VISCOELASTIC BEHAVIOR OF MAMMALIAN DNA

ERIC S. CHASE AND RICHARD H. SHAFER, *Department of Pharmaceutical Chemistry, School of Pharmacy and the Brain Tumor Research Center, University of California, San Francisco, California 94143 U.S.A.*

ABSTRACT The viscoelastic behavior of rat 9L cellular DNA was studied as a function of the detergent used for lysis, the pH and duration of lysis, and gamma ray dose. For nondenaturing lysis conditions, a model of the DNA was proposed to account for the effects of these agents on the viscoelastic retardation time. It was concluded that these agents affect the hydrodynamic radius of the DNA rather than its molecular weight. For denaturing lysis conditions, molecular weights calculated from the relaxation time were consistent with those calculated from alkaline sucrose sedimentation profiles.

INTRODUCTION

The sedimentation coefficient is the most commonly measured hydrodynamic parameter of mammalian DNA. In general, the sedimentation coefficient depends on the hydrodynamic radius as well as the molecular weight. For an independent chain polymer (ICP) form of DNA, it is possible to mathematically model the dilute solution dynamics and develop equations relating the sedimentation coefficient, hydrodynamic radius, and other hydrodynamic parameters to the molecular weight. At the present time, ICP forms of DNA include double- or single- stranded linear, branched, and relaxed circular shapes (Zimm, 1956; Bloomfield and Zimm, 1966; Zimm and Kilb, 1959; Bloomfield et al., 1974). However, there are many cases where the DNA is not in an ICP form, or the form of the DNA is not known. It has been found that the sedimentation coefficient of DNA from mammalian cells lysed under neutral conditions responds biphasically to increasing concentrations of intercalating agents (i.e., the sedimentation coefficient first decreases, then increases with increasing drug concentration) (Ide et al., 1975; Cook and Brazell, 1975, 1976). This certainly must be an indication that first, the DNA is supercoiled and thus in a non-ICP form, and second, that the biphasic response is due to changes in the hydrodynamic radius rather than the molecular weight of the DNA.

Under alkaline lysis conditions sedimentation and S1 nuclease experiments indicate that a non-ICP form of cellular DNA is obtained after brief exposure to high pH, whereas longer exposure times yield single-stranded independent linear chain polymers (Ahnstrom and Erixon, 1973; Cleaver, 1974*a*). In the latter case it is possible to calculate the molecular weight from the sedimentation coefficient, since the DNA is in an ICP form.

The present work was undertaken to characterize the viscoelastic response of neutral and alkaline lysates of mammalian cells to understand the packing of large DNA ($>10^9$ daltons) in the nucleus. The viscoelastic relaxation time, like the sedimentation coefficient, is a function of both the hydrodynamic radius and the molecular weight of the DNA. To calculate the molecular weight from the relaxation time, as from the sedimentation coefficient, the DNA must be in an ICP form. We will show that it is reasonable to conclude that (*a*)

mammalian cells lysed under nondenaturing lysis conditions yield a non-ICP form of the DNA, (*b*) changes in the relaxation time under nondenaturing lysis conditions reflect changes in the hydrodynamic radius rather than in the molecular weight, and (*c*) alkaline lysis induces a transition from a non-ICP form to an ICP form of the DNA with increasing lysis time. A preliminary publication of these results has appeared (Chase and Shafer, 1978).

MATERIALS AND METHODS

Sources

Hanks' balanced salt solution (HBSS), Eagle's basal medium (BME), fetal calf serum, amino acids, antibiotics, and vitamins used for tissue culture were obtained from Gibco, Grand Island, N.Y. Sodium decyl sulfate was obtained from Pflatz & Bauer Inc., Stamford, Conn. and the S1 nuclease and Triton X100 were obtained through Calbiochem, San Diego, Calif. ^{3}HdThd (2 Ci/mmol) and PCS were purchased from Amersham-Searle Corp. Arlington Heights, Ill.

Cell Growth and Treaments

9L cells from an *N*-methylnitrosourea-induced rat brain tumor were grown in monolayer cultures. Normally 6×10^5 cells were seeded into a flask, and allowed to grow for 24 h in a humidified 5% CO_2 atmosphere at 37°C with a solution of 0.25% trypsin and 2×10^{-4} M Na_2EDTA. After resuspension in 15 ml of medium, the cells were shaken vigorously for 10 s to break up clumps, then resuspended and diluted in the appropriate saline solution at 4°C.

For experiments involving irradiation, cells were exposed to a ^{137}Cs source after dilution to their final concentration, and kept on ice to minimize repair of the DNA. The dose rate was 500 rad/min, and the longest exposure time was 2 min.

Viscoelastometry

The viscoelastometer used in this study is similar to the instrument described by Klotz and Zimm (1972*a*), and used in several other studies (Klotz and Zimm, 1972*b*; Kavenoff and Zimm, 1973; Uhlenhopp, 1975). The instrument is essentially a Couette viscometer that utilizes a Cartesian diver as the rotor. In a typical viscoelastic experiment the cassette, which contains the lysate, is placed into the instrument, the rotor is placed in the lysate, and the rotor is made to rotate by an electromagnetically-induced torque. This windup stretches the DNA, and when the windup torque is removed, the rotor recoils exponentially under the influence of the relaxing DNA. The time constant of the recoil is known as the retardation time, and when extrapolated to zero windup rate and concentration, becomes the relaxation time of the DNA contained in the lysate. For a more detailed description of this technique, see Klotz and Zimm (1972*a*).

Nonionic Detergent Lysis

Cells were diluted in a solution containing 1 M NaCl and 0.01 M Na_4EDTA to the desired concentration. 1.5 ml of the suspension was added to a cassette and an equal volume of lysis solution was added containing 1 M NaCl, 0.02 M Na_4EDTA, 0.1 M Na_2SO_4, 2% Triton X100, and 0.02 M Tris, pH 8.8. The cassette was quickly inverted twice, and incubated at 26°C for the desired length of time.

Ionic Detergent Lysis

Cells were diluted in 1 M NaCl and 0.01 M Na_4EDTA, and 1.5 ml of cells was added to a cassette. The cassette was then placed in a 45°C water bath for 1 min, then 1.5 ml of a heavily buffered alkaline solution and 0.35 ml of a detergent solution were simultaneously added to the cells. The buffered solution contained 0.25 M Na_2HPO_4, 0.032 M Na_4EDTA, and sufficient NaOH to adjust the pH to the desired value. The detergent consisted of 4% sodium decyl sulfate in distilled water. The cassette was

then quickly inverted twice to assure complete mixing, and incubated for 5 min at 45°C, then lysed at 26°C for the desired length of time.

Protocol for Viscoelastic Experiments

At the end of the lysis period, the rotor was placed in the lysate, the pressure bar was put into place, and the rotor was forced to the bottom of the cassette by adjusting the downward force exerted by the pressure bar. The downward force was then gradually decreased until the rotor starting moving up. The rotor height servo was then engaged, and the windup was performed. The total windup was 50°, and the shear rate during the windup was approximately $1 \times 10^{-2}\,s^{-1}$. More procedural details may be found in Chase (1978).

Recoil Curve Analysis

The recoil curves obtained from the viscoelastic experiments were analyzed on the PROPHET computer for the retardation times and the magnitude of the recoil. The PROPHET system is a unique national computer resource developed by the Chemical/Biological Information-Handling Program, Division of Research Resources, National Institutes of Health. A detailed description of this system has been published (Raub, 1974). The recoil curves initially appeared on a strip chart recorder, and were digitized into the computer for further analysis. An iterative nonlinear least-squares fitting routine analyzed the curves in terms of one or two exponential decay processes. A semilog plot of the experimental points with the best computer fit was supplied by the program, as well as statistical information about the goodness of fit.

S1 Nuclease Assay

Cells were grown as described above, with the addition of 0.2 μCi/ml ^{3}HTdR at the time of seeding. Periodically, small aliquots were removed from the lysates and added to 3 ml of the S1 buffer, which consisted of 0.3 M NaCl, 0.03 M NaOAc, and 0.003 M ZnCl at a pH of 4.5. Then 25 μg of nonradioactive heat-denatured salmon sperm DNA and 0.025 IU of S1 nuclease were added. The

TABLE I
TYPES OF VISCOELASTIC RESPONSES OF 9L CELL LYSATES

	Observed retardation time	Observed recoil as fraction of windup	Postulated configuration	Calculated* relaxation time	Calculated* hydrodynamic radius
	s			*s*	*μm*
Nucleoid	0	0	•	5	4
Type I	1,500–2,400	0.2–0.4	✱	2,000	26
Type II	3,500–10,700	0.50	✱	10,000	45
Type III	400–800	0.1–0.2	—	—	—
Type IV	70	0.02	Single-stranded linear ICP	57	4
Type V	200	1.0	Gel	—	—

*The relaxation times of the branched forms were found by first calculating the relaxation time of a linear, unbranched DNA polymer of molecular weight 3×10^{12} from the relationship $M = 2.2 \times 10^8\,\tau^{0.60}$ (Klotz and Zimm, 1972*b*). Then $\tau_b/\tau_u = (2/f)^{1.5}$ was used (Zimm and Kilb, 1959), where τ_u is the longest relaxation time of the unbranched DNA, τ_b is the longest relaxation time of the branched DNA, and f is the number of branches. The hydrodynamic radii were found by first calculating the hydrodynamic radius of an unbranched DNA polymer with a molecular weight of 3×10^{12}, and then using $R_b/R_u = 1.88\,f^{-1/2}$ where R_u is the radius of gyration of the unbranched polymer, and R_b is the radius of gyration of the branched polymer. R_u was calculated from the Flory relationship between the intrinsic viscosity and the radius of gyration, using the standard equation for the intrinsic viscosity in terms of molecular weight (Bloomfield et al., 1974).

mixture was then incubated at 50°C for 2 h. Double- and single-stranded DNA controls were run with each experiment. Usually not more than 5% of the double-stranded DNA was digested, while more than 85% of the single-stranded control DNA was digested. The amount of single-stranded DNA in the lysates was determined in the manner described by Linn and Wheeler (1975).

RESULTS

The viscoelastic responses of the cellular lysates fall into five general types. The retardation time and fractional recoil of each type of response are shown in Table I.

Nonionic Detergent Lysis

When cells were lysed with a nonionic detergent with a final DNA concentration of 30 ng/ml, no viscoelastic response was seen for lysis times up to 6 h. The significance of this result will become apparent later.

Ionic Detergent Lysis pH <11.40

When cells were lysed for various times with an ionic detergent at a DNA concentration of 30 ng/ml, the results shown in Fig. 1 were obtained. Between 3 and 5 h of lysis the retardation time had a value characteristic of a type I response. It was found that the pH had to be in the range of 10.00–11.40 to obtain a type I response. Lysis times >8 h usually gave the gel-like type V response; however, lowering the DNA concentration to 15 ng/ml seemed to favor the formation of a type II response at long lysis times. Type V responses were obtained with lytic periods of up to three d.

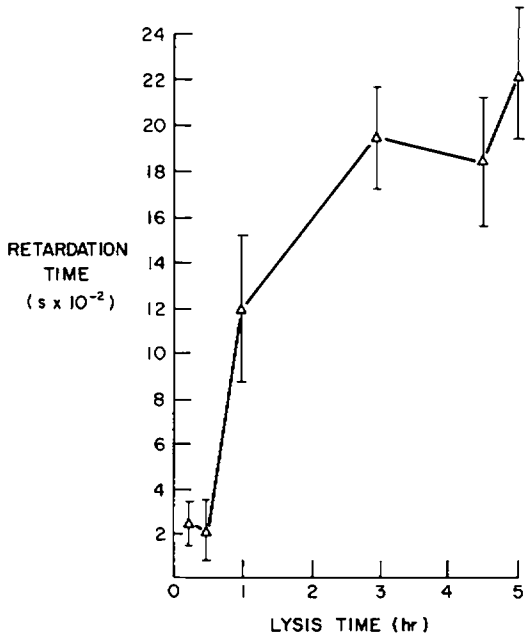

FIGURE 1 Retardation time as a function of the lysis time for ionic detergent lysis, pH < 11.40. The bars indicate standard deviations.

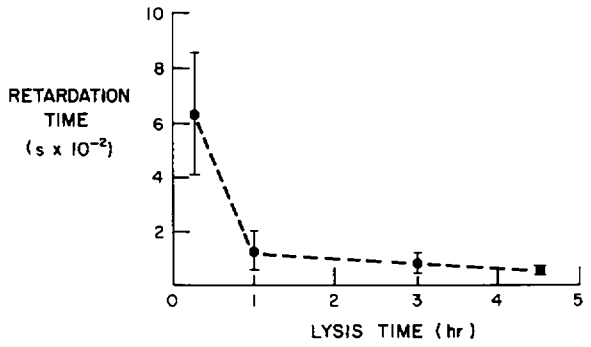

FIGURE 2 Retardation time as a function of the lysis time for ionic detergent lysis, pH < 11.70. The bars indicate standard deviations.

Ionic Detergent Lysis pH >11.70

When the pH of the lytic solution was raised, dramatically different behavior of the retardation time as a function of lysis time was seen. This series of experiments used DNA concentrations from 140 to 200 ng/ml. Fig. 2 shows the behavior of the retardation time as a function of the lysis time for the higher pH. The retardation time decreased from approximately 600 s at short lysis times to 70 s after 4.5 h of lysis. Thus lysis at a pH of 11.70 or greater gave a type III response at short lysis times, and a type IV response at longer lysis times.

Effect of pH at Fixed Lysis Time

When the lysis time was held fixed at 4.5 h, either type I, II, or IV responses were seen depending on the pH of lysis. These results are shown in Fig. 3. With a pH between 10.00 and 11.40, type I behavior was found. With a pH between 11.40 and 11.55, type II responses were found, and with a pH >11.70, type IV responses were seen.

Effect of Gamma Rays at Fixed pH and Lysis Time

The effect of gamma irradiation on the retardation time was studied by lysing previously irradiated cells. For this series of experiments, the DNA concentration was 30 ng/ml, the lysis time was between 4 and 5 h, and the pH was 11.40. The dependence of the retardation time on the radiation dose is shown in Fig. 4. The retardation time rose steeply up to a dose of 150 rad, where it peaked at a value of ~10,700 s. With still higher doses, the retardation time decreased, such that after a dose of 1,000 rad there was a very short retardation time with little viscoelastic recoil. Thus with increasing doses of radiation, type I, II, and IV responses were seen in succession.

S1 Nuclease Assay

The appearance of single-stranded DNA as a function of lysis time for ionic detergent lysis at a pH of 11.60 is shown in Fig. 5. When cells were lysed using a range of pH from 11.10 to 11.90, the appearance of single-stranded DNA as a function of lysis time was similar to that seen in Fig. 5, except for the level at which the plateau occurred. By altering the pH of lysis, it was possible to obtain plateau values from 5 to 95% single-stranded DNA. These plateau values are shown in Fig. 6 as a function of the pH of lysis. Also shown are the retardation

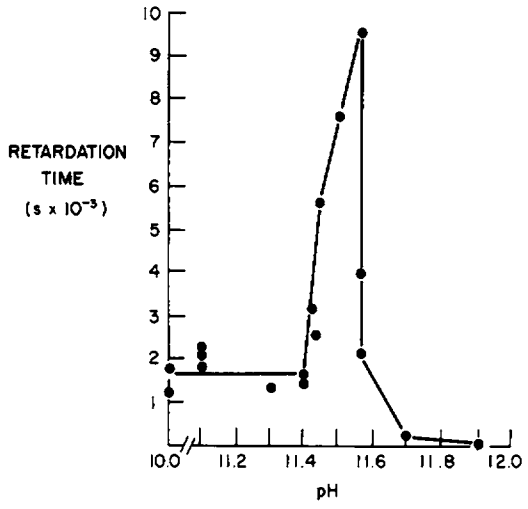

FIGURE 3

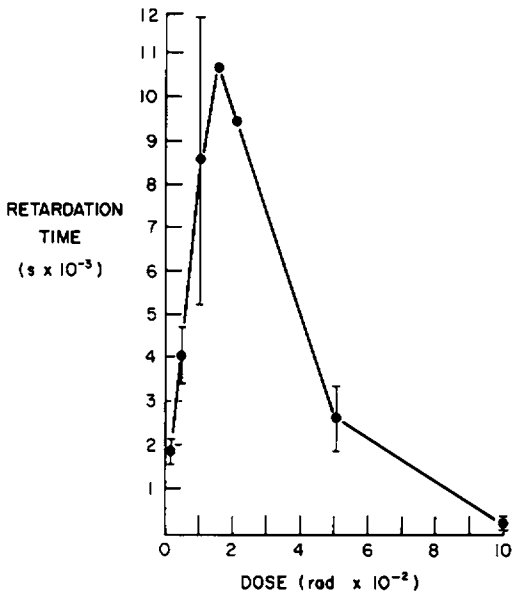

FIGURE 4

FIGURE 3 Retardation time as a function of the pH of lysis for ionic detergent lysis, and lysis time of 4.5 h.
FIGURE 4 Retardation time as a function of the gamma ray dose for ionic detergent lysis for lysis times between 4 and 5 h, pH of 11.40. The bars indicate standard deviations.

times obtained as a function of pH. The amount of single-stranded DNA shown in Fig. 6 has been normalized to control double- and single-stranded standards. Note that regions of DNA that are locally denatured but not strand-separated may renature rapidly upon neutralization in the S1 buffer. Therefore, such regions would be recognized as double-stranded by the S1 assay (Wang and Wheeler, 1978).

DISCUSSION

It is obvious from the above results that gamma irradiation, the detergent used for lysis, and the pH of lysis have a profound effect on the viscoelastic response of mammalian DNA. With the aid of previous sedimentation studies, and the present viscoelastic and S1 nulcease studies, it is possible to gain some insight into the forms of mammalian DNA.

Evidence to be presented indicates that type I, II, and III responses arise from a non-ICP form of the DNA. Since the only relationships that exist between the relaxation time and molecular weight are for ICP forms, we are faced with the problem of correctly interpreting the viscoelastic responses arising from non-ICP forms of the DNA. An approach is to use an ICP model of mammalian DNA that is a reasonable representation of the non-ICP forms. The remarkable electron micrographs of the *E. coli* nucleoid (Kavenoff and Ryder, 1976) show spokes of DNA radiating out from a central core. A reasonable ICP model of the DNA, then, is a branched form with equal sized spokes of double-stranded DNA radiating from a central core (Zimm and Kilb, 1959). With this model, it is possible to calculate the relaxation time and the hydrodynamic radius of the DNA as a function of the number of branches, while keeping the total molecular weight fixed.

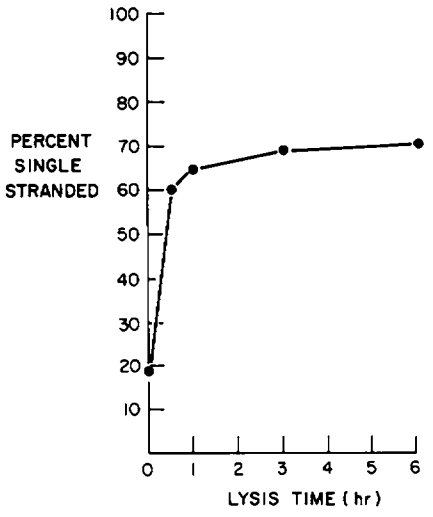

FIGURE 5

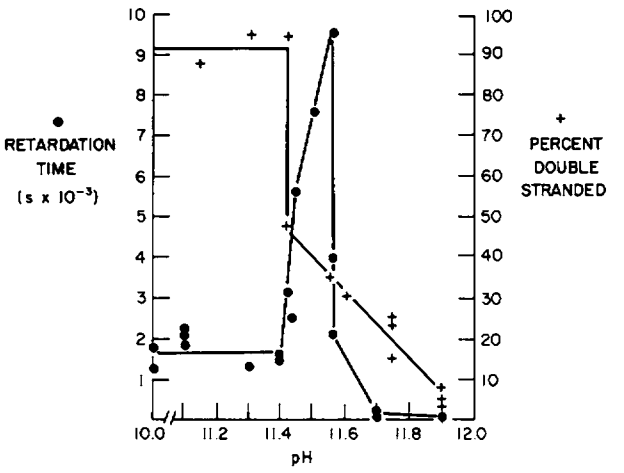

FIGURE 6

FIGURE 5 The percent of DNA digestible by S1 nuclease as a function of the lysis time for ionic detergent lysis, pH of 11.60.

FIGURE 6 The percent of DNA digestible by S1 nuclease as a function of the pH of lysis for ionic detergent lysis. Also plotted are the retardation times obtained as a function of the lysis pH.

We postulate that the DNA of a cell remains in a single hydrodynamic unit under nondenaturing lysis conditions with a molecular weight of 3×10^{12}, equivalent to the DNA content of each 9L cell. The following observations suggest that this may not be an unreasonable assumption. Fluorescence microscopy indicates that even when the nuclear membrane is removed with a nonionic detergent, the cellular DNA remains as a single unit (Hancock, 1974). Micromanipulation experiments with metaphase chromosomes indicate that they are linked together in some fashion (Diacumakos et al., 1971), and nuclease digestion experiments suggest this linker may be DNA (Hoskins, 1968). Previous viscoelastic measurements on lysates of Drosophila (Kavenoff and Zimm, 1973) and the yeast *S. cerevisiae* (Lauer et al., 1977) appear to be consistent with the hypothesis that each chromosome consists of a single piece of DNA. However, in the case of the Drosophila studies, the amount of viscoelastic recoil was inconsistent with the assumption of a linear ICP form for the DNA (Roberts et al., 1977). In the yeast studies, because of the possibility of shear degradation of the DNA, the existence of genome-sized pieces of DNA could not be ruled out.

We wish to point out, however, that the assumption that all the DNA remains in a single hydrodynamic unit mainly serves to facilitate the ensuing discussion. Our conclusions regarding changes in the hydrodynamic radius of the DNA do not depend on this assumption.

Branched configurations used to model the viscoelastic behavior of DNA observed in this study are presented in Table I. The first configuration we deal with is that produced by nonionic detergent lysis. Fluorescence microscopy indicates such DNA is released from cells

in the form of 5–25 μm radius spheres, termed nucleoids (Cook et al., 1976; Hancock, 1974). It is also known that intercalating agents induce a biphasic response in the nucleoid's sedimentation coefficient, and low doses of x-rays decrease the sedimentation coefficient (Cook and Brazell, 1975). This behavior is characteristic of supercoiled DNA arranged in a number of loops; thus nucleoids obviously represent a compact, non-ICP form of the DNA. The highly branched nucleoid configuration shown in Table I is calculated to have a relaxation time of 5 s, which would not be readily observable in a viscoelastic experiment. This may account for the lack of a viscoelastic response from the nucleoids. The calculated hydrodynamic radius of 4 μm is comparable to the nucleoid radius of 5–25 μm observed with fluorescence microscopy.

Ionic detergent lysis at a pH <11.40 yields a type I viscoelastic response. Such DNA is double-stranded, as indicated by the S1 nuclease studies, but it is not in the form of double-stranded independent linear chain polymers. The latter conclusion derives from the amount of recoil seen in the viscoelastic experiments. If the DNA is in the form of independent linear chain polymers, it can be calculated that the fractional recoil compared to the amount of windup is (Kavenoff and Zimm, 1973):

$$\text{Fractional recoil} = kTL\tau/\eta_{sln}, \tag{1}$$

where L is the number of molecules per milliliter with a relaxation time τ and η_{sln} is the viscosity of the lysate. Because of the low DNA concentration, η_{sln} is assumed to be that of the solvent. The average fractional recoil was 0.38 for a type I response when the DNA concentration was 30 ng/ml. It can be calculated from Eq. 1 that there would be 4.5×10^7 molecules/ml of molecular weight 2×10^{10}. However, this would imply a DNA concentration of 1,350 ng/ml, which is 45 times more DNA than was contained in the cells that were lysed. The obvious conclusion is that a type I response arose from a nonlinear chain polymer form of the DNA.

It has been found that mammalian cells lysed with an ionic detergent under denaturing conditions yield DNA whose sedimentation coefficient responds biphasically to increasing concentrations of intercalating agents (Ide et al., 1975). Since this result implies the DNA is supercoiled, it must be concluded that ionic detergent lysis also yields a non-ICP form of the DNA under these conditions. In terms of the model, ionic detergent lysis is shown as yielding a branched form with a hydrodynamic radius of 26 μm. Such a configuration is calculated to have a relaxation time of 2,000 s, which is comparable to a type I retardation time of 1,800 s. The increase in the hydrodynamic radius is accounted for in the model by decreasing the number of branches and increasing the molecular weight of each branch. However, it is possible that in addition to decreased branching, a partial loss of supercoiling contributes to the increase in hydrodynamic radius.

Ionic detergent lysis at a pH of 11.55, or at 11.40 plus 100 rad yields a type II response. It will be shown below that the two lysis conditions give similar numbers of single-strand breaks; thus it is reasonable to attribute the transition from a type I to a type II response to single-strand breaks. Since previous sedimentation experiments indicate that ionic detergent lysis results in a supercoiled form of the DNA (Ide et al., 1975), and it is well known single-strand breaks increase the hydrodynamic radius of supercoiled DNA, it is also reasonable to infer that the increased relaxation time of a type II response is due to an

increased hydrodynamic radius. In terms of the branched model, a type II response is shown as arising from a form with a 45-μm hydrodynamic radius. This configuration has a calculated relaxation time of 10,000 s, which is at the upper range of values seen for a type II response. While the increase in hydrodynamic radius induced by single-strand breaks is probably due mainly to loss of supercoiling, the branched model simulates the loss of supercoiling by decreasing the number of branches and increasing the molecular weight of each branch.

It must be reemphasized that the model of Table I depicts DNA in ICP forms, while nondenaturing lysis of mammalian DNA yields non-ICP forms of DNA. We are approximating the hydrodynamic behavior of a supercoiled loop by a linear chain polymer whose molecular weight is adjusted to agree with the observed viscoelastic behavior. In spite of this simplification, we believe this model adequately represents a first approximation to the transitions in the viscoelastic response of mammalian DNA.

Lysis at a pH >11.70 results in a type III response with a 15-min lysis time, and a type IV response at longer lysis times. S1 nuclease studies indicate a type III response arises from partially single-stranded DNA, while a type IV responses arises from a more fully single-stranded form of the DNA (see Fig. 5). Fig. 7 compares the retardation time to the average sedimentation coefficient obtained at various lysis times under denaturing conditions. It can be seen that a type III response arises from DNA with a sedimentation coefficient of about 300S, and a type IV response arises from DNA with a sedimentation coefficient of about 150S.

It is probable that a type III response arises from a non-ICP form of the DNA that is comparable to the fast sedimenting species obtained on the alkaline sucrose gradient (Cleaver, 1974*b*; Wheeler et al., 1975). The amount of recoil of a type III response is not compatible with a linear chain polymer form of the DNA. From the magnitude of the retardation time, it is reasonable to conclude that the hydrodynamic radius of the DNA giving a type III response is between that of the nucleoid form, and that giving rise to the type I response. It has been proposed that x-rays increase the hydrodynamic radius of the fast sedimenting species (Elkind, 1971,) suggesting that both type I and the fast sedimenting DNA species respond in a similar fashion to single-strand breaks.

A type IV response, obtained at longer lysis times, has a 57-s relaxation time (corrected to

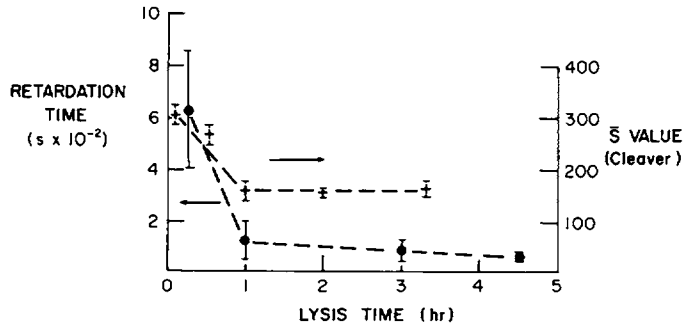

FIGURE 7 The average sedimentation coefficient (Cleaver, 1974*b*) and the retardation times as a function of lysis time under denaturing conditions.

25°C and to a solvent with the viscosity of water), and a recoil of 2% of the windup (with 200 ng/ml of DNA). The number of molecules present in the lysate using Eq. 1 agrees approximately with the amount of DNA known to exist in the cell. Thus a type IV response probably arises from single-stranded, independent linear chain polymers. and thus it is permissible to calculate its molecular weight from its relaxation time. Since the concentration of DNA is so low, we can assume no concentration correction is needed, and use 57 s as the relaxation time. By using a combination of the Scheraga-Mandelkern equation (1952) and Studier's relationship (1965), it is possible to derive the following equation:

$$M = 2.69 \times 10^8 \quad \tau_{25,w}^{0.55}, \tag{2}$$

which is appropriate for 1 M salt. A molecular weight of 2.5×10^9 is calculated for the largest polymers present in the lysate. The weight average molecular weight derived from the 150S profile is usually given as 4×10^8 (Wheeler et al., 1974; Cleaver, 1974*b*; Dingman and Kakunaga, 1976). Although this is six times smaller than the molecular weight obtained from the relaxation time, the discrepancy is to be expected. A weight average calculation from a sedimentation profile is influenced by the entire population of molecular weights, while the calculation of the molecular weight from the relaxation time is dominated by the largest polymers present in the lysate. Examination of the low speed 150S peak profile shows the presence of single-stranded DNA up to a molecular weight of 2.8×10^9 (Palac and Skarsgard, 1972). Thsu the viscoelastic parameters observed under denaturing conditions confirm the results obtained from the alkaline sucrose gradient.

It appears that lysis pH and gamma rays induce a type I → II → IV response with increasing "doses" of either agent (see Figs. 3 and 4). The type I → II transition is obtained by either raising the lysis pH from 11.40 to 11.55, or irradiating cells with 100 rad and lysing at 11.40. From DNA hydrolysis data (Kohn et al., 1976), it is possible to calculate that DNA exposed to a pH of 11.40 for 5 h receives the same number of single-strand breaks produced by 110 rad, whereas DNA exposed to a pH of 11.55 for 5 h receives an equivalent single-strand break dose of 160 rad. Similarly, DNA exposed to 100 rad of gamma rays and lysed at a pH of 11.40 for 5 h receives a total single-strand break dose of 210 rad. This suggests that increasing the total single-strand break dose from 110 to 160–210 rad is sufficient to convert a type I response to a type II response.

The type IV response is obtained by lysing cells at a pH of 11.70 for 5 h, or irradiating cells with 1,000 rad and lysing at a pH of 11.40. Fig. 6 shows that lysis at 11.70 yields 75% single-stranded DNA. With the aid of Eq. 3 below, it is possible to calculate that cellular DNA exposed to 1,000 rad, and lysed at a pH of 11.40 for 5 h is 72% single-stranded. This suggests that lysates containing more than 72% single-stranded DNA yield a type IV response.

The fraction of double-stranded DNA contained in a lysate after 5 h of lysis is predicted to be:

$$F_{ds} = \exp - \{2.3[(OH^-) - 2 \times 10^{-3}][4.5 \times 10^4(OH^-) + D]\}, \tag{3}$$

where (OH^-) is the hydroxide concentration in the lysate in moles per liter, and D is the radiation dose in rads. The form of the equation was suggested by Rydberg (1975). In Eq. 3, $[(OH^-) - 2 \times 10^{-3}]$ represents a DNA unwinding force, $4.5 \times 10^4(OH^-)$ is the number of single-strand breaks (in units of rads) due to alkali hydrolysis, and D is the number of

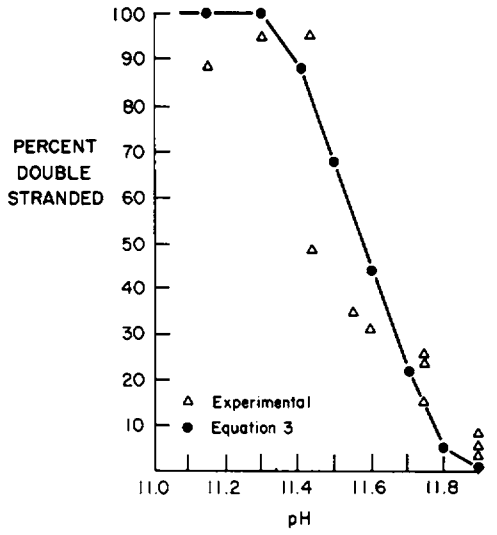

FIGURE 8 The fraction of double-standard DNA predicted by Eq. 3 compared with the S1 nuclease results.

single-strand breaks (in units of rads) due to radiation. The fraction of double-stranded DNA predicted by Eq. 3 is compared with the S1 nuclease results in Fig. 8.

CONCLUSIONS

The viscoelastic behavior of undenatured DNA is most rationally explained in terms of changes in its hydrodynamic radius rather than in its molecular weight. Although ICP configurations were used to model non-ICP forms of DNA, we believe the branched model qualitatively accounts for the large changes in the viscoelastic behavior observed under a variety of lysis conditions. We believe it is unreasonable to interpret changes in the relaxation time of large cellular DNA in terms of molecular weight changes alone. Such an interpretation leads to the highly unlikely conclusion that nonionic detergent lysis gives a low molecular weight DNA species, ionic detergent lysis gives a larger molecular weight species, and irradiation increases the molecular weight still further. This is clearly an unreasonable sequence of events. Finally, we feel these results also serve to caution against the indiscriminate interpretation of changes in neutral sucrose gradient profiles in terms of molecular weight changes alone.

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