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Solid-like character of virus solutions

(coherency/shear stress/thin fibers/yield strength/turnip yellow mosaic virus)

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ABSTRACT **The solid-like behavior of turnip yellow mosaic virus solutions following the extrusion of viral RNA in alkali was observed with a torsion-fiber balance developed for the purpose. This method provided a direct measurement of the yield stresses required to break or liquefy these solutions. The yield stresses were found to increase and to be less time dependent with increasing concentrations of the virus and they were maximal at room temperatures. If the virus had been damaged, as by freeze-thaw, little or no solid-like behavior could be demonstrated. Purified viral capsids, with or without added RNA, were also inactive. The values for the yield stresses were of the same order as the value reported previously with the use of a magnetic suspension viscometer; hence, the apparent coherency appears unrelated to the magnetic fields generated by the latter instrument. These solutions behaved as typical liquids after the required stress was applied [about 0.005 to 0.17 dyne cm^{-2} (0.05 to 1.7 $\mu N\ cm^{-2}$)], these forces being smaller than those usually conferred by ordinary handling.**

By the use of a magnetic suspension viscometer which generated very small shearing stresses [about 10^{-3} dyne cm^{-2} (1 dyne = 10^{-5} N)] anomalously high viscosities and solid-state characteristics were observed with solutions of turnip yellow mosaic virus (TYMV) (1). In these cases, the high pH conditions were sufficient to cause extrusion of the RNA, leaving the isometric protein capsids largely intact in the solution (2). Recently, Ahmed *et al.* (3) have reported that magnetic fields of the order of that which we used for stably suspending the ferromagnetic rotor in these solutions gave rise to susceptibility characteristics in enzyme solutions suggestive of coherency. In order to determine whether the curious solid-like properties which we observed with these virus solutions resulted from the magnetic fields employed, a nonmagnetic, torsion-fiber technique was introduced. By this method, the solid-like behavior of the virus solutions at very low shearing stresses was confirmed. The method permits the direct evaluation of the shearing stress required to break the solid-like solution into a liquid. We describe here the manner in which the torsion-fiber principle was applied and report some values for the yield stresses on solutions of TYMV.

EXPERIMENTAL

The TYMV was purified and treated in the manner described for the experiments reported with the magnetic method (1).

Torsion-Fiber Apparatus. A schematic representation is shown in Fig. 1. Single fibers of DuPont Kevlar fiber, No. 49, about 110–125 cm long, were teased apart from filaments supplied by the manufacturer. These single fibers were about 12 μm in diameter and supported weights in excess of 10 g. (Tungsten fibers about 10 μm in diameter were also used successfully, but these fibers were more fragile.) A single fiber was attached to the center of one end face of a tantalum cylinder (about 1 × 0.28 cm, and weighing about 1 g) via a 27 gauge syringe needle implanted about 0.2 cm into the tantalum and protruding about 0.1–0.2 cm. It was important to center the fiber in the needle shaft (via microscope) when cementing the former in. The cylinder was ruled longitudinally at each 36 degrees of arc so that 0.01 of a revolution, N, could be estimated. The fiber with cylinder attached was first suspended directly above a cylindrical glass cell (about 2 × 0.6 cm inside diameter) for containing the solution (about 0.5 ml). The cell was mounted on a hollow stainless steel shaft connected by flexible tubing to the drive shaft of a small constant-speed motor. In this way the solution could be inserted and removed via a side vent in the hollow shaft without disturbing the fiber above the cell. The motor usually employed was one that rotated the shaft in either direction at a rate of $\frac{1}{3}$ rpm. After lowering the cylinder into the cell containing a liquid, we centered it with fine adjustments to the supporting stage. The rotatable glass cell was enclosed in a thermostated water bath (resting on the support stage) having a cylindrical Plexiglas wall to permit viewing of the suspended cylinder via a microscope (about 25 power). The top center of the water bath was fitted with a circular Teflon sleeve in close contact with a Teflon extension to the top portion of the cell; this arrangement provided for nearly friction-free rotation of the cell around the vertical axis. The circular aperture through the Teflon top of the cell was about 0.3 cm in diameter so that the cylinder could be lifted in and out by grasping the fiber gently. For the purpose at hand, the amount of evaporation was not significant during the measurements. A long Plexiglas chimney (about 100 × 3 cm) surrounded the fiber to prevent distortion by air currents; in addition, it presented a closed chamber so that water placed in a circular groove at the top of the water bath maintained a nearly saturated atmosphere. A narrower Plexiglas tube (about 50 × 2.5 cm), sliding inside of and closing off the top of the main chimney, was fitted with a 1 cm thick disk through which a section of a syringe needle glued to the top of the fiber was centered. By this sliding mechanism, different lengths of fiber could be employed and adjustments to the height of the cylinder in the cell could be made conveniently. The chimney with fiber and cylinder attached was mounted about 15 cm above the water bath and cell to support rods connected to a 250 kg marble table holding the support stand. Damping materials were utilized to reduce vertical and lateral vibrations to the order of 0.5 to 1.5 × 10^{-4} cm. Temperature fluctuations were <0.1° per hr. A short length (about 20 cm) of wider diameter Plexiglas was fitted to slide over the bottom of the main chimney. During measurements, this sliding extension rested on the water bath but still overlapped the bottom 5 cm of the main chimney. Raising the extension made the cell hole and fiber immediately above it accessible (such as for cleaning purposes or for pushing the fiber laterally if the cylinder became stuck to the cell wall during the filling procedure). On occasions, a tungsten cylinder was em-

Abbreviation: TYMV, turnip yellow mosaic virus.

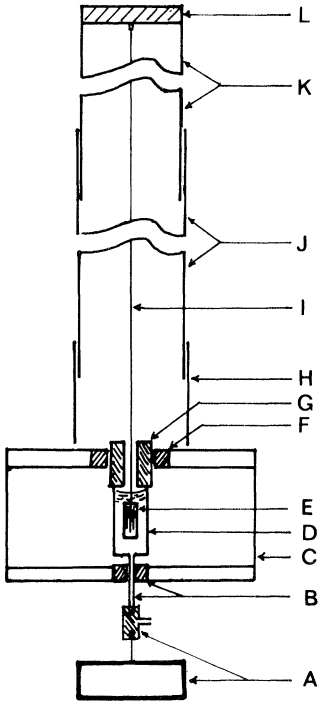

FIG. 1. Schematic drawing for torsion-fiber apparatus. A—reversible, constant-speed motor and shaft to Teflon coupler with side vent for filling; B—hollow shaft to cell and guide bearing; C—thermostated water bath; D—rotatable glass cell; E—immersed cylinder; F—guide bearing for cell top; G—cell extension to fit guide bearing; H—telescoping chimney extension for access; I—thin fiber, J—main chimney; K—telescoping upper chimney for height adjustments; L—fitted disk with center hole for anchoring fiber. Not shown: Support stand with leveling screws for water bath; side support brackets to chimneys; viewing microscope. For materials and approximate dimensions, see *text*.

ployed or a Plexiglas film was evaporated onto the surface of the metal cylinders without resulting in the loss of the solid-like behavior of the virus solutions.

Principle of the Method. When the cell containing a solution rotates about the suspended cylinder, a torque, L_A, is applied to the cylinder. As the latter begins to rotate, its motion is opposed by a restoring torque, L_R, owing to the twist produced in the fiber. The restoring constant of the fiber, C, is determined by the period of oscillation, t, of the cylinder while in air. Thus,

$$t = 2\pi \sqrt{\frac{I}{C}} \qquad [1]$$

in which I is the moment of inertia of the solid cylinder. The value of t was usually 100–120 sec. The creep and relaxation in the fibers were negligible during these experiments. Also,

$$L_R = 2\pi NC \qquad [2]$$

and

$$L_A = 4\pi\omega h\eta \left(\frac{b^2a^2}{b^2 - a^2}\right) \qquad [3]$$

in which N is the number of revolutions of the cylinder, ω is the angular velocity (rad sec^{-1}), η the viscosity (poise) of the solution, h the height and a the radius of the cylinder, and b the inner radius of the cell. This neglects end effects, which were determined or were calibrated out by measurements in distilled water. For these studies, $\omega = 0.349$ rad sec^{-1}. The yield stress, Υ_B, or shearing stress required to break the solid-like solution is given by

$$\Upsilon_B = \frac{NC}{a^2h} \qquad [4]$$

because $\Upsilon = L/(2\pi a^2h)$.

With true liquids, the cylinder turns until $L_R = L_A$. When this equality is obtained, the cylinder remains stationary while the cell is turning. The instrument was tested frequently with water and the calculated viscosities between 18 and 25°, neglecting end effects, were about 3% higher than handbook values. For the present cases studied, the constants of the instrument were such that 4–5 radians were transcribed by the cylinder in water at room temperatures. Owing to imperfections and slight misalignments, the observed rest position of the cylinder was often a fraction of a radian closer to its stationary position in a liquid with the drive turning in one direction than when turning in the reverse direction. The mean value of these two lengths of arc was taken as the number of radians ($2\pi N$) for calculating the viscosity.

In the case of a solid-like solution, the suspended cylinder continues to turn as the cell is rotated until the restoring torque is sufficient to "break" the solution. When this value of N is reached, the solution has liquefied and the cylinder rotates in the reverse direction. The reverse rotation continues until the viscosity of the liquid stops the cylinder or, with drive off, until the cylinder comes back to its starting or rest position (unless thixotropic-like behavior is encountered). The number of forward and reverse rotations before and after breaking a solid-like solution must be the same if the solution remains a liquid during the unwinding process. (Note: the torque on the fiber can be released by unwinding the fiber from above by turning the upper telescoping chimney. In this way, the modulus of rigidity for the solid can be extracted by measuring the relaxation angle transcribed by the cylinder. This measurement has not been achieved with good precision with the instrument as described. A modified apparatus is being tested which should achieve this purpose as well as that of measuring Υ_B without applying distortion to solid-like solutions during the measurements.)

Procedures. In the experiments with TYMV, appropriately diluted samples of stock virus solution were mixed with buffer at time zero to achieve the desired pH jump. The mixture (0.5 ml) was inserted by syringe via the side vent of the hollow shaft into the cell within about 40 sec from time zero. Any bubbles were quickly removed by swinging the cylinder from side to side with the fiber above. The meniscus was about 0.4 cm above the cylinder. After a fixed interval, the drive motor was started and the number of revolutions of the cylinder was counted by observing the distinctive ruled lines on the cylinder passing the vertical cross hair of the microscope. If the rate of rotation of the cylinder steadily decreased, the system was allowed to rest for a further period. When the solution became solid-like, the cylinder continued to rotate at the same rate as the glass cell. Very little warning was given before a breakpoint was reached. Sometimes a slight slippage or lengthening of the period of rotation of the cylinder relative to that of the drive speed was noted prior to the breakpoint. At the breakpoint, the cylinder stopped its forward rotation rather abruptly and reversed its direction promptly, whether the drive remained on or was turned off at that moment. With relatively strong solid-like solutions ($N > 50$), the reverse rotation rates were initially very fast, making it difficult to count the first few revolutions. The resulting disturbance to the solution apparently prevented any recovery of the solid-like character, and the cylinder back rotated to its starting position. As noted previously (1), with gentle

breaking of the solution, the solid reforms and the overall behavior simulates that of a thixotropic system. On some occasions, the back rotation did stop prematurely, and forward rotation was again noted when the drive was turned on. After vigorous stirring, little or no return of the solid-like property has been observed.

RESULTS

The yield stress, Υ_B, was found to increase with TYMV concentration in the manner shown in Fig. 2. These values may not represent the maximum ones for at least 2 reasons: (*i*) small misalignments have been found to affect Υ_B significantly; (*ii*) the values of Υ_B increased with time toward limiting values, longer intervals being required as the concentration was decreased. For example, at 3 mg/ml, the value of Υ_B leveled out only after about 15 hr at this temperature, whereas the values were already constant above 6 mg/ml, whether the cell was rotated immediately after mixing or 1 to 6 hr later. With the present apparatus, it was inconvenient to determine these time constants in a systematic manner owing to the very slow buildup of the restoring torque (20 N/hr). For most of our experiments, each revolution of the cylinder corresponded to about 1.6×10^{-3} dyne cm^{-2} being added to the stress on the solution every 3 min. It is anticipated that the modified apparatus, alluded to previously, will make it possible to follow the change in Υ_B with time more accurately. We point out that for a given preparation and otherwise the same conditions, the value of Υ_B was found to be almost identical when we used a tungsten fiber with a restoring constant nearly an order of magnitude larger than those of the organic fibers usually used (namely, about 10^{-4} dyne cm rad^{-1} versus $2\text{–}3 \times 10^{-5}$ dyne cm rad^{-1} for the latter). In this case, the cylinder was electrically grounded to the metal supporting rods surrounding the entire apparatus. Different preparations, as well as the length of storage at ice temperature, affected the time of onset of the phase change; however, these variables did not greatly affect the "maximum" values of Υ_B. Our studies at present indicate that the maximum value of Υ_B is stable for several hours at 19°, dropping off only slightly after about 1 day. Also, Υ_B was nearly independent of the ionic strength between 0.5 to 2.0 and it decreased to about 40% of the maximum value at ionic strength = 0.03.

A systematic study of the effect of suspended cylinders of different material and of various radii [i.e., the gap $(b - a)$ in Eq. 3] has not been carried out; the solid-like character has been observed, however, with $(b - a)$ varying between 1 to 1.8 mm and with cylinders in which tantalum, tungsten, Permalloy, Kel-F, and Plexiglas faced the solution. We have carried out a limited temperature study between 8 and 33° (4 mg/ml), showing that Υ_B increases from zero to a maximum at about 20°, declining thereafter to about half the maximum value at the higher temperatures. This important study will be taken up when the time constants are better known via the modified apparatus.

TYMV also loses its RNA upon freezing and thawing; the capsids in this case are severely damaged (4). Little or no solid-like character was discernible—whether tested immediately after thawing or after several days—even when the freeze-thaw conditions were adjusted to prevent complete destruction of the capsid (0.02 M sodium acetate). In the ultracentrifuge, this capsid material appeared heterogeneous, but it was still of high molecular weight ($s_{20} \approx$ 37–49 S). Intact capsids that were highly purified following the alkaline treatment (2) also have not exhibited a solid-like behavior, whether alone or in the presence of the appropriate amount of freshly prepared RNA via the freeze-thaw procedure. These experiments were carried out at molarities and conditions comparable to those used for the experiments with intact TYMV.

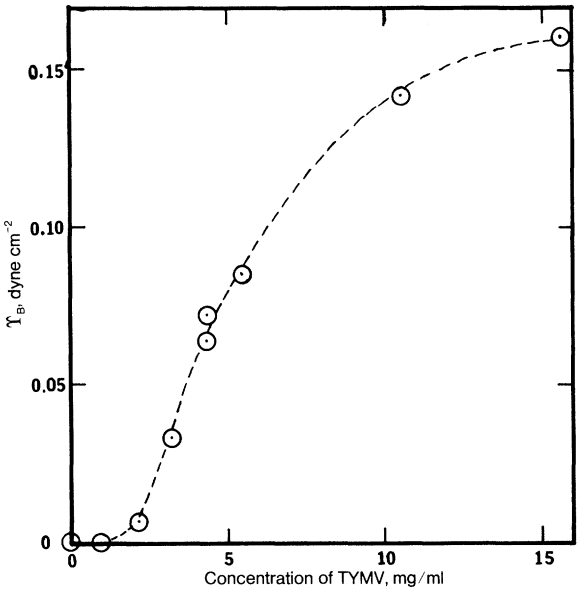

FIG. 2. Shear stress Υ_B required to liquefy the solid-like TYMV solutions at 19° as a function of virus concentration (mg/ml). These data were taken after adjusting the virus solutions to pH 12.6 with KOH; final medium, 0.1 M (K)PO_4/0.5 M KCl. (The broken curve is drawn for convenience in viewing.)

Experiments with another isometric RNA virus (f2 bacteriophage from *Escherichia coli*) that also gives rise to stable capsids have been initiated; we have not found the conditions, however, by which to observe more than a marginal solid-like behavior under the shear stresses applied to liquids (9×10^{-4} dyne cm^{-2}) via the current apparatus. This virus has nearly the same diameter, but is less massive than TYMV (namely, about 3.6×10^6 daltons versus 5.5×10^6 daltons for TYMV) and the capsids possess a correspondingly smaller potential net charge (about 1500 per capsid) as compared to about 2500–2900 for the TYMV capsid in the alkaline region.

DISCUSSION

The values of Υ_B with the torsion-fiber method are of the same order of magnitude as the result observed by means of the magnetic suspension method earlier (1). The absence of magnetic fields in the vicinity of the torsion-fiber instrument was assessed with a sensitive magnetometer. Unknown radiation sources also seem to be ruled out because the observations were performed in two different buildings about 1 km apart. We conclude, therefore, that the observed solid-like behavior is an inherent property of these solutions and is not generated in concert with the measuring techniques and is not initiated by outside forces.

Whether the observed coherency is important in any biological process is not known. In the case of TYMV, small though measurable yield stresses were observed at physiological temperatures (20–25°) at a lower net charge of the capsid, corresponding to the pH of the chloroplast (pH 8–9), the presumed target site of the virus. The time required to achieve the solid-like character was considerably longer (1–3 days).

We credit Mr. T. E. Dorrier for constructing the torsion-fiber apparatus, which required great patience and expertise in balancing the system properly. We also thank Mr. W. Frewer for fabricating the cylinders precisely and Dr. J. M. Kaper (U.S. Dept. of Agriculture, Beltsville, MD) for growing the TYMV and for many consultations.

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