges.

We report that isotopically labeled DNA may have a significantly increased buoyant density, and hence, density determinations based on isopycnic banding in the preparative ultracentrifuge must be carefully controlled.

In the first series of experiments, the effect of different isotopic labels on the density of the same species of DNA was studied. When artificial mixtures of ^3H -thymidine- and ^{14}C -thymidine-labeled DNA of the 117 strain (1) of herpes simplex virus subtype 1 (HSV-1) were centrifuged and analyzed as described in the legend to Fig. 1, the DNA labeled with ^{14}C -thymidine had a buoyant density higher by about 0.0015 g/cm^3 than the DNA labeled with ^3H -thymidine. Preparative centrifugations of five subtype 1 and two subtype 2 isolates of herpes simplex virus gave consistently similar results. This difference in buoyant density was confirmed by centrifugation in the model E analytical ultracentrifuge, a method which should be independent of any artifacts inherent in the counting of radioactive samples from the preparative gradients. Samples of ^3H - and ^{14}C -thymidine-labeled DNA were cocentrifuged in separate cells with SPO1 and *Bacillus subtilis* DNA at 44,000 rpm for 20 h. Analysis of UV absorbance photographs showed

that the density of ^3H -thymidine-labeled DNA was $1.7264 \pm 0.0002\text{ g/cm}^3$ compared with $1.7268 \pm 0.0002\text{ g/cm}^3$ for ^{14}C -DNA. Whereas the results of preparative and analytical centrifugations differ with respect to the magnitude of the effect of isotope on density, both determinations confirm the greater effect of ^{14}C -thymidine incorporation in this phenomenon. Whereas temperature is carefully controlled in the analytical ultracentrifuge, significant changes in temperature, and thus density, as determined by refractive index, can occur when the preparative ultracentrifuge is used. A mathematical treatment of this phenomena is described in the appendix.

A second series of experiments was designed to study the effect of isotopic labeling on the apparent densities of two DNA species known to be different by centrifugation in the model E analytical ultracentrifuge. Previous studies on the DNA of HSV-1 and HSV-2 (3) indicated that they have buoyant densities, as determined in the analytical ultracentrifuge, of 1.726 and 1.728 g/cm^3 corresponding to 67 and 69 guanine plus cytosine mol %, respectively. The difference in guanine plus cytosine content of the DNA was confirmed by the finding of 1 C difference in the melting temperature.

In the present studies, isopycnic centrifugation of artificial mixtures of ^{14}C -thymidine-labeled G (a prototype of HSV-2) DNA and ^3H -thymidine-labeled 117 (HSV-I) DNA in the preparative ultracentrifuge yielded a density difference of 0.0033 g/cm^3 (Fig. 2A), whereas mixtures of ^{14}C -thymidine-labeled HSV-1 DNA and ^3H -thymidine-labeled HSV-2 DNA yielded a density difference of only 0.001 g/cm^3 (Fig. 2B). The values of the densities for the ^3H - and ^{14}C -labeled DNAs are presented in Table 1.

FIG. 1. Isopycnic centrifugation of labeled HSV-1 DNA in the preparative ultracentrifuge. HEp-2 cells were labeled between 4 and 24 h postinfection with either ¹⁴C (C2 or methyl)-thymidine or ³H-methyl thymidine. Cytoplasmic extracts of cells disrupted in hypotonic buffer with a Dounce homogenizer were prepared and solubilized with sodium dodecyl sulfate. Samples of the cytoplasm were freed of protein by gently rolling with phenol and chloroform-8% isoamyl alcohol. Artificial mixtures of ¹⁴C- and ³H-labeled DNA were prepared in CsCl buffered with 0.01 M Tris (pH 7.5) and adjusted to a starting density of 1.72 g/cm³. Centrifugation in the model L centrifuge using an SW-39 rotor was for 46 h at 30,000 rpm and 20°C. Single-drop fractions were collected from the bottom of the centrifuge tube, and the density gradient was monitored by refractive index readings on a Zeiss model A Abbe refractometer. The fractions were precipitated along with carrier DNA in 10% trichloroacetic acid and collected on Millipore filters. Radioactivity was analyzed in a Packard Tri-Carb scintillation spectrometer. Dotted line, ¹⁴C-labeled 117 DNA; solid line, ³H-labeled 117 DNA.

The apparent increase in buoyant density after isotopic labeling of DNA may be readily calculated from its specific activity (see appendix). On the basis of the data, we conclude

that the buoyant density of HSV DNA may be significantly increased by isotopic labeling, particularly with ¹⁴C-labeled precursors. Since this effect is quite appreciable even with HSV DNA, which contains as little as 16 mol % thymidine, density determinations using labeled DNA either in single- or double-label preparations should be carefully controlled. Moreover, as shown in this paper, we would expect that density differences due to isotopic labeling of DNA are also apparent on isopycnic banding in the model E analytical ultracentrifuge.

APPENDIX

Subsequent to our initial finding on the effect of isotopic labeling on the density of HSV DNA described in Fig. 1, we prepared ³H-thymidine- and ¹⁴C-thymidine-labeled DNA from the F prototype strain of HSV-1 for a more analytical treatment of the phenomenon. The rationale of the study was to correlate the difference in the number of isotopic atoms incorporated in ³H-thymidine- and ¹⁴C-thymidine-labeled DNA with an observed difference in density of the DNAs after isopycnic centrifugation.

I. Isotopic content of DNA

A. ³H-HSV-1 DNA

- Decay constant, $\lambda = 0.693/T_{1/2}$
for ³H, $\lambda = 0.693/(12.3 \text{ yr})$
 $(3.16 \times 10^7 \text{ s/yr})$
 $= 1.78 \times 10^{-9} \text{ dps/}^3\text{H atom}$

- Specific activity of ³H-HSV-1 DNA =
 $4.4 \times 10^8 \text{ dps/}\mu\text{g DNA}$
 $4.4 \times 10^8 \text{ dps/}\mu\text{g DNA}$

- $\frac{1.78 \times 10^{-9} \text{ dps/}^3\text{H atom}}{^3\text{H atoms/}\mu\text{g DNA}} = 2.47 \times 10^{13}$

- $\frac{(1.59 \times 10^{-10} \mu\text{g/HSV DNA molecule})}{(2.47 \times 10^{13} ^3\text{H atoms/}\mu\text{g DNA})} = 3.93 \times 10^3 ^3\text{H atoms/molecule } ^3\text{H-DNA}$

Similarly for:

B. ¹⁴C-HSV-1 DNA

- Decay constant λ , for ¹⁴C = $0.693/(5.77 \times 10^3 \text{ yr})$
 $(3.16 \times 10^7 \text{ s/yr})$
 $= 3.8 \times 10^{-12} \text{ dps/}^{14}\text{C atom}$

- Specific activity of ¹⁴C-HSV-1 DNA =
 $1.47 \times 10^8 \text{ dps/}\mu\text{g DNA}$
 $1.47 \times 10^8 \text{ dps/}\mu\text{g DNA}$

- $\frac{3.8 \times 10^{-12} \text{ dps/}^{14}\text{C atom}}{^{14}\text{C atoms/}\mu\text{g DNA}} = 3.87 \times 10^{13}$

- $\frac{(1.59 \times 10^{-10} \mu\text{g/HSV DNA molecule})}{(3.87 \times 10^{13} ^{14}\text{C atoms/}\mu\text{g DNA})} = 6.16 \times 10^3 ^{14}\text{C atoms/molecule } ^{14}\text{C-DNA}$

II. Percentage change in molecular weight of isotopically labeled DNA

From the molecular weight of HSV DNA, 99×10^6 , and the average molecular weight of a nucleotide, 3.4×10^3 , we obtain 2.91×10^5 nucleotides per molecule.

A. ³H-HSV-1 DNA

- $3.93 \times 10^3 ^3\text{H atoms/DNA molecule} =$

FIG. 2. Isopycnic centrifugation of HSV-1 and HSV-2 DNA in the preparative ultracentrifuge. The purification of DNA and analysis of the CsCl gradients were the same as described in the legend to Fig. 1. Left panel, ^{14}C -thymidine HSV-2 DNA (dotted line), cocentrifuged with ^3H -thymidine-labeled HSV-1 DNA (solid line); right panel, ^3H -thymidine-labeled HSV-2 DNA (dotted line), cocentrifuged with ^{14}C -thymidine-labeled HSV-1 DNA (solid line).

TABLE 1. Effect of isotope on density of HSV DNA

Isotopically labeled precursor	HSV DNA subtype 1	HSV DNA subtype 2
^3H -thymidine	1.7257	1.7280
^{14}C -thymidine	1.7270	1.7290

3.93×10^3 ^3H -thymidine nucleotides/DNA molecule.

- Percentage change in molecular weight of ^3H -DNA molecule =
$$\frac{(3.93 \times 10^3 \text{ } ^3\text{H atoms/molecule})}{(2 \text{ daltons/} ^3\text{H atom})(100)}$$
$$\frac{2.91 \times 10^5 \text{ nucleotides/molecule}(344 \text{ daltons/nucleotide})}{(2 \text{ daltons/} ^3\text{H atom})(100)}$$
$$= 7.84 \times 10^{-4}\%$$

Similarly:

B. ^{14}C -HSV-1 DNA

- 6.16×10^3 ^{14}C atoms/DNA molecule = 6.16×10^3 ^{14}C -thymidine nucleotides/DNA molecule
- Percentage change in molecular weight of ^{14}C -DNA molecules =
$$\frac{(6.16 \times 10^3 \text{ } ^{14}\text{C atoms/molecule})}{(2 \text{ daltons/} ^{14}\text{C atom})(100)}$$
$$\frac{2.91 \times 10^5 \text{ nucleotides/molecule}(344 \text{ daltons/nucleotide})}{(2 \text{ daltons/} ^{14}\text{C atom})(100)}$$
$$= 1.23 \times 10^{-2}\%$$

III. Difference in density of ^3H - and ^{14}C -HSV-1 DNA

We assume that the change in molecular weight of isotopically labeled DNA does not affect the specific volume of the molecule, i.e., the change in density is a direct function of the change in mass. Therefore, the percentage change in mass of isotopically labeled DNA equals the percentage change in density.

$$\begin{aligned} \text{Calculated difference in density} &= \\ &= \frac{(1.23 \times 10^{-2} - 7.84 \times 10^{-4})(1.726 \text{ g/cm}^3)}{100} = 0.0002 \text{ g/cm}^3. \end{aligned}$$

Whereas the calculated difference in buoyant density of ^{14}C -thymidine- and ^3H -thymidine-labeled HSV-1 DNA (0.0002 g/cm³) is less than the difference observed by cocentrifugation either in the preparative or analytical ultracentrifuge (0.0015 g/cm³ and 0.0004 g/cm³, respectively), different ^{14}C - and ^3H -DNA preparations were used for the three density determinations. It is clear from the calculations that (i) differences in the specific activities of ^{14}C -DNA could account for the differences between experimental and theoretical results, and (ii), in any event, detectable differences in density between homologous ^3H - and ^{14}C -labeled DNA preparations can be predicted.

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