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THE CONVERSION OF FIBRINOGEN TO FIBRIN

XX. TRANSVERSE WAVE PROPAGATION AND NON-NEWTONIAN FLOW IN SOLUTIONS OF INTERMEDIATE FIBRINOGEN POLYMERS

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INTRODUCTION

The intermediate polymers of fibrinogen formed in the course of clotting with thrombin[1], which can be stabilized against further aggregation by certain inhibitors[1–3] or at high pH[4,5], are believed to be rather stiff long rods of uniform width and varying length. Their cross-section area is twice that of the fibrinogen monomer[6], corresponding to a diameter of the order of 50 A, while their length, though variable[4,7], is at least of the order of 5000 A.

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As would be expected, the viscosity of solutions of such long macromolecules is strongly dependent on shear rate. Some studies of this phenomenon were described in an earlier paper of this series[2], but no quantitative conclusions could be drawn because at that time no adequate theory of non-Newtonian flow had been evaluated numerically; moreover, viscosity measurements by the falling sphere method could not be calculated in terms of homogeneous shear rate. Now, however, theories are available for describing the non-Newtonian flow of elongated rigid ellipsoids (SAITO[8], evaluated numerically by SCHERAGA[9]) and of rigid rods (KIRKWOOD AND PLOCK[10]) in dilute solution. From flow measurements made by the capillary method, calculations can provide the dependence of apparent viscosity on homogeneous shear rate, which then can be compared with theory to determine the relaxation time for orientation.

The present paper reports some preliminary experiments of this sort, together with a more spectacular phenomenon, the propagation of transverse waves in dilute solutions. This demonstrates the presence of an elastic component, as predicted by KIRKWOOD AND AUER[11] for suspensions of rigid rods; it is believed to be the first experimental demonstration of this effect.

MATERIALS AND METHODS

The fibrinogen was refractionated from Armour Fraction I of bovine plasma by methods previously described, most recently by CASASSA[6]. The solutions designated A and B below were derived from lots 210 and 128–163 respectively. The refractionated products contained 83% clottable protein as determined by the usual assay[12]. Stock solutions, dialyzed against 0.5 M sodium chloride-glycinate buffer, were mixed with a stock solution of hexamethylene glycol in the same solvent to provide a glycol concentration of 0.5 M, ionic strength 0.5, and pH about 9.4. The fibrinogen concentration was 4.8 and 7.1 g/l, respectively, in solutions A and B. In this environment, 0.5 unit thrombin per ml causes almost complete conversion of fibrinogen to intermediate polymer within a day without subsequent clotting[5]. In our solutions, sedimentation analysis with the oil turbine ultracentrifuge (operated by Mr. E. M. HANSON) indicated that about 80% of the protein was present as intermediate polymer.

Viscosity measurements were made at 25°C in horizontal zigzag capillaries, using a variable pressure head, as previously described for desoxyribonucleic acid solutions[13]. The rate of shear varied from 0.4 to 33 sec^{-1}.

Measurements of wave propagation were made at 25°C by a photoelastic method previously described[14], using an improved apparatus rebuilt by Mr. D. J. PLAZEK. Transverse waves could be observed at frequencies from 4 to 50 cycles/sec. The damping of the waves was so severe, however, that only one complete wavelength could be measured, and the damping index λ/x_0 could not be determined with any accuracy. Under these circumstances, the real part of the complex dynamic rigidity, G', cannot be calculated, but the results can be expressed in terms of the "wave rigidity", $\tilde{G} = \varrho\nu^2\lambda^2$, where ϱ is the density, ν the frequency, and λ the wavelength.

RESULTS

The nominal viscosity, η_m, was calculated from the usual Poiseuille equation and plotted against the nominal rate of shear, $D = \mathfrak{F}_R/\eta_m$, where $\mathfrak{F}_R$ is the maximum stress at the capillary wall, given by $rP/2l$, r and l being the radius and length of the capillary, and P the driving pressure. To calculate the apparent viscosity, η_a, as a function of homogeneous shear rate, $\dot{\gamma}$, slopes were measured and substituted into the equation[15]

$$\frac{1}{\eta_a} = \frac{1}{\eta_m}\left(1 - \frac{1}{4(1 + d \ln D/d \ln \eta_m)}\right) \qquad (1)$$

The shear rate $\dot{\gamma}$ to which η_a corresponds is given by $\dot{\gamma} = D\eta_m/\eta_a$.

According to theory[8–10], the viscosity increment $\eta_a - \eta_s$ in very dilute solution

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is a function of $\dot{\gamma}\tau$, where η_s is the solvent viscosity and τ the relaxation time; as $\dot{\gamma}$ approaches 0, $(\eta_a - \eta_s)/\eta_s c$ approaches the ordinary intrinsic viscosity. Our solutions are not sufficiently dilute for direct application of the theory, the extrapolated zero shear viscosity being 0.40 and 0.59 for solutions A and B respectively compared with 0.012 poises for the glycol-buffer solvent. We tentatively assume that the relaxation time under these conditions is proportional to η, as has sometimes been done in other frictional problems[16]. Then the normalized increment $(\eta_a - \eta_s)/(\eta - \eta_s)$ should be the same function of $\dot{\gamma}\eta$ at different concentrations. Such a plot does give a single composite curve for the two solutions, as shown in Fig. 1.

The corresponding theoretical curve for rigid rods[10] is a function of $\dot{\gamma}\tau$ alone. That for elongated ellipsoids depends also on the axial ratio but only very slightly when the latter is high as in this case; for the present calculations, the axial ratio was taken as 100. The theoretical curves on a double logarithmic plot can be matched to the experimental data by horizontal adjustment to determine the value of τ. The fit shown in Fig. 1 is fairly good and corresponds to $\dot{\gamma}\eta = 0.50$ and 0.44 for rod and ellipsoid, respectively, when $\dot{\gamma}\tau = 1$; by substituting the value of η for water at 25° we obtain τ reduced to the latter solvent (a common basis of comparison) as 2.95 and $3.4 \cdot 10^{-3}$ sec respectively.

If the same assumption about the concentration dependence of τ is made to evaluate the wave propagation data, then G'/c, as well as $\tilde{G}/c$, should be the same function of $\omega\eta$ at different concentrations. Here ω is the circular frequency. This reduction is again fulfilled by the experimental data, which fall on a single composite curve as shown in Fig. 2.

Although the theories of KIRKWOOD AND AUER[11] and of CERF[17] for rods and ellipsoids, respectively, predict only the shape of G'/c, this can be readily converted to the experimentally measured wave rigidity $\tilde{G}/c$. The theoretical equations for dispersion of G' and $\eta - \eta_s$ (η' being the real part of the complex dynamic viscosity) correspond mathematically to a mechanical model of a Maxwell element with a parallel dashpot[18]. The Maxwell and parallel dashpots have viscosity increments of $(3/4)(\eta - \eta_s)$

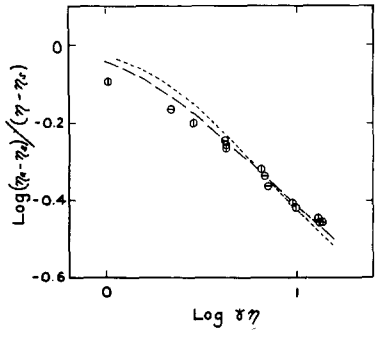

Fig. 1. Relative apparent viscosity increment, $(\eta_a - \eta_s)/(\eta - \eta_s)$, plotted logarithmically against reduced shear rate, $\dot{\gamma}\eta$. Vertical slots, 4.8 g fibrinogen per l; horizontal slots, 7.1 g/l. Long dashes, theory of SAITO for elongated ellipsoids, axial ratio 100, $\tau_w = 3.4 \cdot 10^{-3}$ sec. Short dashes, theory of KIRKWOOD AND PLOCK for rods, $\tau_w = 2.95 \cdot 10^{-3}$ sec.

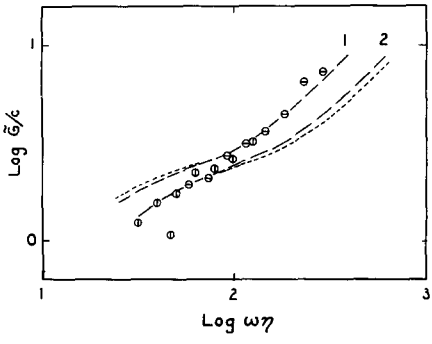

Fig. 2. Wave rigidity per unit concentration, $\tilde{G}/c$, plotted logarithmically against reduced circular frequency, $\omega\eta$. Key to concentrations same as in Fig. 1. Long dashes, theory of SAITO for highly elongated ellipsoids, $M = 5 \cdot 10^6$; 1, $\tau_w = 0.10 \cdot 10^{-3}$ sec; 2, $\tau_w = 0.062 \cdot 10^{-3}$ sec. Short dashes, theory of KIRKWOOD AND AUER for rods, $M = 5 \cdot 10^6$; 1, $\tau_w = 0.13 \cdot 10^{-3}$ sec; 2, $\tau_w = 0.071 \cdot 10^{-3}$ sec.

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and $(1/4)(\eta - \eta_s)$, respectively, for rods, and $(5/7)(\eta - \eta_s)$ and $(2/7)(\eta - \eta_s)$, respectively, for ellipsoids of high axial ratio. This model is in turn equivalent to a model with a slightly different arrangement of components for which the wave dispersion was calculated some years ago[19]—namely, a Maxwell model with a dashpot paralleling the spring alone. In this case the series dashpot is $(\eta - \eta_s)$ and the parallel one is $(1/3)(\eta - \eta_s)$ or $(2/5)(\eta - \eta_s)$ for rods or ellipsoids, respectively. Values of $\tilde{G}/G$, where G is the spring constant of the model, may now be calculated from equation 13a of ref.[19] and plotted against the variable $\omega\tau_s$, where the correspondence of the models specifies τ_s as $(\eta - \eta_s)/G$. The double logarithmic plots for the two molecular types are very similar in shape and have a small characteristic inflection near $\tilde{G} = G$, as seen in Fig. 2.

If either theory is applicable, the frequency dependence of $\tilde{G}$ should have the prescribed shape, and moreover the damping index λ/x_0 (where x_0 is the propagation distance within which the wave amplitude falls off by a factor of $1/e$) would have rather high values lying between 3 and 4, as calculated from equation 13b of ref.[19]. The latter requirement is qualitatively fulfilled, since the wave amplitude vanishes within experimental perception in slightly over one wavelength. The dispersion of $\tilde{G}$ may now be examined graphically.

In principle, double logarithmic plots can be used to match theory with experiment by both horizontal and vertical adjustments, determining both G and τ. We have, however, taken the value of G/c which is specified by both theories[9,11], namely $3RT/5M$, where M is the molecular weight. Although M is not known for the intermediate polymer in alkaline solutions[6], it is perhaps not far from the value of 5,000,000 estimated from light scattering[20] at pH 6.2. Using this value, G/c is calculated to be 3.0 dyn cm^{-2} $(g/l)^{-1}$, fixing the vertical position of the theoretical curve for $\tilde{G}/c$. In Fig. 2, each curve is drawn at two arbitrary horizontal positions corresponding to extreme values of τ: 0.071 to $0.127 \cdot 10^{-3}$ sec for rods, and 0.062 to $0.10 \cdot 10^{-3}$ sec for ellipsoids when reduced to water at 25°C. The experimental points are in fair agreement.

DISCUSSION

The reasonable agreement in form of the shear rate dependence of viscosity and the frequency dependence of wave rigidity with theoretical calculations for rigid rods or elongated ellipsoids lends confidence to the view that the intermediate fibrinogen polymer is indeed a stiff elongated structure, as has been assumed in interpreting various other physical chemical properties. The appearance of elasticity, as evidenced by transverse wave propagation, in a rather dilute solution of such rigid particles is believed to be the first experimental demonstration of the effect predicted by KIRKWOOD AND AUER.

Unfortunately, the relaxation times derived from the non-Newtonian flow and the transverse wave propagation do not agree, the former being much larger. However, the molecular lengths calculated therefrom are not so discrepant. Using the appropriate formulas[9-11] and taking the maximum molecular width (to which the calculation is quite insensitive) as 50 A, we obtain for the molecular length from non-Newtonian flow 7600 A for rod and 7000 A for ellipsoid. From wave propagation we obtain 2100–2600 A for rod and 1800–2100 A for ellipsoid. In magnitude these agree satisfactorily with results from flow birefringence[4,7], which show a distribution of lengths of the order of 4000 to 6000 A. The discrepancies probably reflect different effective

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averages over a distribution. The average should appear lower in wave propagation, where the contributions to rigidity are proportional to the numbers of molecular species, than in non-Newtonian flow, where the contributions to viscosity are weighted, probably with the square of the molecular length. It may be remarked, however, that if the solvent instead of the solution viscosity were used in calculating τ from wave propagation, the molecular length derived therefrom would be about 8000 A.

The detection of the intermediate fibrinogen polymer by purely mechanical measurements suggests the intriguing possibility of assaying this product of incipient clotting in plasma or even whole blood—especially in hypercoagulable states—by sensitive viscoelastic methods.

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SUMMARY

Non-Newtonian flow and transverse wave propagation have been studied in solutions of intermediate polymers of bovine fibrinogen, formed by thrombin at pH 9.4, ionic strength 0.5, in the presence of 0.5 *M* hexamethylene glycol. The results conform approximately to the behavior of rigid rods or elongated ellipsoids as predicted by KIRKWOOD, SAITO AND CERF. The only adjustable parameter in applying these theories is the relaxation time, from which molecular lengths of about 7000 A and 2000 A are calculated from non-Newtonian flow and wave propagation respectively. These agree well in magnitude with values derived from birefringence; the difference between them is probably attributable to a distribution of molecular lengths.

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