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A SMALL DNA RESTRICTION FRAGMENT WITH AN APPARENT PERMANENT DIPOLE MOMENT

Nancy C. STELLWAGEN

Department of Biochemistry, University of Iowa, Iowa City, IA 52242, U.S.A.

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The electric birefringence of two DNA restriction fragments, each containing 147 base-pairs, has been investigated. The decay of the birefringence was the same for the two fragments, with a relaxation time corresponding to the reorientation of fully extended, rod-like molecules. However, the birefringence saturation behavior of the two fragments was markedly different: one fragment oriented by the expected induced-dipole mechanism, while orientation of the other fragment followed the theoretical curve for permanent dipole orientation. This difference in behavior must be due to differences in the base-pair sequences of the two fragments.

1. Introduction

As a continuation of a recent series of birefringence experiments on DNA restriction fragments [1], a study was made of the saturation of the birefringence of two different fragments of the same molecular weight. These fragments were generated by cutting a plasmid of known sequence, pYP4.1 [2], with the restriction enzyme *Msp*I [3]. When the resulting fragments were electrophoresed on an acrylamide gel, the two 147-base-pair fragments were found to migrate as separate bands which could be isolated separately. The results of preliminary electric birefringence studies on these two fragments are reported here.

2. Materials and methods

2.1. Restriction fragments

Plasmid pYP4.1 is a 8464-base-pair plasmid containing a 4.1-kilobase *Hin*dIII fragment of yeast DNA plasmid (2 μ circle) cloned into the *Hin*dIII site of the plasmid pBR322, as shown in fig. 1. The orientation of the 4.1 kilobase fragment with respect to pBR322 was determined from an analysis of the restriction fragments produced by digestion of pYP4.1 with *Msp*I. Since the complete sequences of the yeast plasmid [2] and pBR322 [4] are known, and the nucleotide sequence cut by the enzyme is also known [3], molecular weights of the fragments expected for the two possible orientations of the insert can be calculated. In the region spanning the juncture of the two plasmid segments, *Msp* fragments of $132 + 585 = 717$ and $493 + 69 = 562$, or $132 + 69 = 201$ and $493 + 585 = 1078$, base-pairs would be expected, depending on the relative orientation. As can be seen from the electrophoresis pattern in fig. 2, the latter orientation of the insert, generating fragments of 201 and 1078 base-pairs, was actually observed.

To prepare the restriction fragments used in this study, 950 μg of plasmid pYP4.1 in 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 6 mM β-mercaptoethanol, 6 mM $MgCl_2$, and 100 μg/ml gelatin, total volume 2.5 ml, were incubated at 37°C with 340 units *Msp*I (1.5 units/μl) for 2 h (2.8 × overcut). The fragments were separated by electrophoresis on a $32 \times 40 \times 0.6$ cm, 6% acryla-

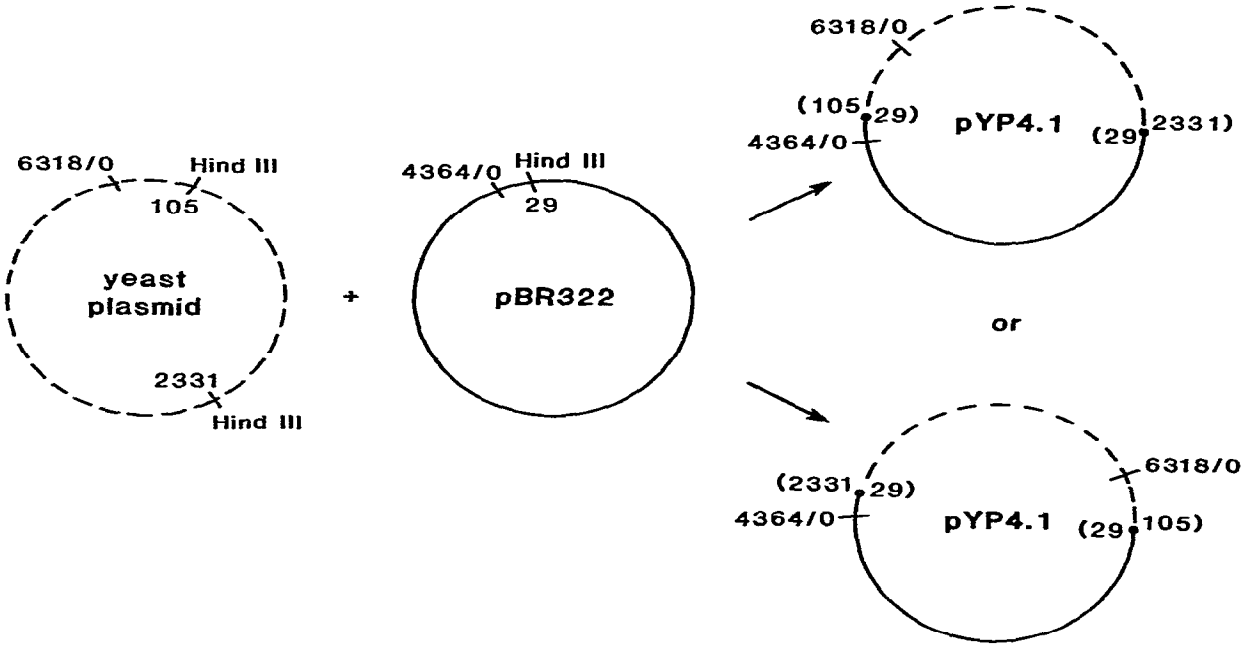

Fig. 1. Schematic diagram of two possible orientations of the *Hin*dIII fragment of yeast plasmid in pBR322.

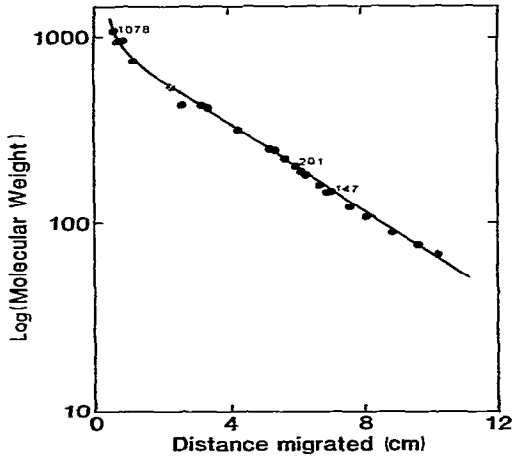

Fig. 2. Analysis of electrophoresis patterns of *Msp*I fragments of pYP4.1. The log of the molecular weight of each fragment is plotted vs. distance migrated on the gel. The eluting buffer was 5 mM Tris-borate, pH 8.3, 1 mM EDTA. The fragments were electrophoresed at 300 V and 60 mA for 23 h at 4°C. The molecular weights of the 147-base-pair fragments and the two fragments spanning the junction of the two plasmid segments are indicated on the figure.

mide slab gel, eluting with 5 mM Tris-borate, pH 8.3, 1 mM EDTA buffer. The gel was pre-electrophoresed at 300 V, 60 mA at 4°C for 2 h before the sample was applied; the restriction fragments were electrophoresed at 300 V and 60 mA, for 23 h at 4°C. The gel was then soaked for about 10 min in approx. 10 μg/ml ethidium bromide, visualized with long-wavelength ultraviolet light, photographed, and the bands containing the different fragments excised. The gel slices were then mashed, covered with 2 vol. of 10 mM Tris-HCl, pH 8.1, 0.1 mM EDTA buffer in large test tubes, and rocked overnight at room temperature. The mixtures were then centrifuged for 20 min at 10000 rpm in a Sorvall R5 refrigerated centrifuge. The supernatants containing the DNA fragments were filtered through glass wool and concentrated on small DEAE-cellulose columns (Whatman DE-52) with 3–4 mm gel beds. After warming for 10 min in a 60°C oven, the fragments were eluted with 250 μl of a solution containing 0.5 M Tris-HCl, pH 7.4, and 1.5 M sodium acetate, ethanol pre-

cipitated, and redissolved in 100 μl of 1 mM NaCl. The solutions were then frozen and stored at −20°C. For the birefringence measurements the solutions were thawed and diluted to 0.33 mM NaCl. Elias and Eden [5] have shown that birefringence relaxation times of small DNA fragments are independent of Na^+ concentration between 0.2 and 2 mM.

The identity and integrity of each fragment were checked by electrophoresis on an 8 × 8 × 0.01 cm 6% acrylamide minigel, or on a 28 × 15 × 0.7 cm horizontal agarose gel. Each fragment exhibited the expected molecular weight and was uncontaminated by fragments of larger or smaller molecular weight, except for the fragments of 927, 950 and 1078 base-pairs, each of which contained some of the adjacent species.

2.2 Determination of the identity of each of the two 147-base-pair fragments

Although the base sequence of each of the two 147-base-pair fragments was known [2,4], it was not known which migrated faster on the preparative acrylamide gel. To identify them, each was separately cloned into plasmid pARA1, a derivative of pBR322 [6]. The details of the cloning procedure will be described separately (N. Stellwagen, to be published). The resulting plasmids, each containing one of the two 147-base-pair fragments, were cut with the restriction enzyme *Eco*RI to excise the 147-base-pair fragments, and then further treated with the restriction enzyme *Alu*I, which would cut one of the fragments but not the other [4].

The reaction conditions were as follows: 2 μg of each plasmid in 30 μl of a solution containing 50 mM NaCl, 10 mM Tris-HCl, pH 7.4, 6 mM β-mercaptoethanol, and 100 μg/ml gelatin were incubated with 1 μl *Eco*RI (9 units/μl) at 37°C for 1 h (18 × overcut). 1 μl *Alu*I was then added to half of each solution (2 units/μl) and all solutions were incubated at 37°C for another hour (4 × overcut). The resulting mixtures were then electrophresed on a 0.8% agarose gel, with the results shown in fig. 3. Both plasmid DNAs generated a 147-base-pair fragment when digested with *Eco*RI. In fig. 3a, the 147-base-pair fragment obtained

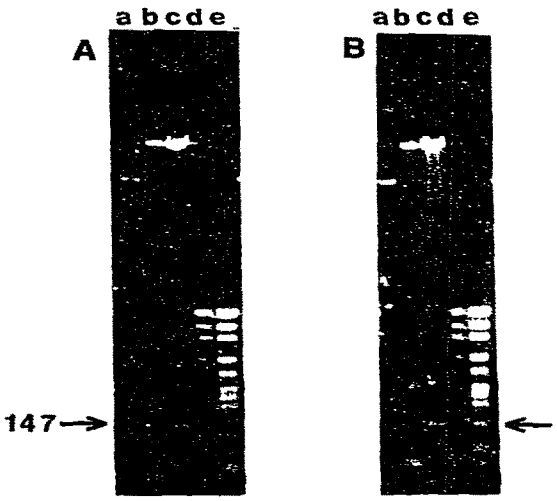

Fig. 3. Electrophoresis of plasmids containing each of the 147-base-pair fragments, after cutting with restriction enzymes. (a) Plasmid pB2 containing the faster migrating 147-base-pair fragment. Lane a: uncut plasmid; lanes b, c: *Eco*RI cut plasmid (note 147-base-pair fragment indicated by arrow); lanes d, e: *Eco*RI and *Alu*I cut plasmid (note absence of 147-base-pair fragment). The extra bands come from *Alu*I sites on the remainder of the plasmid. (b) Plasmid P11t containing the more slowly migrating 147-base-pair fragment. Lane a: uncut plasmid; lanes b, c: *Eco*RI cut plasmid (note 147-base-pair fragment at arrow); lanes d, e: *Eco*RI and *Alu*I cut plasmid (note continued appearance of 147-base-pair fragment).

from the plasmid constructed with the faster migrating 147-base-pair fragment could be cut with *Alu*I (note the absence of the band at the position of the arrow in lanes d and e). In fig. 3b, DNA from a plasmid cloned with the more slowly migrating 147-base-pair fragment gave a 147-base-pair fragment which could not be cut with *Alu*I (note the continued presence of the band at the position marked by the arrow in lanes d and e). Hence, the faster migrating 147-base-pair fragment can be identified with fragment 12B in the pBR322 restriction map of Sutcliffe [4]. The slower migrating 147-base-pair fragment is 12A. The multiple bands observed in lanes d and e in fig. 3a and b are due to the cutting of pARA1 by *Alu*I; these bands can also be used as size markers, since the molecular weight of each band is known [4].

2.3. Enzymes

*Eco*RI and *Alu*I were obtained from New England Biolabs. *Msp*I was prepared by T.S. Gregori following the procedure of Greene et al. [7], and was a gift from J.L. Hartley. pARA1 was also a gift from J.L. Hartley.

2.4 Apparatus and procedures

The birefringence apparatus used in this study was that of Professor C.T. O'Konski, recently described in refs. 8 and 9. In brief, the apparatus consists of a tungsten light source, Glan-Thompson polarizer and analyzer, and fresnel rhomb quarter-wave retardation device. After passing through a photomultiplier and preamplifier circuit, the birefringence signals were stored in a digitizing transient recorder (Biomation 805) and sent through a laboratory-designed interface to a Tektronix PDP 11/10 minicomputer. Single-shot pulses were generated by a Cober 605P pulse generator. The temperature of the measurements was 21°C.

Relaxation times were obtained by computer analysis of the birefringence decay curves [8,9]. Saturation curves were calculated as described previously [1], except that complete orientation was not obtained at the highest field available, 11 kV/cm. The normalized experimental curves were compared with the theoretical curves of O'Konski et al. [10]. More extensive discussions of the theory of electric birefringence are given in several recent reviews [8,9,11].

3. Results and discussion

3.1. Transient birefringence behavior

The relaxation time of the decay of the birefringence of the faster migrating 147-base-pair fragment (12B) was found to be 2.2 ± 0.1 μs, while the relaxation time of the slower migrating fragment was 2.0 ± 0.2 μs. Hence, both fragments had essentially the same overall extension in solution. Assuming a diameter of 26 Å, the average relaxation time of 2.1 μs corresponds to a length of 491 Å, using Broersma's original equation [12], 501 Å using Broersma's revised equation [13], and 508 Å using Tirado and Garcia de la Torre's equation [14]. These values are all in reasonable agreement with the length calculated from the known molecular weight. Assuming full extension of the molecules and a rise per base-pair of 3.4 Å, a blunt-ended 147-base-pair fragment is calculated to have a length of 500 Å. However, *Msp*I cuts the C–C bond of a CCGG sequence, leaving two unpaired bases on the end of each fragment [3]. If these unpaired bases are assumed to have the same perpendicular orientation as the paired bases, the calculated length would be 507 Å. If the two bases are assumed to have a random orientation, the calculated length would be 493 Å. Further studies with DNA fragments having filled and unfilled 'sticky ends' would indicate whether the unpaired bases contribute to the rotation of the macromolecules in solution.

The rise of the birefringence of both 147-base-pair fragments was approximately equal to the decay, as observed previously for a series of DNA restriction fragments of different molecular weights [1].

3.2 Saturation behavior

Both 147-base-pair fragments obeyed the Kerr law at low field strengths, as shown in fig. 4. The

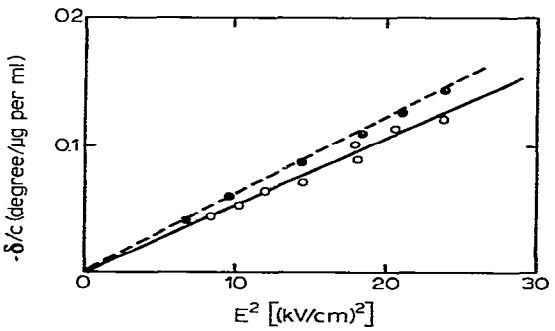

Fig. 4. Dependence of the specific birefringence (δ/c) of the 147-base-pair fragments on the square of the electric field strength. DNA concentration, 5.3 μg/ml in 0.33 mM NaCl. (●) Slower migrating fragment, 12A; (○) faster migrating fragment, 12B.

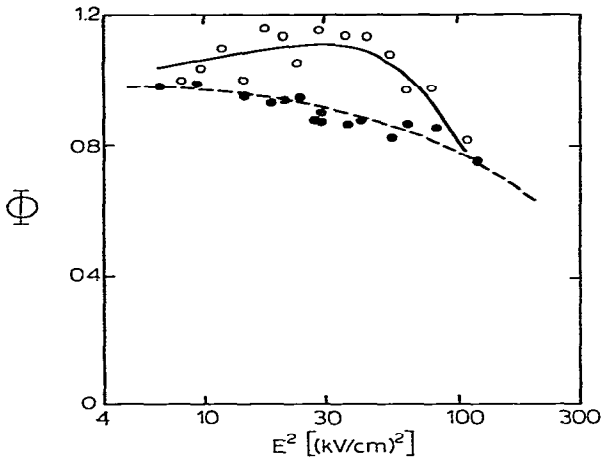

Fig. 5. Theoretical saturation curves of O'Konski et al. [10] compared with experimental data. The orientation function $\Phi = (\delta/E^2)/(\delta/E^2)_{E\to 0}$ is plotted vs. E^2 for: (○) faster migrating fragment, 12B; (————) theoretical curve for induced dipole orientation, $c^{1/2} = 0.32$ cm/kV; (●) more slowly migrating fragment, 12A; (— — —) theoretical curve for permanent dipole orientation, $b = 0.18$ cm/kV. DNA concentration, 5.3 μg/ml in 0.33 mM NaCl.

amplitude of the birefringence of the electrophoretically faster migrating fragment (12B) was approx. 15% smaller than that of the more slowly migrating fragment, even though the birefringence relaxation time of this fragment (12B) was approx. 10% longer. This result is in contrast to previous work, which showed longer relaxation times to be correlated with higher amplitude of birefringence [1].

A significant difference between the two 147-base-pair fragments was found when the saturation curves were compared with the theoretical equations of O'Konski et al. [10], as shown in fig. 5. The saturation curve of the electrophoretically faster migrating fragment (12B) could be fitted by the theoretical curve for induced dipole orientation, as expected for a DNA fragment of this molecular weight [1]. The value of the $c^{1/2}$ required was 0.32 cm/kV, which corresponds to an induced polarizability of 7.6×10^{-16} cm^3. This value is somewhat larger than the value of 3.0×10^{-16} cm^3 observed previously for a 160-base-pair fragment in 0.2 mM Tris-HCl, pH 8.0, 0.02 mM EDTA buffer [1]. A more extensive study would be necessary to determine whether this difference is due to the difference in experimental conditions or to differences in the samples themselves.

The saturation curve of the more slowly migrating 147-base-pair fragment (12A) could only be fitted with the theoretical curve for permanent dipole orientation, as shown in fig. 5. The value of b obtained from the theoretical curve was 0.18 cm/kV, corresponding to a permanent dipole moment of 2150 debye. This is the first time a small rigid monodisperse polyelectrolyte has been shown to follow the theoretical curve for permanent dipole orientation. Previous observations of apparent permanent dipole orientation of high molecular weight DNA and other polyelectrolytes can be attributed to flexibility [1].

The origin of the apparent dipole moment of the slowly migrating 147-base-pair fragment (12A) is not entirely clear, since the rise of the birefringence of this fragment was not appreciably slower than the decay, as would be expected for a 'classical' dipole moment [8–11]. The apparent dipole moment cannot be attributed to the 2-base-pair sticky ends on each end of the fragment, because the faster migrating 147-base-pair fragment, which exhibited induced dipole orientation, also contained the same number of unpaired bases. Both fragments also exhibited essentially the same birefringence relaxation times, and the amplitudes of the birefringence were very similar.

Since the size and shape of the two 147-base-pair fragments in solution were essentially identical, the differences in the mode of interaction of the two fragments with the electric field must arise from differences in the base-pair sequences of the two fragments. The faster migrating fragment (12B, induced moment orientation) contains 40% A + T residues, while the slower migrating fragment (12A, permanent moment orientation) contains 47% A + T residues. Fragment 12A also contains a relatively long stretch of 8 GC base-pairs adjacent to a highly enriched A-T region (9 out of 11 base-pairs). Early et al. [15] have shown that the conformation of AT base-pairs can be perturbed at the junction of AT-GC blocks, even in solutions of moderate salt concentration. Other reports of sequence-dependent variations in the conformation of DNA are beginning to appear in the literature [16–24].

Further studies are now underway in an attempt to explain the differences between these two 147-base-pair fragments. Plasmids have been constructed to yield enriched quantities of these two fragments, and further birefringence measurements will be undertaken as well as studies of other physical properties of these fragments. It is hoped that the origin of the apparent permanent dipole moment can be explained, and structural and/or conformational differences between the two fragments determined.

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