Orientation of DNA in agarose gels

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ABSTRACT An orientation of the lambda DNA during the electrophoresis in agarose gels was measured by a microscopic linear dichroism technique. The method involved staining the DNA with the dye ethidium bromide and measuring under the microscope the polarization properties of the fluorescence field around the electrophoretic band containing the nucleic acid. It was first established that the fluorescence properties of the ethidium bromide-DNA complex were the same in agarose gel and in a solution. Then the linear dichroism method was used to measure the dichroism of the absorption dipole of EB dye bound to lambda DNA. In a typical experiment the orientation of two-tenth of a picogram (2×10^{-13}g) of DNA was measured. When the electric field was turned on, the dichroism developed rapidly and assumed a steady state value which increased with the strength of the field and with the size of DNA. A linear dichroism equation related the measured dichroism of fluorescence to the mean orientation of the absorption dipole of ethidium bromide and to an extent to which the orientation of this dipole deviated from the mean. The observed development of dichroism in the presence of an electric field was interpreted as an alignment of DNA along the direction of the field. The increase in the steady state value of dichroism with the rise in the strength of the field and with the increase of the size of DNA was interpreted as a better alignment of DNA along the direction of the field and as a smaller deviation from its mean orientation.

INTRODUCTION

Large molecular weight DNAs with a mean end-to-end distance of the order of microns are able to migrate under the influence of an electric field through 1% agarose gels (where the pore radius is ~100 nm) because they "reptate" through the gel matrix like a snake making way through the undergrowth (Lumpkin et al., 1985; Slater and Noolandi, 1986). The theory of this motion predicts that during electrophoresis the axis of DNA helix assumes some average orientation with respect to the direction of the electric field (Lumpkin et al., 1985). The present work reports the application of the conventional dichroic technique to measure this orientation.

In solution linear dichroism has been applied to cases wherein DNA has been aligned by an electric field (Fredericq and Houssier, 1973; Mitra et al., 1984; McGhee et al., 1980) and flow (Tjerneld et al., 1982). The introduction of fluorescent labels which bind to DNA was an important improvement, because it increased the sensitivity of detection (Mitra et al., 1984; Kubista et al., 1985; Yoshida et al., 1987). One useful label has been ethidium bromide (ethidiumbromide [EB] LePecq and Paoletti, 1967; Waring, 1970), which binds to DNA by intercalation between the bases with the phenanthridinium plane perpendicular to the DNA helix axis (Sobell et al., 1978; Cantor and Schimmel, 1980). When DNA has been oriented in a flow gradient and the linear dichroism of the bound EB probe has been measured in a Couette cell (Geacintov et al., 1984), orientational changes of microgram quantities of nucleic acid could be measured in seconds (Yoshida et al., 1987).

Here a technique for measuring the dichroism of DNA in gels is reported. It combines microscopic linear dichroic measurements with the sensitivity and speed of the extrinsic (fluorescent) probe approach. It measures under the microscope the polarization properties of the fluorescence field around the agarose gel containing the nucleic acid stained with ethidium bromide. This fluorescence detected linear dichroism (FDLD) technique has been first applied to the case of fluorescently labeled myosin in resting or rigor muscle fibers (Borejdo et al., 1982). More recently, it has been extended to the case of contracting muscle fibers (Burghardt et al., 1983), and a comprehensive theory has been proposed relating the linear dichroism to the orientation of the absorption dipole of the label (Borejdo and Burghardt, 1987). The FDLD method is applied here to determine the geometry of sub-picogram (10^{-13}g) quantities of DNA migrating under the influence of an electric field through agarose gel. Using this method, both the time-dependent and the steady-state behavior of dichroism were measured. Kinetics measurements showed a fast rise and decay of dichroism when the electric field was turned on and off, respectively. The steady-state value of dichroism increased with increasing the strength of the electric field and with increasing the size of DNA.

Recently, a linear dichroism technique based on the

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absorption of intrinsic (Baase et al., 1988) and extrinsic (Holzwarth et al., 1987; Holzwarth et al., 1988) chromophores of nucleic acids has been aplied to measure the local helix orientation of DNA during electrophoresis in agarose gels. The results reported here agree with the conclusions of Holzwarth's work; they supplement it by establishing fluorescence properties of EB-DNA complex in gels, by exploiting the advantages of fluorescence and polarization microscopy to measure the dichroism of minute amount of small DNAs and by using an alternative theory to interpret the data. In an earlier communication we used the same method to measure the orientation of restriction fragments of λ DNA (Borejdo and DeFea, 1988).

MATERIALS AND METHODS

Nucleic acids

Lambda DNA, M13mp18 RF DNA, and high molecular weight DNA markers were obtained from BRL Laboratories (Gaithersburg, MD). Highly polymerized calf thymus DNA was from Sigma Chemical Co., (St. Louis, MO). Electrophoresis grade agarose was from BRL Laboratories.

Electrophoresis of DNA

Electrophoresis was performed on 1% agarose gels in the presence of 40 mM Tris-acetate pH 7.0, 1m M EDTA and 0.5 μg/mL ethidium bromide (EB) per liter in a mini sub gel apparatus (BioRad Laboratories, Richmond, CA). Because agarose gels themselves are dichroic (see below), it was important to make the laser beam path through the gel as short as possible; for this reason gel thickness was fixed at 2 mm. In order to prevent lambda DNA from forming oligomers with itself by basepairing at its sticky ends, it was preheated to 65°C for 10 min. 1–10 μg of DNA (2–10 μL) in a sample buffer containing 40% sucrose and 0.25% bromphenol blue was applied to the wells. The strength of the electric field was varied between 4.8 and 17.2 V/cm.

Lifetime and spectra measurements

Lifetime measurements were made using a single-photon counting instrument assembled from components purchased from PRA, Inc. (London, Ontario, Canada) and from Ortec (Oak Ridge, TN). The data were collected by a multichannel buffer (model 918A; Ortec), stored in an IBM microcomputer, and later transferred to μVaxII computer (Digital Equipment Corp., Maynard, MA) for analysis. Fluorescence decay was deconvoluted by a nonlinear least-squares program (Badea and Brand, 1975). Steady state fluorescence spectra were obtained with an 8000 fluorometer (SLM Instruments, Urbana, IL). Polarization data were obtained on the same instrument equipped with Glan-Thomson prisms.

Linear dichroism measurements

The experimental arrangement (Fig. 1 *A*) is similar to the fluorescence-detected linear dichroism apparatus used with muscle and described earlier (Borejdo et al., 1982). The DNA sample is electrophoresed in an agarose gel in the presence of EB. A gel undergoing migration in the electrophoretic chamber is placed on the scanning stage (0.5 μm resolution) of a light microscope (photomicroscope III; Zeiss, Oberkochen, FRG). A polarized beam of light from an Argon ion laser (model 164-03; Spectra-Physics Inc., Mountain View, CA) is passed through a Pockels cell PC (model 3079FW; Lasermetrics Inc., Englewood, NJ) driven by a wideband amplifier (model GA 21; Lasermetrics Inc.) and an oscillator DR (model 126; Exact Electronics, Hillsboro, OR), and is focused by the auxiliary lens AL (focal length 20 cm). Position of the lens AL is carefully adjusted to focus the illuminating beam precisely at the object plane. The laser beam is attenuated by neutral density filters, and in a typical experiment 1 μW of laser power at 488 nm (2.4×10^{12} photons/s) is incident on the gel. At this power the photobleaching effects were negligible. The Zeiss fluorescein dichroic mirror (model LT460) reflects the light through a Zeiss UD 40x dry objective

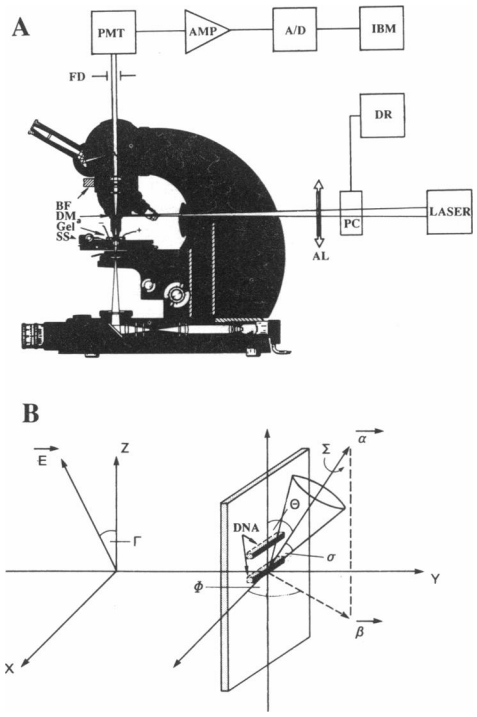

FIGURE 1 The experimental set-up used for measuring the linear dichroism of the EB bound to nucleic acids in gels and the geometry of the dichroism experiment. (*A*) The experimental set-up; Laser—an argon ion laser; PC—Pockels cell; AL—auxiliary lens; DM—the dichroic mirror; BF—a barrier filter; FD—a field diaphragm; PMT—a photomultiplier tube; AMP—an amplifier; A/D—an analog-to-digital converter; IBM—a personal computer. (*B*) A diagram illustrating the geometry of the dichroism experiment; E is the unit vector in the direction of polarization of the exciting light, forming an angle Γ with the *Z* axis in the *X-Z* plane. The gel lies in the *X-Z* plane, with the electrophoretic current running along the *Z* axis (the anode is at the bottom). α is an absorption dipole of the chromophore forming an inclination angle θ with the *Z* axis. Projection of this vector on *X-Y* plane makes an angle Φ with the *X* axis. α can rotate along its own axis by a torsional angle Σ, but this degree of freedom is not considered here. σ is a half angle of a cone within which α rotates and is equal to the sum of an angle describing the disorganization of DNA helix axis and an angle of free rotation of intercalated ethidium bromide.

(numerical aperture NA = 0.65; working distance 7 mm) onto a gel undergoing the electrophoresis. The same objective collects the fluorescence. It was found unnecessary to compensate for the polarization of emitted light by the dichroic mirror (Jenkins and White, 1957). A glass barrier filter (Zeiss LP470) blocks all wavelengths longer than 570 nm. To reject as much as possible the light scattered by the gel, a field diaphragm (FD) limits the field of view to a circular area 30 μm in diameter at the object plane. The fluorescent light is detected by a photomultiplier tube PMT (model R1332; Hamamatsu Phototonics, Hamamatsu City, Japan). In an analogue detection mode, the photocurrent is amplified (AMP, model 110; Pacific Instruments Inc., Concord, CA) and displayed on an analogue recorder (model 2400; Gould Brush, Cleveland, OH) or it is passed through an analogue-to-digital converter A/D (Dascon 1; Omega Engineering Inc., Stamford, CT) and stored on a disk of a personal computer (IBM Co., Boca Raton, FL). In a photon-counting detection mode, the photomultiplier pulses are amplified (video amplifier, model 2A50; Pacific Instruments Inc., Concord, CA), discriminated (discriminator, model 620 CL; LeCroy Research Inc., Spring Valley, NY) and counted (CTM-05 counter, MetraByte, Taunton, MA) which also drives the Pockels cell.

Because a large NA objective is used to collect the emitted light, the fluorescence from a large solid angle is collected and the fluorescence is proportional to the absorption. Therefore the ratio of the fluorescence obtained with the exciting light oriented parallel to a direction of the electric current to the fluorescence obtained when the exciting light is perpendicular to it, is proportional to the dichroism (Borejdo et al., 1982). The measurements are made by alternating the polarization of the exciting light between parallel and perpendicular to the direction of an electric field (by the Pockels cell), and measuring the total fluorescence intensity during each cycle. The ratio of the fluorescence intensities is related to the mean orientation of the absorption dipole, and to the extent to which this orientation deviates from the mean (Eq. 1).

Fig. 1 *B* defines the geometry of the experiment. The linearly polarized excitation light characterized by the electric vector **E** is oriented at an angle Γ with respect to the Z axis. An electric field is also directed along Z axis. Electrophoretic bands containing DNA are marked DNA in Fig. 1 *B*. The polarized light is incident on the dye which has the absorption dipole α oriented at an average angle θ with respect to the Z axis. The dipole α is assumed to be distributed with an equal probability around the mean angle θ. More sophisticated models (Weil and Sturm, 1975; Lumpkin et al., 1985) consider a more realistic fluorophore distribution, but a rectangular one is used here for reasons of simplicity. The variation in θ results from the free rotation of EB between the bases of DNA and from the disorganization of the absorption dipole caused by local deformations in DNA. The half angle of the cone of angles subtended by the vector α is σ. Φ and Σ in Fig. 1 *B* are, respectively, the azimuth angle, i.e., projection of the absorption vector α into X–Y plane, and the torsional angle, i.e., the angle of rotation of the vector α around it own axis.

Data analysis

Eq. 1, derived originally for the case of myosin cross-bridges in the muscle fiber, relates the dichroic ratio R, i.e., the ratio of light absorbed at $\Gamma = 90°$ to light absorbed at $\Gamma = 0°$, to the average value of the angle θ defining the orientation of the chromophore dipole, and to the angular deviation of θ, viz. σ (Borejdo and Burghardt, 1987):

$$1/R = 6a/(2 - 3a), \qquad (1)$$

where

$$a = \tfrac{1}{3} + \tfrac{2}{3}[\sin^2(\theta + \sigma)\cos(\theta + \sigma) - \sin^2(\theta - \sigma)\cos(\theta - \sigma)]/b$$

$$b = [\cos(\theta - \sigma) - \cos(\theta + \sigma)].$$

The azimuthal angle Φ does not enter Eq. 1 because it is assumed that there is cylindrical symmetry of the dipoles around the gel axis (Borejdo et al., 1982). The angle Σ does not appear in Eq. 1 because the torsional movement of the dipole is not considered in the model. Eq. 1 can be solved for θ exactly if σ is known.

RESULTS AND DISCUSSION

The experiments consist in measuring the fluorescence of EB-DNA complexes in agarose gels, i.e., under conditions that are quite different from free solution. In a gel the fluorophore may be immobilized and finds itself in the presence of relatively high concentration of agarose. It was therefore important to establish that the fluorescence properties of EB-DNA were unaffected by the gel. Fig. 2 shows the absorption spectrum of 5 μM EB bound to 50 μM DNA in a low ionic strength buffer in the presence of 0.1% agarose (under these conditions agarose already forms a gel which does not flow when the cuvette is inverted). The spectra show the same two absorption peaks as in solution studies (LePecq and Paoletti, 1967; Burns, 1971). The inset to the Fig. 2 shows the nanosecond decay kinetics of the same gel. The decay was fitted

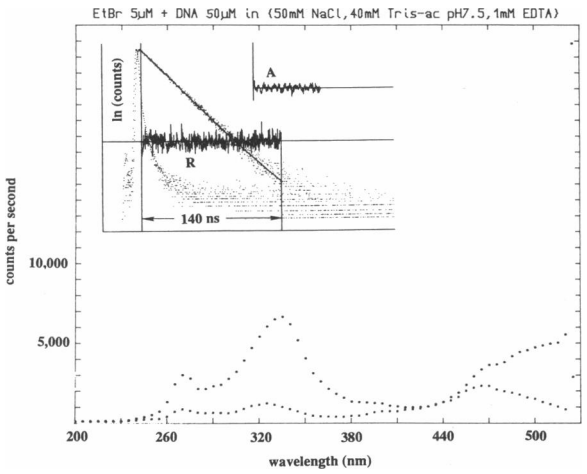

FIGURE 2 Excitation spectra and nanosecond decay kinetics of ethidium bromide in an agarose gel. Main figure, excitation spectra. (*Squares*) 20 μM EB in 50 mM NaCl, 1 mM EDTA, 40 mM Tris-Acetate pH 7.5, 0.1% agarose; circles: 5 μM EB plus 50 μM calf thymus DNA in the same buffer. Emission wavelength: 540 nm room temperature. (*Inset*) nanosecond decay kinetics of EB plus 50 μM calf thymus DNA in 50 mM NaCl, 1 mM EDTA, 40 mM Tris-Acetate pH 7.5, 0.1% agarose. The excitation was at 490 nm, emission was observed through a Corning 3-66 filter. Dots are the experimental points, solid line is the decay calculated in the time interval beginning 40 ns after the beginning of data collection and ending 180 ns after the beginning. The decay was well fitted by a single exponential with $\tau = 22.3$ with the chi-squared value of 2.030. The autocorrelation A and residuals R are also shown.

very well by a single exponential with the lifetime $\tau = 22.3$ ns. For EB in solution $\tau = 23.1$ ns was obtained; Burns (1969) obtained $\tau = 22$ ns. These results show that agarose causes no unexpected quenching of EB. Fig. 3 further demonstrates that the fluorescence properties of EB are little changed by the gel. The polarization excitation of EB bound to DNA is the same at 488 nm regardless of whether the fluorophore is immobilized by 0.5% agarose gel (*squares*) or whether it is immobilized by glycerol at −20°C (*closed circles*). The fact that polarization of fluorescence of EB was higher when it was immobilized by glycerol than when it was bound to DNA (*open circles*) shows that EB has some rotational mobility when bound to DNA. The extent of this mobility is quantitated later (Fig. 9). Together with the excitation spectrum, the excitation polarization spectrum shows that EB fluorescence results from two electronic transitions: one in the UV and the other in the visible wavelengths. Because the excitation polarization spectrum was constant above 440 nm the excitation wavelength in all experiments was chosen as 488 nm. (In our previous experiments [Borejdo and DeFea, 1988] the excitation was at 365 nm because at this wavelength the light scattering by the gel relative to the fluorescence of EB is at a minimum).

Fig. 4 is an experiment in which the orientation of DNA in gel was measured. 10 μg of lambda DNA was electrophoresed at 17.2 V/cm in 1% agarose gel. The 5 μm diameter circular area in the middle of the fluorescent

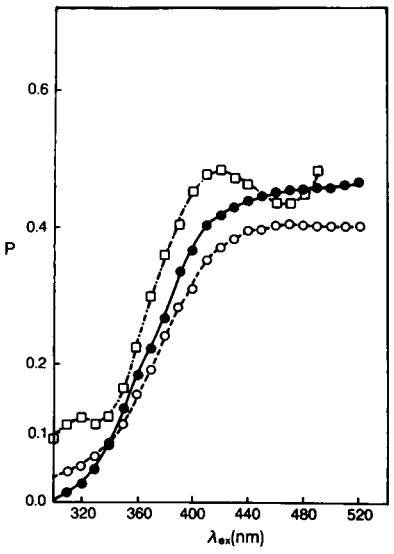

FIGURE 3 Excitation polarization spectra of EB. (*Closed circles*) 20 μM EB in 100% glycerol at −20°C; (*open circles*): 5 μM EB plus 50 μM calf thymus DNA in 50 mM NaCl, 1 mM EDTA, 40 mM Tris-Acetate buffer pH 7.5 at 0°C; (*squares*): 5 μM EB plus 50 μM calf thymus DNA in 50 mM NaCl, 1 mM EDTA, 40 mM Tris Acetate buffer pH 7.5, 0.5% agarose. Emission wavelength 550 nm, channel B filter 546 nm.

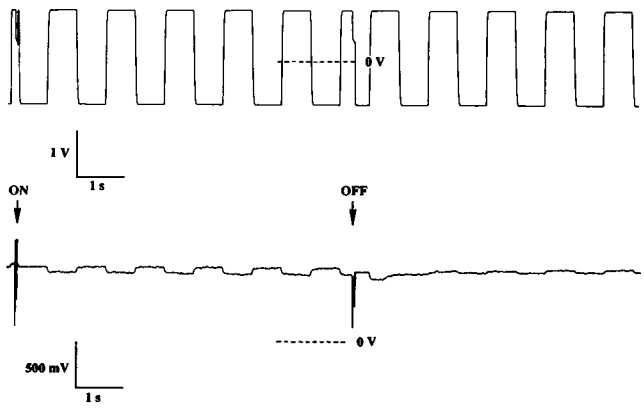

FIGURE 4 Linear dichroism of the DNA in agarose gel. (*Top trace*) the signal to the Pockels cell. The ±1V signal is amplified by a wideband amplifier to 0–260 V used to drive the Pockels cell. (*Bottom trace*) fluorescent light emitted by the EB proportional to the absorption of light. The arrows indicate when the electric field (in this case 17.2 V/cm) was turned on and off. Zero is indicated by the broken line.

gel band was illuminated with 488 nm light from the argon laser. The 1 Hz square wave signal driving the Pockels cell is shown as an upper trace of the Fig. 4. The +1V signal causes the incident light to be polarized in the direction parallel to the electric field, and −1V signal causes the incident light to be polarized in the direction perpendicular to the electric field. The lower trace is proportional to the intensity of the fluorescent light (the background signal, the light contributed by the gel without EB, was <2% of the fluorescence contributed by the EB bound to DNA). Clearly, the fluorescent light has higher intensity when the light is polarized in the direction perpendicular to the electric field than when it is polarized in the direction parallel to the electric field, i.e., the fluorescent light is dichroic. The positive dichroism (i.e., $R > 1$) is present only when the electric field is turned on. When the field is turned off, the dichroism decays to 1, and eventually assumes a constant value smaller than 1. This residual dichroism, present in the absence of the electric field, is of the opposite direction to the one exhibited in the presence of the field. It is caused by the dichroic properties of the agarose gel itself (it is also present when a solution of EB is gelled by adding agarose, e.g., when a gel such as the one used in Figs. 2 or 3 is examined) and by the residual polarization of the incident beam by the inclined microscope mirrors. This residual dichroism has a constant value of 0.958 and all the measurements have been corrected for it (i.e., 1 − 0.958 = 0.042 has been added). Fig. 5 summarizes the results of the experiments such as the one shown in Fig. 4 where the strength of the electric field was varied between 4.8 V/cm and 17.2 V/cm. A corrected dichroism is plotted against time (Fig. 5 *A*) and against the strength of

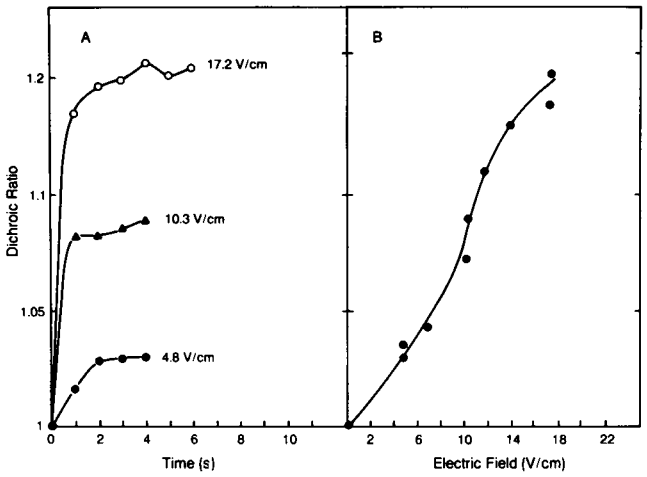

FIGURE 5 The dependence of the dichroic ratio on time (*A*) and the value of the electric field (*B*). The ratio was obtained from the experiments such as shown in Fig. 4, and has been corrected for the intrinsic dichroism of the 1% agarose gel (0.958).

the electric field (Fig. 5 *B*). It is clear that the rate of the development of dichroism and the steady state value of dichroism increase with the value of the electric field. This is in agreement with the results of Holzwarth et al., 1987 and with the predictions of a theory of reptational motion of DNA through the gel (Lumpkin et al., 1985). On the other hand, Fig. 5 *A* shows that in contrast to the earlier work, the dichroism exhibits no over-shoot of the rising phase of orientation. This was true regardless of the size of DNA or the strength of the electric field. I have no explanation for this small but consistent discrepancy.

The present method can be used to follow the kinetics of the development of dichroism (orientation). Fig. 6 is the same as Fig. 4, except that the time scale is 10 times faster. The time course of the development and relaxation of dichroism is shown in Fig. 7 *A*. The rapid changes in dichroism can be better visualized when the changes in the intensity of the fluorescent light are detected in photon counting mode. The counts are stored on a disk of a computer, and the ratio of the alternate counts is equal to the linear dichroism. Fig. 7 *B* shows a typical record of the development of dichroism of Lambda DNA when the electric field of 14 V/cm is switched on. Digital collection of data allowed me to directly compute signal-to-noise (S/N) ratio by dividing the change in a level of dichroism induced by the electric field to the standard deviation of its steady state value. For the electric field at 14 V/cm the S/N ratio was 14. At this level of sensitivity there was never any signal over-shoot.

Fig. 8 shows the dependence of dichroism on the molecular weight of the DNA. The electric field was constant at 14 V/cm. In the case of the high molecular weight markers, only the dichroism of the most intense (19.4/Kbp) band was measured. The inset shows the appearance of the various DNA preparations under a broad UV illumination. Fig. 8 demonstrates that it is possible to measure the dichroism of low molecular weight macromolecules (e.g., of the small restriction fragments of DNA; Borejdo and DeFea, 1988). M13mp18 RF DNA is shown in the Fig. 8 even though it is supercoiled, the fact that may affect its orientational behavior in the electric field. We are at present investigating the question of whether supercoil-linear transformation of DNA, which results in large difference in the Stokes radius but which does not change the mass or the change of DNA, affects *R*.

From the measured dichroism one can estimate with Eq. 1 an average angle that the absorption dipole of EB makes with the direction of the electric field, provided that σ is known. σ is a measure of the extent to which the orientation of the absorption dipole of EB deviates from its mean value, and is a measure of the local motions of the dye. It contains the contribution from free rotations of EB between the bases of DNA and the contribution

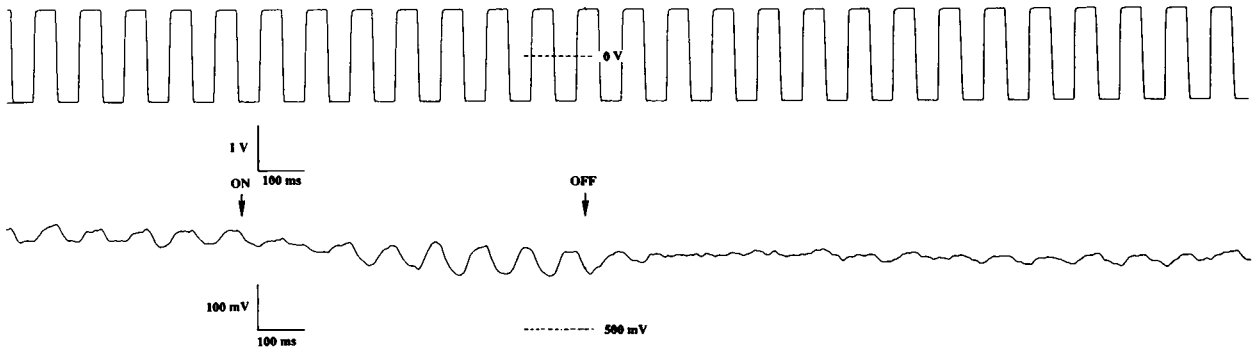

FIGURE 6 Linear dichroism of the DNA in agarose gel at fast time resolution. Top and bottom traces as in Fig. 4. The dichroic signal has been smoothed by the 15 Hz low pass filter.

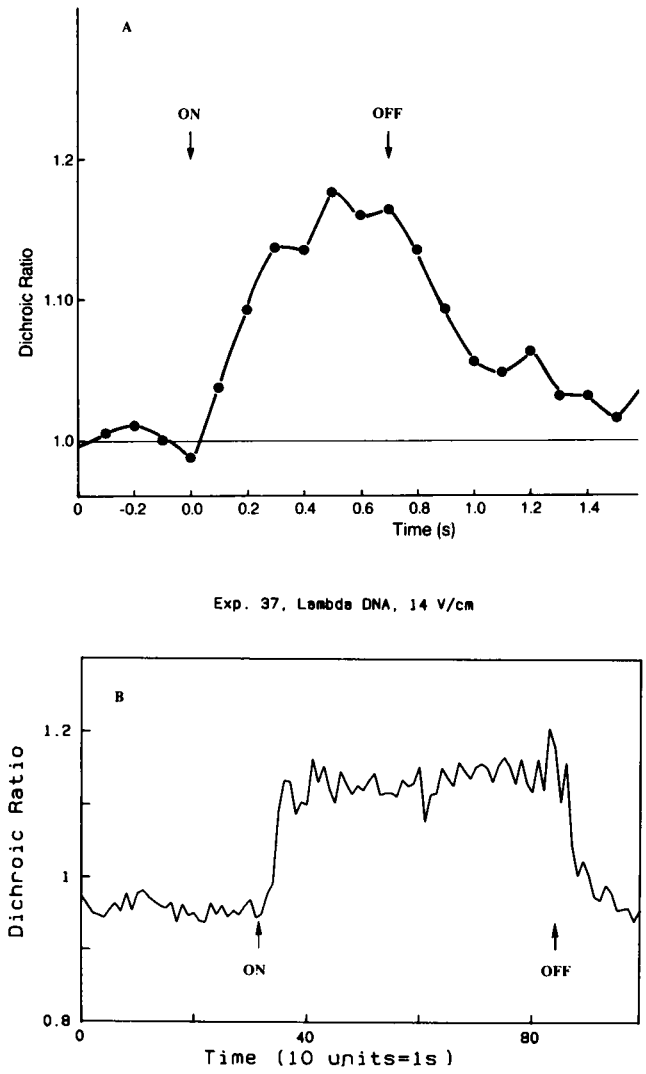

FIGURE 7 (*A*) The time course of the change in dichroic ratio computed from Fig. 6. The arrows indicate when the electric field (14 V/cm) was turned on and off. (*B*) The time course of the change in dichroic ratio measured by photon counting electronics. The arrows indicate when the electric field (14 V/cm) was turned on and off.

resulting from the disorganization of the absorption dipole caused by the local motions of DNA. Its value can be estimated from the steady-state polarization of fluorescence measurements as follows: at high viscosity, the dye cannot rotate (even in the absence of DNA) and polarization of fluorescence is high. In the limit of infinite viscosity the polarization extrapolates to the p_0, the "true" limiting polarization of fluorescence. At lower viscosities, the overall rotation of the DNA is inhibited, but the rotation of bound EB caused by either the motion of the intercalated EB or by the local deformations of DNA is not. The polarization of fluorescence at these lower viscosities extrapolates to the p_x, the "false" limiting polarization of fluorescence. The ratio of these limiting polarizations is related to the angle σ by (Weber, 1966):

$$p_x/p_0 = (3\cos^2\sigma - 1)/2. \quad (2)$$

The polarization of fluorescence of EB bound to DNA was measured as a function of solvent viscosity (ν). The reciprocal polarization of fluorescence was plotted versus T/ν; the resulting (Perrin) plot for 5 μM EB bound to 50 μM DNA is shown in Fig. 9. The T/ν ratio was changed by increasing the glycerol concentration from 10 to 90%. The excitation was at 514 nm and the emission at 550 nm. The extrapolated value the limiting true polarization of fluorescence was $p_0 = 0.465$, and the limiting false polarization of fluorescence $p_x = 0.336$; from Eq. 2, $\sigma = 25.5°$. In the presence of the electric field this value is likely to be smaller.

The angle θ is then obtained from Eq. 1: for lambda DNA migrating in an electric field of 17.2 V/cm, $\theta = 50°$. The orientational function f as commonly defined (Holzwarth et al., 1987) is 0.12. It is believed that the angle between the ethidium chromophore and the DNA axis is either 90° (Fredericq and Houssier, 1973), or that it is close to 70° (Hogan et al., 1979). It is therefore concluded that the DNA molecules orient with their helix axes largely parallel to the electric field. The average angle between the helix axis of DNA and the direction of the electric current (for a field of 17.2 V/cm) is between 20° and 40°.

The increase in dichroism with an increase in the strength of the electric field or with an increase in size of the DNA (Figs. 5 and 8) can arise because, (*a*) The average value of angle θ decreases (i.e., the average orientation of the DNA helix axis becomes more parallel to the electric field); (*b*) angle σ decreases, or (*c*) both of these effects contribute. The present technique cannot distinguish between these possibilities, but it is likely that both angles decrease. In any event, the decrease in σ must be caused by the decrease in the local deformations in DNA rather than from the decrease in a free motion of the intercalated dye: intercalated EB moves very little, extensive evidence indicates that intercalated EB is held rigidly between the bases (LePecq and Paoletti, 1967).

In the first application of the fluorescence detected linear dichroism method, striated muscle, the essential advantages of the technique were discussed (Borejdo et al., 1982). For the purposes of the present work, two of them are of particular importance: the ability to measure the orientation of the very small amounts of material, and the rapidity with which measurements can be made. Both of these can be further improved by increasing the NA of the microscope objective, because a larger NA decreases experimental volume and increases the geometrical col-

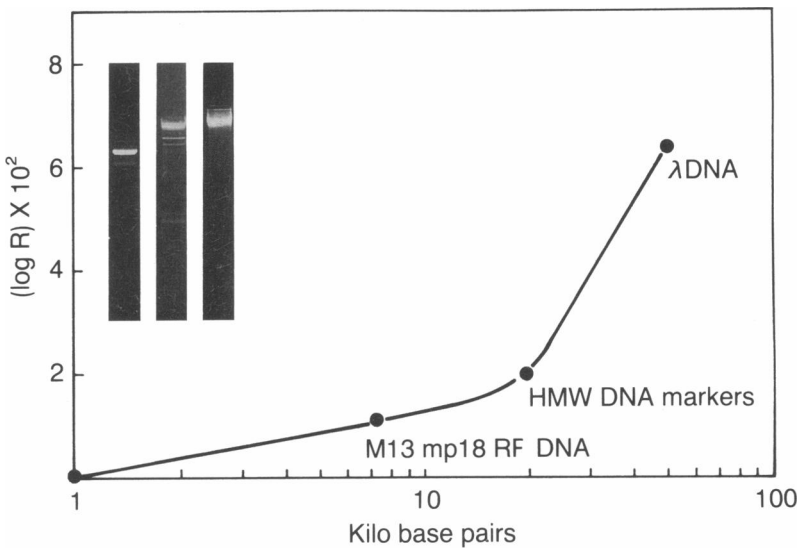

FIGURE 8 The dependence of the dichroic ratio on the molecular weight of the DNAs. (*Inset*) appearance of the DNAs under a broad UV illumination; from left to right: M13mp18 RF double-stranded supercoiled DNA, 7.250 Kbp; high molecular weight DNA markers, 19.4 Kbp band is most intense; lambda DNA, 48.5 Kbp. The 100 log R value of 0 (R = 1) for 1 Kbp DNA is an extrapolation.

lection efficiency of the microscope. But already with the existing optics it should be possible to see the individual molecules of DNA present in the experimental volume. There were 10 μg of DNA in the gel from which data of Fig. 4 was obtained. The area from which the signal is collected by the microscope objective is ~80 μm^2. The depth of focus of 40× objective is 5 μm. Therefore the sample volume is 400 μm^3, which is ~5 × 10^7 times smaller then the band volume. The amount of DNA in a sample volume is thus 0.2 pg. Taking the average molecular weight of a DNA base pair as 618, then for a relatively large DNA such as 48.5 Kbp lambda DNA, there are only 4,000 molecules in the illuminated volume. This number can be made smaller by using a higher NA objective. We are at present trying to see the individual molecules of DNA in an electrophoretic band.

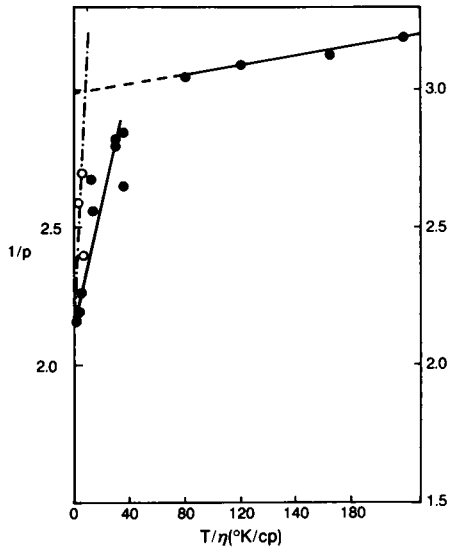

FIGURE 9 Perrin plots of EB and of EB together with DNA in the presence of 10–90% glycerol. (*Open circles* and *dashed line*) 20 μM EB in 50 mM NaCl, 1 mM EDTA, 40 mM Tris Acetate buffer pH 7.5; Closed circles and solid line: 5 μM EB plus 50 μM calf thymus DNA in 40 mM NaCl, 1 mM EDTA, 40 mM Tris-Acetate pH 7.5, 0°C. Excitation wavelength 514 nm, emission wavelength 550 nm, background filter 546 nm.

The use of the microscopic detection of DNA in gels during the electrophoresis has the additional advantage in that it reduces the global inhomogeneities of the gels which can affect the macroscopic measurements of orientation.

An obvious extension of fluorescence detected linear dichroism study is to investigate the electric dichroism of other DNAs, such as single-stranded or nicked DNAs. Another intriguing possibility is to study the electric dichroism of proteins. A prominent candidate is F-actin, because it also is a long polymer that must reptate through the gel. We are at present trying to achieve the electrophoresis of F-actin in agarose gels (J. Borejdo and H. Ortega, manuscript submitted for publication).

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