
Size fractionation of double-stranded DNA by precipitation with polyethylene glycol*see also Meth Enz*

John T. Lis and Robert Schleif*65 347*

Brandeis University, Waltham, Mass. 02154, USA

Received 24 January 1975

ABSTRACT

We show that DNA molecules of differing molecular mass are separable by selective precipitation with polyethylene glycol (PEG[†]). Higher molecular mass DNA precipitates at lower PEG concentrations than lower molecular mass DNA. Double-stranded DNA can be fractionated at least in the range of 3×10^7 to 1×10^5 daltons. The effects of PEG concentration, sodium chloride concentration, DNA concentration, pH, divalent ions, precipitation time, and centrifugal force have been determined. These studies show PEG precipitation offers a size fractionation method for DNA which is convenient, of high capacity, and applicable over a wide range of conditions. However, resolution is not high and separation of two species approaches 100% only if they differ in molecular mass by at least a factor of two.

*150-56,050-5p***INTRODUCTION**

Many problems presently being studied in molecular biology require fractionation of DNA according to size. Fractionation of DNA in the range of 10^5 to a few hundred base pairs can be achieved by electrophoresis on agarose gels (1), while DNA in the range of several thousand base pairs to oligonucleotides can be fractionated by electrophoresis on acrylamide gels (2). These procedures allow high resolution, separation of two species differing in size by 10%, and have a capacity of up to five or ten milligrams.

Here we describe another method for fractionation of DNA according to size. The method is based on the fact that the size of DNA molecule precipitated by PEG is dependent on the concentration of PEG. The method is simple, virtually unlimited in capacity, and inexpensive, but possesses the following limitations: it works efficiently only at DNA concentrations above 10 $\mu\text{g/ml}$, it can only fully resolve two species of DNA whose sizes differ by more than a factor of two, and this degree of resolution has only been demonstrated in the range of 100-2000 base pairs. Thus the method appears useful in some applications requiring a high capacity fractionation step.

MATERIALS AND METHODS

Materials

Polyethylene glycol (PEG) was from Union Carbide and sold under the trade name Carbowax 6000. Salmon DNA was from Sigma. The λ DNA was from transducing phage, either λ plac5 (3) or λ paraB107 (4) and was prepared from whole phage as described previously (5).

The PEG precipitations were performed in either Solution A or Solution B. Solution A consisted of 0.55 M-NaCl, 0.01 M-Tris HCl, pH 7.5, 2mM-EDTA and contained a mixture of lightly and heavily sonicated λ paraB107 DNA at 50 or 100 μ g/ml and whole length λ paraB107 at 1.2 or 2.4 μ g/ml respectively. Solution A was used for studies on the effect of PEG concentration, stepwise precipitation, and pH. Solution B consisted of 0.35 M-NaCl, 0.01 M-Tris HCl, pH 7.5, 2 mM-EDTA, and contained a mixture of lightly and heavily sonicated salmon DNA at 100 μ g/ml and whole length λ plac5 DNA at 0.5 μ g/ml. Solution B was used for studies on the effect of salt (NaCl) and DNA concentrations, divalent ions, time of incubation, and centrifugal force.

The standard assay

Solid PEG was added to a 2 ml DNA solution, the mixture was chilled at 0°, and the precipitated DNA was collected by a 10 min centrifugation at 8,000 x g. The precipitate was resuspended in 0.2 ml of 0.01 M-Tris borate pH 8.3, 0.1 mM EDTA, 5% glycerol, and 0.02- 0.1% bromophenol blue. A 20 μ l aliquot of the sample was loaded onto a slab gel (0.15 X 14 X 16 cm) containing 4% acrylamide, 0.33% methylene-bisacrylamide, 0.1 M Tris borate, pH 8.3, and 1 mM-EDTA. Although a 4% gel solidifies 20 min after pouring, we allowed a minimum of 10 hours before use. After this time the polymerization of the UV absorbing acrylamide monomer to non UV absorbing polymer is sufficient that the absorbance of the gel itself does not interfere with the viewing of the DNA by ethidium bromide fluorescence described below. The electrophoresis was run at 20 mA (250 V) for about 2 hr, until the bromophenol blue marker reached the end of the gel. After removing one of the two glass plates, the gel atop the remaining glass plate was stained 15 min in a solution of 1 μ g/ml ethidium bromide. The glass with gel was placed on top of the UV Products long wavelength (365 nm) transilluminator and photographed on Polaroid type 107 film using the contrast filter provided with the illuminator and a 2 mm Corning glass filter (CS-73). DNA bands containing more than 20 ng are detected with exposures of f/8 for 5 sec. A tenfold increase in sensitivity is obtained by lowering the ethidium bromide concentration to 0.1 μ g/ml, using the short wavelength, 260 nm, transilluminator, and substituting a quartz plate (obtained from Quartz

Scientific Inc.) for the glass plate. Glass is used neither below nor on top of the gel when using short wavelength illumination since the glass not only absorbs short wavelength light, but also fluoresces weakly on illumination and contributes significantly to background. Only 365 nm illumination is used when biological activity is to be recovered.

The migration velocity of DNA with respect to bromophenol blue, R_f , was measured from photographs of gels and compared to the R_f of standards obtained by digestion of λ DNA with Hemophilus influenzae restriction endonuclease (6). The following relationship of mobility to the length of DNA in base pairs was observed:

$$\text{base pairs} = 2540 e^{-3.81 R_f}$$

The efficiency of the precipitation was estimated by eye from the photographs.

Assay incubation times

Centrifugation of samples was after 18-25 hr incubation at 0° except for studies on the effect of divalent ions and centrifugal force which were after 2 hr incubations. In the stepwise precipitation experiment incubation was for 23 hr prior to the centrifugation at 5% PEG, 12 hr for centrifugations at PEG concentrations 6% through 8.5%, and 3 hr for centrifugations at PEG concentrations of 10, 12, and 15%. In the study of the effect of time of incubation, incubations were at 0° for 10 min, 1, 2, 5, 9, and 25 hr.

RESULTS

Effect of PEG concentration

Upon addition of PEG to a DNA solution containing a range of DNA sizes. from 46,500-100 base pairs, we find the sizes of DNA molecules collected by low speed centrifugation is strikingly dependent on the amount of PEG added. The DNA sizes precipitated at different PEG concentrations were analyzed by gel electrophoresis (Figure 1). The track labeled "before" shows the distribution of the DNA molecular sizes before precipitation; the arrows mark the location of known molecular mass standards, presented in base pairs, obtained by digestion of λ DNA with Haemophilus influenzae restriction endonuclease (6). The minimum PEG concentration required to precipitate λ length DNA in Solution A was 5%. Increasing the PEG concentration to 7% precipitated, in addition to λ length DNA, DNA as small as 700 base pairs, while 15% PEG precipitated essentially all the DNA from 46,500-100 base pairs.

The resolving power of PEG precipitation as a method of fractionating DNA on the basis of size is demonstrated in the results presented in Figure 2. In this experiment PEG was added to 5% and after centrifugation the supernatant was poured off and additional PEG added to the supernatant to give a

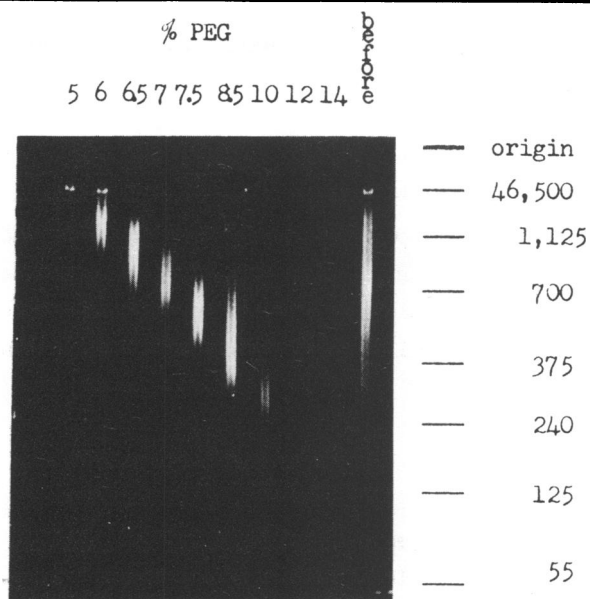


Figure 1. Parallel precipitations of DNA at the indicated PEG concentrations.

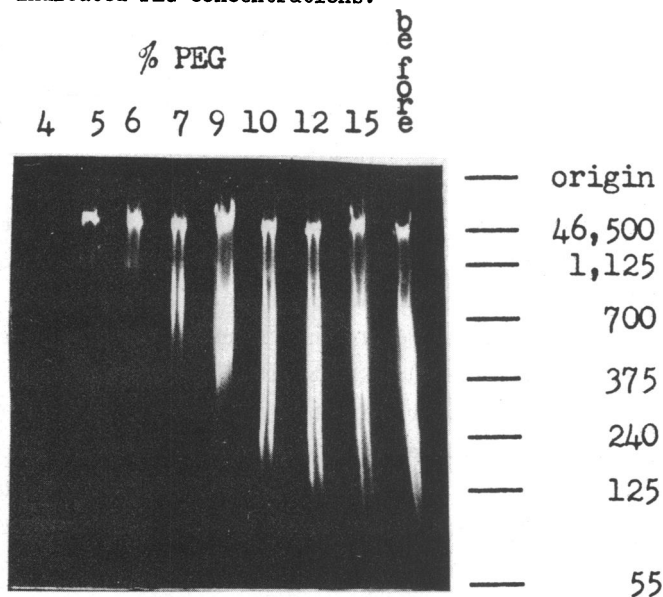


Figure 2. Sequential precipitations on a single DNA sample. In this experiment the small volume loss on purging off the supernatant after each centrifugation was ignored when calculating the amount of PEG to add to produce the next PEG concentration step.

final concentration of 6%. After a second centrifugation the supernatant was again poured off and to it was added PEG to 6.5% and the procedure repeated at each of the PEG concentrations indicated in Figure 2. The tracks of Figure

2 show the sizes of DNA precipitated at each stepwise increase in PEG concentration. By this procedure DNA of λ length is separable from 1,125 base pair DNA, 1,125 from 700, and 700 from 240 base pair DNA, etc.

Effect of salt concentration

If the salt concentration in the standard precipitation buffer was lowered from 0.55 M to 0.2 M NaCl, no DNA of any size range was detected in the pellet following precipitation by 6.5% and 12% PEG. At 0.35 M NaCl no detectable DNA was precipitated by 6.5% PEG, but DNA from 46,500 to 240 base pairs was quantitatively precipitated by 12% PEG. At salt concentrations of 0.55 M and 1.1 M, DNA in the size range of 46,500 to 700 and 46,500 to 375 base pairs respectively were precipitated by 6.5% PEG. Precipitation by 12% PEG at these salt concentrations showed much less variation; DNA from 46,500 to 125 and 46,500 to 80 base pairs were precipitated at salt concentrations of 0.55 M and 1.1 M respectively.

Effect of DNA concentration

Although we find the sizes of the DNA molecules precipitated by PEG (only concentrations of 7.5 and 11% were tested) to be relatively independent of DNA concentration in the range of 10-1000 $\mu\text{g/ml}$, below 50 $\mu\text{g/ml}$ the efficiency of precipitation begins to decrease from 100%. At 10 $\mu\text{g/ml}$ the precipitation efficiency for those sizes which are precipitable is 50%. Further reduction in DNA concentration causes further decrease in efficiency, the decrease being more noticeable for lower molecular mass DNAs.

Effect of divalent ions

Preparation of specific fragments of DNA frequently requires digestion with restriction endonucleases (6) or single-strand specific nucleases (3,4). Both classes of enzymes require the presence of divalent ions. DNA size fractionation by PEG could be applied directly to DNA digests containing these ions if they don't interfere with the selective precipitation. We find that the addition of MgSO_4 , CaCl_2 , MnCl_2 , or ZnSO_4 to 0.01 M has little effect on the size fractionation abilities of PEG precipitation. The salts, ZnSO_4 and MnCl_2 , were tested at 9% PEG while MgSO_4 and CaCl_2 were tested both at 6.5 and 15%. Each of the four salts extends the range of DNA precipitated to about 1/2 the size precipitated in the absence of divalent ions.

Effect of time of incubation at 0°

The experiment whose results are shown in Figure 2 was repeated reducing the incubation between steps from several hours to 5 min. Fractionation by size was still obtained, but with less resolution, especially for high molecular mass DNA. Furthermore, precipitations at PEG concentrations of 6.5, 9, and 15% after 10 min, 1, 2, 5, 9, and 25 hr incubations at 0° show the efficiency and size ranges of DNA precipitated to increase with incubation time especially

for DNA between 46,500 and 1,125 base pairs. For example, addition of 6.5% PEG quantitatively precipitated λ length DNA after a 25 hr incubation, over 70% was precipitated after 9 hr, while incubations of 2 hr or less precipitated less than 10% of the λ length DNA.

Effect of centrifugal force

After an 8,000 x g centrifugation for 10 min the PEG precipitated DNA is in a pellet with a firmness approximately that of Escherichia coli cells centrifuged similarly. After precipitating DNA with 9% PEG 10 min centrifugations were performed at 480, 1,900, 4,300, 14,600, and 27,000 x g force and the DNA in the pellet of each tube was analyzed by gel electrophoresis. DNA pellets collected by centrifugation between 1,900 to 27,000 x g all had identical amounts of all DNA sizes. Reducing the centrifugal force to 480 x g resulted in about a 50% loss of the DNA in all sizes.

Effect of pH

The size fractionation by PEG was found insensitive to pH at least in the range of pH 5.0-8.3. Precipitations were performed at 7, 9, and 15% PEG at pHs 5.0, 7.5, and 8.3. Buffers were at 0.01 M and sodium acetate was used for the lowest pH and Tris HCl buffer for the higher pH solutions.

DISCUSSION

Precipitation by PEG is of general utility as a method of concentrating a host of biological macrostructures: bacteriophage, plant and animal viruses, ribosomes, DNA, and bacterial cells (7,8,9,10). These studies show the minimally required amount of PEG necessary for precipitation decreases for very asymmetric or large structures. Highly asymmetric bacteriophage Fd, whole bacterial cells, and the asymmetric and large TMV, tobacco mosaic virus, require no more than 1% PEG for quantitative precipitation, while smaller more symmetrical structures, such as ribosomes and phage ϕ X, require 10% PEG (7,10). If the quantity of PEG necessary for precipitation is a function of size and asymmetry of the molecule precipitated, then a chemically homogeneous class of molecules that differ only in molecular mass should prove separable into sized subclasses by precipitation at different PEG concentrations. We have found this to be so for double-stranded DNA. We have investigated the precipitation of DNA by PEG in order to develop a large scale method for fractionating DNA on the basis of molecular mass. This study included investigation of the effects of a number of parameters and demonstrates the range and limitations of the method.

Some uses of the DNA purified by the PEG fractionation method may require the removal of PEG. Bacteriophage that have been precipitated by PEG and collected by low speed centrifugation contain about 2% of the input PEG. This is a result of simple trapping of solvent in the pellet (7).

This residual PEG can be removed by electrophoresis of charged DNA polymer from uncharged PEG (11). For DNA at least in the size range of 150-1000 base pairs, we have found that chromatography on DEAE cellulose conveniently and effectively removes PEG (4).

REFERENCES

1. Sharp, P.A., Sugden, B., and Sambrook, J. (1973) *Biochem.* 12, 3055-3063
2. Danna, K.J., Sack, G.H., and Nathans, D. (1973) *J. Mol. Biol.* 78, 363-376
3. Shapiro, J., Machattie, L., Eron, L., Ihler, G., Ippen, K., and Beckwith, J. (1969) *Nature* 224, 768-774
4. Lis, J.T. and Schleif, R. (1975) To be published
5. Lis, J.T. and Schleif, R. (1973) *J. Mol. Biol.* 79, 149-162
6. Maniatis, T., Ptashne, M., and Maurer, R. (1973) Cold Spring Harbor Symposium Quant. Biol. 38, 857-868
7. Yamamoto, K.R., Alberts, B.M., Benzinger, R., Lawhorne, L., and Treiber, G. (1970) *Virology* 40, 734-744
8. Herbert, T.T. (1963) *Phytopathology* 53, 361-366
9. McSharry, J. and Benzinger, R. (1970) *Virology* 40, 745-746
10. Eshenbaugh, D.L., Sens, D., and James, E. (1974) *Anal. Biochem.* 58, 390-394
11. Albertsson, P.A. (1960) *Partition of Cell Particles and Macromolecules*, Wiley, New York

†Abbreviation used: PEG, polyethylene glycol