whether or not this peak splitting reflected heterogeneity in the DNA molecules.

A 789-bp fragment was purified from a plasmid by use of preparative RPC-5 column chromatography.[16] Numerous peaks were associated with this fragment in the preparative column profile. We chromatographed this DNA on our previously described analytical system at 43° using a KCl gradient. Figure 7A shows that a single sharp peak was observed.

If the same fragment was chromatographed at 13° using a sodium acetate gradient, multiple peaks were observed (Fig. 7B). In similar experiments, as many as 19 peaks were observed under these chromatographic conditions. Also, these same conditions produced multiple peaks for other pure fragments (data not shown) which, like the 789-bp DNA, were otherwise found to be virtually homogeneous by a variety of criteria.[16]

When a single peak from the 789 fractionation in sodium acetate solution was pooled (see bar in Fig. 7B for pooled fractions) and then rechromatographed under the same conditions (Fig. 7C), the peak did not run true but instead the entire population of peaks reappeared. Moreover, the extent of heterogeneity suggested by RPC-5 chromatography with the sodium acetate gradients cannot be due to nicks or damaged ends because our previous results showed that at least half of the fragments were completely undamaged.[16]

Thus, we believe this peak splitting is not due to heterogeneity in the DNA fragments but is due to a column artifact.

[42] Fractionation of DNA Fragments by Polyethylene Glycol Induced Precipitation

By JOHN T. LIS

Precipitation by polyethylene glycol (PEG) has proved to be a general method of concentrating a variety of biological macrostructures. Bacterial cells, bacteriophage, plant and animal viruses, ribosomes, proteins, and DNA are all precipitable by similar procedures; however, the minimally required concentration of PEG in general is less for larger and/or more anisometric structures. Bacterial cells and rod-shaped viruses (the large tobacco mosaic virus and the filamentous bacteriophage fd) require only 1% PEG for quantitative precipitation[1,2]; structures of intermediate size

[1] D. L. Eshenbaugh, D. Sens, and E. James, *Anal. Biochem.* **58**, 390 (1974).

[2] K. R. Yamamoto, B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber, *Virology* **40**, 734 (1970).

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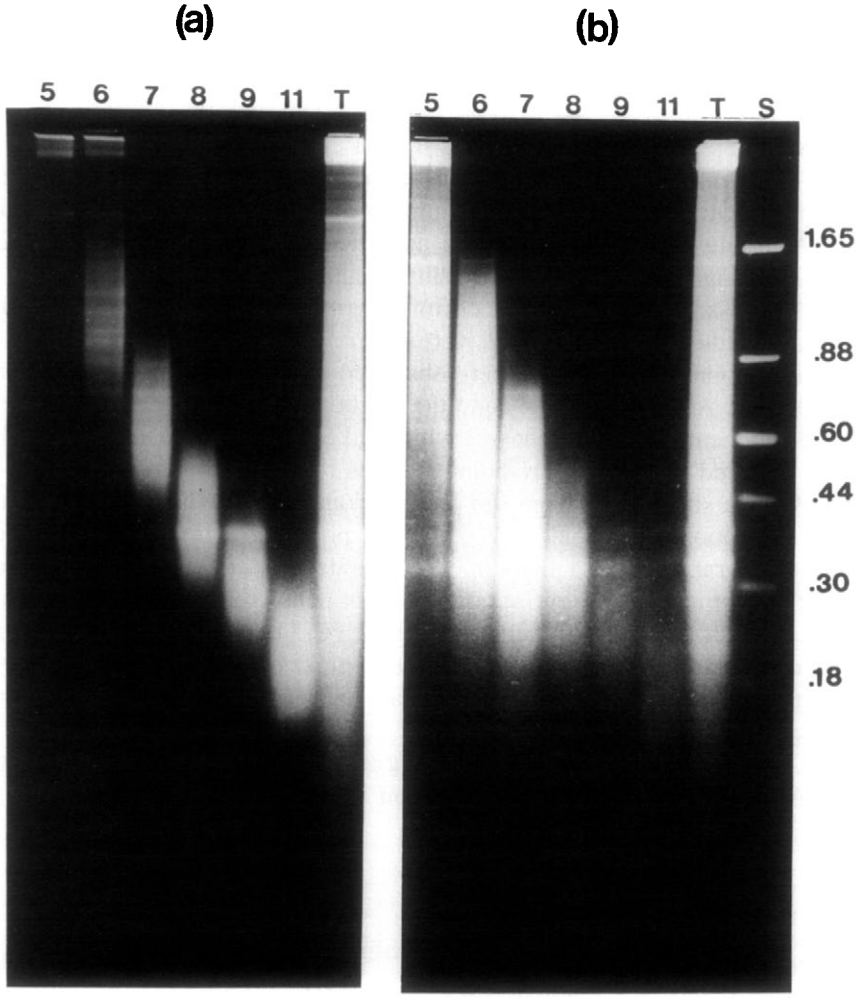

FIG. 1. Size analysis of PEG precipitated DNA by agarose gel electrophoresis. The numbers above each track correspond to the percentage PEG used to obtain the respective precipitates. (a) and (b) show the size distribution of precipitated *Hae*III and *Mbo*I cleaved total *D. melanogaster* DNA, respectively. The total mixture of DNA fragments before fractionation is shown in tracks labeled T. Track S contains size standards, *Hha*I cleaved ColE1, and the size of each band is written in units of kilobase (1000 base pairs). The values 0.60, 0.30, and 0.18 kb each represent the average size of an unresolved pair of fragments. DNA was precipitated as described in under Method from a 500-μl solution containing 0.5 *M* NaCl, 10 m*M* Tris-HCl, pH 7.4, 10 m*M* EDTA, 4.4 m*M* $MgCl_2$, DNA at 100 μg/ml, and PEG at 5%. The incubation was at 0° for 24 hr and the precipitate was collected by centrifugation for 2 min in a microfuge (15,000 *g*). Additional PEG was added to the supernatant to give a final concentration of 6%. The process was repeated for each of the indicated PEG concentrations. The precipitated DNA pellets from (a) and (b) were resuspended in 10 m*M* Tris-HCl, pH 7.4, 1 m*M* EDTA, and 6% of the total precipitate was fractionated by electrophoresis on a 2.5% agarose gel.

and anisometry (ribosomes and bacteriophage ϕX174) require 10%[2]; while some proteins require PEG concentrations exceeding 20%.[3] The relationship of macromolecular size and anisometry to the threshold PEG concentration is only approximate for this chemically diverse collection of macrostructures (for exceptions, see Table 1 of Yamamoto *et al.*[2]). However, a chemically homogeneous set of macromolecules, native DNA fragments, do show a clear inverse relationship of size to the threshold PEG concentration.[4] A statistical thermodynamic basis for these recent empirical observations resides in a treatise put forth by Onsager in 1949[5] on the effects of shape on the interaction of colloidal particles.

The striking dependence of the PEG concentration required for precipitation on the length of DNA allows a mixture of different size DNAs to be fractionated into classes by selective precipitation.[4,6,7] The ability of the method to fractionate DNA is demonstrated by the results shown in Fig. 1a. In this experiment the starting material is the *Hae*III generated restriction fragments of *Drosophila melanogaster* which provide the size spectrum of DNA fragments shown in track T. These fragments are fractionated by size using successive precipitation steps. After each step the DNA precipitate is collected and additional PEG is added to the supernatant. The addition of PEG at a concentration of 5% causes precipitation of DNA fragments larger than 1650 base pairs (track 5). The smaller fragments, as well as the PEG,[2] remain in the supernatant. Another size class of fragments is precipitated by increasing the PEG concentration to 6%, track 6. Repetition of this process in steps of PEG concentration yield the DNA classes shown in Fig. 1.

The method possesses several virtues: high capacity, simplicity of both the fractionation and recovery of DNA fragments, and applicability to crude lysates. The high capacity of the method makes it an attractive first step in large-scale preparations of specific fragments, since precipitations can be performed at DNA concentrations at least as high as 1 mg/ml.[4]

The method requires no sophisticated equipment, expensive chemicals, or complex procedures as can be seen from the standard protocol presented in the discussion of Method below. The separated fractions that result are in the form of DNA precipitates which can be resuspended in a buffer suitable for subsequent use of the DNA. The precipitates contain

[3] W. Hönig and M. Kula, *Anal. Biochem.* **72**, 502 (1976).

[4] J. T. Lis and R. Schleif, *Nucleic Acids Res.* **2**, 383 (1975). (Correction, The photograph of Fig. 1 should be interchanged with the photograph of Fig. 2.) J. T. Lis and R. Schleif, *Nucleic Acids Res.* **2**, 757 (1975).

[5] L. Onsager, *Ann. N.Y. Acad. Sci.* **5**, 627 (1949).

[6] J. T. Lis and R. Schleif, *J. Mol. Biol.* **95**, 409 (1975).

[7] R. Ogata and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4973 (1977).

small amounts of PEG resulting from solvent trapped in the pellet. If this needs to be removed, the pellet can be resuspended in 0.2 *M* NaCl and the DNA reprecipitated with 2 volumes of ethanol. Polyethylene glycol is efficiently removed from DNA by several other procedures as well: chromatography on DEAE–cellulose,[6] gel electrophoresis,[8] or CsCl density gradient centrifugation.[2]

Size fractionation of DNA fragments by PEG precipitation should prove applicable even in the presence of crude cell lysates. The ability of the method to separate DNA fragments by size under such conditions has not been reported; however, *E. coli* plasmid DNA is precipitated from crude lysates at the expected threshold concentration (unpublished observations). Although a multitude of structures in a crude lysate are precipitated by PEG, the minimal concentration of PEG required varies considerably for different structures. It, therefore, should be possible to separate DNA fragments not only from other size fragments but also from other types of cellular components. Indeed, the PEG precipitation protocol has been included as a step in a rapid *E. coli* plasmid purification procedure.[9] After this step the plasmid DNA is usually suitable as a substrate for restriction endonucleases and for transformation.[9]

The size fractionation of DNA by PEG precipitation does possess limitations: the resolution is only fair in comparison with gel electrophoresis and RPC-5 chromatography[10,11]; fractionation of fragments above 2000 bp is at present poor; and at least a moderate DNA concentration is required for efficient precipitation. In the stepwise precipitation shown in Fig. 1a the resolution of two species is essentially complete when their sizes differ by more than a factor of 2.5. In this experiment increments of 1 or 2% PEG were used; however, decreasing the size of the increment should increase resolution. Reprecipitation should also improve resolution as well as remove low levels of contaminating fragments (not detectable in this figure) arising from solvent trapped in the pellet.

A special problem occurs when fragments possess cohesive ends such as those generated by restriction endonucleases *Eco*RI or *Mbo*I. This problem arises because the PEG precipitation is usually performed by incubating high concentrations of the DNA fragments and salt at 0° for several hours. These conditions permit the joining of fragments via their cohesive ends to form an assortment of multimers. Multimers could be-

[8] P. A. Albertsson, "Partition of Cell Particles and Macromolecules." Wiley, New York, 1960.

[9] A. Rambach and D. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5041 (1977).

[10] A. Landy, C. Foeller, R. Reszelbach, and B. Dudock, *Nucleic Acids Res.* **3**, 2575 (1976).

[11] S. C. Hardies and R. D. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3117 (1976).

have like single fragments of equivalent size in response to precipitation by PEG. To test whether this occurs, the PEG induced precipitation of fragments with cohesive ends was compared to the precipitation of fragments possessing flush ends. *Mbo*I was used for this test since it makes single-strand breaks adjacent to a specific 4-bp sequence thereby generating cohesive ends. The spectrum of fragment sizes resulting from the cleavage of total *D. melanogaster* DNA with *Mbo*I is shown in track T of Fig. 1b and is similar to the spectrum generated by *Hae*III (Fig. 1a, track T). However, the *Mbo*I fragments precipitated by 5% PEG (Fig. 1b track 5) cover a broad range of sizes from the largest down to 300-bp fragments. This precipitation of the small fragments is in striking contrast to the size dependent precipitation of *Hae*III generated, flush-ended fragments (Fig. 1a, track 5). It should be noted that the electrophoretic separation of DNA fragments is performed above the melting temperature of the cohesive ends. The results of Fig. 1b demonstrate that small fragments, which possess cohesive ends, do indeed contaminate fractions that contain larger fragments; however, the larger fragments do not contaminate fractions that are expected to contain only smaller fragments (Fig. 1b tracks 6–11). Thus, the method can be used to remove large fragments from preparations of smaller fragments, albeit with reduced yield.

A solution to this problem associated with cohesive ends is to perform the incubation step for the PEG precipitation at temperatures above the melting temperature of annealed cohesive ends. The melting temperature of cohesive ends of fragments produced by known restriction endonucleases is well below room temperature and depends on the length of the cohesive ends, base composition, and salt concentration. Although I have routinely incubated the PEG–DNA mixture at 0° to minimize degradation of DNA by contaminating nucleases, Lerman has precipitated DNA with PEG at room temperature.[12] Furthermore, the partitioning coefficient of calf thymus DNA between PEG and dextran phases (the partitioning system from which simplified PEG precipitation method evolved) is the same at both 4° and 20°.[8]

Method

The following specific directions should be regarded as a recipe that can be modified in accordance with information in the Appendix.

1. The DNA solution is prepared for precipitation by adding NaCl to 0.5 *M* and Tris, pH 7.4, and EDTA to 10 m*M* each. The DNA concentration should be greater than 10 μg/ml, preferably 100 μg/ml. Add an amount of PEG 6000 (Union Carbide) required to fractionate the DNAs of

[12] L. S. Lerman, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1886 (1971).

interest using the results of Fig. 1a and the results in Lis and Schleif[4] as guides. Polyethylene glycol is conveniently added as a 50% (w/w) solution ($p = 1.05$) on a volume-to-volume basis.

2. Incubate the mixture at 0°. Incubations lasting 12 hr or more are best for DNA in the size range of 500 base pairs and larger. However, 1-hr incubations are sufficient for fractionation of lower molecular weight species which are precipitated at PEG concentrations greater than 7.5%.

3. Collect the precipitated DNA by low speed centrifugation; 8000 *g* for 5 min is more than sufficient to sediment the precipitated DNA into a firm pellet.

4. The supernatant is carefully removed with a hand-controlled pipetting devise or by decanting.

If another size class of DNA is to be precipitated, additional PEG is added to the supernatant, and operations 2–4 are repeated.

Appendix

A summary of data on the sensitivity of the PEG precipitation method to various parameters is as follows.

1. PEG Concentration. The minimum PEG required to precipitate DNA fragments (the threshold concentration) shows an inverse dependence on fragment size as is demonstrated in Fig. 1a and in a previous publication.[4]

2. Salt Concentration. Efficient precipitation requires high salt concentration, 0.5 *M* or higher. If the NaCl concentration is lowered to 0.2 *M*, no DNA of any size is detected as a rapidly sedimenting form following attempted precipitations by PEG concentrations as high as 12%.[4] At 0.35 *M* NaCl no DNA is detected following precipitation by 6.5% PEG; however, 12% PEG causes precipitation of DNA from 49,000 to 240 bp. Very high salt concentration, 1.1 *M*, allows precipitation of DNA down to 375 bp by as little as 6.5% PEG.[4]

3. DNA Concentration. The size of DNA fragments precipitated by PEG is relatively independent of DNA concentration in the range of 10–1000 μg/ml. However, below 50 μg/ml the recovery of DNA is less than quantitative and is 50% at 10 μg/ml.[4]

4. Incubation Time. Incubations as short as 5 min allow fractionation of fragments into size classes; however, the resolution is less than with longer incubations, especially in the high molecular weight range. This stems from the fact that the efficiency of precipitation of high molecular weight DNA near the threshold PEG concentration increases with time. λ DNA is precipitated with 10, 70, and 100% efficiency after incubation for 2, 9, and 25 hr, respectively, at a PEG concentration which was determined to be threshold in an 18-hr incubation.[4]

5. Centrifugal Force. Both the size of the DNA fragments and the efficiency with which they are precipitated is independent of the centrifugal force used to collect precipitated DNA at least in the range of 1900 to 27,000 *g* for a 10-min centrifugation.[4] Centrifugation at 480 *g* can result in a 50% loss of fragments of all sizes.

6. Divalent Ions. Preparations of specific DNA fragments usually require digestion with either of two classes of enzymes, restriction endonucleases, or single-strand-specific nucleases. The presence of divalent ions at concentrations normally required by both classes of enzyme, ≤ 10 m*M*, do not interfere with the size fractionation. However, divalent ions at 10 m*M* extend the size range of the precipitated DNA to include fragments that are approximately one-half the size of those precipitated in the absence of divalent ions.[4]

7. pH. The method is insensitive to pH at least in the range of pH 5.0–8.3.[4]

[43] A Photographic Method to Quantitate DNA in Gel Electrophoresis

By ARIEL PRUNELL

The purpose is to determine the relative amount of DNA in bands obtained upon electrophoresis in polyacrylamide or agarose gels.

The procedure[1] is, in outline, as follows: The gel is stained with ethidium bromide and photographed under ultraviolet illumination. The photograph is traced with a microdensitometer, and pen deflections converted into fluorescence intensities (which are proportional to the amounts of DNA; see below) with the use of the *characteristic curve* of the film. This curve relates optical densities of the film to light intensities or exposures. Practically, a wide range of exposures is obtained with a *step tablet,* and the characteristic curve consists of a plot of densities in a photograph of the step tablet (this photograph is termed *sensitogram*) against the logarithm of exposures.

Procedure

Staining. Gels are stained at room temperature for 2 hr in electrophoresis buffer[2] supplemented with 2 μg/ml of ethidium bromide

[1] A. Prunell, F. Strauss, and B. Leblanc, *Anal. Biochem.* **78,** 57 (1977).
[2] U. E. Loening, *Biochem. J.* **102,** 251 (1967).

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