Nucleic Acids Research

Pulsed-field gel electrophoresis of circular DNA

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ABSTRACT

Mobility of supercoiled (form I) and nicked circular (form II) plasmid DNAs was determined on two major forms of pulsed-field electrophoresis, CHEF and OFAGE. Plasmids with molecular lengths ranging from 2.30 to 17.8 kilobase pairs (kb) were used with *Saccharomyces cerevisiae* chromosomes as standards. Agarose gel concentrations were varied from 0.3 to 2.0 percent, with higher percentage gels resolving forms I and II of smaller plasmids. The pulsing range of 3.7 to 240 seconds resulted in quite variable Saccharomyces chromosomal mobilities on both 0.5 and 1.0 percent gels, while both form I and II of all plasmid DNAs showed relatively constant mobilities with some increase at the shortest pulse times. Using a 30 second pulse time and gel concentrations of at least 1.0 percent, the usual order of migration of plasmid forms for a 17.8kb plasmid could be changed. We interpret this result as an increase in the relative mobility of form II in our pulsed-field gel conditions.

INTRODUCTION

Pulsed-field gel electrophoresis (PFGE) has revolutionized the genetic mapping of fungal and protozoan genomes as their intact chromosomal DNA molecules are readily resolved on gels (1–4). These cells usually contain both linear and circular forms of DNA. In the earliest experiments that used PFGE, small circular DNA molecules such as the 6kb (2μ) plasmid of *Saccharomyces cerevisiae* were found to migrate within the same range as the linear chromosomes with molecular lengths of hundreds of kilobases (1). In several systems, new plasmids introduced by DNA transformation are replicated as concatameric forms that are not readily separated from uncleaved chromosomal DNA on conventional gels. As physical mapping methods are rapidly displacing genetic methods for chromosome assignment, it is essential to understand the behavior of circular DNA species on pulsed-field gels.

On conventional (single field) agarose gels, supercoiled plasmid DNA (form I) will generally show higher mobility than the nicked circle (form II). The relative mobility of

residual amounts of linear (form III) DNA of a plasmid will vary with the electrophoresis conditions when compared to the circular plasmid forms. In addition, on a single field gel at high voltages, form II may not enter the gel at all (5, 6).

Under PFGE conditions, several differences are observed. First, form III of a plasmid generally has a high mobility relative to the other forms. Also, pulsed field gels are usually run in the absence of ethidium bromide and then stained after completion of the separation. The compression of species with different numbers of superhelical turns into a single band (as seen on a conventional gel run with the dye) need not occur (7). Several studies have found relatively constant mobilities for highly supercoiled plasmids in the range of pulse times used for chromosome separation (7–8).

We have systematically investigated the behavior of plasmids on two of the major forms of PFGE, OFAGE (Orthogonal Field Alternation Gel Electrophoresis, ref. 2) and CHEF (Contour Clamped Homogeneous Electrophoresis, ref. 4). We have concentrated on the CHEF system as the uniform fields are easier to interpret and larger numbers of samples can be run on the same gel. The two variables we have examined are pulse time and agarose concentration. Pulse time is the variable usually employed when separations in different size ranges are required. In our study, we have examined short pulse times in addition to the ones used for separation of large linear DNA. We have studied the effect of agarose concentration on the mobility of circular DNA, which is of limited use in improving the separation of linear DNA. Also, in high percentage agarose gels, we found deviations from the usual order of mobility of plasmid forms. This information should be of value in designing experiments for the electrophoresis of large, circular DNA species.

MATERIALS AND METHODS
Agarose Gels

All single field gels used the Tris-acetate-EDTA buffer system and submerged gels (9). Pulsed field gels used 50mM Tris-Borate, 2.5mM Na_3EDTA as running buffer. The buffer was circulated and cooled to 11°C. Buffer was replaced regularly as a weak correlation was seen between an increase in conductance and an increase in DNA mobility. The measurement of DNA migration was taken from the trailing margin of the band.
Electrophoresis Equipment

Orthogonal Field Alternation Gel Electrophoresis — The OFAGE device is essentially identical to that originally described (2). Gels were run at 10 V/cm which produced a current of about 170mA. Approximation of the potential gradient across the gel was

performed by dividing the supply voltage by the perpendicular distance separating an active pair of electrodes.

Contour Clamped Homogeneous Electrophoresis — The CHEF device is as described by Chu *et al.* (4). The same electrophoresis buffer was used as in the OFAGE experiments and the field strength calculated in the same fashion. Gels were run at 5.82 V/cm and a corresponding 170-185mA (except as noted).

Size standards

Saccharomyces cerevisiae chromosomes were prepared from our strain #327 as described by Schwartz and Cantor (1). The smallest chromosome was used as a size standard and is estimated to be 260kb (3).

Plasmid DNA

The plasmids used were pBR322 monomer and dimer, 4.36kb and 8.72kb; 86 (our stock number), 2.3kb; YCp50, 8.05kb; 831 (again our number) monomer and dimer, 8.9kb and 17.8kb; and pMB9-Sc2601, 16kb. In cases where both monomer and dimer of the same plasmid were used, the plasmid species were resolved on a gel and transformed into a *recA* mutant prior to plasmid isolation. All the plasmids used in this study have in common a sequence in pBR322.

Plasmid DNA was prepared by CsCl ethidium bromide gradient centrifugation. During the subsequent purification process, a portion of form I DNA converted to form II DNA. To verify that minor species seen on gels were plasmid derived and not a result of contamination with linear chromosomal DNA, two representative gels (0.5% and 1% agarose, 30 second pulse time) were transferred to nitrocellulose and probed with labelled, highly purified pBR322 DNA. The 1% percent gel demonstrated the anomalous migration of form II DNA (see results). All bands labeled at an intensity roughly proportional to their appearance in the fluorescence pattern.

RESULTS

Variation in Pulse Time

Figure 1 shows the effects of variation in pulse time on migration of form I and form II plasimd DNAs in the CHEF system. In the range of pulse times examined (3.7 to 240 seconds), there was a relatively modest effect on plasmid mobility with all forms showing higher migration at the shorter pulse times. The relative increase for form I reached 25% with somewhat larger effects on form II.

In contrast, the largest *S. cerevisiae* chromosomes showed an almost four fold variation
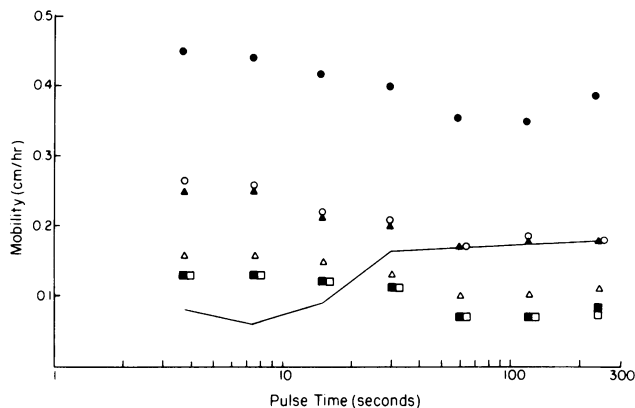
Figure 1. Mobility of plasmid DNA and *Saccharomyces cerevisiae* chromosomal DNA as a function of the logarithm of pulse time in the CHEF system. The gels were run at 1% agarose, 5.82 V/cm, 11°C. The symbols for form I plasmid DNA are: 16kb (■), 8.05kb (▲), 4.36kb (●). Identical open symbols are used for form II. The mobility of the smallest Saccharomyces chromosome, 260kb, is shown as a solid line.

in mobility. It is important to note that with the longer pulse times, which are the ones used for separations of yeast chromosomes, varying this parameter had little effect on the mobility of either form I or form II plasmid DNA.

Similar experiments were conducted on OFAGE using 1.0 and 0.5 percent agarose gels. Results were similar to the CHEF experiments shown in figure 1 with absolute mobilities of all DNA species being greater on the lower percentage gels (not shown).

Variation in Agarose Concentration

Figures 2 shows the effects of variation of agarose concentration on the mobility of supercoiled and nicked plasmid forms in CHEF electrophoresis. Agarose concentration was varied between 0.3 and 2.0 percent. The gels were run under the same voltage and temperature conditions as the previous experiment, with the pulse time being held constant at 30 seconds.

Two major effects were seen on the relative mobility of plasmid forms and chromosomal DNA. First, at the lowest concentrations tested, all of the plasmid species showed higher mobility than the smallest Saccharomyces chromosome while at the highest concentration examined, all but the 2.3kb plasmid forms ran with the yeast chromosomes.

Second, as agarose concentration was increased, good resolution of form I from form II was achieved for progressively smaller plasmids. At the same time, form I and form II of larger plasmids began to run together. For the largest plasmid tested, 17.8kb, the two
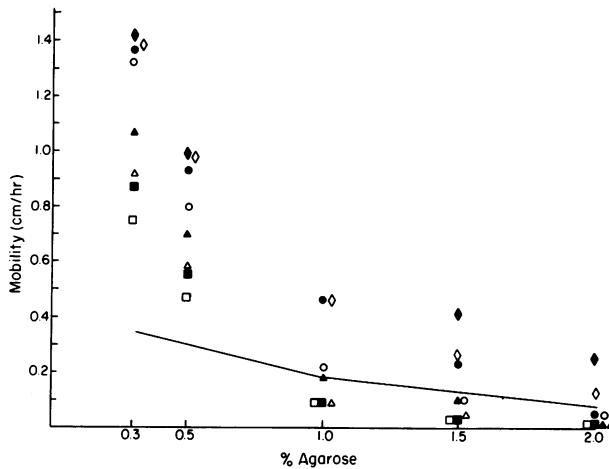
Figure 2. Mobilities of form I and form II plasmid DNA and *S. cerevisiae* chromosomal DNA in the CHEF system as a function of agarose concentration. The gels were run at 5.82 V/cm, 11°C, with a 30 second pulse time. The symbols for Plasmid DNA are: 16kb (■), 8.72kb (▲), 4.36kb (●), 2.30kb (♦). Filled symbols are for form I; open symbols are for form II. The mobility of the smallest Saccharomyces chromosome, 260kb, is shown as a solid line.

forms appeared to pass each other on gels of 1% agarose or higher. Also, form II DNA did not show smooth decrease in mobility with increasing plasmid size as was seen with form I. For these reasons, we verified the identity of the plasmid bands seen in the mixtures being electrophoresed.

Relative Mobility of Plasmid Forms

To determine the relative mobility of plasmid forms on a CHEF gel (1% agarose, 30 second pulse time), the 4.36 and 8.05kb plasmids were cleaved with *Eco*RI and closed with T4 DNA ligase at 0°C. As expected for larger plasmids, the linear form of the 8.05kb plasmid ran faster than either circular form in the purified plasmid DNA. The 4.36kb linear had a mobility between form I and form II. As was previously seen on OFAGE (7), plasmids with small numbers of superhelical turns produced in the ligation were resolved with mobilities between form I and form II (not shown).

Discrimination of form I versus form II was done by relying on the higher mobility of form I on conventional gels. The gel conditions for the single field gel were 0.5% agarose and 1.22 V/cm. Under these conditions, each plasmid preparation would give two well separated bands, with the majority isolated as form I. To verify the anomalous migration seen with the 17.8kb plasmid, forms I and II of the 16, 17.8 and 8.9kb plasmids were

cut from a single field gel and the gel slices transferred to the wells of a CHEF gel. The conditions for the second gel were as described for the previous experiment. When the purified plasmid forms were run on CHEF, the bands from the 16kb plasmid had essentially the same mobility. The trailing band of 17.8kb from the conventional gel ran ahead of the leading band on the pulsed-field gel, while the 8.9kb sample showed no change in the order of band migration.

As the bands showing anomalous migration had mobilities equal to or less than that of the smallest yeast chromosomes (on the same gels), they were not due to traces of linear DNA in the plasmid preparations. The plasmid showing this unusual behavior was a cloned dimer molecule. None of the bands in the dimer preparation comigrated on conventional gels with any of the species produced by our cloned monomer.

DISCUSSION

In comparison to yeast chromosomal DNA, plasmid forms I and II were affected minimally by pulsing the electric field in a CHEF gel. Forms I and II of all plasmids tested had greater mobility than all chromosome-sized linear DNAs at short pulse times. Higher agarose concentrations promoted the comigration of both forms of plasmid DNA with yeast chromosomal DNA. On CHEF gels of agarose concentrations of 1% or greater, we found changes in the order of migration of forms of a 17.8kb plasmid.

For yeast chromosomes, the effective separation conditions for the higher molecular length linear DNAs (580kb and greater) required agarose concentrations between 0.5 and 1.0 percent and a pulse time in the range of 1-5 minutes. Since form I and form II plasmid DNA mobility was relatively unaffected by changes in pulse times compared to the large linears, it was relatively simple to develop conditions to separate plasmids from the chromosomes as a group. For the sizes of linear and circular DNA tested here, this was seen with pulse times of 15 seconds or less. As agarose concentration was increased at a constant pulse time, large linear DNA began to migrate faster than the form I circular DNA. Form II mobility was inhibited at a lower agarose concentration than form I DNA of the same size.

Several factors may be combining to produce the unusual effects seen with the mobility of form II plasmid DNA. At high voltages and agarose concentrations, form II plasmid DNA may become trapped on the gel fibers. The role of the pulsing would be to free the DNA from the matrix by producing the equivalent of a backward diffusion. This effect is maximized in field inversion gel systems (6). At reduced voltages and increased pulse

time, we have observed an apparent increase in the relative mobility of form II DNA of the largest plasmids we have examined, 39 and 41kb (not shown). The lower voltage would facilitate backward diffusion for the higher molecular length plasmids by reducing their entrapment on the gel matrix. Form II DNA appears to behave in a manner similar to that of linear DNA in that different pulse time/voltage combinations have their largest effect on DNAs of different sizes. However, form II plasmid DNA behaves differently in PFGE from linear DNA in that the pulsed-field appears to be increasing the mobility of the DNA rather than retarding it.

One method to examine large circular DNAs on pulsed-field gels uses γ-irradiation to produce double-strand breaks so the molecules can be electrophoresed as linears (9). Our findings suggest that a combination of short pulse times, reduced agarose concentration, and lower field strengths can be used to promote the mobility of circular DNA on CHEF gels.

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