STRUCTURAL TRANSITION PRODUCED BY ELECTRIC FIELDS IN AQUEOUS SODIUM DEOXYRIBONUCLEATE

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ABSTRACT It was found that the birefringence of aqueous solutions of sodium DNA is anomalous when electric fields of high intensity ($\geq 10^4$ v/cm) are applied. The magnitude of the birefringence first rose upon application of the orienting pulse, then fell as the field was sustained above a critical value. The occurrence of the effect depended upon macromolecular and electrolyte concentrations. Upon removal of the field, the birefringence was rapidly restored and then it decayed with an increase of the reorientational relaxation times, relative to those observed below the critical field. It is proposed that the electric field may cause aggregation of the macromolecules and then produce a structural transition concomitant with the electric field orientation effect. This transition may correspond to the "B" $\rightleftharpoons$ "A" structures identified in x-ray studies, or to a "B" $\rightleftharpoons$ "V" structure change, where "V" is a postulated new helical form stabilized by cooperative interactions of base and dipoles in the electric field. Field induced transitions of this type would be of interest in connection with molecular mechanisms of transport through membranes, nerve impulse transmission, or information storage.

INTRODUCTION

The unique structure of the deoxyribonucleic acid (DNA) macromolecule, with the orientation of the purine and pyrimidine base pairs perpendicular to the helix axis, (1) gives unusual interest to the question of its electro-optic properties. In a recent study of transient electric birefringence, (2) it was noted that the birefringence of aqueous solutions of sodium DNA was anomalous when electric fields of high intensity were applied. In this report the high field effects are described and an explanation is given in terms of a field induced macromolecular structural transition. Its possible biological implications are briefly outlined.

EXPERIMENTAL

The apparatus (3–5) and techniques (3, 4, 6) used for the measurement of electric birefringence have been described previously. The macromolecular birefringence

is characterized by the optical retardation, δ, after subtracting the birefringence of the solvent. The solvent contribution ranged from 0.4 per cent to about 20 per cent of the observed signals. Birefringence decay times were obtained as follows: t_0 is the intercept of the tangent to the initial birefringence decay curve with the time axis, where time is zero at the instant the field is removed; t_1 is the time required for the birefringence to decay to $1/e$ of its initial value; and t_i is the time required to decay to $1/e^i$, divided by i. This is an arbitrary, but convenient, procedure for characterizing a distribution of relaxation times; all the decay times (or "apparent relaxation times") are equal for the case of a single relaxation time (3).

The DNA sample, called DNA-3 was a Sigma Chemical Co. (St. Louis, Missouri) sample of calf thymus DNA, lot No. D79-51, kindly supplied by Dr. D. F. Bradley. The DNA was dissolved in Tris-HCl buffers of 10^{-2} to 10^{-4} M total Tris concentration, pH = 8.0. Tris was "Sigma 121," primary standard grade buffer from Sigma Chemical Co. Measurements of the hyperchromic effect (7) and melting temperature (8) of the DNA solutions showed the DNA to be in its native state under the conditions used here. Details of the preparation of the DNA solutions and the characterization of DNA-3 are described elsewhere (9). All birefringence measurements were carried out at 25°. The optical path length was 1 cm.

RESULTS

The birefringence signals observed when high voltage pulses (E = 10,700 v/cm) of two different pulse lengths were applied to a solution of DNA-3, 0.0074 gm/liter in 10^{-4} M Tris buffer, are shown in Fig. 1. Although the birefringence of DNA oriented by a pulse of 0.15 msec. appeared to be quite normal (Fig. 1*a*), increasing the pulse length to 0.30 msec. (millisecond) caused the absolute magnitude of the birefringence to pass through a maximum and decrease while the pulse was still

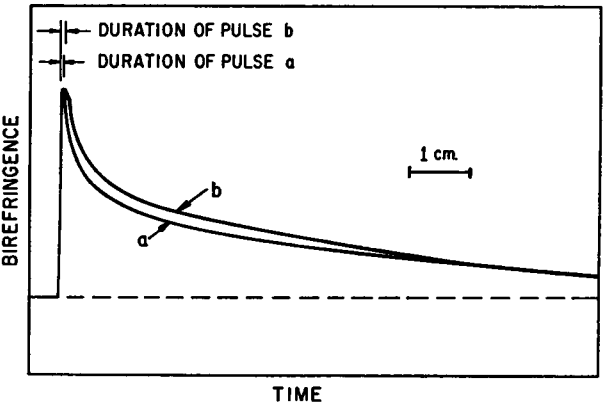

FIGURE 1 High field birefringence of DNA-3, 0.0074 gm/liter in 10^{-4} M Tris buffer, E = 10,700 v/cm (tracings of oscillograms). Sweep speed, 2 msec./cm. (*a*). Pulse length = 0.15 msec. (*b*). Pulse length = 0.30 msec.

being applied (Fig. 1*b*). In addition, the decay of the birefringence exhibited a distinct "hump," visible between 7 and 13 msec. after removal of the pulse. These differences are quantitatively described by the decay time values in Table I. Similar results were observed for several other DNA samples studied under the same conditions.

The effects of variations in the DNA concentration and Tris buffer concentration on the birefringence decay curves observed at high field strength are shown in Table II. The pulse length was 0.30 msec. in all cases.

The accuracy of the decay times in Tables I and II was about ±20 per cent for the t_1 and t_2 values and ±50 per cent for the t_0 and t_3 values.

TABLE I

EFFECT OF PULSE LENGTH ON BIREFRINGENCE DECAY TIMES
DNA-3, 0.0074 gm/liter IN 10^{-4} M TRIS BUFFER, E = 10,700 v/cm

Pulse length	t_0	t_1	t_2	t_3
msec.	*msec.*	*msec.*	*msec.*	*msec.*
0.15	0.23	3.8	8.4	—
0.30	0.8	6.0	7.5	12

TABLE II

DEPENDENCE OF BIREFRINGENCE DECAY TIMES ON DNA AND BUFFER CONCENTRATIONS $E \simeq$ 10,000 v/cm PULSE LENGTH = 0.30 msec.

DNA-3 concentration,	Tris concentration	t_0	t_1	t_2	t_3	
gm/liter	M	*msec.*	*msec.*	*msec.*	*msec.*	*"Hump"*
0.0071	1×10^{-4}	0.70	6.1	6.9	12.	yes
0.0071	2×10^{-4}	0.45	4.3	6.0	7.7	no
0.0140	2×10^{-4}	1.3	6.7	6.5	11.	yes
0.0142	1×10^{-3}	0.21	1.4	3.2	5.4	no

The decrease in the absolute magnitude of the birefringence while the pulse was being applied is illustrated clearly in Fig. 2, using an expanded time scale and higher field strength (E = 21,200 v/cm). The negative optical retardation, $-\delta$, rose to 0.047, then decreased to 0.017 while the pulse was being applied. After removal of the field, the negative retardation increased almost immediately to 0.039 before it began to decay slowly as the macromolecules became disoriented.

DISCUSSION

When electric fields equal to or greater than about 10,000 v/cm were applied for 0.3 msec. to dilute solutions of DNA in Tris buffer, the decay of the birefringence was found to exhibit a distinct hump, as shown in Fig. 1*b*. Quantitatively, this

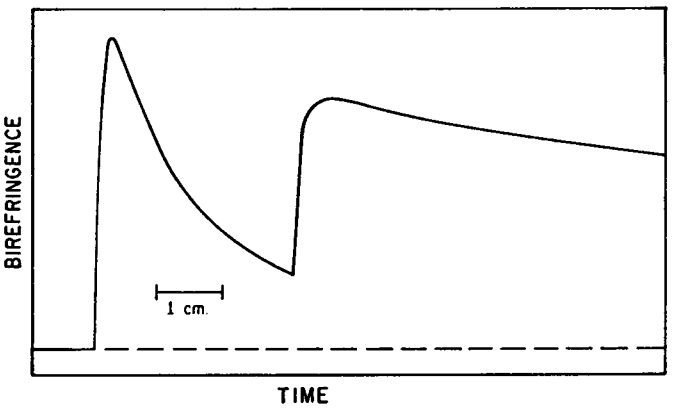

FIGURE 2 Birefringence signal and and applied pulse for DNA-3, 0.0074 gm/liter in 10^{-4} M Tris buffer, $E = 21{,}200$ v/cm (tracing of oscillogram). Sweep speed, 0.1 msec./cm. The birefringence scale is linear and the maximum corresponds to $\delta = 0.047$ radians cm^{-1}.

hump was manifested by larger t_0 and t_1 values and smaller t_2 values than observed when sufficiently short pulses were used so that no hump was produced. The decay times in Table II show that the hump was observed for DNA solutions of 0.007 gm/liter in 10^{-4} M Tris buffer and 0.014 gm/liter in 2×10^{-4} M Tris buffer, but not for DNA solutions of 0.007 gm/liter in 2×10^{-4} M Tris buffer or 0.014 gm/liter in 10^{-3} M Tris buffer.

The appearance of a hump in the birefringence decay curves upon increasing the DNA concentration at constant buffer concentration, upon decreasing the buffer concentration at constant DNA concentration, and upon increasing the length of the high voltage pulse applied to the solution all suggested that the hump was due to intermolecular interactions between the DNA molecules. It is known that a homogeneous electric field can produce attractive forces between particles (10), and that the effective polarizabilities of large anisometric polyions are very high due to ionic polarization effects (11).

The absolute magnitude of the birefringence was observed to go through a maximum and then decrease at constant applied field whenever a hump was observed in the birefringence decay curves. Upon removal of the electric field, the magnitude of the birefringence increased relatively rapidly to a value close to its maximum negative value, before decaying slowly as described above. This decrease in the absolute magnitude of the birefringence during the pulse can be explained by the onset of a positive birefringence component having a much slower rise time and much faster decay time than that of the normally observed negative birefringence. The possibility was considered that this positive component might be due to electrophoretic migration of some of the molecules out of the light path during the

pulse. However, this cannot explain the results because of the rapid restoration of the negative signal upon removal of the electric field, and because a calculation showed the electrophoresis effect would have been too small to account for the observed decrease in the birefringence. Taking the mobility of DNA to be 125×10^{-5} cm^2/v second (12), the DNA molecules could have moved 0.008 cm, or 4 per cent of the distance between the electrodes, during the most extreme pulse applied to the solution ($E = 21{,}200$ v/cm, pulse length = 0.30 msec.). However, during this pulse the absolute magnitude of the birefringence decreased more than 60 per cent, as shown in Fig. 2.

Because the positive birefringence signal appeared only when a hump was observed in the birefringence decay curves, it seems reasonable to assume that interactions between the DNA molecules were necessary for its occurrence. The slow buildup of this signal could then be attributed to the finite time required for the molecules to diffuse together. However, because the positive signal disappeared rapidly after removal of the electric field, it appears that the DNA molecules may still be interacting as the signal decays; this is supported by the presence of the humps seen later in the decay curves.

The suggestion arose that the positive birefringence signal might be due to the orientation of aggregated DNA molecules perpendicular to the direction of the applied field. However, a gross macromolecular orientation effect is unlikely because of the fast disappearance of the positive signal; t_1 was estimated to be ≤ 20 μsec. from Fig. 2.

A more likely explanation of the high field anomalies is that the electric field produces a structural transition in the macromolecule, following the concentration dependence process of aggregation in the electric field. Any more polarizable structure than the normal Watson-Crick or "B" structure would tend to be stabilized by the presence of an electric field, through the interaction term $-\mu \cdot E$, which contributes a negative increment to the electric free energy of the system. Here μ is the electric dipole moment and E is the local electric field. The electric energy is known to be large, under the experimental conditions, compared to the thermal energy, kT, because the macromolecular orientation is strongly saturated (9). An obvious possibility of a transition of the DNA molecule to a random coil from does not seem a likely explanation, in view of the fact that the birefringence relaxation time increases in the anomalous high field effect. In separate experiments (2, 9) on "melted" and recooled DNA solutions, very large decreases in birefringence relaxation times were observed. The field may produce a transition from structure B to structure A, which has the bases tilted an angle of 65° with respect to the helix axis (13), or it could produce a bending of the planes of the base pairs into a "V" configuration, as illustrated schematically in Fig. 3.

A somewhat similar electro-optic phenomenon known to us is the anomalous behavior observed in moderately concentrated solutions of tobacco mosaic virus

(14, 15). There, a reversal of the initial signal was observed, and changes in relaxation times occurred, but the anomaly was most prominent at low fields and disappeared at high fields. The similarity is that both effects appeared to be enhanced by increasing the concentration. Both phenomena may provide interesting means to study the nature of interactions between macromolecules.

An electric field-induced structural transition of the type discussed here may be of interest in connection with the molecular mechanisms of nerve impulse transmis-

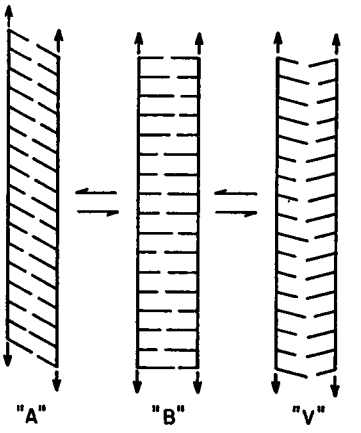

FIGURE 3 Schematic illustrations of possible DNA transitions which could produce birefringence anomalies of the type seen in high electric fields.

sion and of information storage in the central nervous system. It is known that action potentials in nerves are about 0.1 volt, applied across nerve membranes which are of the order of 100 A thick (16). This corresponds to electric fields of the order of 10^5 v/cm. If macromolecule structure changes can be produced by fields of this order of magnitude, it is conceivable that the changes observed in ion permeabilities of the nerve membrane during transmission of the nerve impulse could be due to macromolecular structural transitions produced by the electric fields. It also seems possible that the transcription of nerve impulses into sensory response, and the recording of nerve impulses, *i.e.*, the memory mechanism, might involve the direct action of the electric field on some special macromolecular structure, perhaps a nucleic acid. The electric field structure effect may be an even more widespread phenomenon controlling the permeability of cell membranes; if this proves to be the case, it may be a basic mechanism of all life forms above the virus level.

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