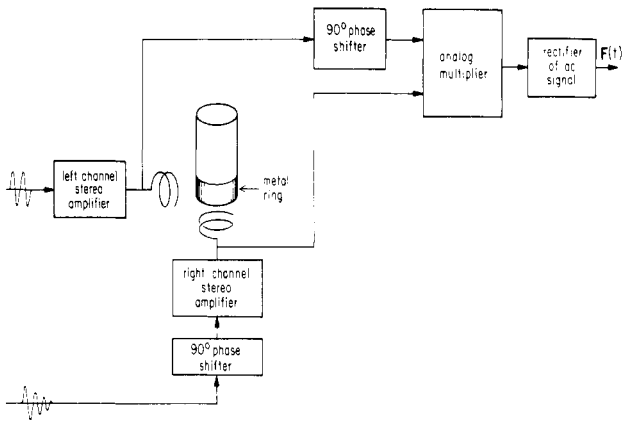

Figure 3. The torque-producing and -measuring system. See text.

Torque Measurement

As shown in Figure 3, the torque is measured by sampling the voltages applied to the drive coils. After phase shifting them back into register, they are multiplied by an analog multiplier (Motorola, MC1595). The output from this stage is a 20 kHz signal whose amplitude is proportional to the product of the input amplitudes. This amplitude is measured by rectifying the alternating current component of the 20-kHz signal (the DC level of the multiplier output is capacitively blocked). The output of the rectifying circuit is filtered with a fourth-order Butterworth circuit and applied to a strip-chart recorder, which displays the torque.

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Dynamics of Polymer Solutions. 4. Shear-Stress-Relaxation Experiments on Solutions of DNA from Bacteriophage T2

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ABSTRACT: We have previously described a stress-relaxation viscoelastometer which applies very small shear stresses to dilute solutions of large polymer molecules. Here we present experimental results. We show that in the limit of zero applied shear stress, and zero concentration, the primary retardation time is equal to the primary stress-relaxation time. We show that various stress-relaxation parameters are more sensitive to smaller molecules in the solution than are strain-relaxation parameters. One stress-relaxation parameter, a dimensionless measure of the primary relaxation amplitude, is found to be sensitive to the heterogeneity of the distribution of molecular weights, even at nonzero shear stress and concentration.

Dilute solutions of very large polymer molecules demonstrate remarkable effects due to their properties of viscoelasticity. For example, suppose a rotor is suspended in such a solution and is rotated in one direction by an external force. Then upon removal of the force there is enough energy stored in the elasticity to impart a recoil to the rotor—it reverses its direction of motion, even when the polymer concentration is only a few parts per million.[1,2] The angular velocity of the rotor decays exponentially to zero in that direction, and the time constant of this decay can be related directly to the molecular weight of the largest polymer molecules in solution.[1,2] This technique has been successfully applied to the measurement of chromosomal-sized DNA molecules in sizes ranging from 20 million to 40 billion daltons, a difficult size range for other techniques. The experimental procedure is called "creep recovery", and the decay times are known as "retardation" times. The instrument is a modified Couette viscometer and is capable of measuring intrinsic viscosities as well as these retardation times.[1,2] In parts 1 and 3,[3,4] we have described the theory and design for a substantially modified instrument which can measure shear-stress relaxation as well as creep-recovery dynamics. In this paper, we present experimental data from this instrument on solutions of DNA from bacteriophage T2. The advantage of having this additional stress-relaxation capability is in the potential for getting simple information about the molecular weight distribution. Creep-recovery dynamics are insensitive to all but the largest such molecules, while stress relaxations give data more biased toward the lower molecular weight components in the solution. Here, we demonstrate that effect. Also we present a stress-relaxation parameter, F_{11}/F_0, which is simple to get from the experimental curves and which appears to be a reasonably good measure of molecular weight heterogeneity, even without extrapolation to zero concentration and zero applied shear. With our measured values of this parameter and of the primary stress-relaxation time, we have confirmed the results from several other studies. First, the theory of Chapman et al.[1] predicts that the primary retardation time and the primary relaxation time are the same at zero concentration. We find this to be true so long as these decay times are also extrapolated to zero applied shear stress. In those limits, our stress-relaxation times are the same as the retardation times of Bowen and Zimm, who predict a molecular weight of 1.26×10^8 daltons for T2 DNA.[5] Using our parameter which is more sensitive to heterogeneity than the ones used by Bowen and Zimm,

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we find that these solutions contain only a single molecular weight species of DNA, to within experimental error. Finally, we show qualitative agreement with the predictions of Adam and Zimm, who have shown that the rate of shear degradation of these large molecules decreases to an asymptotic limit with time of shear.[6]

Theory

The polymer solution is prepared for either stress or strain relaxation by a process we call windup. We apply a constant external electromagnetic force which causes the rotor to turn, thus imparting a fixed shear stress to the solution. During this time we measure the strain by following the rotor angle, $\theta(t)$, as a function of time. In this way, we get the relative viscosity of the solution, so long as a similar measurement had been made on the solvent alone. After an appropriate windup time, t_w, has elapsed, we can measure either stress or strain relaxation. To measure strain relaxation (creep recovery), we simply remove the external force. The rotor then reverses direction to recover the elastic component of the strain. The angular velocity of the rotor then decays exponentially to zero as the polymer molecules in the solution relax to their equilibrium configurations. On the other hand, for a stress-relaxation experiment, a feedback loop is connected into the circuit at time zero. The feedback electronics sense small variations in $\theta(t)$ and correct by applying a restoring torque to hold the rotor fixed at that angle, θ_0. Thus the strain is fixed, and we measure the torque required to balance the molecular relaxation of the stress in the solution. In both the stress- and strain-relaxation cases, the measured variable decays as a sum of exponentials. The difference, as shown in the expressions below, is in the weighting of the exponentials.[3] For a strain relaxation

$$\theta(t) - \theta(\infty) = (\text{constant 1}) \sum_{i,k} f_i \tau_{ik}^2 e^{-t/\tau_{ik}} [1 - e^{-t_w/\tau_{ik}}] \quad (1)$$

and for a stress relaxation

$$F(t) = (\text{constant 2}) \sum_{i,k} f_i \tau_{ik} e^{-t/\tau_{ik}} [1 - e^{-t_w/\tau_{ik}}] \quad (2)$$

where f_i is the number fraction of molecules of molecular weight species i, t_w is the windup time, k is the internal relaxation mode index, t is the time after the start of the relaxation, and τ_{ik} are the stress-relaxation times at zero concentration and zero applied shear (and are therefore theoretically equal to the retardation times). The main point is that in the same instrument, on a single solution, we can measure specific viscosity, stress-relaxation times, retardation times, and amplitudes of exponentials from each kind of experiment. These parameters all depend differently on the molecular weight distribution. Thus these values can in principle be combined to give simple information about the molecular weight distribution of the DNA.

Experimental Section

Materials and Methods. We followed the procedure of Bowen and Zimm[5] for growing the T2 phage and for performing the hot phenol extraction of the DNA. The resultant DNA stock solutions contained 100–300 μg/mL of DNA in a phosphate buffer (BPES). Our experimental solutions were then made up in either 75 or 80% glycerol depending on the viscosity of interest. For an 80% glycerol solution (η_0 = 39 cP), we put 0.154 mL of ten times concentrated BPES buffer, 4.8 g of glycerol, and a volume of the DNA stock solution which would result in an appropriate final concentration into a polypropylene test tube. The solution was brought to 5.0 mL with distilled water. The tube was rotated slowly for about 30 min to mix, then the sample was gently poured into a glass cassette. This viscoelastometer cassette was then covered with parafilm and stored at 4 °C overnight. To run an experiment, we gently inserted the rotor into the cassette, which was then put into the viscoelastometer chamber and allowed to come to thermal equilibrium at 25 °C for about 1 h. The first experiments on a solution were measurements of the rotation time of the rotor as a function of applied shear stress. The range of shear stresses was from 4.2×10^{-3} to 6.5×10^{-5} dyn/cm^2. From these data, we could later compute relative and specific viscosities. We found these viscosity measurements to be the least reproducible of all the viscoelastic measurements we made. Next, a series of creep-recovery experiments were done and in most cases our data were consistent with that of Bowen and Zimm,[5] although we did not do such an extensive analysis as they did. Finally, we performed shear-stress-relaxation experiments, at various shear rates, for various windup times, at various feedback loop gains, and at various concentrations. Each measurement was made three times in the same manner. If at least two of the three identical runs did not show reasonable agreement, we did not use the data. The DNA concentrations were determined beforehand by dilution and afterwards by measurement of the optical density at 260 nm. On some solutions we purposely degraded the DNA by shearing it. We did so by pouring the liquid into the gap between two concentric cylinders in a Rao flow birefringence machine. The radius of the outside cylinder was 1.85 cm, and the gap width was 0.05 cm. The outside rotor was spun at 6.6 rev/s, creating a shear stress of 599 dyn/cm^2. This shear degradation proceeded for times ranging from 500 to 1500 s. Approximate shear breakage rates of the largest molecules were consistent with and can be calculated from the work of Adam and Zimm.[6]

Results

In Figure 1, we show a typical shear-stress-relaxation curve alongside a creep-recovery curve on the same solution. The data are digitized at the same time they are recorded as an analogue voltage. This digital information is then analyzed by a nonlinear least-squares computer program, which also produces the semilog plots shown in Figure 1. We have modified a Levenberg–Marquardt best-fit procedure to fit six exponentials, corresponding to the four slowest internal modes of relaxation of the molecules and to two independent molecular weight species. From this we get time constants and amplitudes of the primary decay modes and goodness of fit parameters. This program is available in ref 10. From the figure it is evident the primary stress-relaxation time and the retardation time are about the same, in accord with the theory. Each of these depends somewhat differently on DNA concentration and applied shear stress, but as we show later, when extrapolated to zero shear and zero concentration, the primary relaxation time is the same as the primary retardation time, within experimental error. The data are displayed as points in the figure, and the two curves are the computer-calculated best-fit lines. The straight line represents only the single slowest exponential, and the other curve represents the complete six-exponential fit. Although it is clear from the semilog plot that there are faster relaxing components in each curve and that these are adequately modeled by the theory as it is manifested in the computer program, nevertheless it is also clear that there is a distinctly measurable longest component. This is demonstrated by the linearity of the long time end of the semilog plot.

We have discovered several practical differences in running stress- vs. strain-relaxation experiments. First, the stress always relaxes to a final value of zero applied force whereas for creep recoveries $\theta(t)$ relaxes to an arbitrary $\theta(\infty)$. If there is a small constant instrumental torque on the rotor, then this would show up in a creep recovery as a linear base line drift whereas it would only be a small constant offset in a stress-relaxation curve. Thus since the stress-relaxation base line is always flat, we have found that we only need run those experiments until about 3–4 decay

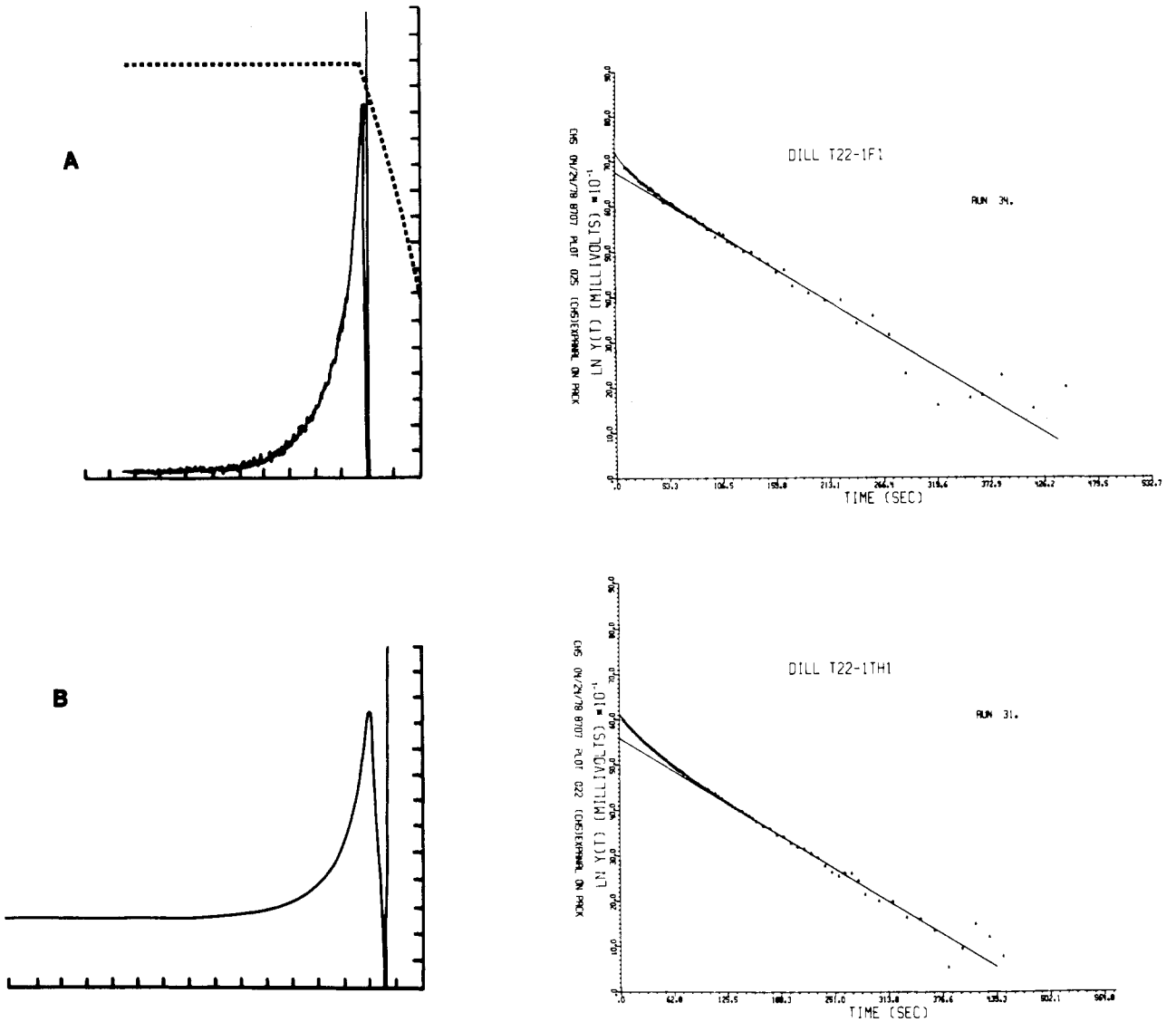

Figure 1. Comparison of stress-relaxation and creep-recovery experiments. Experimental decay curves on the left, computer semilog plots on the right: (a) stress relaxation, solid line shows the relaxation of torque on the rotor, dotted line is rotor angle, $\theta(t)$; (b) creep recovery, solid line is rotor angle, $\theta(t)$.

times have elapsed whereas creep recoveries must be allowed to proceed for about 10 times the primary retardation time. Another difference is that stress-relaxation curves are inherently noisier. This is due to the requirement that the feedback loop gain, A, must be made large in order that the dynamics follow the theoretical stress-relaxation behavior. Thus a small fluctuation in rotor angle, $\Delta\theta_n$, causes the feedback loop to produce a restoring noise torque, $T_n = -A\Delta\theta_n$. Since the torque which the DNA produces on the rotor is

$$T_{DNA} = (\text{constant})L\omega\tau \tag{3}$$

where L is the number of molecules per unit volume, ω is the shear rate, and τ is the relaxation time, then the signal-to-noise ratio for the applied torque depends on

$$\frac{T_{DNA}}{T_n} = \frac{(\text{constant})L\omega\tau}{A\Delta\theta_n} \tag{4}$$

Thus there is an optimum value for the feedback loop gain—it must be large enough to satisfy the theoretical criterion, but small enough to keep the noise level low. As we show in the next section, we can easily do this, but with the result that there is more noise than in a creep-recovery experiment. Also the frequency of the noise depends on the relaxation time of the DNA.

The first series of experiments was to test the reproducibility of both creep recoveries and stress relaxations. We alternately performed six of each type of experiment under identical conditions on a single solution. The DNA was in 75% glycerol; the shear stress was 6.488×10^{-3} dyn/cm^2. The retardation times ranged from 20.37 to 22.9 s with a mean of 21.65 and a standard deviation of 1.047 s. The primary recoil was 5.55 ± 0.18. For the stress relaxations the range of relaxation times was 20.32–21.75 s with a mean of 20.99 and a standard deviation of 0.638. The primary component of the force amplitude F_{11} was 15.55 ± 1.43. Both amplitudes are in arbitrary chart units. This reproducibility depends on not removing the rotor from the chamber between runs and is just a measure of the reproducibility of the identical experimental procedure applied to a single solution.

Loop Gain

The loop gain, A, determines whether an experiment will be a creep recovery or a stress relaxation. When $A = 0$, there is no restoring torque applied, and we measure strain relaxation. It is in the limit of infinitely large loop gain

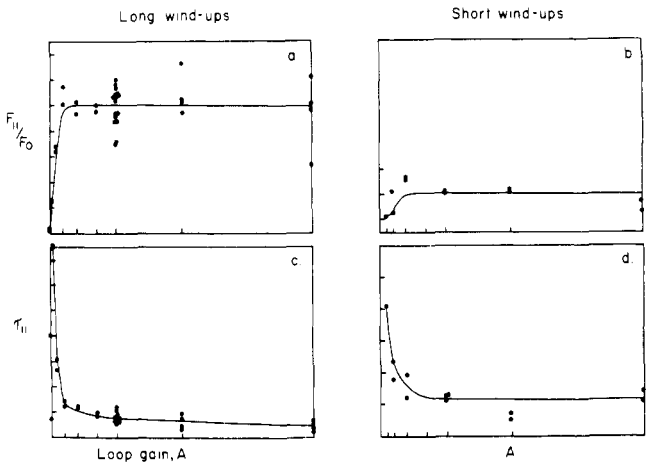

Figure 2. Dependence of stress-relaxation parameters on loop gain: (a, c) long (steady-state) windups; (b, d) short (twitch) windups; (a, b) reduced primary exponential amplitude, F_{11}/F_0; and (c, d) relaxation time. Note that at large loop gain, all parameters become independent of loop gain, A.

that we get stress-relaxation dynamics. Dill and Zimm[3] have described this in Laplace transform notation as follows:

$$\tilde{\theta}(s) = \frac{-c_1}{s\left(s + \frac{1}{\tau_{rtd}}\right) - c_2 A\left(s + \frac{1}{\tau_{rlx}}\right)} \tag{5}$$

$$\tilde{F}(s) = A\tilde{\theta}(s) \tag{6}$$

where c_1 and c_2 are constants, s is the Laplace transform variable, τ_{rtd} is the retardation time, τ_{rlx} is the stress-relaxation time (we have ignored all but the primary decay times and assume those are not equal at finite concentration and shear stress), and $\tilde{\theta}(s)$ and $\tilde{F}(s)$ are the Laplace transforms of the rotor angle $\theta(t)$ and applied torque $F(t)$, respectively. We ignore rotor inertia. For $A \to 0$,

$$F(t) = 0,\ \theta(t) = -c_1\tau_{rtd}e^{-t/\tau_{rtd}} \tag{7}$$

and for $A \to \infty$,

$$\theta(t) = 0,\ F(t) = \left(\frac{c_1}{c_2}\right)e^{-t/\tau_{rlx}} \tag{8}$$

In Figure 2, we show the effect of the loop gain on the measured relaxation times and the exponential amplitudes. In that figure F_0 is the drive force applied during windup, which is constant. The amplitude for the exponential of the slowest mode of the largest molecules is F_{11}. It is taken from the intercept of the linear part of the semilog plot of the decay curve. From these definitions it is clear that F_{11}/F_0 is a dimensionless measure of the primary relaxation amplitude. With the use of eq 5 and 6, it is possible to show that F_{11}/F_0 should increase linearly with loop gain for small values of A and become constant as A gets larger. Indeed this is the result we get for both steady-state ($t_w \to \infty$) and "twitch" ($t_w \to 0$) windups. Although it is more difficult to calculate an effective relaxation time and its dependence on loop gain, nevertheless it is evident from eq 5 and 6 that for large loop gain $F(t)$ should become independent of A. Indeed it appears that the relaxation time asymptotically approaches a limit. So even though we cannot in practice attain infinitely large loop gain, it appears to be unnecessary to do so, as the effect is probably no more than a few percent. In addition to the loop gain, a velocity feedback has been built into the electronics for

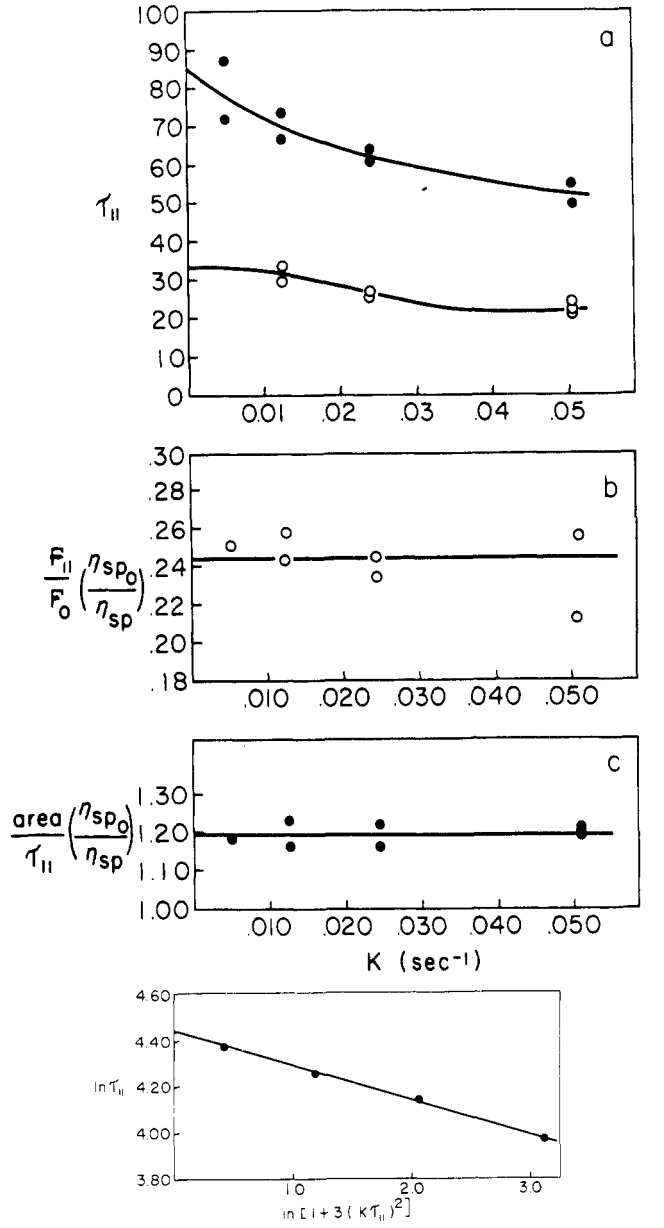

Figure 3. Shear-rate dependence of stress-relaxation parameters: (a) relaxation time, (●) intact T2 DNA, (○) half-molecule DNA produced by shearing; (b, c) both show curves of intact T2 DNA; (d) dependence of ln τ_{11} on ln $[1 + 3\ (\kappa\tau_{11})^2]$. We have found plots such as these to be linear, which allows us to extrapolate τ_{11} to the value it would have at zero applied shear stress.

the event that additional stabilization would be necessary for transients. In this case the restoring torque is

$$F(t) = -A(\theta(t) - \theta_0) - B\frac{d\theta}{dt} \tag{9}$$

In a series of experiments, we determined that the velocity gain had no effect on the relaxation time, so long as the loop gain is large enough. However, for the experiments reported here in high viscosity solvents, we have found this additional feedback to be unnecessary altogether, so $B = 0$ hereafter.

Shear Rate Dependence

The stress-relaxation times are somewhat dependent on the shear stress which had been applied during windup. A simple dimensionless measure of the molecular deformation of the molecules during windup is provided by the product of the shear rate and the relaxation time, $\kappa\tau_{11}$.

Bowen and Zimm[5] found that many of the strain-relaxation parameters were increasingly affected as $\kappa\tau_{11}$ increased. They studied these effects for a range of $\kappa\tau_{11}$ from 0 to 6. Our maximum $\kappa\tau_{11}$ was about 3. Following their procedure, we have plotted ln τ_{11} against ln $[1 + 3(\kappa\tau_{11})^2]$ and find this to be reasonably linear, making it simple to extrapolate to zero shear. A typical such curve is shown in Figure 3d.

In addition to the relaxation time of the primary exponential, we are also interested in its amplitude, the dimensionless measure of which is F_{11}/F_0. It has been shown[7] that this parameter can be used as an indication of the molecular weight heterogeneity of a solution, since

$$\frac{F_{11}}{F_0} = \frac{\eta_{sp_1}}{S_1(1 + \eta_{sp})} \tag{10}$$

where η_{sp_1} is the specific viscosity of the largest molecules, $1 + \eta_{sp}$ is the relative viscosity of the solution, and S_1 is a constant = 2.041. Equation 10 suggests that F_{11}/F_0 might have the same shear rate dependence as η_{sp}. Indeed, plots of $F_{11}\eta_{sp_0}/F_0\eta_{sp}$ are reasonably independent of shear rate, as can be seen in Figure 3b. Here η_{sp_0} is the value of η_{sp} extrapolated to zero shear. It has been shown[7] that the heterogeneity of the solution can be measured by a parameter of the form

$$\frac{F_0\eta_{sp}}{S_1F_{11}\eta_{rel}} - 1$$

which is zero for a homogeneous solution and is 0.315 for a solution of 50% full-sized molecules and 50% half-sized ones. This parameter too would be independent of shear rate, so its validity as a measure of heterogeneity should extend even to nonzero shear rates (at least up to $\kappa\tau_{11}$ = 3). In a similar fashion, we found that the area under the stress-relaxation curve can be normalized to be independent of shear rate, and this is shown in Figure 3c.

Dependence on Windup Time

It can be seen from eq 1 and 2 that the dynamics of both stress and strain relaxations depend on the windup time, t_w. For long windup times, as $t_w \to \infty$, $(1 - e^{-t_w/\tau_{ik}}) \to 1$, and the relaxation behavior will become independent of the windup time. However, in the limit of very short "twitch" windups

$$\lim_{t_w \to 0} (1 - e^{-t_w/\tau_{ik}}) = t_w/\tau_{ik} \tag{11}$$

so that now the amplitude of relaxation will depend linearly on t_w. It is more convenient to consider the dimensionless amplitude parameters Γ_{11}/Γ which we get from creep-recovery experiments and F_{11}/F_0 which we get from stress-relaxation experiments. For very short windups

$$\lim_{t_w \to 0} \frac{\Gamma_{11}}{\Gamma} = \frac{f_1\tau_{11}}{\sum_{i,k} f_i\tau_{ik}} \tag{12}$$

where Γ is the total recoil in a strain decay, Γ_{11} is the primary component of it, and

$$\lim_{t_w \to 0} \frac{F_{11}}{F_0} = (\text{constant } 2)\frac{f_i t_w}{\eta_0} \tag{13}$$

Thus F_{11}/F_0 should be linear in t_w whereas Γ_{11}/Γ should be independent of t_w for these short windups. Our experiments confirm this. (Note, however, that the data in Figures 4 and 7 are not plotted to show this linearity, as the time axis is logarithmic.) This linearity, and the assumption that the windup is short, are no longer valid when t_w/τ_{11} gets larger than about 0.2–0.4.

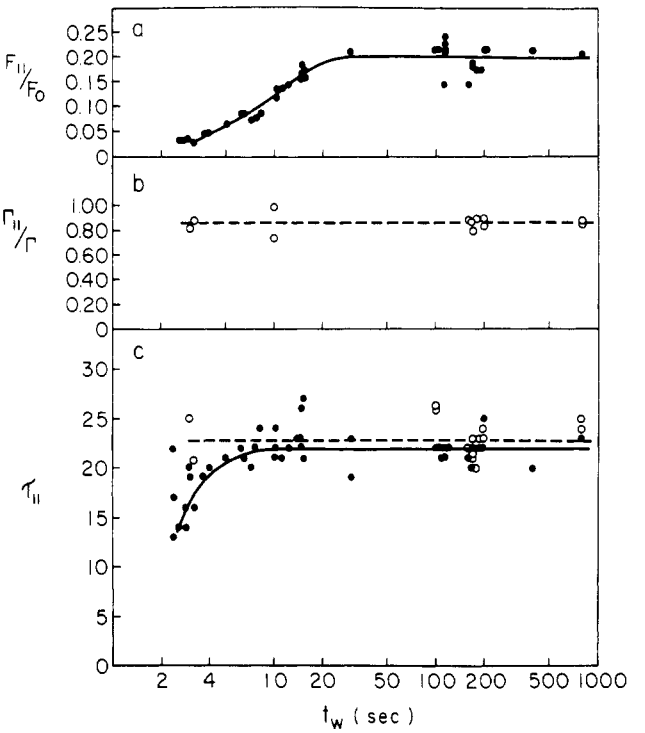

Figure 4. Comparison of windup time dependence of stress relaxations vs. creep recoveries: stress relaxation (●, solid line), creep recovery (○, dashed line). Intact T2 DNA, 75% glycerol. Note that for short windups, stress-relaxation parameters are dependent upon windup time, t_w.

There is a second consequence of very short windup times. The effect of short windups (eq 11) is to reduce the amplitude of the exponentials in each of eq 1 and 2 by one power of τ_{ik}. The slowest relaxation modes have the largest τ_{ik}, so the effect of dividing out a power of τ_{ik} is to increase the relative amplitudes of the faster modes of relaxation. Under these twitch conditions, a stress-relaxation curve now loses all dependence on the eigenvalues altogether, so that the exponentials are all equally weighted for each single molecular weight class of DNA. Thus the apparent relaxation time is no longer that of the slowest mode alone but is now a combination with the faster modes and is thereby effectively shortened. In these cases, we have found that it is not always simple to get a linear semilog plot, but nevertheless it is quite clear that the observed relaxations become faster under twitched stress decay conditions. Some of these data are shown in Figures 4 and 7. The relative amplitudes of the exponentials are therefore also quite sensitive to the distribution of molecular weights in the solution. If there exists a substantial fraction of molecules which are smaller, thus faster relaxing, then creep recoveries should be insensitive to it whereas stress relaxations should show a shorter apparent τ_{11}, and F_{11}/F_0 should be much smaller than its value for a homogeneous solution. We return to this point in the last section.

Concentration Dependence

In order to get a reduced relaxation time, from which a molecular weight can be calculated, we first extrapolate the stress-relaxation times to zero shear stress on plots such as Figure 3d. This gives a single reduced relaxation time for each solution. According to the theory of Chapman et al.,[1] if these values are extrapolated to zero concentration, the primary retardation time should be equal to the

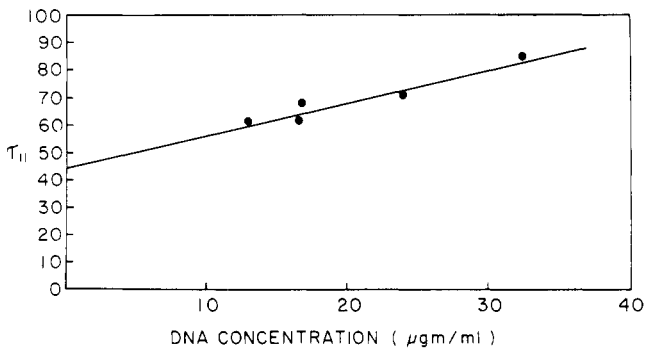

Figure 5. Dependence of primary relaxation time on DNA concentration. The τ_{11}'s have previously been reduced to their zero-shear values. τ_{11} is given in seconds.

primary relaxation time. Bowen and Zimm[5] have shown that the reduced retardation time for homogeneous solutions of T2 DNA in 75% glycerol and 0.06 M Na^+ is 20.0 s. When this is corrected to a solvent of water at 20 °C, the retardation time is 0.82 s. For our 80% glycerol solutions, the corresponding stress-relaxation time is 44 s. This is the zero-concentration intercept of Figure 5. Similarly reduced to an assumed solvent of water at 20 °C, this gives a relaxation time of 1.0 s. For our 75% glycerol solutions, the zero shear and zero-concentration primary relaxation time is 20.3 s, which reduces to 0.83 s in water. Finally, the value of $\tau c[\eta]/\eta_{sp}$ which is reasonably independent of concentration extrapolates to 0.75 s in water. Therefore, within experimental error, we take these data to indicate reasonable agreement with the creep-recovery data of Bowen and Zimm.

Finally, we note that the slope of the plot of relaxation time vs. concentration (Figure 5), which is reasonably linear, is 1.2×10^6 (s mL)/μg. We have shown elsewhere[9] that this slope can be predicted by a recent theoretical model of Muthukumar and Freed.[8]

Molecular Weight Heterogeneity

To some of the 80% glycerol DNA solutions, we have applied high shear stresses in order to break the DNA (see Materials and Methods). We have sheared these solutions for different amounts of time, after which creep-recovery and stress-relaxation measurements were made. As the time of shear degradation increases, the fraction of full-sized molecules decreases. Thus an increasing fraction of the molecules are smaller broken fragments of the original whole DNA. The primary relaxation and retardation times and amplitudes are plotted in Figure 6. These values appear to reach an asymptotic limit with increasing shear degradation. Adam and Zimm[6] found similar asymptotic behavior from relaxation measurements of sheared DNA solutions. This limit of the decay times is indicative that the degraded solutions contain primarily half-sized molecules. In theory, the stress-relaxation parameters should be more sensitive to this molecular weight heterogeneity than the creep-recovery parameters, and this too can be seen in Figure 6. This point is reinforced by a comparison of Figures 4, 7, and 8. The data in Figure 4 are representative of DNA which has not been degraded. In Figure 8 the data were taken from DNA which was partially degraded by usage and age but not by extensive applied shearing. The rotor had been added to and removed from the solution many times over the course of about 2 weeks, after which these experiments were done. Finally, for Figure 7, the DNA had been sheared for 1050 s.

For the case of homogeneity (Figure 4), and in the limit of long windups, stress and strain decays have about the same relaxation time. But in the cases of more extensive

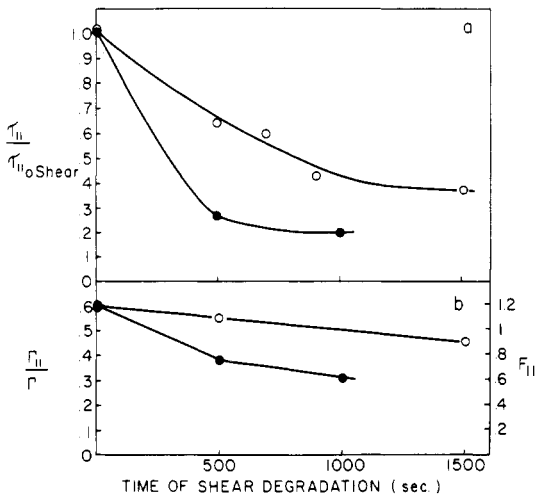

Figure 6. Dependence of stress- and strain-relaxation parameters on time of shear degradation, scaled to coincide at zero shear degradation: stress relaxation (●), creep recovery (○). Note that stress-relaxation parameters are more sensitive indicators of shear degradation than creep-recovery parameters.

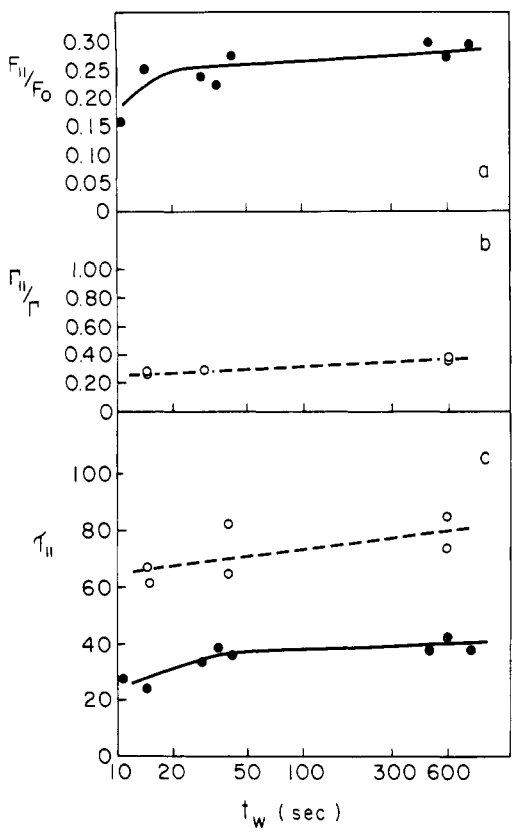

Figure 7. Windup time dependence of viscoelastic parameters for half-T2 DNA molecules: stress relaxation (●), strain relaxation (○). Note that relaxation times are smaller for stress relaxations. DNA is in 80% glycerol. Note that Γ_{11}/Γ is small, indicating the presence of a small amount of full-sized molecules. The large value of F_{11}/F_0 indicates the presence of a large proportion of half-molecules and a small proportion of quarter-sized molecules.

shearing (Figures 7 and 8), the stress-relaxation time is shorter than the retardation time. This is due to the fact that higher modes and smaller molecules contribute more significantly to stress relaxations, resulting in faster apparent primary relaxation times. We can see also that the steady-state value of Γ_{11}/Γ from the creep-recovery curves is not sensitive to the degree of degradation shown in the progression from Figure 4 to Figure 7. On the other hand,

Table I
Heterogeneity Measurements on Three Solutions

soln	$(F_{11}/F_0)/(F_{11}/F_0)_{homog}$	$\left(\frac{F_0\eta_{sp}}{S_1F_{11}\eta_{rel}} - 1\right)$	DNA concn, μg/mL
1. 80% glycerol/whole DNA	0.323/0.320 = 1.01	−0.01	36
2. 75% glycerol/whole DNA	0.20/0.20 = 1.00	0.00	21
3. 75% glycerol/degraded soln (see Figure 8)	0.10/0.17 = 0.59	0.69	15

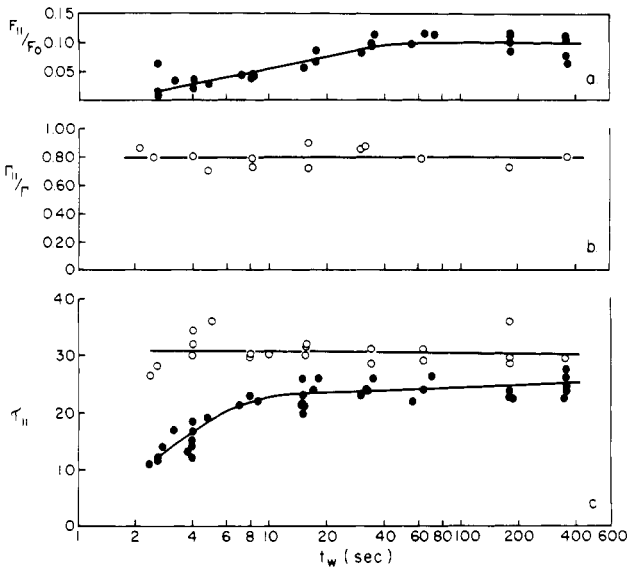

Figure 8. Windup time dependence of viscoelastic parameters for a deteriorated solution of T2 DNA molecules: stress relaxation (●), strain relaxation (○). This is an intermediate case of degradation—compare to Figure 4, intact T2, and Figure 7, T2 sheared to half-molecules.

F_{11}/F_0 is obviously smaller for the data in Figure 7 than for those in Figure 4.

Dill and Zimm[7] have shown that one of the moments of the molecular weight distribution is

$$\sum_{i=1}^{P}\left(\frac{C_i}{C_1}\right)\left(\frac{M_i}{M_1}\right)^{\alpha} = \frac{F_0\eta_{sp}}{S_1F_{11}\eta_{rel}}$$

where C_i is the concentration of the ith species of molecular weight M_i, and C_1 is the concentration of the largest molecular species M_1. For DNA in 0.2 M Na^+, α = 0.665. Therefore a useful measure of the molecular weight heterogeneity of the solution is given by the parameter

$$\frac{F_0\eta_{sp}}{S_1F_{11}\eta_{rel}} - 1$$

In Table I we show the value of this parameter for several solutions. In a previous section we have shown this parameter to be reasonably independent of shear rate, and here the indication is that it is also independent of concentration. Thus this should be a reasonably good predictor of heterogeneity, and one which need not be extrapolated to zero shear and zero concentration to give a reliable estimate.

Conclusions

We have performed shear-stress-relaxation experiments on solutions of T2 DNA. We have compared them to our own and previous creep-recovery experiments. We conclude that when stress-relaxation times and creep-recovery times are extrapolated to zero applied shear stress and zero concentration, they converge to the same reduced relaxation time. Thus our data agree with the creep-recovery data of Bowen and Zimm[5] which estimate the molecular weight of T2 DNA to be 1.26×10^8 daltons. We have found that stress-relaxation parameters are more sensitive to molecular weight heterogeneity than are creep-recovery parameters. Finally, we have shown there is a concentration-independent, shear-rate-independent parameter, $F_0\eta_{sp}/S_1F_{11}\eta_{rel} - 1$ which we get from viscosity and stress-relaxation measurements, which provides a sensitive indicator of molecular weight heterogeneity. With it we have shown that these solutions can be routinely prepared to be homogeneous.

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