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Quasi-elastic behavior of solutions of viral capsid and RNA at very low shearing stresses

(viscosity/density/thixotropy/modulus of rigidity)

M. G. HODGINS*, O. C. HODGINS†, D. W. KUPKE‡, AND J. W. BEAMS*

* The Department of Physics, and the ‡ Department of Biochemistry, The University of Virginia, Charlottesville, Virginia 22901; and † Instituto Nacional de Energia Nuclear Mexico, Mexico City, Mexico

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ABSTRACT By the application of shearing stresses on the order of 10^{-3} dyne cm^{-2} (10^{-2} μN cm^{-2}), via the magnetic viscodensimeter, extremely high relative viscosities (>500) were observed when turnip yellow mosaic virus was degraded in alkali into its capsid and RNA. The solutions, however, possessed a watery consistency at this stage and exhibited a quasi-elastic character by rotor-recoil experiments. The development of this curious behavior was concentration and temperature dependent; it was not seen <0.5% nor at 8°, and appeared sooner at 30° than at 20°. The time of appearance was delayed as the pH was lowered; however, the effect was still observed when the pH was as low as 9. Whereas reversibility was demonstrated when the shearing stresses exceeded the elastic resistance [0.17 dyne cm^{-2} (1.7 μN cm^{-2})], thorough mixing usually resulted in a normal behavior of the solutions thereafter. Values for the modulus of rigidity at 20° for about 1% virus concentration was $< 2 \times 10^{-2}$ dyne cm^{-2} rad^{-1} (0.2 μN cm^{-2} rad^{-1}), which, while extremely small, was reproducible. A porous structure, possibly involving a capsid and RNA complex, is envisioned.

A regulatory role has been suggested for the coat protein in the infection and replication of certain viruses (1). Not excluded are those viruses characterized by having a stable protein shell or capsid which are found as such in the infected cell. Recently, Aposhian *et al.* (2) presented evidence for the existence of bound complexes of polyoma capsid and the viral DNA strands; the complex also offered some protection to the nucleic acid (oral communication). We are interested in the physical properties of such solutions wherein virus has ejected its nucleic acid, and have attempted to follow their viscosity and density changes. Turnip yellow mosaic virus (TYMV) offered a good isometric model because it has a stable capsid and the chemistry of the extrusion of the viral RNA as a function of pH and ionic strength has been studied in detail (3). In this communication, we report primarily the viscometric results which, at abnormally low shear stresses, reflect a puzzling property of those solutions wherein TYMV is degraded into RNA, capsid, and some capsid subunits. At these low stresses (about 10^{-4} that of conventional capillary viscometers), very high viscosities, often changing with time in a bizarre manner, were observed. By controlling the conditions, the rate of shearing reached zero and remained so indefinitely while under the applied stress. In this condition the solutions retained a watery consistency despite showing a relative viscosity value in excess of 500; an elastic behavior, however, could be demonstrated. At higher stresses these effects disappeared, and the viscosities were normal. Although tests with other probes have not been completed, the strange viscosities appear not to be an artifact of the measuring system. We omit the details here from a number of other kinds of studies that are more designed to look into the molecular nature of the solutions, and we also omit other viscometric data, which while interesting, do not add substantially to the main purpose of this report.

By the use of the magnetic viscodensimeter described previously (4), which yielded accurate results on 0.2-ml samples for a variety of protein systems (5), the simultaneous change in the viscosity and density of a sealed solution during a reaction can be followed conveniently. (Modifications designed to enhance the kinetic capabilities are under continuing development; the original instrument, however, was found suitable for the purpose at hand.) A second feature of the instrument is that the completely immersed, magnetically suspended cylinder can be rotated by remote means to produce unusually low shearing stresses [<10^{-3} dyne cm^{-2} (<10^{-2} μN cm^{-2})]. This is accomplished with high precision via the production of a constant amplitude, rotating magnetic field by field coils suitably situated and controlled. Heating effects have not been observed. Thus, the search for metastable, long-range forces in solutions of biological interest may be feasible with this probe.

EXPERIMENTAL

TYMV was isolated and purified as described (5) from frozen chinese cabbage leaves, infected and grown at the U.S. Department of Agriculture (Beltsville, Md.). Stock solutions containing approximately 16–26 mg/ml of the virus were maintained at ice temperature in 0.02 M Na acetate, pH 6; 0.2 M (K)PO$_4$, pH 7; or in 1 M KCl, pH 6. For the viscosity-density experiments, 100-μl samples of either the stock solutions or dilutions of them were mixed rapidly with 100 μl of high pH buffer. The latter buffer was 0.2 M (K)PO$_4$, pH 7 to which 5 M KOH had been added to reach the desired pH. On occasions, a small volume of KOH was added directly with stirring to 200 μl of the virus solution, since this procedure also produced a so-called elastic condition. In most of the experiments, the high pH buffer was such that the initial pH of the reaction mixture was 11.9; substantial decreases in pH (0.3–0.5 units) at this and all lower pH reactions (to pH 9) were observed.

The magnetic viscodensimeter used for these experiments was the original instrument as described (4). The magnetically suspended rotor for most of the experiments was a permalloy core within a Kel-F cylinder, 2.8 mm diameter and 7 mm long. The diameter of the glass cell containing the solution was 5 mm. Four electric, manually controlled timers were utilized so that two data points per rotation could be obtained from two vertical lines (about 180 degrees apart)

Abbreviations: TYMV, turnip yellow mosaic virus.

on the rotor; one timer was started and another stopped simultaneously as each mark passed the cross hair in the microscope. Only one data point for density per revolution could be obtained with this rotor (this limitation is being corrected). Unless the elastic barrier of the solution was to be challenged, the usual speed of the rotor in pure solvent solutions was such that less than 0.1 mm of rotor circumference passed the cross hair per sec [shearing stress, $\tau \simeq 2 \times 10^{-3}$ dyne cm^{-2} (0.02 μN cm^{-2})]. On occasions, rotors of smaller diameter (to 1.4 mm) and greater density (8.7 instead of 1.3 g/ml) were used. The gap between rotor wall and cell wall was varied between 1.1 and 1.8 mm. The development of the unusual viscosity behavior was unaffected by these variations in the measuring system.

The reaction was initiated by rapid addition, via gas-tight syringes, of 100 μl of the high pH buffer to 100 μl of a virus solution. This mixture was immediately withdrawn into another syringe (to ensure mixing) and was delivered into the viscodensimeter cell without introducing bubbles. This part of the operation required about 30 sec. The time required for suspending the rotor and for demonstrating stable rotation was about 1 min. Added to these intervals was the variable time elapse before a reference mark on the rotor passed the cross hair (at which point timing was begun). Hence, the initial average relative viscosity (seconds per one rotation divided by the time elapse between the same reference marks for the unreacting solution) was obtained after 3 or more minutes from mixing. In general, if the initial concentration of the virus in the mixture was sufficient (>0.5%), the value for the rate of shearing, G, reached zero; the interval depending upon the temperature, pH, and to some extent on the age of the TYMV preparation (shorter intervals with older preparations). G is related to the shearing stress, τ, by the coefficient of viscosity, η, so that $\tau = \eta G$. Relative viscosities, $(\eta/\eta^{\circ})_0$, as applied in these experiments represent the ratio of the average time per revolution during the reaction relative to the time of one revolution by the mixture at zero time. The value at zero time was obtained from the observed value of the virus sample after appropriate dilution with the reacting buffer at neutral pH; a small correction was then applied for the observed increment resulting from the addition of the required amount of the 5 M KOH to the reacting buffer. This correction was insignificant compared to the effects described in this report.

RESULTS

Fig. 1 shows the character of the viscosity response seen with TYMV solutions when the pH is raised to produce a rapid accumulation of capsid at the expense of the whole virus. The early small rise in viscosity, suggested by the first few measurements after mixing, cannot be followed with the procedure currently used; on occasions, an apparent crest has been observed which is not much higher than the first data point recorded in the figure. The relative viscosity (i.e., relative to that at zero time) invariably declines. After a shorter or longer interval, the profile may or may not exhibit upward undulations before climbing to very high relative values; at sufficient virus concentrations the rotor stops completely and remains so. With this preparation, the concentration was just insufficient to completely stop the rotor, and a characteristic sharp increase in rotation rate was then seen. After this precipitous decline in the viscosity, smaller undulations were usually observed until the viscosity reflected a fairly normal behavior for macromolecular solutions. We cannot properly evaluate the maximum relative viscosity at the peak in these cases because the rate of rotation changes drastically during a single revolution in this region. Visual inspection of the slowest rate indicated that the relative viscosity was in excess of that observed for 70% w/w of sucrose in water, which at 20° has a relative viscosity of about 500. In those experiments where the solution fully stopped the rotor, no further changes were observed after several hours (e.g., overnight). Moreover, rotor stoppage was observed even if the drive coils were not activated until the approximate time when the solution was known to possess this ability via control experiments. (In this case, the rotor turned only a quarter turn or so before stopping altogether after the drive coils were powered.)

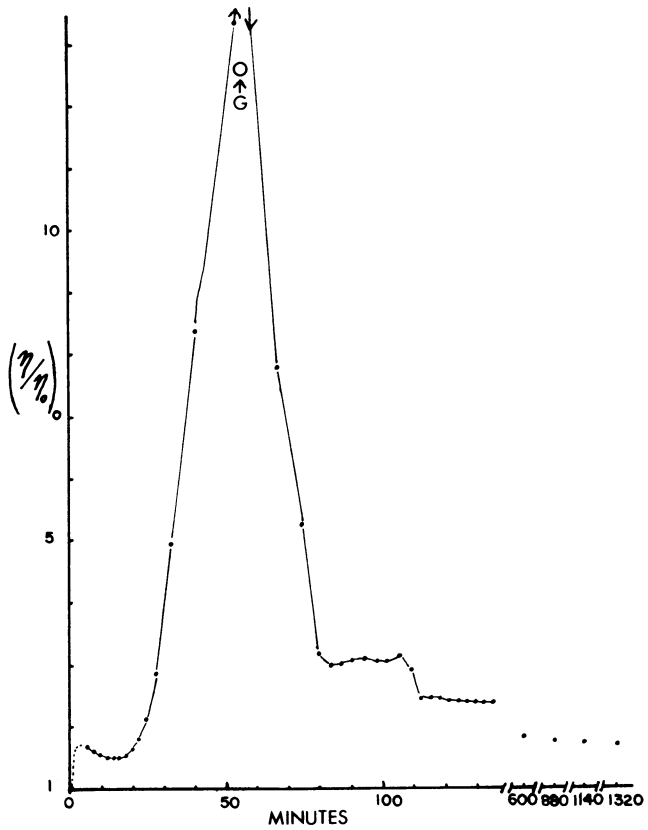

FIG. 1. Viscosity relative to that at time zero (see *text*) against minutes (20°). 7.9 mg/ml of TYMV in 0.2 M (K)PO$_4$, pH 11.9. τ = 2.8 × 10^{-3} dyne cm^{-2} (28 × 10^{-3} μN cm^{-2}). Rotation at peak region ($G \rightarrow 0$) was barely perceptible.

During the final revolution when G approaches zero, the density of the solutions was observed to decrease sharply, on the order of 10–20 ppm. Better precision than this was not expected because the reference mark on this rotor, used for the density determination, passed the cross hair at longer or shorter times before the rotor came to rest in the different experiments.

Fig. 2 shows a display of the relative viscosity–time profiles as a function of concentration with another preparation. After mixing to a final concentration of 8 mg/ml of this virus, the relative viscosity quickly approached $G = 0$ under the stress applied. At 6 mg/ml, an interval of bizarre behavior (about 2½ hr) preceded the approach to $G = 0$. Below this concentration, solutions of TYMV at this stress, pH, and temperature have not stopped the rotor; the bizarre behavior, however, was clearly evident even at 2 mg/ml, which is not obvious in the figure owing to the scaling of the ordinate.

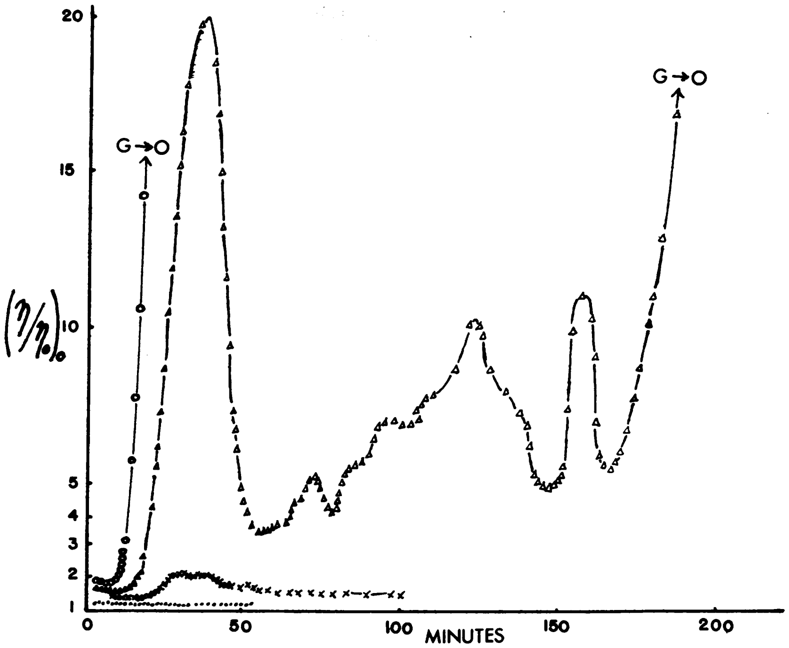

FIG. 2. Effect of virus concentration on viscosity-time profile after starting the reaction (20°). Final medium; 0.2 M (K)PO$_4$, pH 11.9. *G* → 0 refers to those solutions where rotor stopped indefinitely after the last data point. ○, 8 mg/ml of TYMV; △, 6 mg/ml of TYMV; ×, 4 mg/ml of TYMV; . . . . , 2 mg/ml of TYMV.

An abbreviated temperature study is shown in Fig. 3. At 30°, the time of approach to zero rate of shearing after mixing is clearly less than at 20°. At 8.2°, however, the solution did not stop the rotor, and also, the bizarre effects were greatly suppressed.

Elasticity

Whenever the power to the drive coils was cut off after the rotor was stopped by the reacting solution, the rotor recoiled between 8 and 9 degrees at 20° (this precision can be greatly improved upon). At 30°, the recoil was 11–12 degrees of arc.

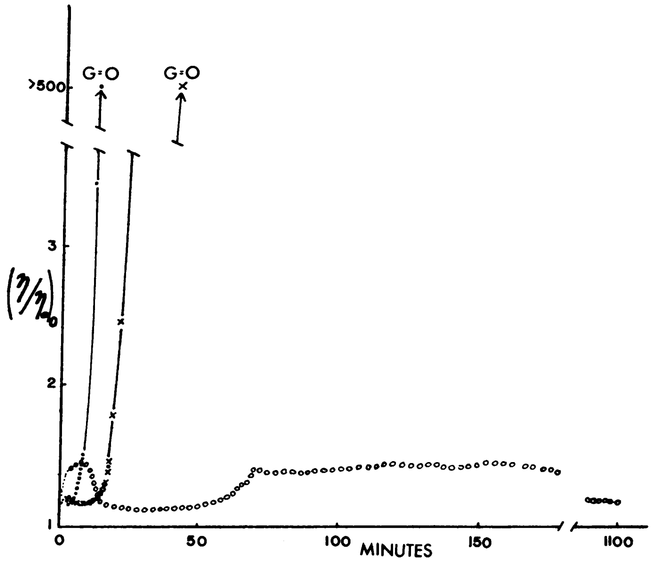

FIG. 3. Effect of temperature on viscosity-time profile. 8.3 mg/ml of TYMV in 0.75 M KCl–0.05 M (K)PO$_4$, pH 11.9. The rotor stopped indefinitely ($G = 0$) after 13 min at 30°, and after 42 min at 20°. ○, 8.2°; ×, 20°; . . . ., 30°.

On reapplying the same power again, the rotor turned until it had reached its former static position. This cycle was repeated on occasions without any apparent change in the recoil values. Moreover, when the drive was reversed, the rotation proceeded in the reverse direction about the same amount of arc as in the forward direction. The interval for the recoil effect lasted between 18 and 27 seconds at 20°, and between 12 and 18 seconds at 30°, an exponential type of decay to zero movement being observed; therefore, it was difficult to determine the time at which the rotor had fully stopped. We have not studied the recoil as a function of concentration or other variables, pending improvements in the precision. When the power to the drive coils was gradually increased, stresses of approximately 60-fold were required in order to cause the rotor to begin turning. The breakthrough stress was found to be reversible; i.e., upon reducing the power, the rotor would stop until the power was increased again to the breakthrough point.

Although the relative viscosity appears to be greater than that of thick syrup ($\eta/\eta^\circ > 500$) when $G = 0$ at these low shearing stresses, the solution always exhibited a typically watery consistency when withdrawn via a syringe. Indeed, this thorough mixing usually destroyed the ability of the solution to stop the rotor, although the bizarre effects were sometimes in evidence for minutes or hours. On the other hand, gentle mixing (as with a syringe needle around the rotor several times) allowed the solution to recover the ability to stop the rotor and exhibit recoil after a few minutes.

Other notes

We have observed also that apparently the same quasi-elastic behavior is attained by solutions at lower pH (20°). In these cases the time to achieve zero shearing rate was greatly lengthened. At about the lower limit in pH for the elastic effect noted thus far [pH 9 to 9.5 in 0.1 M (K)PO$_4$], several hours were required to stop the rotor. Interestingly, when the pH was lowered to about 9–9.5 (with 2 M HCl) *after* initiating the reaction at pH 11.9, the onset of the quasi-elastic behavior followed very quickly. The phenomenon was seen whether or not the pH was lowered after being 2, 3, or 60 min at the high pH. Surprisingly, these solutions regained their ability to stop the rotor after thorough stirring; such a solution could be withdrawn via a syringe and returned repeatedly without losing this capacity, unlike the solution that had been at this lower pH throughout.

Thus far, we have not observed this quasi-elastic behavior with various combinations of the viral degradation products. None of the strange behavior was observed in solutions of appropriate concentrations of purified capsids, or RNA obtained by the freeze-thaw method (6), nor with the mixtures of capsids and RNA, or of capsid subunits (6) before and after RNA was removed.

In passing, no obvious change in direct scatter at 330 nm was observed during the formation of the quasi-elastic condition. Electron microscopy (courtesy Dr. Carl A. Schnaitman) has revealed the presence of large amounts of clustered capsid in samples taken from quasi-elastic solutions, which amount was not consistent with the small proportion of free capsid seen by velocity ultracentrifugation. [A very slow boundary comprising the great majority of the refractive material is seen at speed after the reaction has taken place in the centrifuge cell during evacuation of the rotor chamber; the sedimentation rate (1–3 S) is somewhat dependent on the velocity. This aspect will be communicated separately.] Also, polystyrene latex spheres (1.30 μm diameter) exhibited much more difficulty in sedimenting through the reacted solutions than through equivalent concentrations of the unreacted virus under the force of gravity. Finally, high resolution nuclear magnetic resonance experiments (courtesy of Dr. Ronald P. Taylor) have shown that the mobility of water is not at all reduced when the paired samples exhibited the very high relative viscosity ($G = 0$) in the viscodensimeter, in agreement with the observation of a watery consistency at the quasi-elastic state.

DISCUSSION

The torque on the inner cylinder was determined accurately by calibration in reference liquids. From this measured torque and the dimensions of the cylinder, the shearing stress τ was evaluated. Proceeding on the assumption that the medium is quasi-elastic, the modulus of rigidity, M, can be calculated from τ and the angle of shear, θ, which was evaluated from the recoil angle when $G = 0$. In the recoil experiments at 20°C, $\tau = 2.85 \times 10^{-3}$ dyne cm^{-2} (28.5×10^{-3} μN cm^{-2}). The average value of θ was 14.8×10^{-2} rad (11 mg/ml of TYMV) which gives 1.9×10^{-2} dyne cm^{-2} rad^{-1} (19×10^{-2} μN cm^{-2} rad^{-1}) for the modulus of rigidity, M. At higher temperatures, M decreases. This value of M is very small compared to that of most elastic substances. The shearing stress required to start the rotor spinning at this temperature was found to be 0.17 dyne cm^{-2} (1.7 μN cm^{-2}). By reducing the torque in various amounts and waiting a short while, the rotor again stopped, but it was caused to start repeatedly when the torque was increased to give a stress of 0.17 dyne cm^{-2} (1.7 μN cm^{-2}).

In rheological studies (7) many materials have been found which apparently have visco-elastic properties in some ways similar to those encountered in this work, except that they occurred at much higher shearing stresses. This phenomenon is called thixotropy and is attributed to the formation of a structure that can be altered or destroyed by shearing forces of sufficient magnitude, but that again reforms when the forces are sufficiently reduced. The virus solutions studied in this work, although "thixotropic-like," have an apparently porous structure through which water and large molecules can pass, at least with some degree of freedom. The increase observed in the volume when the structure forms is noteworthy. It is premature to infer that the quasi-elastic behavior observed for this *in vitro* system is operative in the infective or replication processes of the virus. The pH and ionic strength used in most of the experiments to obtain the above values are much too high for biological systems. A study of these and other variables with respect to this quasi-elastic phenomenon, however, is indicated.

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