ABSTRACT

A series of closed circular (I) plasmid DNAs has been derived from drug resistance factor R12, and the nicked circular (II) and linear (III) derivatives of these molecules prepared by irradiation in the presence of ethidium bromide and by treatment with restriction enzyme EcoRI, respectively. These DNAs encompass the molecular weight range 3.6 to 61 megadaltons. The base compositions range from 45% to 51% (GC) as estimated by buoyant density determinations. The smaller plasmids are significantly less supercoiled (9-10%) than are the larger (12-13%). The gel electrophoretic behavior of the three DNA structural forms was determined as a function of molecular weight in agarose gels of concentrations ranging from 0.7% to 1.6% and at electrophoresis salt concentrations from 0.02 M to 0.08 M sodium acetate. The mobilities of DNAs I and III undergo a reversal relative to each other at a molecular weight which decreases with increasing agarose gel concentration. The molecular weight at which DNA II fails to enter a gel depends upon the ionic strength during electrophoresis but not upon the gel concentration.

INTRODUCTION

We have previously reported the isolation, by TC* selection, of a series of plasmid DNAs derived from the drug resistance factor R12 in E. coli K-12 (1). These plasmids are covalently closed duplex circular molecules and range in molecular weight from 3.6 to 61 megadaltons. The selection procedure is sufficiently general so that plasmid DNAs of essentially any size within these limits may be obtained. The plasmids were shown to be homologous with R12 and, in general, plasmid DNAs of increasing size represent direct extensions of those of the next lower molecular weight. An exception is plasmid pSM1, the smallest member of the series, which contains an internal deletion.

Heteroduplex experiments among several of these plasmid DNAs, the results of which will be reported in detail elsewhere (2), strongly support an IS1-mediated intramolecular recombination mechanism for plasmid formation. These plasmids are incompatible with the original R12, hence rapidly segregate and form stable systems in E. coli K-12. The yields
of DNA are large, approximately 1.0 mg DNA per liter of lysate. The presence of antibiotics, such as CM, is unnecessary to attain this amount of DNA (corresponding to approximately 100 copies per bacterium in the case of plasmid pSM1). The plasmid DNA is easy to isolate from stationary phase cells and the yield of DNA is comparable to that obtained from bacteriophage PM-2 (3), for which the isolation procedure is much more complex (4) and for which the amount of DNA per liter tends to be highly variable.

This series of molecules is therefore extremely useful for physical and chemical studies of the properties of closed circular DNAs, as well as of the nicked and linear molecules which can be produced from them. In the present communication we document many of the physical characteristics of these plasmid DNAs, including the molecular weights, buoyant densities, and superhelix densities. We also present the agarose gel screening procedure used to obtain the pSM series of plasmids.

In the process of performing agarose gel electrophoresis with the DNA molecules corresponding to these closely related plasmids, we noticed that for the larger plasmids the ccc DNA migrates more slowly than the linear DNA of the same length. For smaller plasmids, on the other hand, the ccc DNA migrates faster than the derived linear DNA. We also observed that the crossover point, at which the linear DNA migrates at the same rate as the ccc DNA of the same size, depends upon the experimental conditions. A systematic study was undertaken to examine the effect of the percentage agarose on the electrophoretic behavior of the plasmids and of their nicked and linear derivatives. The effects of varying the composition of the electrophoresis buffer or the voltage gradient were also examined. We found that all these factors can affect the relative rates of migration of the various DNA species. The information so obtained is generally useful in the identification of DNA components in gels and in the selection of optimal conditions for the electrophoresis of DNAs over a wide range of size and of various structural types.

MATERIALS AND METHODS

Bacterial strains. The E. coli strains containing the various plasmids derived from R factor R12 were described previously (1). E. coli strain 904, containing the colicinogenic factor Col El (5), and strain W1485, a prototrophic strain containing the F factor (6), were obtained from Dr. E. Ohtsubo. P. mirabilis strains Pml5 (7) and Pml5/NR1 (8) were gifts from Dr. R. Rownd.
Materials. Electrophoresis grade agarose, Tris and EDTA were obtained from the Sigma Chemical Co., St. Louis, Mo. SDS, BDH "specially pure" grade, was obtained from Gallard Schlesinger, Carle Place, N. Y. Optical grade CsCl was purchased from the Harshaw Chemical Co., Solon, Ohio; and EtBr was supplied by Calbiochem, Los Angeles, Ca. All other chemicals were of reagent grade.

Agarose gel electrophoresis. All gel electrophoresis experiments were performed in the following standard (E) buffer, except as otherwise noted: 0.04 M Tris-acetate, 0.02 M sodium acetate, and 2 mM EDTA, pH 8.1 made from stock solutions 1 M tris-acetate pH 8.3, 1 M sodium acetate and 0.5 M sodium EDTA pH 8.13. Gels for the plasmid screening were made of 1% agarose (w/v) prepared in E buffer. The agarose solution was solubilized by partially immersing the bottle containing it in boiling water for 20-30 min, with intermittent mixing until a homogeneous solution was obtained. Failure to properly mix at this stage results in the formation of artifacts in DNA band shape during slab gel electrophoresis. This solution was then pipetted into 14 x 0.8 (o.d.) cm sealed glass tubes, partially constricted at the bottom. After 20-30 min the gels were ejected from the tube with a syringe and cut to 10 cm with a razor blade. The gels were placed in a Buchler electrophoresis apparatus and E buffer placed in the chambers. Sample application is described in the next section. The slab gels for multiple samples were prepared as described previously (1).

Screening of single colonies for plasmids. Single colonies of bacteria were inoculated into 5 ml of Penassay broth (Antibiotic Medium 3, Difco) in 18 x 150 mm culture tubes and grown overnight at 37° with vigorous shaking so that they had been in stationary phase for 10-12 hr when harvested, thus providing an opportunity for plasmids to continue and complete replication. Then 0.25 ml of cells were spun down at 8,000 rpm, 0°C for 10 min in a Sorvall SS34 rotor. The cells were resuspended by vortexing at room temperature in 1 ml of TE buffer (0.01 M tris-HCl, 0.001 M EDTA pH 8.6) and again spun down. The cells were resuspended in 0.25 ml TE buffer and 0.020 ml of 25% (w/v) SDS added, following which the tube was gently tilted to mix and placed at 63°C, 10 min, for lysis to occur. An aliquot of 30-40 µl of lysed bacteria was mixed on a small square of parafilm with 10 µl of 50% sucrose, 0.02% bromphenol blue and the resulting solution gently stirred and layered using a 0.1 ml pipet and a propipet with minimal dead space through the E buffer onto the
surface of the gel. The samples were run at 100 V, 5 mA/gel at 25°C for 2 hr. The gels were then removed from the tubes and stained in E buffer containing 0.5 μg/ml EtdBr. The DNA bands were visualized with either long wave or short wave ultraviolet light (Ultraviolet Products, San Gabriel, Ca.) and photographed with Polaroid type P/N film through a 23A orange filter (Tiffen Photar). Short wave illumination allows the detection of less than 0.05 μg of DNA (9).

Buoyant density determinations. Buoyant densities of all plasmids were determined in the analytical ultracentrifuge, employing bacteriophage 2C as a marker. This latter DNA, a generous gift of Dr. Jacques Pène, has a buoyant density of 1.7378 g/ml as determined in our laboratory, taking E. coli DNA to have a standard buoyant density (θ) of 1.7035 g/ml (10). The buoyant density gradient was used in all calculations (11), employing the pressure coefficient Ψ = 23.3 x 10^-12 cm sec^2 g^-1 (12).

RESULTS
Assay for plasmids on agarose gels. Single colonies of bacteria were lysed with SDS and treated as described above, following which samples were layered onto 1% (w/v) agarose gels prepared in E buffer. Figure 1 shows representative gels, following EtdBr staining, of strains containing (a-d, f, h) or not containing (e, g) plasmids. The band designated C, present for all samples, probably consists of large, fragmented chromosomal DNA. The bright bands towards the bottom of the gels are RNA. These can be eliminated either by the 63°C treatment prior to cell lysis (Fig. 1 a'-e'), which may allow ribonucleases to remain active while inactivating other heat-sensitive enzymes, or by a prior treatment with a mixture of T₁ ribonuclease and pancreatic ribonuclease A during lysis (data not shown). Three main RNA bands are present for P. mirabilis strains, two for E. coli strains. The other bands present in gels a-d, f, and h correspond to plasmid DNA. In all of these except gel a the bands correspond to ccc DNA, no nicked circular DNA being detectable. If the lysis step is performed at 37°C a band of nicked circular DNA is also observed, particularly if incubation is continued for longer than 10 min. In addition, lysis is often incomplete at this lower temperature. For the Col El-containing cells (Fig. 1a) the result is different. The predominant plasmid band just below the C band corresponds to nicked circular Col El DNA, since Col El DNA is largely present as a protein–DNA relaxation complex (13). Subjecting this complex to SDS treatment results in the introduction of a strand-specific nick. A 65° heat treatment prior to SDS treatment inactivates
Figure 1. Analysis by agarose gel electrophoresis of whole cell lysates prepared from cultures of single colonies of bacteria. Electrophoresis on 1% cylinder gels was for 2 hr at 25° and 10 V/cm in E buffer and the bands were visualized by staining with EtBr (0.5 μg/ml in E buffer). The cells employed are: a, E. coli 904/Col El; b, E. coli W677/pSM2; c, W677/pSM1; d, E. coli W1485/F; e, E. coli CSH2; f, CSH2/R12; g, P. mirabilis Pml5; h, Pml5/NR1. Gels a'-e' contain the same strains as a-e, except that the cells were heated at 63° for 15 min. prior to the addition of SDS. C marks the position of large linear DNA.

the relaxation complex, hence the closed circular DNA chain remains intact (13). For that reason the 63°C incubation was performed prior to cell lysis (Fig. 1a'-e'). More of the Col El DNA is present as ccc molecules in this case, but a diminution in the amount of DNA seen for the larger plasmid bands probably indicates the occurrence of nicking.

This direct lysis technique permits the rapid screening of a large number of bacterial colonies for plasmid DNAs, whether or not they are associated with a protein of the relaxation complex type. By selection of the appropriate gel conditions, as described below, the method is applicable to plasmids over a wide range of molecular weights.
Superhelix densities of the pSM plasmid DNAs. Superhelix densities were determined in buoyant CsCl/EtdBr (14, 15), using PM-2 DNAs I/II as a reference pair and correcting for the superhelix density shift of this marker relative to SV40 DNA I/II (16). Approximately 1 µg of DNA per nicked and ccc DNA band was added to solutions of initial density 1.56 g/ml containing 328 µg/ml EtdBr. The cognate nicked DNAs were produced by mixing a solution containing 5 µg EtdBr (1 µl) with the appropriate closed DNA sample (100 µl) and incubating in a sealed melting point capillary against a white background with a tungsten lamp (Hamilton Industries, Chicago, Il.; Model HC-18, No. 93 bulb). A sample of 2 µg of pSM1 DNA was approximately 50% nicked after a 10 min exposure at a height of 8 mm and a "low" lamp setting. Exposure times for the other plasmids were inversely proportional to the ratio of the DNA length to that of pSM1. The extent of nicking was determined by electrophoresis of the samples on agarose slab gels, following by staining with EtdBr and microdensitometry. The samples were centrifuged at 40,000 rpm at 20°C for a minimum of 36 hr in a Beckman SW50.1 rotor.

The superhelix densities of several of the pSM plasmids are listed in Table 1, along with the buoyant densities determined analytically as described above. All superhelix densities are referred to PM-2 DNA, for which $\sigma_0 = -0.126 \pm 0.006$ (15). The superscript 28 denotes the value in degrees of the EtdBr unwinding angle used in the calculation of $\sigma_0$ from the measured critical dye binding ratio, $v_c$ moles EtdBr per mole nucleotide, at which the closed DNA is relaxed. Each photograph was measured ten times by three different methods: microdensitometry, a Nikon microcomparator, and directly with a stainless steel ruler and a magnifying ocular. The errors stated represent standard deviations and include the uncertainty in the PM-2 DNA reference value. The Table also includes the buoyant densities, determined as described above, and the calculated base compositions (17, 18). The molecular weight measurements, the results of which are listed here, are reported in detail elsewhere (1,2).

The effect of the agarose concentration upon the relative electrophoretic mobilities of the pSM plasmid DNAs. A typical 1% agarose slab gel, 10 cm in length, which was subjected to a voltage gradient of 8 V/cm for 2.5 hr is shown in Figure 2. The distances the various bands migrated from the origin (sample slot) at the top of the gel were measured and normalized relative to the migration position of linear R12 DNA (channel k, the band farthest into the gel). The identification of the bands corresponding to
Table 1
Physical Properties of pSM Plasmid DNAs

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Length, k Bases</th>
<th>Mol. Wt., Megadaltons</th>
<th>θ g/ml</th>
<th>% G+C</th>
<th>νc (X 100)</th>
<th>28σc (X 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSM1</td>
<td>5.42*</td>
<td>3.59*</td>
<td>1.7016</td>
<td>48.2</td>
<td>6.0 ± 0.5</td>
<td>9.3 ± 0.8</td>
</tr>
<tr>
<td>pSM15</td>
<td>6.26</td>
<td>4.14</td>
<td>1.6984</td>
<td>45.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pSM2</td>
<td>6.64*</td>
<td>4.40*</td>
<td>1.6989</td>
<td>45.7</td>
<td>6.8 ± 0.5</td>
<td>10.6 ± 0.8</td>
</tr>
<tr>
<td>pSM3</td>
<td>7.30*</td>
<td>4.89*</td>
<td>1.6990</td>
<td>45.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pSM4</td>
<td>8.29</td>
<td>5.49</td>
<td>1.6990</td>
<td>45.8</td>
<td>6.3 ± 0.5</td>
<td>9.8 ± 0.8</td>
</tr>
<tr>
<td>pSM5</td>
<td>17.7*</td>
<td>11.4*</td>
<td>1.7033</td>
<td>49.8</td>
<td>8.0 ± 0.5</td>
<td>12.4 ± 0.5</td>
</tr>
<tr>
<td>pSM6</td>
<td>23.6</td>
<td>15.6</td>
<td>1.7015</td>
<td>48.1</td>
<td>7.7 ± 0.2</td>
<td>12.0 ± 0.3</td>
</tr>
<tr>
<td>pSM7</td>
<td>38.5</td>
<td>25.5</td>
<td>1.7020</td>
<td>48.6</td>
<td>7.7 ± 0.3</td>
<td>12.0 ± 0.5</td>
</tr>
<tr>
<td>pSM8</td>
<td>56.6</td>
<td>37.5</td>
<td>1.7017</td>
<td>48.3</td>
<td>7.7 ± 0.3</td>
<td>12.0 ± 0.5</td>
</tr>
<tr>
<td>pSM9</td>
<td>83.0</td>
<td>55.0</td>
<td>1.7040</td>
<td>50.5</td>
<td>7.8 ± 0.3</td>
<td>12.1 ± 0.5</td>
</tr>
</tbody>
</table>

*The molecular weight of these plasmids was measured by electron microscopy, in collaboration with Dr. E. Ohtsubo (2). The molecular weights of the remaining species were determined by agarose gel electrophoresis following cleavage with restriction enzyme EcoRI (1).
nicked molecules migrate into the gel as a discrete band. For the 23.6 kb pSM6, channel h, the nicked band is spread from the diffuse visible front back toward the origin. The filled circles in Figure 3 represent the occurrence of this phenomenon.

Two conclusions can be drawn from Figure 3. First, the molecular weight (crossover point) at which linear and ccc DNAs comigrate increases as the percentage agarose is decreased. This is shown in the lower curve of Figure 4, in which the logarithm of the crossover molecular weight is plotted as a function of the gel concentration. This former quantity is
linearly related to the agarose percentage below about 1.2%. At yet higher gel concentrations the relative mobilities of DNAs I and III become insensitive to molecular weight. The upper curve in Figure 4 represents the size of ccc DNA which would migrate at the same rate as linear R12 DNA. This is an approximation of the plasmid size class which is obscured by chromosomal DNA in the bacterial whole cell lysis method discussed above if the gels were subjected to 8 V/cm for 2.5 hr. Smaller plasmids would migrate faster than the chromosomal DNAs, larger plasmids slower. The second conclusion which may be drawn from Figure 3 is that, for gels run at 8 V/cm in standard E buffer, the size cutoff for the exclusion of nicked circular DNA molecules from the gel is the same for all agarose percentages.

We have assessed the reproducibility of the data obtained with the gel experiments by examining the same DNAs on duplicate gels and with various gel holders and power supplies. The absolute mobilities (see below) for pSM1 ccc DNA vary from about 6-10 cm$^2$/volt/sec with changing conditions. When normalized relative to the limiting (constant) mobility of large linear DNAs present on the same slab gel, however, the relative mobilities are remarkably reproducible. The variation in absolute mobilities may be ascribed to lack of adequate temperature control or to some irreproducibility in the preparation of the gels.

The effect of the voltage gradient. Figure 5 shows data from two 1% agarose 10 cm slab gels. Electrophoresis was conducted for 2.5 hr at 80 V or for 5.0 hr at 40 V. The absolute mobility, $U$, is plotted as a function of plasmid molecular weight in each case. The distance migrated was determined in cm for each band and divided by the voltage gradient (V/cm) and time (sec). The mobilities were significantly lower at the lower voltage. The crossover point for ccc and linear molecules was shifted toward higher molecular weights at the lower voltage and the cutoff for nicked circular molecules was slightly higher at the lower voltage; nicked pSM6 enters the gel forming a discrete band at the lower voltage.

The effect of varying the sodium acetate concentration. Electrophoresis buffers containing either 0.05 M or 0.08 M sodium acetate were employed and 10 cm slab gels were run in parallel at 40 V. Data from these gels are plotted in Figure 6. The standard E buffer (0.02 M sodium acetate) gel, which was run separately, is shown at the right of Figure 5. At lower ionic strengths higher voltages may be employed without producing the characteristic gel deformations which probably result from coulombic heating at higher solution conductivities.
Several effects of varying the sodium acetate concentration can be seen. The first is that the relative mobilities of the various DNA species are differently altered. In particular, at higher sodium acetate concentrations the crossover point for ccc and linear molecules is shifted toward higher molecular weights. Second, the exclusion size cutoff for nicked circular molecules is shifted toward higher molecular weights at the lower voltage gradient. Third, the relationship for ccc DNA between \( U \) and the log of the molecular weight is approximately linear over a larger
Figure 3. The relative mobilities of a series of pSM plasmid DNAs in gels of various concentrations of agarose (indicated in each panel). All experiments were conducted as in Figure 2, and the DNA molecular weights are listed in Table 1. The symbols represent DNA I (□), II (○) and III (▲). The filled circle indicates that the nicked DNA was extremely broad (see text). The quantity $U_{rel}$ is the ratio of the distance migrated by each band to that measured for linear R12 DNA on the same gel.

range (up to 10 or 12 million daltons) at 4 V/cm than at 8 V/cm. It is also clear that the plot of log (M) versus mobility for linear DNA is nonlinear under these conditions for the DNA size range of this study. Finally, the bands seemed equally sharp at all sodium acetate concentrations.

DISCUSSION

Significance of superhelix density variability among the plasmids. The pSM plasmid DNAs listed in Table 1 may be divided into two classes on the basis of superhelix density. Plasmids pSM1, 2, and 4 are approximately
Figure 4. Analysis of electrophoretic behavior of DNAs I and III as a function of molecular weight and percent agarose (data taken from Figure 3). The open circles represent the points of intersection of the $U_{rel}$ vs $M$ curves for these DNAs. The open squares indicate the DNA I molecular weight which would produce comigration with R12 (taken as representative of a very large linear DNA).

Figure 5. The electrophoretic mobility (cm$^2$ V$^{-1}$ sec$^{-1}$) plotted as a function of plasmid molecular weight for DNAs I, II and III and for two different voltage gradients. For symbol definitions see the legend to Figure 3.

9.5-10.5% supercoiled, whereas plasmids pSM5-9 are 12.0-12.5% supercoiled. In order to confirm the reality of this distinction, the Student t test was applied to ascertain the significance of the difference between several of the mean values both within and between the classes. The difference in means between the most extreme cases, that of pSM1 and pSM5, is significant at the 99.999% level. The least extreme case between classes, that of pSM2 and pSN7 or 8, involves a difference in means significant at the 99.99% level. Within the smaller size class, the difference between pSM1 and pSM4 is significant only at the 62% level; that between pSM1 and pSM2 and pSM4 at the 85% level; and that between pSM1 and pSM2 at the 95% level. Variations among the means for the larger plasmids are clearly
Figure 6. The electrophoretic mobility ($cm^2 V^{-1} sec^{-1}$) plotted as a function of plasmid molecular weight for DNAs I, II and III and for sodium acetate concentrations of 0.05 M and 0.08 M. All other buffer components and concentrations were the same as in standard E buffer. For symbol definitions see the legend to Figure 3.

statistically insignificant. These considerations lend strong support to the conclusion that a real variation in superhelix density occurs among the members of this homologous series of plasmids, even though they were grown under identical conditions in E. coli K-12 as host strain.

It is striking that the members of the low superhelix density class are also the plasmids of low molecular weight (< 8.3 kb), whereas those of the high superhelix density class represent the larger DNAs (> 17 kb). It is possible that a causal relationship exists between DNA size and superhelix density, since the smaller molecules are certainly less flexible than the larger. A distinction of this type is unlikely to be important, however, because even the smallest of these plasmids, pSM1, contains about 28 persistence lengths of 650 Å (19). The range in number of persistence lengths in the lower class is 28-43 and in the larger class 92-430. Although molecules of the larger class are clearly more flexible, both classes appear to be well above the size range in which stiffness is expected to be an important factor in limiting the attainable superhelix densities. It should also be stated that in no other system has a correlation between molecular weight and superhelix density been observed. The E. coli 15 plasmid, for example, has $\sigma_o = 0.089$ (20) and is 1.45 megadaltons (21); chick liver mitochondrial DNA has $\sigma_o = 0.061$ (22) and is 10.7 megadaltons (23); and λCI857 from sensitive cells has $\sigma_o = 0.063$ (20) and is 32.8 megadaltons (20). Other such examples have been tabulated (24).
As an alternative interpretation, it is perhaps noteworthy that all members of the high superhelix density class are of relatively high G+C content whereas the opposite is true of the low class, with the exception of pSM1. It has been reported (25) that the duplex rotation angle of very AT-rich DNA is reduced in solution from 33.4° (determined with calf thymus DNA) to 28.6°. Since duplex unwinding is associated with a reduction in superhelix density, it is possible that the origins of the class differences reported here lie in differences in base composition.

The overall base composition is clearly too crude a discriminator to permit quantitative predictions, however, and it is also probable that local base sequence differences play a role. The case of pSM1 is especially interesting in this respect. This plasmid differs from pSM2 by a deletion of 1.22 kb (20%) immediately following the replication origin (1, 2). By comparison with the denaturation map of the parental NR1 DNA (26), this deleted sequence corresponds to a very high AT stretch. This localized deletion accounts for the elevated buoyant density of pSM1 compared to pSM2. The remainder of the base sequences are identical, however, thus accounting for the similarity in superhelix densities. Plasmid pSM4 extends to the terminus of a region relatively high in AT and includes all of pSM2. Plasmids pSM5 and larger are sufficiently long so that the average base compositions are similar and any especially high AT regions represent a small fraction of the molecule. A more detailed interpretation awaits the availability of detailed sequence information (H. Ohtsubo, in preparation).

As a final comment, an alternative explanation based upon unwinding by a specific protein associated with the replication origin may be ruled out. Since these plasmids all possess a single origin, the generation of supercoiling by binding of an unwinding protein, closure, and subsequent removal of the protein would lead to the opposite dependence of $\sigma$ upon $M$ to that observed here.

Electrophoresis behavior of closed and linear DNAs. The data presented in Figures 3 and 4 reveal that DNAs I and III undergo a reversal of relative mobilities at a critical molecular weight, the value of which decreases substantially as the % agarose is increased. Failure to take this effect into account can clearly lead to the incorrect identification of components. A related phenomenon, also shown in Figure 3, is that the relative mobilities of both components become insensitive to molecular weight at progressively lower values as the gel concentration is increased. This loss of molecular
weight dependence occurs at much lower gel concentrations for III than for I. In the former case the plateau in U begins at about 15 megadaltons at 0.7% agarose but at only 5 megadaltons at 1.6% agarose. In the latter case the plateau does not become apparent until the gel concentration has reached 1.2%, and at higher concentrations the flattening appears to occur at about 12 megadaltons regardless of further gel concentration increases. In no case was either component excluded from a gel regardless of the agarose concentration.

A rational explanation of the above behavior must be based upon both the structures of the DNAs and upon that of the gel matrix. Arnott and co-workers (27) have shown that agarose gels consist of associated double helices which are probably organized into a network of rods containing large voids through which macromolecules may pass. If the motion of III is essentially via "snaking," the mobility of this form should diminish with molecular weight until the limit is reached beyond which further distal encounters with the matrix provide no additional contribution to retardation. As the gel concentration increases so does the encounter frequency, hence the molecular weight insensitivity limit is reduced. DNA I, having no free ends, cannot move by "snaking." At low gel concentrations the reduced radius of gyration of I relative to III is probably responsible for its relatively greater mobility at low molecular weights, the implication being that the DNA I radius of gyration is here comparable to the matrix pore size. As the agarose concentration increases the pore size decreases, resulting in increased DNA/gel encounter. Eventually, further increases in the size of the DNA I species provides no significant increase in encounter frequency hence the mobility becomes molecular weight-insensitive. The physical significance of the crossover point is difficult to explain, the structures of the two molecules being so different. Presumably this point represents a balance between the relative advantages of "snaking" and of a reduced radius of gyration. As shown in Figures 5 and 6, however, this interpretation is clearly incomplete.

Electrophoretic behavior of nicked circular DNAs. The analyses presented in Figure 3 reveal that a nicked circular DNA invariably migrates more slowly than either the corresponding linear or closed circular species. The mobility of DNA II decreases with M over the very low molecular weight range, becoming independent of size above about 5-6 megadaltons. This DNA completely fails to enter all gels, regardless of percentage agarose, at molecular weights in excess of 14-16 megadaltons. This DNA,
also having no ends, cannot move by the "snaking" mechanism. In the standard E buffer, 0.02 M NaOAc, these molecules are expected to be quite stiff (and therefore extended) due to charge repulsions (28). As demonstrated in Figure 6, the molecular weight exclusion limit for this DNA may be shifted to much higher values by increasing the salt concentration in the electrophoresis buffer. (The behavior of DNAs I and III is little affected by this procedure.) We attribute the salt effect to a decrease in stiffness of DNA II, thus allowing it to enter the gel at an increased M. The mechanism of the exclusion itself is not known. It is possibly associated with a "hoop and stick" effect, in which nicked circular DNA becomes looped around an agarose rod. In such an event the only mechanism for DNA II to continue migration would be by reverse diffusion against the field and off the end of the rod. Such a reverse diffusion would become increasingly difficult at higher fields, possibly explaining the slightly higher exclusion limit at 4 V/cm compared to that at 8 V/cm (Figure 5).

Whole cell lysis method. The whole cell lysis method which we present here is a remarkably easy way to screen large numbers of colonies of bacteria for plasmids as well as to estimate their size, since no prior DNA purification is necessary. Visual results can be obtained in approximately three hours starting with 0.25 ml of fresh stationary phase bacteria from a liquid culture. Other typical gels obtained using this procedure have been published previously (1) along with a description of the origin of the R12-derived plasmids. If duplicate samples are loaded on both a 0.7% and 1% agarose gel, no plasmids should be obscured by comigration with the chromosomal DNA. Two similar independently derived methods utilizing SDS and 60-70°C lysis have recently been published (29, 30). These methods differ from ours in that whole colonies were directly lysed and that electrophoresis buffers and thus mobilities differed. Electrophoresis in both cases was at much lower voltages for longer times. The method of Barnes (29) seems suitable for small plasmids only, probably due to the high ionic strength of the electrophoresis buffer. Under these conditions large plasmids would barely enter the gel. The method of Telford et al. (30) appears to result in considerable nicking, possibly due to their 30 min lysis procedure. In our hands samples are more readily applied to cylinder than to slab gels, and substantially enhanced band smearing often occurs in the latter case. Meyers et al. (31) have also published a procedure for plasmid detection on gels, but this requires
much more time and labor than the other two methods cited. Undoubtedly single colonies could be suspended in buffer and lysed under our conditions, but then the advantage of the relatively constant amount of cells of stationary culture for optimal lysis conditions would be lost. Considerably poorer results are obtained if either too many or too few cells are lysed or layered onto a gel.

Meyers et al. (31) stated that their procedure, which also utilizes SDS for lysis, was effective on many different types of clinical isolates. It seems likely that the whole cell lysis method presented here will be effective for any strain which can be disrupted by SDS. We have not attempted to assess the utility of the method for plasmids larger than those employed in this study or for plasmids present in fewer copies per cell. The method of Meyers et al. (31), in which chromosomal DNA is eliminated prior to electrophoresis, might be advantageous in some cases.

ACKNOWLEDGMENTS

We are grateful for the valuable assistance and advice of Drs. E. and H. Ohtsubo, and we express our thanks to Mrs. Lenora Pong for her indispensable assistance in the preparation of the manuscript. This research was supported by Public Health Service grant GM-21176 from the National Institute of General Medical Sciences. S. M. was a postdoctoral trainee of the Public Health Service, with Training Grants CA-05243 and CA-09121 from the National Cancer Institute.

REFERENCES

\* This paper is dedicated to the memory of Professor Jerome Vinograd, our dear friend and teacher.

\*Abbreviations: TC, tetracycline-HCl; CM, chloramphenicol; DNA I, covalently closed circular (ccc) DNA; DNA II, nicked circular DNA; DNA III, linear DNA; Tris, tris(hydroxymethyl)aminomethane; EDTA, disodium salt of ethylenediaminetetraacetic acid; EtdBr, ethidium bromide. The superhelix density, \( \sigma \), is defined as the number of superhelical turns per ten base pairs.

5. This strain was originally obtained from the laboratory of Dr. M. Oishi.