Reorientation Time of DNA Molecules in Pulsed-Field Gel Electrophoresis

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ABSTRACT: The precise determination of the influence of an electric field strength E on the resolution of DNA molecules during a pulsed-field gel electrophoresis shows that the maximal molecular size N_{max} of still resolved DNA molecules is described by the equation $N_{max} = k\tau E^{\alpha}$, where k is a coefficient, τ is a pulse time, and α is an exponent (calculated as $\approx 3/2$). We assume that the best estimation of the reorientation time τ_R for each DNA fragment is such a pulse time in which this DNA molecule is the largest separated one.

The work of Schwartz and Cantor (1984), who described DNA molecule electrophoresis in a pulsed electric field, has opened a new perspective for 10^4–10^7 base pair DNA fragment separation. A theoretical description of the DNA molecules' motion during electrophoresis in agarose gel is presented in the reptation theory reported by Lumpkin et al. (1985), Slater and Noolandi (1986), Slater et al. (1987). According to this theory the molecules move, under the influence of the electric field, with one of their ends ahead. An electric field stretches and aligns the molecules during their motion through the polysaccharide network. This simple "snakelike" or "worm-like" motion is probably correct only for short fragments (up to several thousands of base pairs). During periodical changes of the electric field direction the molecules undergo the process of reorientation before they start moving according to the field direction. The reorientation time τ_R[1] (Mathew et al., 1988b), also called the stretching time τ_{str} (Slater & Noolandi, 1986), depends on the size of a molecule. Smaller molecules need less time for the reorientation, and during one pulse they will cover a longer distance than larger molecules.

Theoretical considerations on the reorientation time in the reptation theory are inaccurate and lead to an unsatisfactory analysis of the experimental data. Migration of a phage λ DNA monomer and concatemers in agarose gel seem to be more complicated as indicated by Cantor et al. (1988) and Serwer (1988). As was reported by Stellwagen (1988), the 48.5 kb λ DNA undergoes merely 14% of its full stretching under the effect of the electric field strength of over 0.5 kV cm^{-1}. Bancroft and Walk (1988) noticed that the distance covered by the mass center of the λ concatemer in the area of the inflection point during one pulse is shorter than half of the total length of this DNA chain. The reorientation, therefore, cannot produce a full stretching of the DNA molecule in a tube as assumed in the reptation model. Moreover, the activity of the polysaccharide network during the electrophoresis in agarose gel (Stellwagen & Stellwagen, 1990) should also be taken into account in any theoretical model. Other theoretical models have been also proposed, e.g., the bag model described by Chu (1991) and Noolandi and Turmel (1992). Further comprehension of the motion mechanism of a large DNA in gel during PFGE is possible thanks to experimental studies such as these of Mathew et al. (1988a,b) and other papers which investigated physical parameters characterizing this experiment. Significant attempts to measure physical parameters other than those applied in a simple electrophoresis involved optical methods and were described by several authors (Stellwagen, 1985, 1988; Akerman et al., 1989; Akerman & Jonsson, 1990; Whitcomb & Holzwarth, 1990).

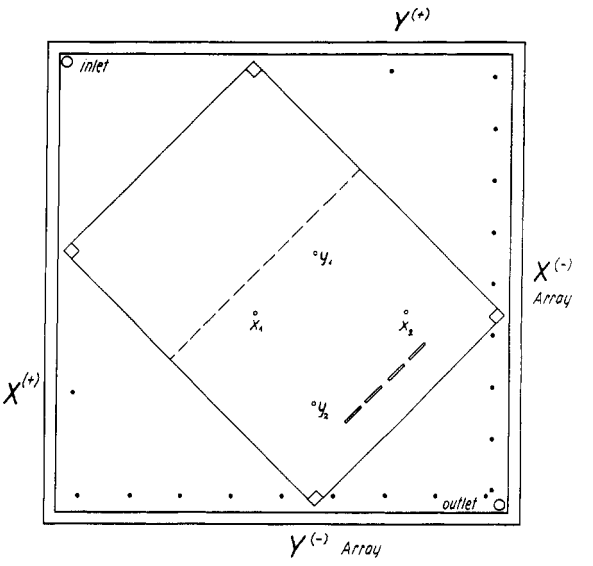

FIGURE 1: Top view of the electrophoresis apparatus. Dots indicate electrodes connected to the diodes in the arrays $X(-)$ and $Y(-)$. The electric field is switched alternately between the X and Y sets of electrodes. Position of the glass plate and inlet and outlet of the circulating electrophoresis buffer are indicated. Time of the electrophoresis was chosen in such a way that electrophoretic patterns did not cross the dashed line. The value of the electric field was measured between points x_1 and x_2 or between points y_1 and y_2.

An important parameter for PFGE is the reorientation time τ_R determined in our experiments by means of a simple method. It can be assumed from theoretical considerations that the reorientation time of the longest concatemer λ DNA containing n genomes, which yet undergoes separation during PFGE, is $\tau_R(n) < \tau(n)$, where $\tau(n)$ is a pulse time, for which the λ ladder has n steps. For the λ DNA concatemer containing $n + 1$ genomes $\tau_R(n+1) \geq \tau(n)$. Thus, it is the shortest

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[1] Abbreviations: E, electric field strength; PFGE, pulsed-field gel electrophoresis; kbp, kilobase pairs; N_{max}, maximal molecular size of the still resolved DNA molecule during PFGE; k, coefficient; τ, pulse time; α, exponent; τ_R, reorientation time; τ_{str}, stretching time; $\tau(n)$, pulse time for which λ ladder has n steps; $\tau_R(n)$, reorientation time for λ phage DNA concatemer containing n genomes; TBE, 0.1 M Tris, 0.1 M borate, and 0.2 mM EDTA, pH 8.4; N, molecular size; N_c, size of the smallest DNA species in the compression zone; N_i, size of DNA species at the inflection point; τ_{biref}, time constant; $R_{1/2}$, time required for birefringence to rise to half of its steady-state value; FDLD, fluorescent-detected linear dichroism; f, orientation function determined in FDLD experiment; θ, angle between DNA helix axis and field direction E.

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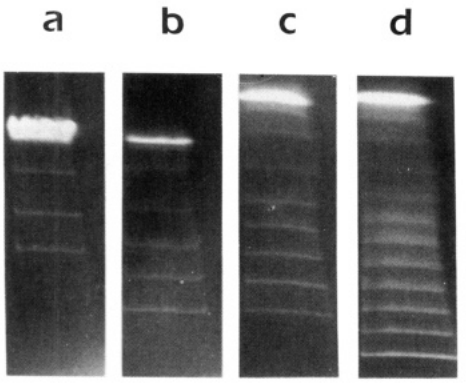

FIGURE 2: Electrophoretic patterns of the λ L47 DNA concatemers in 1.5% agarose obtained in the square apparatus at swich time equal to 10.75 s and different electric fields: (a) 5.03, (b) 7.55, (c) 10.1, and (d) 12.6 V cm^{-1}. It is visible that λ ladders have 3, 5, 8, and 11 steps from (a) to (d), respectively. Compression zones are clearly visible. The starting points are not shown.

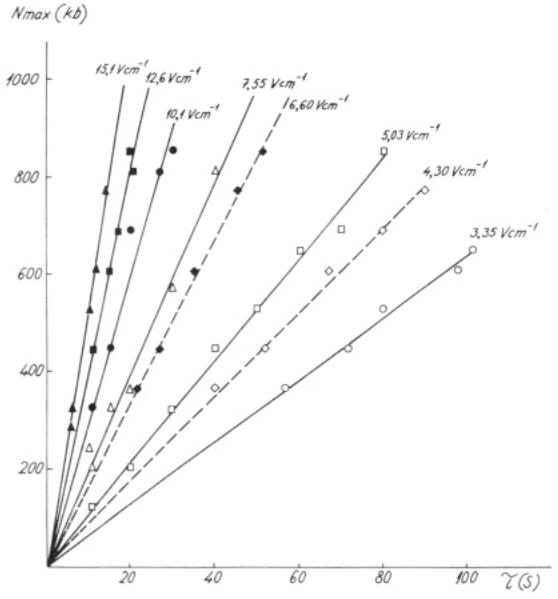

FIGURE 3: Length N_{max} of the greatest resolved λ L47 concatemer as a function of pulse time at constant electric field. Field strength indicated at the ends of the straight lines defines the point sets. The lines have been drawn through the beginning of coordinates and correspond to a linear least-squares fit of the data. Continuous lines are connected with data from the square apparatus, dashed lines with data from the hexagonal apparatus (for comparison).

fragment which cannot reorient during the pulse time $\tau(n)$ and therefore migrates in the gel with the other longer fragments, forming a compression zone. Accordingly, we can accept the pulse time $\tau(n)$ as the best experimental estimation of the reorientation time $\tau_R(n)$ for a λ concatemer containing n genomes. Such reasoning can be extended to DNA of any origin and size, if we choose such a pulse time and field strength which yet allow a given DNA molecule to separate from the compression zone.

MATERIALS AND METHODS

The electrophoresis was carried out in a square apparatus constructed in our laboratory (shown in Figure 1), similar to that described by Smith and Cantor (1986). Vertical platinum electrodes were connected to diodes. The distance between an anode and an array of cathodes was 14 cm. Calbiochem A grade 1.5% agarose gel was placed on a 1.5 mm thick glass plate with a catch at each corner. The agarose concentration was chosen according to the guidelines of Mathew et al.

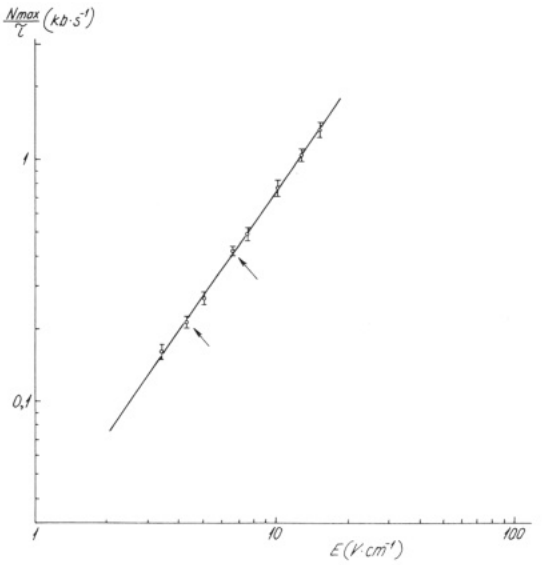

FIGURE 4: Determination of exponent α and constant k used in eq 1. The exponent α is equal to the slope of the straight line. The bars indicate the position of log (N_{max}/τ) standard deviation. The line corresponds to a linear least-squares fit of the data. Data from the hexagonal apparatus are arrows indicated and presented for comparison.

(1988a) and corresponds to a constant relative mobility of DNA of various sizes. The range of gel concentrations between 1% and 2% fulfills conditions of constant resolution. The applied buffer was 1 × TBE. Its circulation (400 mL/min), for cooling purposes, was propelled by a rotation pump. The buffer temperature in the box was 20 °C. The electric field switch control unit was a device of our construction. The field strength was measured between the points shown in Figure 1. The meter we used was of a high internal resistance. Two platinum electrodes embedded in a plexiglass stand were immersed in the buffer. The electric field in the area in which the gel with DNA samples was placed had no more than 2% nonhomogeneity and appeared to be 0.59 U/d, where U is the voltage connected to the electrodes and d (equal to 14 cm) is the distance between the anode and the array of cathodes. The given empirical dependence is correct only for the square apparatus used. It is clear, however, that in the area surrounding the electrodes the field is nonhomogeneous. The actual measured value of the field strength allows us to compare the results obtained in the apparatuses of various geometry. Thusly, several electrophoreses were carried out in a hexagonal apparatus constructed by us according to the method of Chu et al. (1986). The conditions of the electrophoresis regarding the gel, buffer, cooling, and field strength measurement rules were the same as for the square apparatus.

The sample material was a 40620 bp long bacteriophage λ L47 DNA and its concatemers obtained by ligation and partial digestion by *Xho*I endonuclease (Maniatis et al., 1989). A chromosomal DNA obtained from *Saccharomyces cerevisiae* strain X2180-1B was also applied. After the electrophoresis, the gels were stained with ethidium bromide (1 μg/mL) and photographed in UV.

RESULTS

Figure 2 shows an example of four gels of the phage λ L47 DNA. The dependence between the size of the largest separated concatemer N_{max} and the pulse time τ for six different electric fields E measured in the square apparatus is shown in Figure 3. The same figure also shows, for comparison, two series of electrophoreses for two different field strengths in

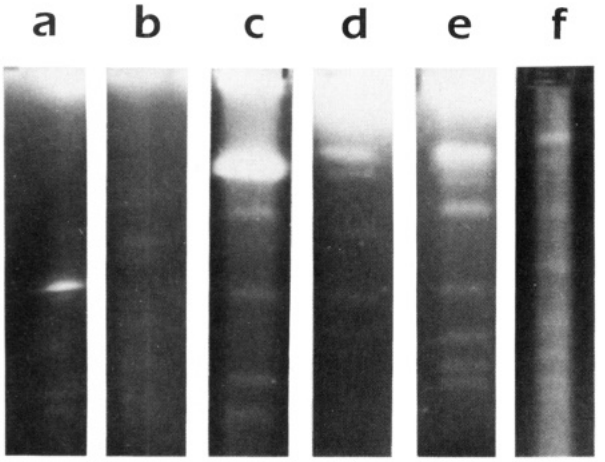

FIGURE 5: Field strength and pulse time dependence of PFGE resolution for *S. cerevisiae* strain X2180-1B chromosomal DNA and its comparison with our calculations. Separation was performed in 1.5% agarose in the square apparatus (a, b) and in the hexagonal apparatus (c–f). Size N_{max} of the greatest separating band [data from Anand (1986)], experimental conditions of electrophoresis, i.e., field strength E and pulse time τ, and also pulse time calculated from eq 1 for experimental value N_{max} and E are as follows: (a) band 4, chromosome IX 450 kb, 10.2 V cm^{-1}, experimental pulse time 15 s, calculated 14.7 s; (b) band 5, chromosomes V and VIII 600 kb, 12.6 V cm^{-1}, experimental pulse time 15.1 s, calculated 14.5 s; (c) band 4, chromosome IX 450 kb, 4.3 V cm^{-1}, experimental pulse time 52 s, calculated 51.0 s; (d) band 5, chromosomes V and VIII 600 kb, 4.3 V cm^{-1}, experimental pulse time 67 s, calculated 68.0 s; (e) band 7, chromosome X 770 kb (band 6 is clearly visible, band 7 is just starting to separate), 4.3 V cm^{-1}, experimental pulse time 90 s, calculated 87.3 s; (f) band 8, chromosome XIV 800 kb, 6.7 V cm^{-1}, experimental pulse time 50 s, calculated 47.9 s.

the hexagonal apparatus. The linear dependence between the length of the largest separated fragment and the pulse time was reported by Bancroft and Wolk (1988) and many other authors. The relations presented in Figure 3 are described by the empirical equation

$$N_{max} = k\tau E^{\alpha} \quad (1)$$

where k is a constant and α is a power exponent. It is clear that expression kE^{α} is the slope coefficient of straight lines presented in Figure 3 and can be determined on the basis of the experimental data as a ratio N_{max}/τ, different for each specified field strength. We can easily count $\alpha = 1.44 \pm 0.02$ and $k = 1.08 \pm 0.03$ kb s^{-1} (V/cm)$^{-1.44}$, plotting log (N_{max}/τ) against log E in Figure 4. Using these parameters for a given field strength and pulse time, we can find the size of the largest DNA molecule that will still undergo separation during PFGE. Another way of applying eq 1 allows selection of appropriate field strength and pulse time conditions to obtain separation of a molecule of a specified size.

Figure 5 presents electrophoretic patterns of *S. cerevisiae* DNA. It is clearly visible how precisely eq 1 determines the conditions at which any DNA molecule of a specified size will separate from the compression zone.

Our results and argumentation presented in the introduction allow us to transform eq 1 into

$$N = k\tau_R E^{\alpha} \quad (2)$$

It shows the relation between the size of the DNA molecule N, the reorientation time τ_R, and the field strength E. From eq 2 the reorientation time will be

$$\tau_R = (1/k)NE^{-\alpha} \quad (3)$$

Figure 6 presents plots of the reorientation time as a function

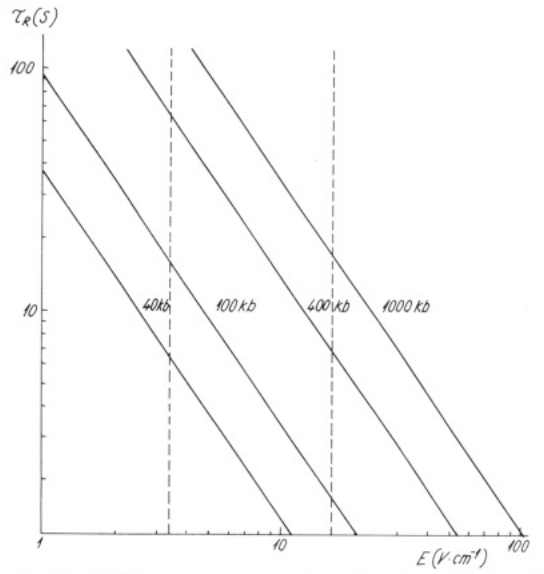

FIGURE 6: Straight lines represent reorientation time calculated from eq 3 for four DNA fragments. Range of field strength in our experiment is marked by dashed lines. Presented relations confirm data from other experiments (see text).

of the field strength for DNA molecules of a chosen size.

DISCUSSION AND CONCLUSIONS

In our opinion the best way to evaluate the reorientation time τ_R for a given field strength E is to find a pulse time τ for which $N = N_{max}$. We feel that the most important physical characteristic of PFGE is the size of the largest DNA molecule separated from the compression zone. The pulse time and the field strength depend on the size of the molecule. The DNA mobility is less significant. We understand that a similar idea can be found in the papers of Mathew et al. (1988b), Gunderson and Chu (1991), and Chu (1991). However, the definition by Mathew et al. (1988b) stating that N_c is the size of the smallest DNA species in the compression zone, if taken strictly, means that it is a nonmeasurable value. Similarly, windows of the size resolution function W, given by Gunderson and Chu (1991) and Chu (1991), cannot be defined precisely (we refer to these papers in a greater detail later).

Equation 3 is not consistent with the theoretical considerations which assume that the reorientation time is proportional to NE^{-2} (Slater & Noolandi, 1986; Fessjian et al., 1986). Noolandi et al. (1989) predicted yet another relation. Our results suggest that the exponent value at the field strength E is near $-3/2$.

It is interesting to compare our results with the reorientation time measured with other methods. It should be noted, however, that the reorientation time applied by us and the parameters used for the description of the dynamical behavior of a DNA chain in a changing electric field in other experiments can be compared only approximately. Stellwagen (1985) described a method of measuring the birefringence relaxation time of DNA in agarose gel after switching off the electric field. Changes in birefringence signal after switching on the electric field should give the possibility of defining the relationship between the reorientation time, electric field, and molecule size. We assume that an increase of the signal is proportional to $1 - \exp(-t/\tau_{biref})$. For calculating the time constant τ_{biref} after switching on the electric field, we used curves obtained by Stellwagen (1985). The results presented in Table I showed a surprising consistency between the time constant obtained from Stellwagen's data and the reorientation

Table I: Comparison of Time Constant τ_{biref} with Reorientation Time τ_R

length of DNA fragm (bp)	field strength E (kV cm^{-1})	time const[a] τ_{biref} (μs)	reorientn time[g] τ_R (μs)
622	3.6	8.5[b]	4.5
1426	4.3	12[c]	8
2936	4.5	19[d]	15
1426	2.2	24[e]	20
2936	1.1	114[f]	111

[a] Time constant τ_{biref} of the increasing birefringence signals of DNA fragments in agarose gels calculated as follows: [b] Figure 1a, [c] Figure 3a, [d] Figure 5a, [e] Figure 4a, [f] Figure 6a from Stellwagen's (1985) results. [g] Reorientation time calculated from our eq 3.

Table II: Comparison of the Time $R_{1/2}$ with Reorientation Time τ_R

kind and length of DNA fragm	$R_{1/2}$[a] (μs)	reorientn time[b] τ_R (μs)
phage λ 48.5 kbp	420	2160
pBR 322 lin 4363 bp	200	194
fragment 2936 bp	160	131

[a] Time required for the birefringence to rise to half of its steady-state value, $R_{1/2}$, calculated on the basis of Stellwagen's (1988, Figure 6A) paper. Calculations were performed for field strength 1 kV cm^{-1} and only for linear fragments. [b] Reorientation time τ_R calculated from our eq 3.

time calculated from our eq 3, although the electric field used by Stellwagen exceeds by over 2 orders of magnitude the field strength in our experiment.

The reorientation time values for three fragments at the field strength of 1 kV cm^{-1} were counted on the basis of eq 3 and then compared to Stellwagen's (1988) data (results in Table II). One can proceed conversely and by extrapolation of Stellwagen's (1988, Figure 6A) results calculate $R_{1/2}$ for the 48.5 kb λ DNA monomer at the field strength 10 V cm^{-1} ($R_{1/2}$ is the time required for the birefringence to rise to half of its steady-state value). The obtained value $R_{1/2}$ = 42 ms is smaller by 2 orders of magnitude than the reorientation time τ_R = 1.4 s calculated for this field strength from eq 3. Thus, it is evident that the −1.0 slope given by Stellwagen (1988, Figure 6A), which would correspond to the exponent $\alpha = -1.0$ in our eq 3, is not a universal value. A family of straight lines shown by us in Figure 6 describes both our and Stellwagen's results in a better way.

Table III: Comparison of Time Constant τ_f and Time To Reach f_{max} with Reorientation Time τ_R

kind and length of DNA fragm	field strength E (V cm^{-1})	time const[a] τ_f (s)	reorientn time[c] τ_R (s)	time to reach f_{max}[b] (s)
λ DNA 48.5 kb	4	1.8	6.1	8.3
	6	1.5	3.4	6.4
	8	1.1	2.3	4.7
	10	0.8	1.6	3.2
T4 DNA 170 kb	4	7.5	21.5	24
	6	6.6	12.0	20
	8	4.7	7.9	14
	10	3.8	5.7	10
G DNA 700 kb	4	30	88	110
	6	26	49	82
	8	20	33	61
	10	15	24	45

[a] Time constant τ_f and [b] time to reach f_{max} calculated on the basis of Whitcomb and Holzwarth's (1990, Figure 6) paper. [c] Reorientation time calculated from our eq 3.

A significant confirmation for our eq 3 was found in a paper of Akerman and Jonsson (1990). These authors investigated reorientational dynamics of the T2 DNA by a linear dichroism (LD) in a pulsed field in 1% agarose. They found that the total reorientation time, defined as the time between when the field is switched and the LD, has reached within 5% of the new steady level, $t_{reo} \propto E^{-1.4\pm0.1}$ for 60°, 90°, and 120° reorientation angles between steady states. This is the first paper reporting an exponent value so close to ours. In an earlier paper Akerman et al. (1989) presented a slightly lower value of the exponent, −1.32, obtained in the same experimental conditions.

A similar method of measuring the reorientation time was proposed by Whitcomb and Holzwarth (1990). They observed stretching and alignment of DNA during PFGE by a fluorescent-detected linear dichroism (FDLD) experiment. The authors measured an orientation function f, determining an angle θ between the DNA helix axis and the field direction E during field changes. Table III shows the values calculated on the basis of Whitcomb and Holzwarth's (1990) paper of the time to reach f_{max} and the time constant for this experiment τ_f [analogous to that used by us in the case of our calculation from Stellwagen's (1985) data] and the reorientation time calculated from our eq 3.

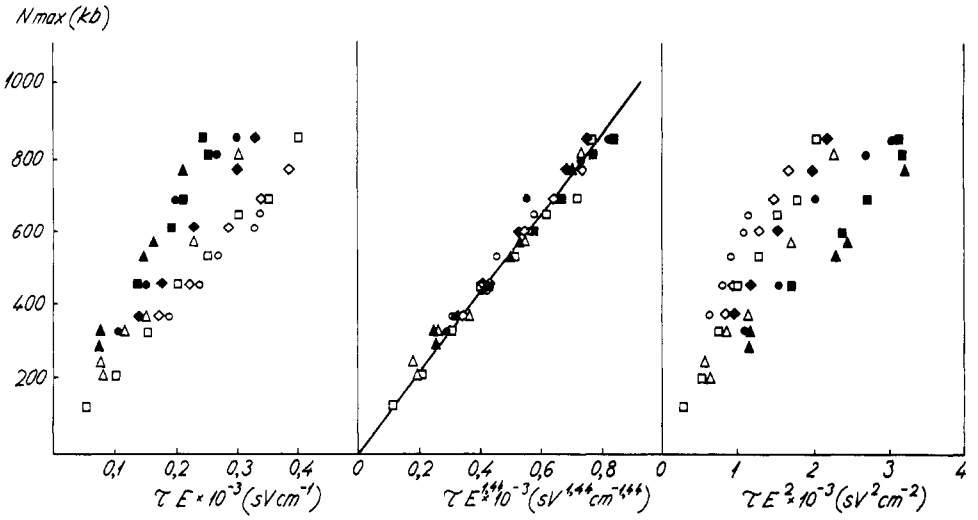

FIGURE 7: Dependence of the size of the longest separated fragment, N_{max}, on pulse time and electric field strength. The data set of Figure 3 was used and replotted as a funcion of τE, $\tau E^{1.44}$, and τE^2, respectively. Uniformity with power exponent 1.44 is clearly visible. The line corresponds to a linear least-squares fit of the data.

A comparison of the presented results with the published experimental data in common PFGE experiments, especially, where λ concatemers were used as the DNA length markers is not always possible because only a few authors give all of the essential experimental data. The actual field strength in the gel area is usually the concern. Our results were consistent with those obtained in the following studies: Van Ommen et al. (1986), Gardiner et al. (1986), and LKB (1986).

The parameter defined in the paper by Mathew et al. (1988b), i.e., the size of the smallest species in the compression zone N_c, is very similar to our N_{max}. The identity of N_c and N_{max} depends on whether Mathew et al. (1988b) connect their parameter with the gel image in such a way that it is still the longest separately visible DNA fragment or the shortest not visible separately and remaining in the compression zone with high DNA molecules of various sizes. Moreover, Mathew et al. (1988b) admit that defining N_c produced difficulties, which was confirmed by the electrophoretic patterns published in their paper.

We compared our results with those of Mathew et al. (1988b). The data set of Figure 3 was used and replotted as a function of τE, $\tau E^{1.44}$, and τE^2 in three frames, respectively, (Figure 7). The conformity of our results with eq 1 and the 1.44 exponent is visible. An attempt to present the results obtained by Mathew et al. (1988b, Figure 9) in scale $\tau E^{1.44}$ gives similarly unsatisfactory results as that in the scale τE and τE^2 applied by these authors. The presumable reason of divergence between our results and those of Mathew et al. (1988b) is the difference of accuracy in determining the size of the largest separated DNA fragment. These authors attempted to use the parameter N_i, defined as the size of the DNA molecule at the inflection point between the two zones of the linear resolution, for estimating the DNA reorientation time τ_R depending on the DNA size N_i and the electric field strength E. This approach gives rise to some doubts. The authors did not point out a physical connection between the pulse time corresponding to a given N_i value and the reorientation time.

The results obtained by Mathew et al. (1988b, Figure 8) seem to confirm the dependence $N \propto \tau_R E^2$ to the same extent as the results of Kölble and Sim (1991, Figure 3) fulfill the dependence $N \propto \tau_R E$. We think that these divergences arise from a lack of precision in determining the basic measured quantity and the notions related to DNA motion in agarose gel.

Gunderson and Chu (1991) argued for a dependence between the size range of resolution or "window of resolution" and the pulse time and field strength $W = E^{1.4}T_p$. They, however, do not present a precise definition of the function W or the way the 1.4 exponent was calculated but refer to the paper of Akerman and Jonsson (1990) mentioned earlier. In his next paper, Chu (1991) presented the following equation: $T_R = g(E/E_0)^a(L/L_0)^b$, where T_R is the reorientation time, E is the field strength, L is the DNA size, E_0 is a reference field strength, L_0 is a reference DNA size, and g, a, and b are three independent parameters. The parameters g and b are determined by Chu (1991, Figure 4A) from a set of three single experimental points on a diagram presenting the dependence of the migration distance during the electrophoresis, on the DNA molecule size. No precise determination of the parameter a was presented by the author. For the experimental conditions described in the paper the values given were $a = -1.4$ and $b = 1.17$. As proposed by the author, by means of transformation of the equation mentioned above we have $L \propto T_R^{1/b}E^{-a/b}$. If we assume that L could equal size N_{max} of the largest separated DNA molecule, then by our denotations $N_{max} \propto \tau_R^{0.85}E^{1.2}$, i.e., a result different from ours.

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