ISOLATION OF HIGH-MOLECULAR-WEIGHT DNA FROM PLASMIDS FB1 AND FB1 drd OF SEROTYPED STRAINS OF Escherichia coli (AP1 AND AP2)

A. P. Kalyuzhnaya, N. I. Matvienko, V. P. Shchipkov, and A. P. Pekhov

UDC 576.851.48.098.396.332

A method of isolating high-molecular-weight DNA from plasmids FB1 and FB1 drd of conditionally pathogenic strains of Escherichia coli has been developed. By electrophoresis of the DNA fragments in agarose gel and by sedimentation in a neutral glycerol concentration gradient the molecular weights of the isolated plasmid DNA were found to be of the order of $30 \cdot 10^6$ and $40 \cdot 10^6$, assuming two different plasmids, or $70 \cdot 10^6$ if only one.

KEY WORDS: plasmid DNA; molecular weight; electrophoresis; sedimentation; restriction.

Plasmids FB1 and FB1 drd were discovered during a study of the conjugation properties of cells of a conditionally pathogenic strain of Escherichia coli belonging to the serotype 06:K54 (L):H10 [2]. Whereas plasmid FB1 is a repressed variant of the sex factor F of serotyped strains of E. coli, plasmid FB1 drd is a derepressed mutant of that factor.

The object of this investigation was to isolate plasmid DNAs from cells of conditionally pathogenic strains of E. coli containing plasmids FB1 and FB1 drd and to investigate some of their physicochemical properties.

EXPERIMENTAL METHOD

Plasmid DNA was isolated by modified methods of Guerry et al. [7] and Clewell and Helinski [5], using cultures of E. coli (AP1 and AP2) whose cells contain plasmids FB1 and FB1 drd, respectively. These cultures were grown in 200 ml nutrient broth at +37°C to a titer of $5.6 \cdot 10^8$ cells/ml and then centrifuged. The cell residues were resuspended in 12 ml of a cold 25% solution of sucrose in 0.05 M Tris–HCl buffer, pH 7.2, Each cell suspension was treated with 2.4 ml of a solution of lysozyme (5 mg/ml in 0.25 M Tris-HCl, pH 8.0), and 5 min later, with 4.8 ml of 0.25 M EDTA, pH 8.0. The spheroplasts thus formed were lysed by the addition of a 10% solution of sodium dodecyl sulfate to a final concentration of 1%. Chromosomal DNA was precipitated by adding 5 M NaCl solution to the lysates to a final concentration of 1 M and then allowing the lysates to stand at 4°C overnight. The supernatants were separated from the residue, their volumes were measured, and they were treated with 200 μg/ml ethidium bromide and dry CsCl at the rate of 0.75 g/ml to a density of 1.57 g/cm^3, equivalent to a refractive index of 1.398 on the IRF-22 refractometer. Equilibrium centrifugation of these supernatants was carried out on the Beckman L-5-50 ultracentrifuge in a Ti-50 rotor at 44,000 rpm and 15°C for 50 h. The resulting plasmid DNAs were dialyzed against 100 volumes of 0.01 M Tris–HCl buffer, pH 7.2, and 0.05 M NaCl.

The molecular weights of the plasmid DNAs was determined by two methods: by sedimentation in a neutral glycerol concentration gradient and by electrophoresis in an agarose gel of DNA fragments obtained by treating plasmid DNAs with the enzyme EcoR1.

In the first case a neutral glycerol gradient was made up by the method of Ihler and Kawai [8], using a gradient of glycerol (10-30% w/w) in a solution containing 0.02 M Tris-HCl, pH 8.0; 0.001 M EDTA; 1.0 M

Department of Biology and General Genetics, P. Lumumba Peoples' Friendship University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1492-1494, December, 1976. Original article submitted May 10, 1976.

NaCl. A linear gradient was formed by means of the device described by McConkey [1]. To each gradient 3 μg of plasmid DNAs was applied. The specimens were centrifuged on the L-5-50 ultracentrifuge in the SW-41 rotor at 38,000 rpm and at 4°C for 7 h. After centrifugation, the contents of the nitrocellulose centrifuge tubes were passed through a highly sensitive "Analabs" UV-densitometer (flow rate 4 ml/h, sensitivity of the instrument 0.04 μg). The molecular weight was calculated by the equation of Burgi and Hershey [4]:

$$D_1/D_2(L_1/L_2)^{0.35},$$

where D_1 and D_2 are the distance traveled in the centrifuge tube by the DNA fractions and L_1 and L_2 are the molecular weights of the DNA fractions. Plasmid ColE1, the molecular weight of which is $4.2 \cdot 10^6$ daltons, was used as the marker.

In the second case plasmid DNAs were treated with EcoR1 restrictase by the method of Greene et al. [6] with slight modification in an incubation mixture with a total volume of 50 μl, containing 100 mM Tris-HCl, pH 7.3, 5 mM $MgCl_2$, 50 mM NaCl, 2.5 μg plasmid DNA, and 7 μl EcoR1 restrictase. The mixture was incubated at 37°C for 70 min, the reaction was stopped by heating the mixture to 70°C for 5 min, after which it was cooled in an ice bath. The mixture was then applied to the upper end of a tube (0.6 × 10 cm) containing 0.65% agarose gel in the proportion of 1.5 μg/20 μl DNA, and the tubes were placed in a Reanal apparatus for electrophoresis. The tubes were covered with electrophoresis buffer E (0.004 M Tris-acetate buffer, pH 7.8; 0.02 M CH_3COONa; 0.2 M EDTA). Electrophoresis was carried out at room temperature with a voltage of 20 V for 14 h. The molecular weight of the fragments was determined from their relative electrophoretic mobility compared with fragments of phage λi 434, whose DNA fragments have molecular weights of $13.7 \cdot 10^6$, $3.7 \cdot 10^6$, $3.5 \cdot 10^6$, $3.2 \cdot 10^6$, $3.0 \cdot 10^6$, $2.1 \cdot 10^6$, and $1.0 \cdot 10^6$, respectively.

EXPERIMENTAL RESULTS

After equilibrium centrifugation in a CsCl gradient two bands were found in the tube.

Bauer and Vinograd [3] showed previously that a preparation of DNA from virus SV 40 contained two clearly distinct zones after centrifugation in a CsCl gradient in the presence of ethidium bromide. After investigating the material from each zone, these workers concluded that the lower zone contained supercoiled molecules of plasmid DNA, which bound the dye more weakly, whereas the upper zone contained linear and open-ring molecules of plasmid DNA and residues of chromosomal DNA, which are more accessible for interaction with ethidium bromide. On interaction with the different forms of DNA, the ethidium bromide thus reduced their density to a different degree. On the basis of these findings the present writers concluded that the upper zone formed in the tube after equilibrium centrifugation consisted of linear and open-ring molecules of plasmid DNA and chromosomal DNA of E. coli, whereas the lower band consisted of supercoiled molecules of plasmid DNA. Material from the lower band was accordingly used in the subsequent investigations. By the method described above DNA was isolated from each F-like plasmid tested, in amounts of 250 and 300 μg from 1 liter of the E. coli culture (AP1 and AP2) with a titer of $5.6 \cdot 10^8$ cells/ml. DNA from plasmids ColE1, ColE 12124, and also RI and RII, which were used as markers, also were isolated by the method described above.

As was pointed out above, the molecular weight of the plasmid DNAs tested was determined by sedimentation in a neutral glycerol concentration gradient and by electrophoresis in agarose gel of fragments obtained from these DNAs after treatment with EcoR1 restrictase. The results are illustrated by curves in Fig. 1 (a and b) and Fig. 2. Clearly, after sedimentation of the plasmid DNAs in a neutral glycerol gradient and after passing the material from the centrifuge tube through the UV-densitometer, several homogeneous peaks were found (4 peaks in Fig. 1a and 3 peaks in Fig. 1b), indicating distribution of the DNA among the fractions. The molecular weight (in daltons) of the resulting fractions was calculated by the use of plasmid DNA ColE1 with a molecular weight of $4.2 \cdot 10^6$ daltons as the marker in a parallel gradient in accordance with the equation of Burgi and Hershey [4]. The calculations showed that for fractions 1, 2, 3, and 4 of DNA illustrated in Fig. 1a the molecular weights were $27.2 \cdot 10^6$, $41.3 \cdot 10^6$, $61.4 \cdot 10^6$, and $127.9 \cdot 10^6$, respectively; whereas for fractions 1, 2, and 3 shown in Fig. 1b the molecular weights were $27.4 \cdot 10^6$, $42.6 \cdot 10^6$, and $62.4 \cdot 10^6$, respectively.

The results of electrophoresis of restriction fragments of plasmid DNAs after treatment with EcoR1 restrictase are shown in Fig. 2. The number of bands is about 10, and this evidently reflects the number of DNA fragments formed.

To determine the molecular weight (in daltons) of the hypothetical fragments of plasmid DNAs, the standard curve plotted for DNA fragments of phage λi 434 with known molecular weights was used. As the calcula-

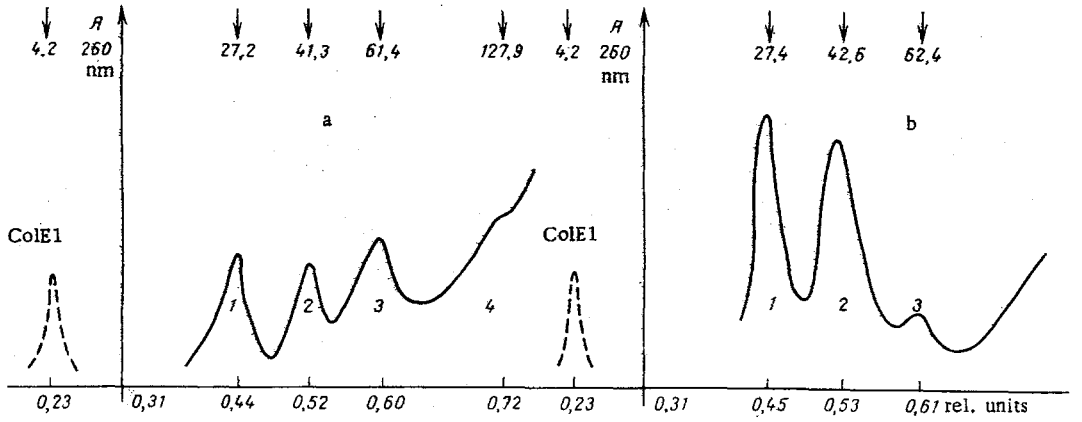

Fig. 1. Centrifugation of DNAs of plasmids FB1 and FB1 drd in a neutral glycerol concentration gradient (420 min, 38,000 rpm, 4°C). Vertical arrows indicate dimensions of DNA fragments calculated by equation [4]. Molecular weight of marker plasmid ColE1 taken as $4.2 \cdot 10^6$ daltons: a) DNA of plasmid FB1, strain AP1; b) DNA of plasmid FB1 drd, strain AP2. 1, 2, 3, 4) DNA fractions. Abscissa, distance from beginning of gradient, relative units.

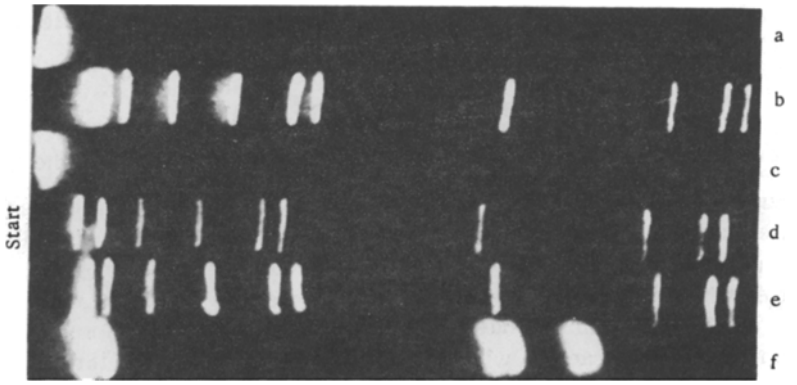

Fig. 2. Electrophoresis of restriction fragments of plasmid DNAs in 0.65% agarose gel: a) DNA of plasmid FB1 without treatment with EcoR1 restrictase; b) the same DNA after treatment with enzyme; c) DNA of plasmid FB1 drd without restrictase treatment; d, e) the same DNA but after enzyme treatment; f) DNA of phage λi 434 after enzyme treatment.

tions showed, fragments of isolated DNA of plasmid FB1 had the following molecular weights (in daltons): $13.7 \cdot 10^6$, $13.3 \cdot 10^6$, $11.7 \cdot 10^6$, $10.0 \cdot 10^6$, $8.4 \cdot 10^6$, $8.1 \cdot 10^6$, $3.7 \cdot 10^6$, $2.4 \cdot 10^6$, $2.2 \cdot 10^6$, $1.6 \cdot 10^6$. Fragments of DNA isolated from plasmid FB1 drd had molecular weights (in daltons) of $13.7 \cdot 10^6$, $12.3 \cdot 10^6$, $12.1 \cdot 10^6$, $9.8 \cdot 10^6$, $9.3 \cdot 10^6$, $7.8 \cdot 10^6$, $4.4 \cdot 10^6$, $2.7 \cdot 10^6$, $2.4 \cdot 10^6$, $2.2 \cdot 10^6$. Summation of the molecular weights found for the DNA fragments shows that the molecular weight of the whole DNA molecules of plasmid FB1 was $75.1 \cdot 10^6$ daltons, whereas the molecular weight of the whole DNA molecules of plasmid FB1 drd was $76.7 \cdot 10^6$ daltons. The molecular weights of DNAs of plasmids FB1 and FB1 drd were thus of the order of $70.0 \cdot 10^6$ daltons when determined by the sedimentation method in a neutral glycerol gradient, and between $75.1 \cdot 10^6$ and $76.7 \cdot 10^6$ daltons in the case of determination of the molecular weight of the fragments of the plasmid DNAs after their treatment with EcoR1 restrictase.

Two hypotheses can be put forward on the basis of these data: 1) The plasmid DNAs in the preparations studied were present as monomers and dimers. The mean molecular weight of one form of plasmid DNA, in this case, was about $30.0 \cdot 10^6$ daltons and that of the other form $40.0 \cdot 10^6$ daltons; 2) plasmid DNAs in the preparations tested were present in only one form with a molecular weight of about $70.0 \cdot 10^6$ daltons, but existed as supercoiled, linear, and open-ring molecules, possibly formed as a result of the manipulations with the DNA preparations. The quantitative ratio between the different molecules in the DNA preparations from plasmids FB1 and FB1 drd was different.

Further investigations are required to prove which of these hypotheses is correct.

The authors are grateful to Candidate of Physico-Mathematical Sciences V. A. Zaginaiko for help with the mathematical analysis of the data and to D. A. Vorozheikina for technical help with the investigation.

LITERATURE CITED

1. E. McConkey, in: Methods of Investigation of Nucleic Acids [in Russian], Moscow (1970), p. 127.
2. A. P. Pekhov, V. P. Shipkov, and N. I. Buyanova, Dokl. Akad. Nauk SSSR, 220, 711 (1975).
3. H. W. Bauer and J. Vinograd, J. Mol. Biol., 33, 141 (1968).
4. E. Burgi and A. D. Hershey, Biophys. J., 3, 309 (1963).
5. D. B. Clewell and D. R. Helinski, Proc. Nat. Acad. Sci. U.S.A., 62, 1159 (1969).
6. P. M. Greene, M. Betlach, H. M. Goodman, et al., Methods Mol. Biol., 7, 87 (1974).
7. P. Guerry, D. J. Le Blanc, and S. Falkow, J. Bacteriol., 116, 1064 (1933).
8. G. Ihler and Y. Kawai, J. Mol. Biol., 61, 311 (1971).