The Use of Ethidium Bromide in Detecting Banded DNA in Cesium Chloride-Polyacrylamide Gels

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Abstract

Previous studies have shown that DNA banded in cesium chloride-polyacrylamide gels can be detected by staining with methyl green or pyronin B. The time-consuming step of destaining can be eliminated by introducing a trypanocidal drug, ethidium bromide, into the DNA-containing solution. This drug binds to the DNA and is visualized with ultraviolet light. An ultraviolet filter permits photographing of the DNA-ethidium bromide complexes.

Introduction

Previous reports from this (1) and other laboratories (5, 6, 7) detail the technique of immobilization of proteins after centrifugation in a sucrose density gradient. The immobilization process involves the photopolymerization of acrylamide and N, N′-methylene-*bis*acrylamide according to the procedure for generating a large pore gel as used in disc electrophoresis (4). Such a photopolymerizable, large pore gel is slightly opaque. The development of a clear, photopolymerizable gel that is compatible with high concentrations of cesium chloride and can be used in staining nucleic acids has been reported by Cole and Middendorf (2). Immobilization, staining and destaining of *E. coli* DNA in centrifuged solutions of cesium chloride and acrylamide-*bis*acrylamide have been reported by us (3).

Methods and Materials

Preparation of Cesium Chloride-polymerizable Solutions, Centrifugation and Polymerization

Cesium chloride (SC11352) was purchased from the Sargent-Welch Company or Henley and Co. (New York, N. Y.). The appropriate amount of cesium chloride was dissolved in distilled water to give a solution of desired density. For example, water was added to 14.5 g of cesium chloride until 12.3 ml of solution was produced. Ethidium bromide was added to a final concentration of 60 μg/ml. The cesium chloride solution was then added to 1.71 ml of solution B and 2.50 ml of solution C. Solution B consisted of 11.4 g of "tris buffer" (tri(hydroxymethyl)aminomethane (free base)), 1.2 ml N,N,N′,N′-tetramethylethylene-diamine (TEMED, practical grade, Matheson Coleman and Bell 8563) and 33 ml of distilled water. The pH was adjusted to 6.9 with 85% phosphoric acid. Solution C is 24 g of acrylamide; 0.735 g N, N′-methylene-*bis*acrylamide (Bis, Eastman 8383) and distilled water to give a final volume of 50 ml. Addition of 12.33 ml of cesium chloride and ethidium bromide to the indicated volumes of solutions B and C gave 16.5 ml of cesium chloride-ethidium bromide

polymerizable (CEBP) solution with a density of about 1.7 g/ml at room temperature. The CEBP solution is stable and may be stored at room temperature.

For centrifugation 5 ml of CEBP solution was pipetted into 5 x 1.5 cm cellulose nitrate tubes. The nucleic acid samples may be mixed with the CEBP solution. A small (about 0.2 mg) amount of riboflavin was added at the same time. The tubes and buckets were weighed together and adjusted to within 0.1 g of each other. A 50SW swinging bucket rotor was used in a Spinco Model L preparative ultracentrifuge in these studies.

When the run was completed, the tubes were removed from the buckets and overlayered with about 2 mm of water with a fine capillary pipette. The tubes were photopolymerized in 10 to 20 minutes before a 14 watt fluorescent lamp.

DNA Samples

Escherichia coli, *Clostridium perfringes* and *Micrococcus lysodeikticus* DNAs were purchased from the Sigma Chemical Company, St. Louis, Missouri. The DNA samples were dissolved in dilute saline citrate (0.015 M sodium chloride and 0.0015 M sodium citrate) at a concentration of 1 mg/ml. The desired volume of DNA was mixed into the CEBP solution.

Visualization

The ethidium bromide is concentrated by the banded DNA. It can be seen as a faint red line under white light. Ultraviolet light (UVL-21 Lamp, Longwave 320-380 nm, Ultraviolet Products, San Gabriel, California) produced a bright fluorescence of the banded DNA-ethidium bromide complex. The direct long wave length UV light may be removed by a contrast filter (Ultraviolet Products). The light transmitted can be photographed with Kodak Ektachrome B film.

Results

DNAs from different bacteria, *Escherichia coli*, *Micrococcus lysodeikticus*, and *Clostridium perfringes* have been banded in the presence of ethidium bromide in cesium chloride-polymerizable solution. These DNAs of different densities can be located in ultraviolet light and the regions containing the DNA can be cut out without the use of any staining-destaining routine.

Discussion

The isolation of DNA from biological material is often a critical part of an investigation. Since cells often contain heterogeneous DNAs (nuclear and satellite) or DNAs in different physical states (circles and linear strands of different densities), a routine method of isolation is useful. This method may aid in detecting various conformations and kinds of DNA. The concentration of ethidium bromide is critical. With the DNA's studied in this investigation, a

concentration of 60 μg/ml has been found useful. Above 100 μg/ml the unbound ethidium bromide gives an interfering background. Below 50 μg/ml, the detection of the ethidium bromide fluorescence is difficult. The kinetics of binding of DNA and ethidium bromide in CEBP solution have not been investigated as well as the usefulness of this technique to determine molecular weight.

Acknowledgments

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