SODIUM IODIDE DENSITY GRADIENTS FOR THE PREPARATIVE BUOYANT DENSITY SEPARATIONS OF DNA MIXTURES

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SUMMARY

Preparative buoyant density separations of a DNA mixture have been achieved in sodium iodide density gradients. Better separations are obtained in sodium iodide than in cesium chloride gradients. The separation is relatively unaffected by the addition of the dye ethidium bromide, thus making sodium iodide gradients convenient and economical.

Cesium chloride density gradients are widely used for analytical characterization and preparative buoyant density separations of DNA mixtures. Although cesium chloride is satisfactory on the analytical scale, it suffers two disadvantages on the preparative scale. Firstly, cesium chloride gives a relatively large gradient at high centrifugal fields, thus causing two DNA species with a small buoyant density difference to band close together (Ifft, Voet and Vinograd, 1961). This is especially so when binding with the dye ethidium bromide (2,7-diamino-9-ethyl-10-phenylphenanthridinium bromide) is used to locate banded DNA (Radloff, Bauer and Vinograd, 1967). Secondly, cesium chloride is costly.

We report the use of sodium iodide gradients for the preparative buoyant density separations of a mixture of Escherichia coli DNA ($\rho_{B,CsCl}$ = 1.710) and Micrococcus lysodeikticus DNA ($\rho_{B,CsCl}$ = 1.731) (Schildkraut, Marmur and Doty, 1962). It is shown that better separations of the two species are obtained in sodium iodide than in cesium chloride at the same centrifugal field. In contrast to cesium chloride, the separation is relatively unaffected by the presence of added dye. Thus, sufficient dye (e.g. 50 μg/ml) can be used to make DNA bands

visible under ordinary light, making drop-collecting procedures highly convenient. The low cost of sodium iodide (less than one-tenth that of cesium chloride) makes sodium iodide gradients attractive for preparative separations.

MATERIALS AND METHODS

DNA was extracted (Marmur, 1961) from a culture of Escherichia coli 15 T$^-$ grown in presence of ^{3}H-labeled thymidine (New England Nuclear Corp.) and from a culture of Micrococcus lysodeikticus grown in presence of ^{32}P-labeled inorganic phosphate.

Preparative centrifugations were carried out on a Beckman-Spinco Model L centrifuge at 27°, 36,000 r.p.m. for 96 hours, using a Beckman-Spinco fixed angle 40 rotor. Cesium chloride solutions were prepared by adding a weighed amount of cesium chloride to a mixture of about 300 μg each of the two DNA's in 0.01 M EDTA-Tris (pH 8). The densities of the cesium chloride solutions were calculated from the measured refractive index (Messelson, Stahl and Vinograd, 1957) and the pH was measured and found to be 8.2. Additions of the dye were made from a stock solution (1 mg/ml) of ethidium bromide (Calbiochem).

Sodium iodide solutions were prepared by adding a known volume of a saturated solution of sodium iodide at 25° (ρ = 1.9) containing a few crystals of sodium sulfite (sodium sulfite was added to stabilize the stock solution against oxidation) to a mixture of the two DNA's (about 600 μg). The final solutions were 0.01 M in EDTA-Tris (pH 8), their density as measured gravimetrically was 1.55 and the pH was 8.3. In contrast to cesium chloride (Radloff, Bauer and Vinograd, 1967), no change in density was necessary when separations were carried out in presence of the dye.

Sodium iodide solutions (5.6 ml) and cesium chloride (5.8 ml) were topped with mineral oil and in each centrifugation experiment, a sodium iodide tube was paired with a corresponding cesium chloride tube. The gradients were collected in 12 drop fractions (100 drops/ml) in 4 ml of 0.015 M sodium chloride - 0.0015 M sodium citrate (Baldwin and Shooter, 1963). Each fraction was precipitated with 1 ml of 3 M trichloroacetic acid near 0° and the DNA collected

on a 0.45 μ millipore filter. The millipore filters were dried and dissolved in 10 ml of Bray's solution (POP, POPOP and naphthalene in dioxan). The radioactivity due to ^{3}H and ^{32}P was measured on a Packard Tri-Carb Liquid Scintillation Counter.

The gradients were photographed using illumination with ultraviolet light and a high contrast panchromatic film.

RESULTS AND DISCUSSION

The preparative buoyant density separation of a mixture of equal quantities of ^{3}H-labeled Escherichia coli DNA and ^{32}P-labeled Micrococcus lysodeikticus DNA in a sodium iodide gradient was compared with the separation of the same mixture in a cesium chloride gradient. Both gradients were collected from the same centrifugation experiment. Since iodide ion absorbs at 260 mμ (O. D. of sodium iodide of $\rho = 1.55$ is > 2), the gradients were analyzed by measurement of radioactivity. The radioactivity profiles are shown in Fig. 1.

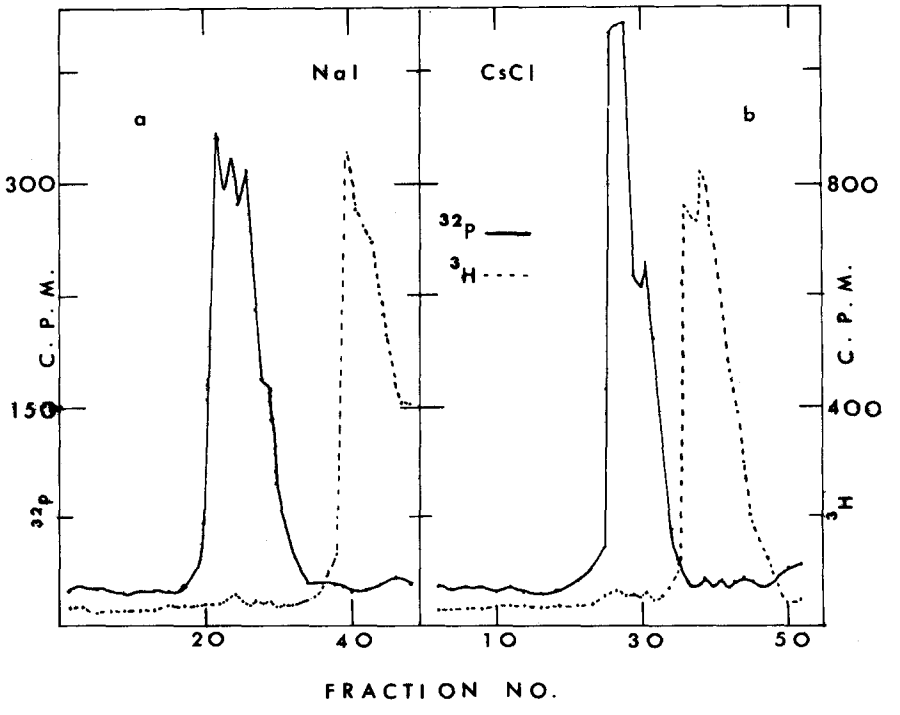

Fig. 1. Comparison of the separation of ^{3}H-labeled E. coli DNA (-----) and ^{32}P-labeled M. lysodeikticus DNA (———) in a) sodium iodide ($\rho = 1.55$) and b) cesium chloride ($\rho = 1.72$) solutions.

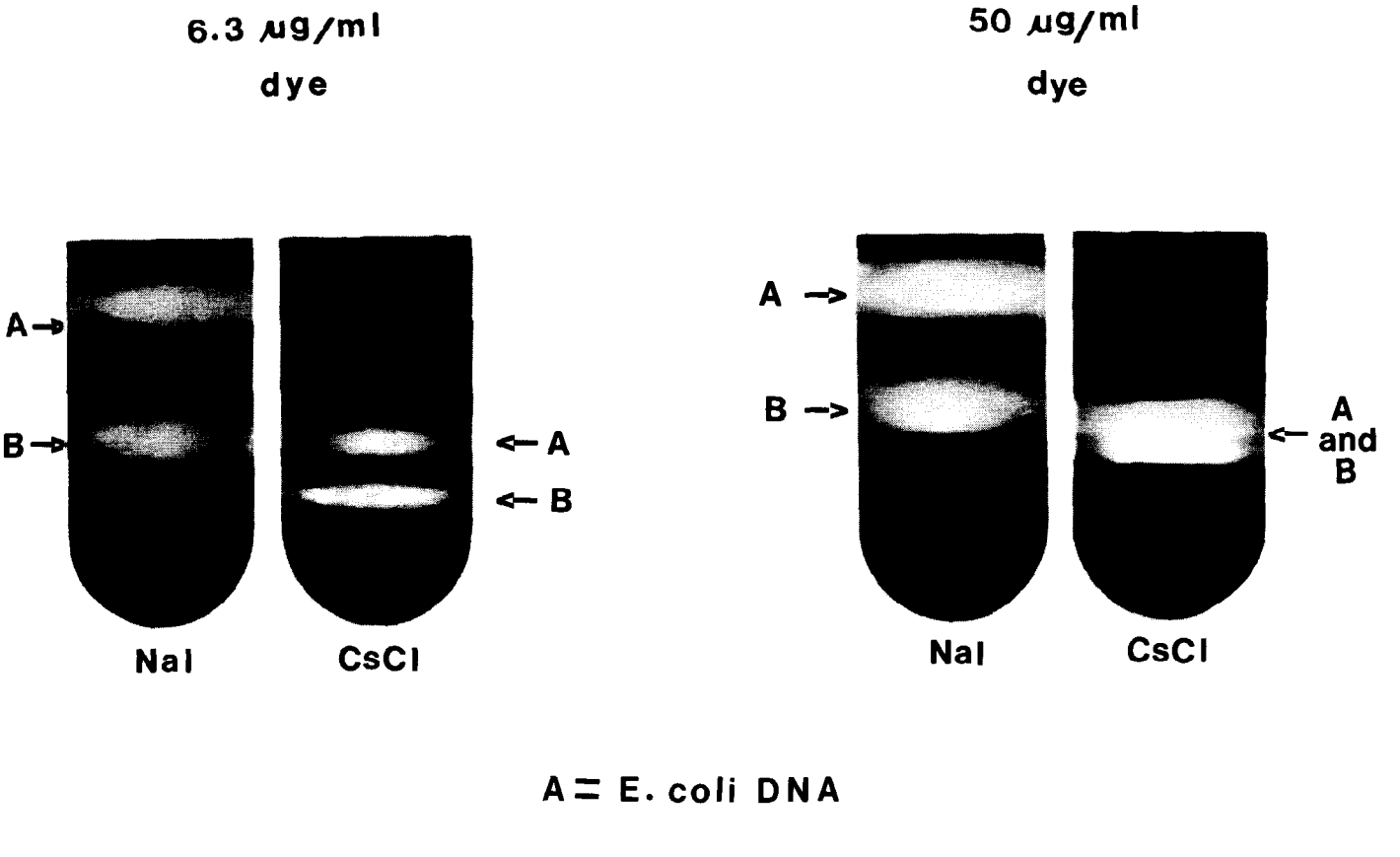

Fig. 2. Photographic comparison of the separation of ^{3}H-labeled E. coli DNA (A) and ^{32}P-labeled M. lysodeikticus DNA (B) with 6.3 and 50 µg/ml dye, in sodium iodide and cesium chloride solutions. The radioactivity profiles of the collected gradients are shown in Figs. 3 and 4.

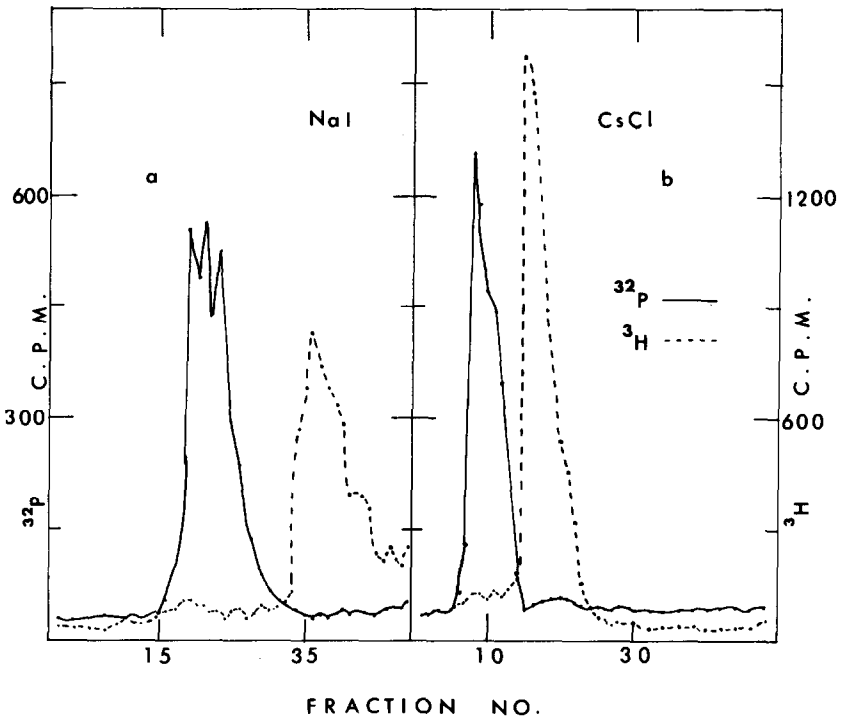

Fig. 3. Comparison of the separation of ^{3}H-labeled E. coli DNA (-----) and ^{32}P-labeled M. lysodeikticus DNA (———) in a) sodium iodide (ρ = 1.55) and b) cesium chloride (ρ = 1.67) solutions with 6.3 μg/ml of dye.

The separation of the same mixture in presence of the dye is shown photographically in Fig. 2. Figure 3 shows the radioactivity profiles of the separation in sodium iodide (Fig. 3a) and cesium chloride (Fig. 3b) solutions in presence of 6.3 μg/ml of the dye. Figure 4 shows the corresponding separation of the same mixture in presence of 50 μg/ml of the dye. All four tubes were obtained from the same centrifugation experiment, photographed (Fig. 2) and collected for measurement of radioactivity in the same way. It is clear that both the separation and the buoyant densities of the two DNA species are relatively unaffected in the sodium iodide gradients (Figures 1a, 3a and 4a). Thus, the buoyant density of the Na-DNA: ethidium iodide complex must be very close to that of Na-DNA. The situation is different in the case of Cs-DNA: ethidium chloride complex and Cs-DNA (Figures 1b, 3b and 4b).

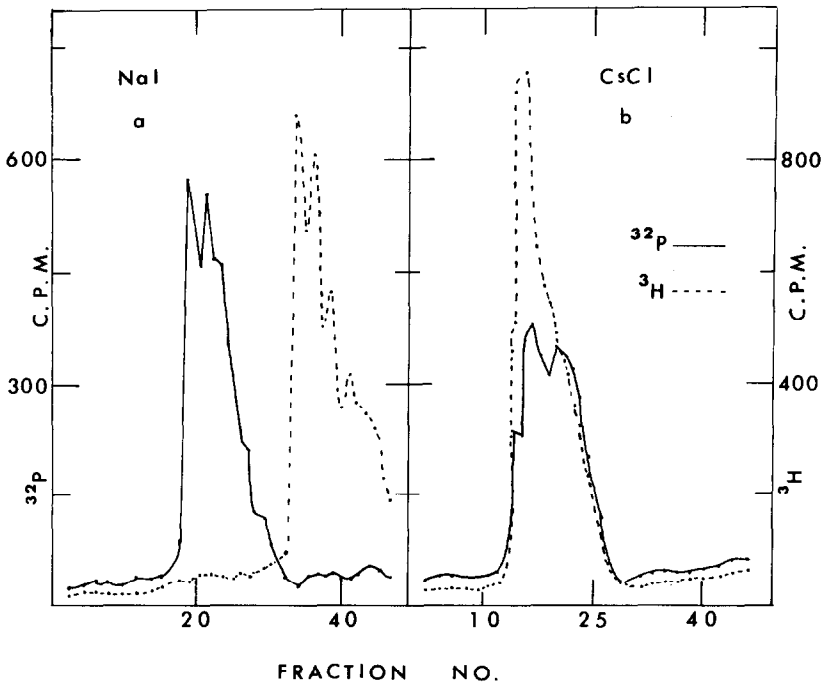

Fig. 4. Comparison of the separation of ^{3}H-labeled E. coli DNA (-----) and ^{32}P-labeled M. lysodeikticus DNA (———) in a) sodium iodide (ρ = 1.55) and b) cesium chloride (ρ = 1.61) solutions with 50 μg/ml of dye.

The separations obtained in sodium iodide solutions can be attributed to two factors: a) the density gradient in sodium iodide is smaller than the density gradient in cesium chloride at the same centrifugal field, b) the buoyant density difference between the two species is larger in sodium iodide than in cesium chloride. The relative contribution of these factors has not been ascertained, but the band-widths of the DNA species (cf. Figures 1a, 3a and 4a with 1b, 3b and 4b) suggest that the density gradient is smaller in sodium iodide than in cesium chloride. A preliminary experiment done with a range of initial densities of sodium iodide appears to support this suggestion; however, no precise information on the density gradient is yet available. Variations in density gradient with different alkali salts are not unexpected: Vinograd has calculated that varying both the anion and the cation in an alkali salt can be expected to yield different density gradient constants (Ifft, Voet and Vinograd, 1961; Hearst and Vinograd, 1961).

A further extension of the sodium iodide gradients is to combine them with the density gradient relaxation method (Anet and Strayer, 1969). This may be of advantage in cases where larger quantities of DNA with smaller density differences need to be separated.

Finally, Prof. W. R. Romig (Dept. of Bacteriology, University of California, Los Angeles) has checked the effect of the sodium iodide solutions used in the present work on the biological activity of DNA. His results show that the ability of bacterial DNA isolated from Bacillus subtilis 168 (ind^- leu^+) to transform competent Bacillus subtilis 168B (ind^- leu^-) to leucine independence is not impaired on storage of the transforming DNA in a sodium iodide solution at 24°C for 4 days.

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