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Increase in ϕX174 DNA Radiation Sensitivity Due to Electric Fields

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The application of an external electric field simultaneously with γ irradiation to an aqueous suspension of ϕX174 DNA (in the RFI form) is shown to increase significantly the number of strand breaks. Tritiated DNA allowed the number of single-strand breaks to be estimated from changes in the scintillation of electrophoretic gel band associated with the fastest mobility moiety. At 400 V ($\approx$2400 V cm^{-1}) the corrected increase (corrected for phoresis of DNA on the stainless-steel plates) in the *G*-value yield is 38%. The increase in damage with field strength appears to follow the increase in reduced dichroism. Dichroism results correspond at 400 V to approximately 10% of the maximum orientation. Our results support the conjecture that this significant increase in DNA–radiation interaction with an electric field is due to field-induced conformation changes in the molecule.

INTRODUCTION

The object of the research reported here was to establish whether or not orientation of DNA in electric fields would result in a significant increase in its sensitivity to damage by ionizing radiation. It is well known that linear polyelectrolytes, particularly polynucleotides and polypeptides, are electrically and optically anisotropic and exhibit the phenomena of birefringence and dichroism (*1*). Basic information on the macromolecular dimensions, size, and shape can be inferred from relaxation kinetics of the field-induced changes. It has been shown that the very large dipole moments which are induced in DNA by external electric fields via displacement of the counterion atmospheres are responsible for its orientation along the field direction (*2*, *3*). The induced dipole moments of linear polyelectrolytes may become very large and depend on the length of the molecule and the ionic density of the medium. For rod-like molecules with lengths the order of 1000 Å, at low ionic density (<10 mol d m^{-1}) induced moments of up to 10^5 D have been determined. The induced dipole moment of an individual linear polyelectrolyte dependence on electric field displays a Langevin-

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like behavior (*4*). Saturation of the polarization occurs at moderately high electric fields; when the induced moment becomes independent of the external field and the molecules behave as possessing permanent dipoles. Intramolecular dipole reorientations in polyelectrolytes induced by electric fields can also occur; an excellent example illustrating this is the rapid helix/coil transition of a polypeptide in a viscous solution (*5*).

The overall rotation of a macromolecule in solution is very slow compared to the fast orientation of dipolar helical sequences. In the case of ϕX174 DNA the normal form is a supercoiled circle which has rod-like characteristics and therefore will orient in an external field. In addition to orientation there will be considerable conformational changes as the field strength increases. With significant orientation and conformational effects of electric field on DNA in solution it would not be unexpected to find a corresponding large change in the interaction of ionizing radiation with DNA via the indirect mechanism. A considerable literature exists on the effects of radiation on DNA in aqueous solutions. We have selected ϕX174 DNA because of the well separated and simple band pattern which it produces in gel electrophoresis for the damaged and undamaged forms.

ELECTRIC DICHROISM OF MACROMOLECULES

Orientation of a suspension of macromolecules in solution can be produced by either static or pulsed electric fields. The applied field-induced orientation results from interactions of the field with the permanent molecular electric dipole, the molecular polarizability, and the transition dipoles of the molecules. In general, the polarizability contribution to the orientational interaction energy is a complex function of the ionic atmosphere of the polyion. Model calculations of the effects of the polarization of the surrounding counterions on the macroions have been performed by Rau and Charney (*6*, *7*). Electric linear dichroism refers to the difference in the selective absorption of light polarized perpendicular to the direction of the external field and the absorption of light polarized parallel to the applied field. By analogy with the definition of birefringence the reduced parallel dichroism (*8*) is defined as

$$\Delta A_{11}/A = (A_{11}(E) - A)/A \tag{1}$$

where A is the isotropic absorbance and A_{11} is the optical absorbance of the solution for light polarized parallel to the applied electric field. The isotropic absorbance is taken to be field independent, that is where dichroism arises only from orientational induced anisotropy. The reduced dichroism, $\Delta A_{11}/A$, is concentration independent. For an ensemble of macroions the reduced parallel dichroism can be written as the product of an optical term, $(G(\theta))$, and an orientation function Φ (*9*)

$$\Delta A_{11}/A = G(\theta)\Phi \tag{2}$$

where θ denotes the angle between the field direction and the transition moment of the absorbing moiety. For optical transitions of the nucleic acid bases at $\lambda = 260$ nm, $G(\theta)$ will approach -1 and Φ will approach unity at high fields (saturation orientation). Charney and Yamaoka (*9*) have determined $G(\theta)$ for DNA in aqueous solution at different salt concentrations (0.09 to 1 m*M* NaCl) and found that at $\lambda = 270$ nm, $G(\theta)$ varied between -0.58 to -0.67 depending on the molecular weight of the DNA.

Our measurement of the specific reduced dichroism dependence on field voltage for a dilute aqueous solution of ϕX174 DNA with 0.8 m*M* Tris buffer is shown in Fig. 1.

The optical density of the solution was 0.15 at 260 nm and 0.077 at 280 nm. In the Kerr cell used for the measurement, the electrode spacing was 0.15 cm; thus it is seen that saturation sets in at about 15,000 V cm^{-1} (2400 V). A least-squares fit of the arc tan function is shown in Fig. 1; however, several other expressions can be shown to fit equally well. A theory of optical activity for circular molecules is currently lacking. There is evidence that unfolding of the coiled molecule occurs as part of the orientation effect (*10*). Transient electric dichroism measurements offer a means of studying conformational changes. In general, one expects the decay of the dichroic signal to exhibit at least two decay constants: a fast transient time constant arising from internal molecular reorientation and a much slower time constant due to reorientation of the entire molecule. This has been confirmed by Revzin and Neumann (*11*) in their studies of the effects of electric field impulse of 30 μs on *E. coli* ribosomal RNA in solution. Transient dichroic measurement by Chen and co-workers (*12*) allowed for the determination of the physical extension of the dG-dC polymer on its transition from its B to Z form. Our transient dichroic measurements on ϕX174 DNA will be reported in a separate publication.

EFFECT OF IONIZING RADIATION ON DNA IN SOLUTION

The types of damage induced in DNA by exposure to ionizing radiation include single-strand breaks, double-strand breaks, cross-linking, and base damage. It is known

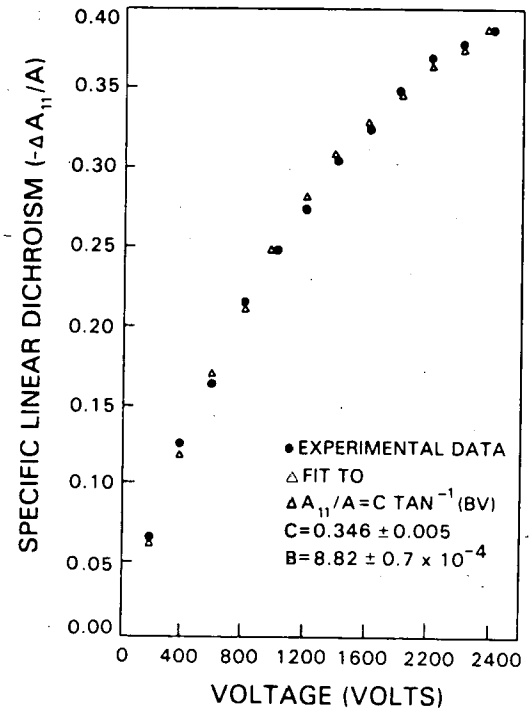

FIG. 1. Specific reduced dichroism versus electric field voltage for ϕX174 DNA in 0.8 m*M* Tris at pH 7.5 with OD = 0.15 at 260 nm.

that DNA damage in aqueous solutions is predominately caused by the indirect action of $OH^{\cdot}$ radicals initially produced by radiolysis of water (*13*). Consider the electrophoretic gel-band pattern for ϕX174 DNA as illustrated in Fig. 2. The normal conformation is that of the supercoiled circle—the RFI form—designated as Band I in Fig. 2. A single-strand break is sufficient to release tension in the RFI form, thereby forming the "nicked" circular form represented in the gel pattern by Band III. This form has the lowest mobility in the gel. A double-strand break causes the circle to unfold and gives rise to the linear form, represented by Band II. In this latter form the DNA would be devoid of any biological activity. If the DNA is tritiated the intensity in each band can be monitored by scintillation counting. Postulating that single hits by γ irradiation produce the "nicked" circular form, then from target theory it can easily be shown that the number of single-strand breaks n_{ssb} (for low total doses) is given by

$$n_{ssb/mol} = \ln \frac{RFI_0}{RFI_D} \tag{3}$$

where RFI_0 denotes the counts/minute in the RFI band for the unirradiated sample and RFI_D is the counts/minute in the RFI band for a total dose of D (rad).

After irradiation of DNA in dilute aqueous solution the number of single-strand breaks per molecule is proportional to the dose,

$$B_{ssb/nT} = kD \tag{4}$$

where k is the probability of a break per nucleotide (nT) per rad. At a DNA concentration of 0.2 mg/ml the value found for k was 4.15×10^{-7} rad^{-1} (*14*). The molecular weight of ϕX174 DNA is 3.4×10^6 Da, corresponding to 3.5×10^2 Da/nT or approximately 10^4 nT per RFI molecule. Hence the theoretical number of single-strand breaks in ϕX174 DNA per molecule is

$$B_{ssb/mol} = 4 \times 10^{-3} D. \tag{5}$$

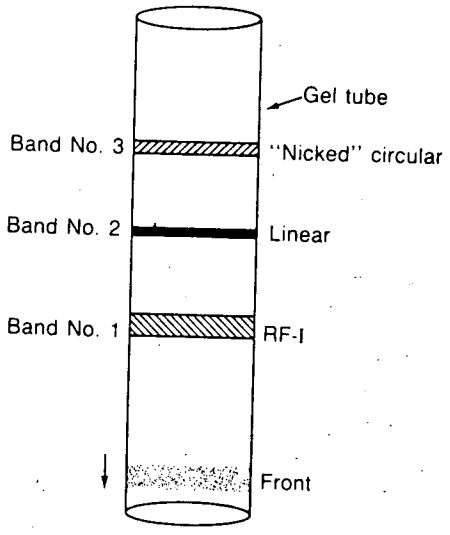

FIG. 2. Gel pattern for ϕX174 DNA.

An alternative expression for the yield of single-strand breaks is the G value, given by

$$G_{ssb/mol} = 9.65 \times 10^4 C/MD_{37} \quad (6),$$

where C is the concentration of DNA in grams per milliliter, M is the molecular weight in daltons and D_{37} is the 37% survival dose. At a concentration of 0.75 mg/ml the 37% survival dose is the order of 400 rad (*15*).

EXPERIMENTAL DETAILS

Kerr Cell

The same Kerr cell employed in dichroism measurements was used for exposing dilute aqueous solutions of ϕX174 DNA with Tris buffer, EDTA (pH 8.0) to intense ^{60}Co irradiation. A diagram of the cell is shown in Fig. 3. The cell is a high quality Lucite cylinder, out of which a rectangular cavity was cut along the equatorial plane. The cavity was lined by stainless-steel plates spaced 0.15 cm apart. The cavity ends were sealed by Lucite plates screwed down to impinge on Teflon seals. Other fittings included two filling holes, two steel pins which contacted the steel electrodes and connected them via external cables to the power supply. To compute the dose to the solution in the cavity the tissue to air (TAR) ratio was determined both experimentally and theoretically (using the Bragg–Gray principle governing small cavity ionization). With the cell/source configuration used in the experiment, the computed TAR value was 0.94, a value confirmed experimentally. Thus for an air dose rate of 7005 rad/min this gave a solution dose rate of 6505 rad/min. There was an additional dose of 487 rad due to the finite time it takes to raise and lower the cobalt source. The maximum total exposure time was 2 min giving a total dose of 13,627 rad.

Electric Power Supply

The mobility of DNA in solution is quite high, of the order of 5×10^{-4} cm^2 s^{-1} V^{-1} at a sodium ion concentration of 10 mM. With electrode separation of 0.15 cm at 400 V, it is estimated that all DNA would be phoresed on the electrodes in about 125 ms. DNA molecules bind covalently to the metal and field reversal unfortunately does not remove it. The power supply could deliver a maximum of 2000 V at 100 mA. The vacuum relay which accomplished the field reversal had an "ON" time of 140 ms with a "dead" period between reversal of about 50 ms. Figure 4 reports the

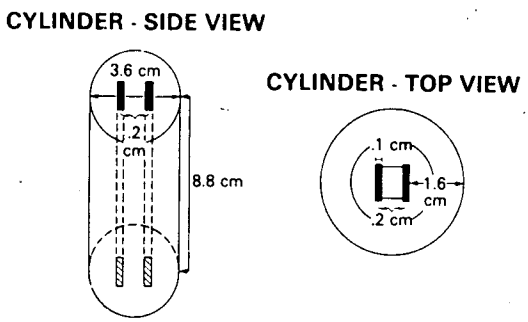

FIG. 3. Schematic of Kerr cell. Cylinder made of high quality Lucite with stainless-steel electrodes.

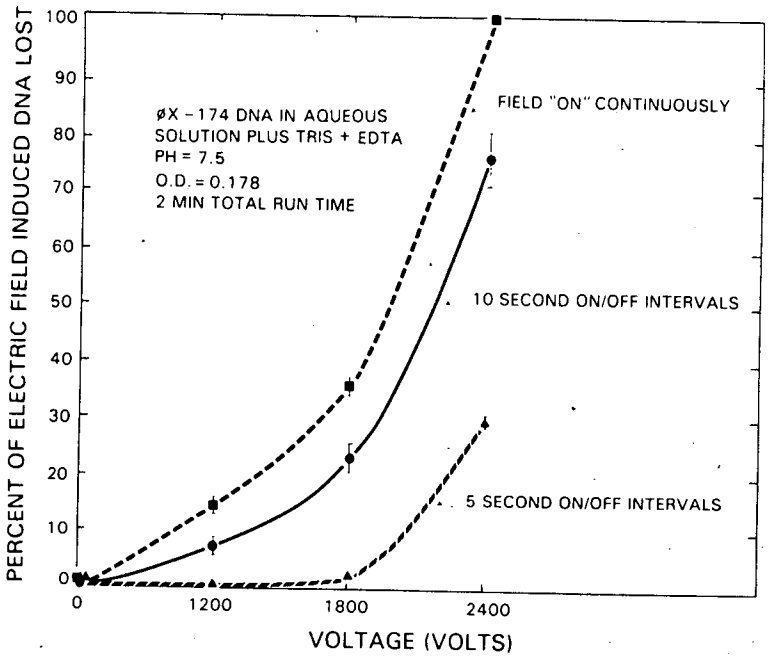

FIG. 4. Electric field-induced loss of DNA by electrophoresis in the Kerr cell.

results of measuring DNA loss by electrophoresis in the cell. The percentage loss of DNA was determined by taking 2 μl of the exposed DNA (to the field dose for periods of 2 min) and adding them to 10 ml of scintillation "cocktail" for counting (the DNA was tritiated). It is evident that there is an unacceptably large loss of DNA even at 400 V. These losses can be reduced by switching the field ON and OFF every 10 s and even more if the interval was reduced to 5 s. In the second radiation experiment, this tactic was resorted to and allowed a field voltage of up to 400 V to be utilized. Under these conditions the total exposed time to the electric field was reduced to 1 min. In our first experiment, the field was applied continuously, but the maximum voltage used was 250 V. In all cases, corrections to computed yield values for electrophoresis losses were made.

ϕX174 DNA

The ϕX174 DNA used was supplied in the Replicative Form I (Genex Corp., Gaithersburg, MD). Some nicked circular form was present. The tritiated DNA allowed for quantitative assessment of damage by scintillation counting as discussed above. The solution concentration was 0.75 mg/ml for the experiment. This was diluted to produce an optical density between 0.18 to 0.20. The solution was buffered with Tris and EDTA to pH 8.

Gel

Gel pattern for the solutions used is shown in Fig. 5 (background—0 V/0 radiation). One percent agarose tube gels were formed and ethidium bromide dye was used after the electrophoresis. The gel tubes were examined under uv light and photographed. The bands (I, II, and III) were dissected out of the gels placed in containers with 20 ml of scintillation "cocktail," and the relative DNA contents were measured in an automatic scintillation counter unit.

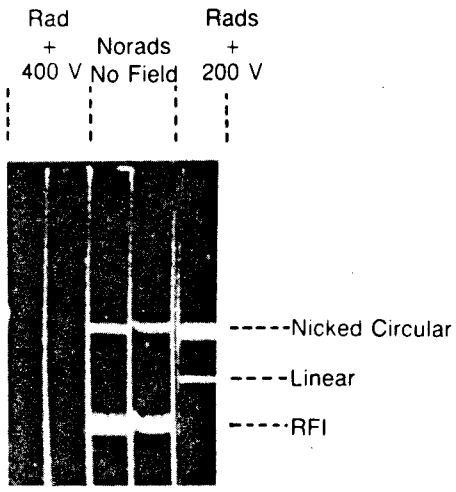

FIG. 5. Gel bands for radiation experiment No. 2.

EXPERIMENTAL RESULTS

First Radiation Experiment

In this experiment three exposures of the ϕX solution in the Kerr cell to the ^{60}Co source were made. The exposures were 2 min long, with a total absorbed dose of 13,622 rad for each exposure. The electric field was ON continuously. The conditions of the three exposures were

Number 1	Radiation + 250-V field
Number 2	Radiation + 200-V field
Number 3	Radiation only.

In addition three control measurements were made:

Number 4	250-V field only
Number 5	200-V field only
Number 6	Background—0 radiation/0 field.

The production of the nicked circular form (Band No. 3) and linear form (Band No. 2) through radiation interaction is clearly seen for the "rad only" case, and these bands are more pronounced in the presence of the electric field (200 and 250 V). A fourth band is evident and corresponds to low mobility material considered to be a contaminant.

Table I summarizes the relative intensities in the various bands in terms of scintillation counts/minute. It is seen that there is a substantial loss of DNA through electrophoresis even at the 250- and 200-V level.

Using Eq. (2) the yield of single-strand breaks (or more) per ϕX molecule, $G_{ssb/mol}$ was computed from Table I for the three radiation runs. These are given in column 1 of Table II and designated as "uncorrected." The corrected values, obtained by subtracting out the electrophoretic losses, are given in column 2. The percentage increase in radiation damage (single-strand breaks and more), attributable to the electric field effect, is also computed and is 40% at 250 V (1800 V/cm) and 27% at 200 V (1200 V/cm).

TABLE I

Scintillation Counts per Minute—Experiment No. 1

Run No./Band No.	*4*	*3*	*2*	*1*
1. rad + 250 V	810	7,100	806	1,612
2. rad + 200 V	680	12,700	218	2,238
3. rad	640	25,400	4,500	5,455
4. 250 V	791	1,100	484	18,621
5. 200 V	740	950	640	20,500
6. Background	868	7,140	545	31,221

Second Radiation Experiment

In this experiment, voltages of 200, 250, 300, 350, and 400 were used. As mentioned earlier, to minimize electrophoretic loss of DNA, the field was switched ON for 5 s and OFF for 5 s sequentially over the 2-min exposure interval. Some reduction in field effect would be expected, and so the results of Experiments 1 and 2 are not exactly comparable. The gel patterns obtained for the 400 V, 300 V, and background cases are shown in Fig. 5. The increase in nicked circular and linear form can be seen at 200 V. Very faint bands were apparent at 400 V and most of the effect here is due to electrophoretic loss.

Table III shows the background, 400 V, and 400 V with γ-radiation results in counts/minute, the uncorrected and corrected single-strand break yields, and the percentage increase in single-strand breaks.

It is seen that at 400 V the corrected increase in the value of the yield, $G_{\text{ssb/mol}}$, is 38%. The predicted number of ssb for ϕX174 DNA molecules at a concentration of 2 mg/ml is

$$G_{\text{ssb/mol}} = 4.15 \times 10^{-3} D$$

where D is the dose in rad. For a total dose of 13,627 rad this relationship predicts a yield of about 56 ssbs per ϕX molecule. The concentration used here was about 0.008 mg/ml and scaling linear, a yield value of about 2 would be expected. Values obtained in the "rad only" cases of experiments 1 and 2 are in the vicinity of 1.7. Within experimental errors (estimated at about ±20%), the correspondence is close enough to confirm the reliability of the results obtained in these experiments.

Figure 6 shows the percentage increase in damage plotted versus field strength. The corrected and uncorrected curves are shown and the specific reduced dichroism curve is superposed. The increase in damage with field strength appears to follow the increase

TABLE II

Single-Strand Break Yields in Experiment No. 1

Run No.	*Uncorr.*	*Corr.*	*% Increase*
1. rad + 250 V	2.96	2.44	40
2. rad + 200 V	2.63	2.21	27
3. rad	1.74	—	—

TABLE III

Radiation Results—Experiment No. 2

Run	Band No.			G		% Inc. in G	
	3	2	1	Uncorr.	Corr.	Uncorr.	Corr.
1. Background	1500	560	8300	—	—	—	—
2. Radiation	6350	1250	1500	1.71	—	—	—
3. 400 V	1250	940	3800	—	—	—	—
4. rad + 400 V	4620	850	358	3.14	2.36	83	38

in reduced dichroism. At a field strength of 2400 V/cm (400 V) the increase is about 38%. Dichroism results correspond at 400 V to only about 10% of the maximum orientation. This observation supports the conjecture that this significant increase in DNA/radiation interaction is due to field-induced conformation changes in the molecule.

CONCLUSIONS

The results demonstrate a significant electric field-induced increase in ^{60}Co γ-ray damage to ϕX174 DNA in dilute aqueous solution, even at field strengths of 2400 V/cm and below. It is likely that the conformational changes in the ϕX molecule are

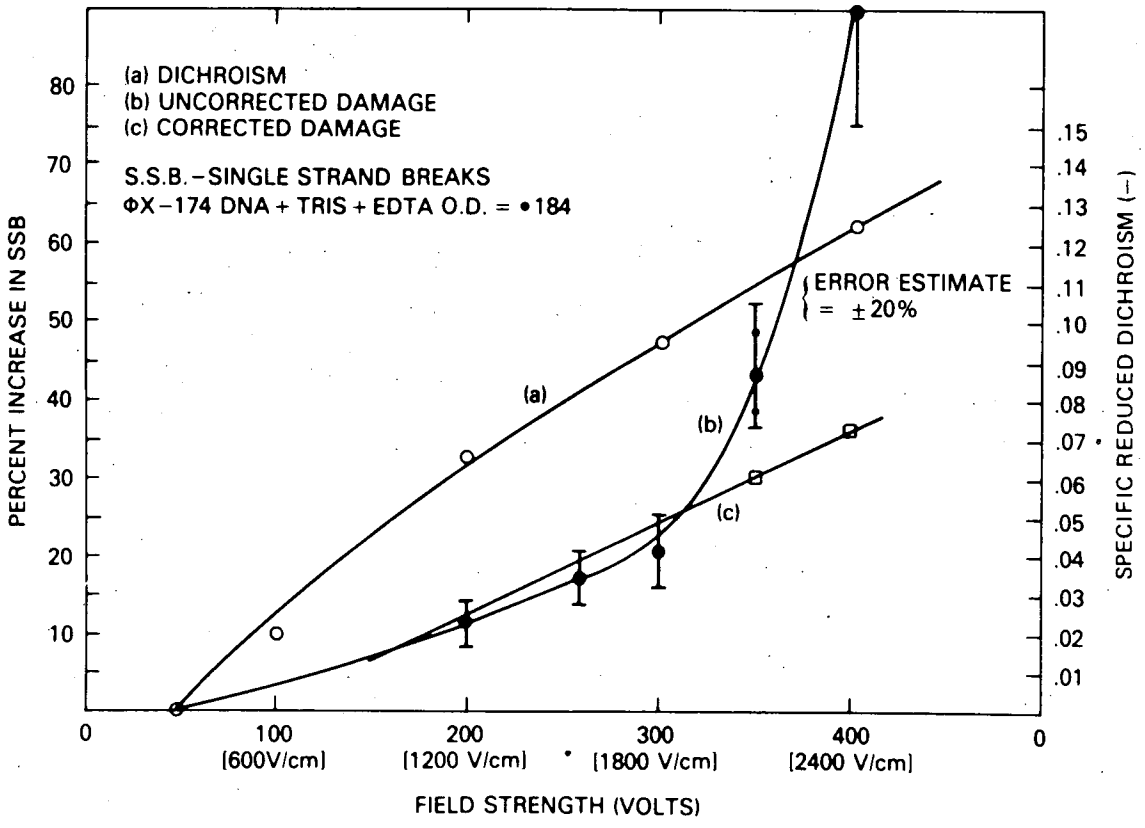

FIG. 6. Increase in radiation damage of ϕX174 versus field strength (V/cm). Open and solid circles and open boxes denote data points. Lines drawn for clarity.

the source of this increased interaction, leading to considerable exposure of reactive sites on the DNA molecule to the action of the $OH^{\cdot}$ radicals. Linear extrapolation of the damage versus field strength curve, to a saturation value of 15,000 V/cm, would predict that the percentage increase in damage would rise to over 250%. This large predicted increase needs to be verified by further experiments. The present results are promising enough to warrant a similar investigation using intracellular DNA. In this connection it must be noted that the phenomena of electric dichroism has already been established for chromatin subunits (*16*, *17*).

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