A rapidly-banding component of animal DNA preparations that interferes with buoyant density studies

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ABSTRACT

Rapidly-banding components of purified animal cell DNA's have been observed which have abnormal buoyant densities and show anomalous band profiles when centrifuged to equilibrium in cesium salts. These components interfere with the separation of animal DNA fractions and with the analysis of animal cell DNA's by buoyant density techniques. They can be detected by examining photographs of analytical buoyant-density ultracentrifugations before equilibrium has been reached. These rapidly-banding components are removed by phenol treatment.

INTRODUCTION

Preparations of purified animal cell DNA's have been obtained in which the DNA sediments rapidly, shows abnormal buoyant densities in CsCl and Cs₂SO₄ density gradients, and bands extremely rapidly and sharply during equilibrium density-gradient centrifugation. These rapidly-banding components (RC's) result in altered buoyant density profiles and interfere with the fractionation of animal DNA's (Ref. 1 and unpublished observations). Our laboratory has observed such RC's after the preparation of animal cell DNA's from a variety of animals and animal tissue culture cells. Similarly, an abnormal component of chloroplast DNA has been observed with an unusual buoyant density (1.697 g/ml rather than 1.693 g/ml) which "probably is an aggregate of the native DNA"^{2,3}. The RC's may be aggregates of DNA and protein since, as will be shown here, they are eliminated by phenol treatment.

METHODS

DNA was prepared by a modification of Marmur's method^{4,5}. Kung et al.², who also obtained DNA preparations with rapidly-sedimenting components, used a similar modification of Marmur's method to prepare their chloroplast DNA. These modifications of Marmur's method utilize chloroform-isoamyl alcohol rather than phenol extractions to deproteinize. Analytical density-gradient

centrifugation in a Spinco model E ultracentrifuge was as described previously⁴. Enzymes were obtained from the following sources: pancreatic RNase and pancreatic DNase I from Worthington, pronase from Calbiochem, and trypsin 1:250 from Difco.

RESULTS

The presence of RC's was routinely monitored by examining photographs of the purified, native, animal cell DNA after 4 hr of centrifugation at 44,770 rpm in the Spinco Model E ultracentrifuge. At 4 hr, DNA free of the RC's appeared as a broad, diffuse band (Fig. 1a), whereas preparations containing an RC formed a sharp band at this time (Fig. 1b). An attempt was made to separate the RC from the bulk of the DNA. 5 ml of the monkey DNA sample represented in Fig. 1b were centrifuged at 36,000 rpm for 4 hr at 4° C in a Spinco SW 39 rotor. The DNA was in Tris-EDTA (10 mM Tris-HCl plus 1 mM EDTA (pH 8.4)) at a concentration of 100 µg/ml. At the end of the centrifugation, the supernatant was decanted and the pellet was redissolved in Tris-EDTA buffer. Approximately 20% of the total DNA was recovered in the pellet, which was markedly enriched in the abnormal component (Fig. 1c). The supernatant was nearly free of the RC (Fig. 1d). Thus the component sediments more rapidly than the bulk of the native animal cell DNA prepared by the Marmur method.

Fig. 1(a)-(e). Photographs of Spinco Model E CsCl analytical density-gradient centrifuge runs after 4 hr at 44,770 rpm. (a) Purified native monkey DNA, no RC present. (b) Purified native monkey DNA, RC present. This monkey DNA sample was centrifuged in a SW 39 rotor at 36,000 rpm for 4 hr. (c) Pellet from this centrifugation after resuspension in Tris-EDTA buffer. (d) Supernatant from this centrifugation. (e) Sample (c) after 2 deproteinizations with buffer-saturated phenol. The sample was in Tris-EDTA plus 0.1 M NaCl to prevent loss of DNA fractions rich in alternating deoxyadenylate-deoxythymidylate sequences¹¹. After deproteinization, the sample was dialyzed against Tris-EDTA buffer.

The preparation shown in Fig. 1b had been deproteinized by shaking with chloroform-isoamyl alcohol (24/1 v/v) several times until proteinaceous material no longer appeared at the interface. However, when this DNA or the pellet from the preparative centrifugation (Fig. 1c) was deproteinized with

an equal volume of buffer-saturated phenol at room temperature, a thick white interface was seen. This indicated that the chloroform-isoamyl alcohol treatment had not completely deproteinized the DNA. When this DNA was treated twice with phenol, dialyzed against Tris-EDTA buffer, and centrifuged in the Spinco Model E ultracentrifuge for 4 hr, the hypersharp band disappeared (Fig. 1e).

Although phenol extraction eliminated the RC, prolonged treatment (up to 24 hr) at 37°C with 1 mg/ml of pronase or 100 µg/ml of trypsin in the presence of 10 mM EDTA did not eliminate it. The RC was eliminated after heat or alkali treatment sufficient to denature the DNA or after a 1 min treatment with 1 µg/ml of pancreatic DNase I at 37°C. This DNase treatment broadens, but doesn't eliminate, the major band of DNA. In contrast, the RC was not sensitive to 100 µg/ml of pancreatic RNase treatment for 30 min at 37°C.

Contamination of DNA preparations by RC's results in partial to complete failure of separation of satellite DNA's or other specific DNA fractions in both analytical and preparative cesium salt density gradients. For example, the mouse L cell DNA represented in Fig. 2 is contaminated by an RC and shows a satellite band corresponding to 6% of the total DNA, less than the usual total of about 9-10%. Contaminated preparations have been ob-

Fig. 2. Mouse DNA contaminated by a rapidly-banding component. Fig. 2 is a microdensitometer tracing of the photograph from a Spinco Model E CsCl analytical density-gradient centrifugation after 24 hr at 44,770 rpm. The DNA sample was mouse L cell DNA contaminated by a rapidly-banding component as evidenced by the presence of a sharp band after 4 hr of centrifugation. After 20 hr of centrifugation, the rapidly-banding component could still be detected as a hypersharp shoulder on the heavy side of the main band DNA (arrow). The amounts of main band and satellite DNA's were taken to be proportional to the areas under the main band and satellite DNA peaks which were measured by cutting out and weighing the peaks.

tained in which as little as 2-3% of the mouse DNA separates as a light satellite band. Further, the RC's can give rise to anomalous hypersharp bands which interfere with the fractionation of animal cell DNA by

preparative density-gradient centrifugation. It has been shown that animal DNA's can be fractionated according to guanine plus cytosine content by CsCl preparative density-gradient centrifugation⁶. Whereas the purity of fractions isolated from these gradients usually ranges from 80-95% giving a single distinct band, contamination by RC's can yield additional bands and thereby greatly reduce the purity (Fig. 3). It should be noted that contamination of this fraction due merely to inadequate preparation and handling of the gradient would have resulted in a broad peak centered about a density of 1.699 g/ml rather than the pattern observed in Fig. 3. Therefore, one must screen for the presence of RC's when assaying either the presence or absence of distinct DNA components by density-gradient centrifugation.

Fig. 3. Light fraction of HeLa DNA contaminated by a rapidly-banding component. HeLa DNA was fractionated in a preparative CsCl density gradient⁶. The DNA sample in Fig. 3 is a light fraction from such a preparative CsCl density gradient. This light fraction (density=1.696 g/ml) is contaminated by a heavier rapidly-banding component (density=1.707 g/ml). Fig. 3 is a microdensitometer tracing of the photograph from a Spinco Model E CsCl analytical density-gradient centrifugation run after 20 hr at 44,770 rpm.

DISCUSSION

The mechanism of RC formation is unknown: RC's were observed in some, but not all, preparations of animal cell DNA's. In general, decreasing the number of cells per ml of lysis buffer appeared to minimize the occurrence

of these components. The salient point is that animal DNA purified by one of the standard isolation procedures, the Marmur method⁵, often contains rapidly-banding components which can interfere with buoyant density studies, resulting in little or no separation of satellite DNA's and in the presence of anomalous bands. Fortunately, RC's are readily detected using analytical density-gradient centrifugation and can be removed by phenol treatment.

Since phenol extraction eliminates RC's, they could result from protein contamination. However, it is not certain that RC's are due to protein contamination since they are not removed by pronase, trypsin, or chloroform-isoamyl alcohol treatments. It is unlikely that RC's result from polysaccharide contaminations; the DNA's in this report were double-stranded and were obtained from isolated nuclei which have little contaminating polysaccharide. In contrast, DNA-polysaccharide complexes result from the interaction of denatured DNA with high concentrations of polysaccharides⁷. Further, although polysaccharides can band in CsCl density gradients at densities characteristic of DNA⁸⁻¹⁰, the sensitivity of RC's to DNase and to phenol distinguished them from such polysaccharides⁹.

In summary, rapidly-banding components of animal cell DNA's have been observed which are sensitive to phenol and brief DNase treatments and are resistant to pronase, trypsin, chloroform-isoamyl alcohol, and RNase treatments. It is appropriate to be aware of these components when performing DNA buoyant density studies in cesium salts since they can interfere with the separation of DNA fractions and the accurate determination of DNA buoyant densities and band profiles.

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