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ANALYSIS OF GAMMA-RAY DAMAGE TO SUPERCOILED DNA
FROM ELECTROOPTICAL BIREFRINGENCE RELAXATION TIMES

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Electrooptical birefringence (EO) has been used to analyze changes in the conformation of DNA resulting from the interaction of various forms of physical and chemical agents [1]. In the majority of the EO studies conducted to date, the characterization of altered DNA conformations has been based on changes in the mean relaxation time, $\bar{\tau}$. By using a computer-based, nonlinear regression analysis, we have now been able to accurately resolve the decay portion of the EO signal amplitude into a series of 3 exponential terms, thereby providing a more detailed picture of the changes in molecular rotational parameters that result from DNA structural alterations.

EO measurements were performed on covalently closed, supercoiled Simian Virus 40 (SV40) DNA both in its native form, and after graded doses of ^{60}Co γ -irradiation ranging from 2.5 to 104.6 Gy. Fig. 1 shows the characteristic relaxation times and population parameters for native and γ -irradiated SV40 DNA in solution. The decay of the EO signal amplitude for native DNA can be represented as a sum of 3 exponentials with characteristic relaxation times $\tau_1 = 12\mu\text{s}$, $\tau_2 = 90\mu\text{s}$, and $\tau_3 = 650\mu\text{s}$. Based on studies in our laboratory with DNA fragments, the shortest relaxation time can be attributed to internal molecular motion. The longer relaxation times, τ_2 and τ_3 , are associated with two major modes of molecular rotation. All 3 characteristic relaxation times are unchanged by γ -ray doses up to 105 Gy, and the subpopulation of molecules, a_1 , exhibiting the relaxation time τ_1 also undergoes little change as a function of the absorbed radiation dose. However, the population parameter a_2 decreases from approximately 50% to 40%, and a_3 increases from 17% to 27% as the γ -ray dose is increased up to 50 Gy. The increase in the fraction of DNA molecules exhibiting the longest relaxation time would be expected insofar as the accumulation of radiation damage produces a progressively larger number of double-strand breaks, with a resultant increase in the number of linear

molecules. The approximately twofold increase in the mean relaxation time, $\bar{\tau}$, as a function of radiation dose is primarily due to the increase in the population of molecules with the characteristic relaxation time τ_3 . It is interesting to note that the additional damage produced by absorbed doses in excess of 50 Gy does not lead to further changes in the relaxation parameters, and hence the molecular rotational modes, of the irradiated DNA. This type of information can be gained only by a direct determination of the individual relaxation times by the method described here, and could not be obtained by the conventional procedure of estimating a single mean relaxation time, $\bar{\tau}$, using the assumption of a logarithmic normal distribution of component relaxation times.

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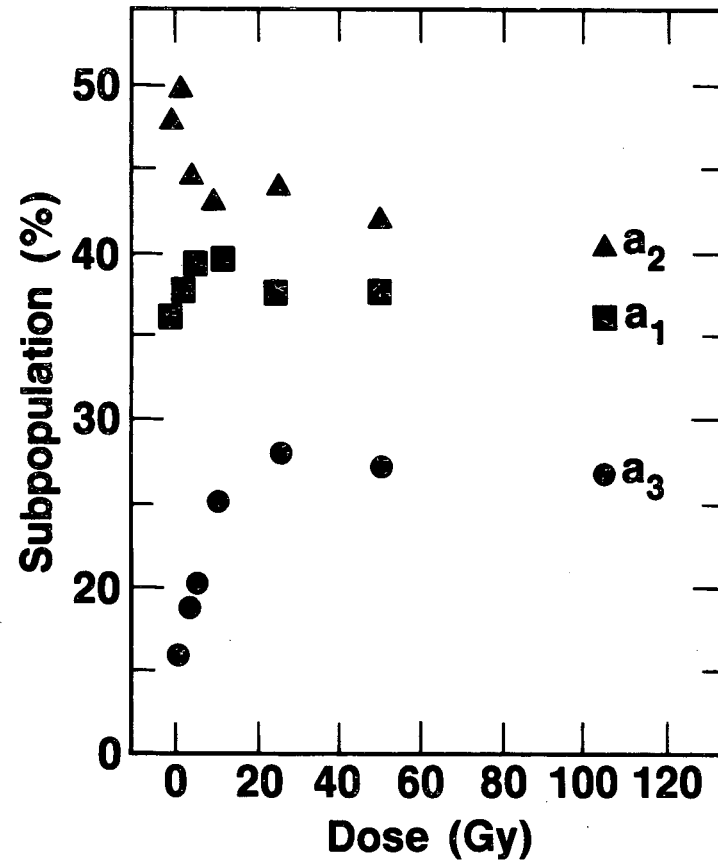
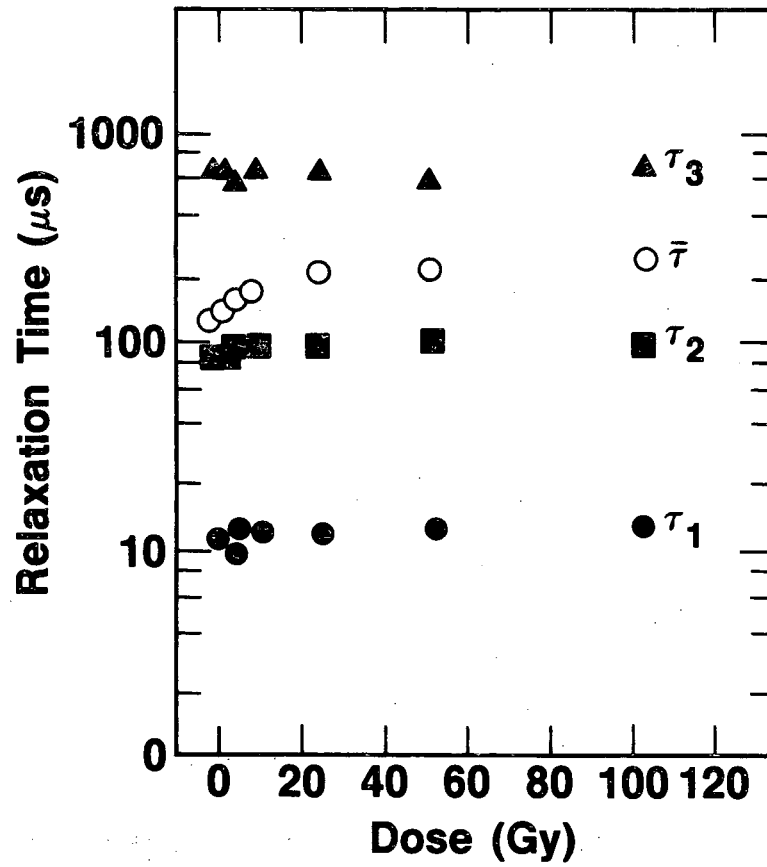
LEGEND TO FIGURE 1

EO relaxation parameters for SV40 DNA are plotted as a function of absorbed γ -ray dose. The plotted points are the mean values of 2 determinations, in each of which 3 repeat measurements were made of the relaxation times. The standard deviations of the relaxation parameters determined in the 2 independent series of measurements were smaller than the plotted points. The DNA samples (20 $\mu\text{g}/\text{ml}$ in 0.5 mM Tris, 0.1 mM MgCl_2 buffer, pH 7.9) were subjected to 200 μs , 9.25 kV/cm square-wave pulses in an EO spectrometer described previously [2]. The transient birefringence signal was captured in a Biomaton 805 recorder and read into a PDP 11/10 on-line computer equipped with an AED 2500 flexible disk storage system. Computer programs were developed to analyze EO signal amplitudes and to fit the decay portion of each curve to a sum of 3 exponentials by means of a nonlinear least-squares algorithm. In equation form, the amplitude $S(t)$ was represented as:

$$S(t) = \sum_{i=1}^3 a_i e^{-t/\tau_i}$$

where each coefficient, a_i , is the fraction of the molecular population with the relaxation time τ_i . The signal to noise ratio in the EO signal amplitude limited the fit of the decay curve to 3 exponential terms. The 3 relaxation times obtained for each fit were used to compute the mean relaxation time, $\bar{\tau}$, as the weighted sum of the τ_i values:

$$\bar{\tau} = \left(\sum_{i=1}^3 a_i \tau_i \right) / \left(\sum_{i=1}^3 a_i \right)$$



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FIGURE 1

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