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Collagen as a Biomaterial: Modulus Jump and Degradation of Collagen Gels*

CARL W. ZVANUT and FERDINAND RODRIGUEZ, *School of Chemical Engineering, Olin Hall, Cornell University, Ithaca, New York 14853*

Synopsis

The effect of temperature on the storage modulus G' of UV crosslinked collagen has been investigated. Collagen solutions, 0.5%, at pH 2.2 were irradiated at 4°C to a maximum modulus, after which they were stored at 4°C. In order to study the effect of temperatures above 4°C, the gels were placed in a constant-temperature bath and the change in modulus with time was recorded using a torsion pendulum. The temperatures studied ranged from 25° to 60°C. The results show an initial jump in modulus immediately on heating presumably due to the helix–coil transition. The modulus goes through a maximum value. There is then a decay of modulus with time which is linear on a semilog plot. This could indicate a first-order chain-scission reaction, since the storage modulus is directly proportional to the number of crosslinks. A simple kinetic expression can be written for which the value of the rate constant can be determined from the data.

INTRODUCTION

Dilute aqueous collagen solutions can be converted to crosslinked gels by the action of UV light under an inert atmosphere.[1,2] The dilute gels (0.1% to 1% collagen) themselves have potential application as burn dressings and as replacements for certain body fluids.[3] In addition, the mechanism of crosslinking can be studied more conveniently than in concentrated solutions. Crosslinking of films and tubes (5% to 20% collagen) is a method of toughening and insolubilizing reconstituted collagen for use in various prosthetic applications and for use in dialysis membranes.

When a dilute collagen solution which has been gelled at 4°C is heated above about 25°C, the shear modulus increases rapidly, goes through a maximum, and then decays in exponential fashion. In the present work, the time and temperature dependences of shear modulus have been studied. In order to help elucidate the mechanism, an examination of the changes in optical activity of gels and a parallel study of the degeneration of solution viscosity for uncrosslinked collagen have been added.

EXPERIMENTAL

Apparatus and Procedures

As described previously,[1] a collagen solution in a coaxial-cylinder geometry is centered in a chamber with eight 15-watt germicidal lamps which radiate

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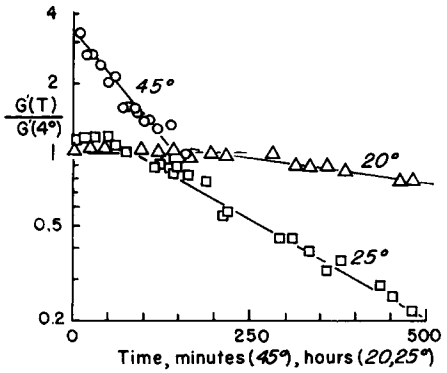

Fig. 1. Storage modulus G' of 0.5% collagen solutions at pH 2.2.

primarily at 254 nm. The solution, when gelled, acts as the restoring element in a torsion pendulum. In order to handle a number of gels simultaneously during prolonged observations, several simple torsion pendulums were constructed to supplement the more elaborate one previously described.[1] Each simple pendulum consists of a glass rod (radius r_1 = 0.20 cm, immersed length L = 7.1 cm) in a quartz tube (radius r_2 = 1.40 cm). The pointed lower end of the rod acts as a bearing. The upper end of the glass rod has a cross arm providing a constant moment of inertia. Modulus is calculated from the frequency of oscillations (ω, radians/sec) and the moment of inertia (I, g-cm^2) of the pendulum from the formula

$$G = \omega^2 I[(r_1)^{-2} - (r_2)^{-2}]/(4\pi L) \quad (1)$$

In general, ω was in the range of 6 to 18 rad/sec. The changes in modulus from the original value at 4°C as measured in these small pendulums checked well with those obtained in the more elegant, quartz-metal model previously described.

Viscosity of uncrosslinked collagen solutions was measured in Ubbelohde viscometers. For the measurement of specific rotation, collagen gels were prepared in quartz tubes with flat ends and a path length of 10 cm. Although the polarimeter used is capable of resolving angles ±0.5 minutes, turbidity of the gels caused some scattering of results.

The material used was twice enzyme-treated, telopeptide-poor collagen similar to that previously described.[1]

RESULTS AND DISCUSSION

Modulus–Time Behavior

Collagen solutions (pH 2.2) were irradiated at 4°C to a maximum modulus in 240 min, after which they were stored at 4°C. In order to study the effect of higher temperatures, the gels were placed in a constant-temperature bath and the modulus was measured at intervals. Typical behavior at three temperatures (Fig. 1) shows the jump and degradation phenomena. At temperatures above 25°C, there is an initial jump immediately on heating. The modulus goes through a maximum value, G'_T, after which there is a linear decay

of log modulus with time. The decay rate is what can be expected for a first-order chain-scission reaction, since the storage modulus is believed to be directly proportional to the number of crosslinks.[4]

A simple kinetic expression can be written for which the value of the rate constant can be determined from the data:

$$-\frac{dN}{dt} = kN \text{ or } -\frac{dG'}{dt} = kG' \tag{2}$$

since

$$G' \sim N \tag{3}$$

then,

$$-\ln G/G_0 = k(t - t_0) \tag{4}$$

or

$$k = -\left(\frac{\ln G/G_0}{t - t_0}\right) \tag{5}$$

where G' = storage modulus, dynes/cm^2; t = time, min; k = rate constant, min^{-1}; and N = number of crosslinks.

The rate constant is the slope of the lines in Figure 1. At low temperatures, a direct dependence of the logarithm of G' on time is obtained only after an initial "induction period" during which modulus is almost constant. This effect is typified by the 20°C line. The length of this "induction period" is longest at the lowest rates of reaction. Furthermore, the dimensionless product of this "induction period" and the rate constant is, for the three cases for which it can be measured, independent of temperature. This would indicate that although the scission reaction may occur during the "induction period," it does not affect the modulus until a certain minimum number of bonds are broken. This would be reasonable for collagen since it is in a triple helix, and one bond could be broken without affecting the gel modulus. Another explanation could be that parts of the collagen molecule that are not directly involved with the gel network are broken, and this also would not affect the modulus.

Activation Energies From Modulus Data

The activation energy for the above reaction can be determined using the Arrhenius temperature dependence for the rate constant. The natural log of the rate constant versus reciprocal temperature is plotted in Figure 2. There is some scatter in the data, but it can be presented adequately as a straight line above 40°C. The activation energy for the reaction above 40°C is 28.5 kcal/mole. This value is close to the 25 kcal/mole that was found by Weaver[5] for the hydrolysis of acid-soluble, rat-tail tendon collagen above 40°C and by Scatchard et al.[6] for the hydrolysis of bovine ossein gelatin. It is quite likely that hydrolysis does occur since the solution is acidic, and peptide linkages are hydrolyzed under the influence of acids. In the 40–30°C region, the rate constant value falls rapidly. This temperature range corresponds to the collagen–gelatin transition region, so one would expect this shift to be linked to

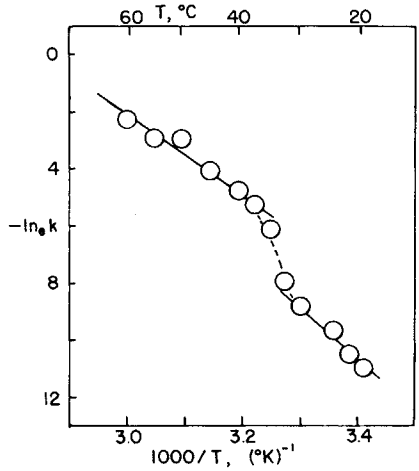

Fig. 2. Temperature dependence of rate constant k derived from plots like Fig. 1 (0.5% collagen, pH 2.2). Energy of activation derived from straight-line portions is 28.5 kcal/mole above 40° and 38 kcal/mole below 30°C.

that transition. In the 30–20°C region, the data can again be represented as a straight line, but with a somewhat greater slope than in the above 40°C region. This yields an activation energy of 38 kcal/mole. Considering the difficulty of establishing the activation energy at lower temperatures, the fact that the sub-30°C value and over-40°C value differ by only 30% may mean that the same basic process is involved in both ranges.

Viscosity–Time Behavior

In order to better understand the hydrolysis of the collagen gel, the same reaction was studied for collagen solutions. The viscosity of 0.1% solutions of collagen was followed with time at different temperatures. The viscosity of the solution, η, is converted to the specific viscosity:

$$\eta_{sp} = \frac{\eta}{\eta_s} - 1 \tag{6}$$

where η_{sp} = specific viscosity and η_s = viscosity of the solvent.

The specific viscosity can be related to the rate of chain scission if the assumption is made that the number-average chain length is proportional to the specific viscosity.[7] This is probably not a bad assumption for a rod-like polymer such as collagen for which the specific viscosity is almost directly proportional to the molecular weight. The number-average chain length is proportional to the molecular weight, provided the molecular weight distribution is not large. The collagen is a monodisperse polymer to begin with. The distribution broadens inevitably as degradation occurs. The specific viscosity is taken to be proportional to the intrinsic viscosity, which it should be at such a low concentration. Although the denatured collagen is a random coil, it has a rod-like shape, and this analysis is assumed to hold for it, too. The rod-like shape is demonstrated by the fact that, if the molecule is considered as a rigid ellipsoid, the ratio of length to diameter is about 25 at the

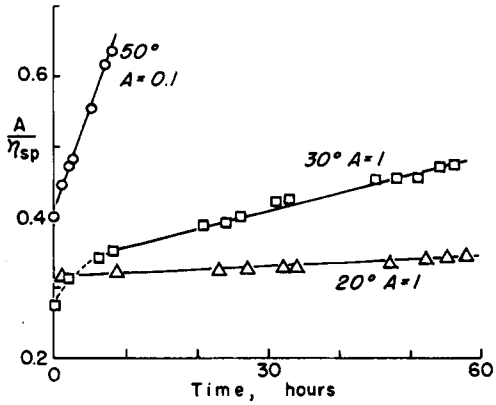

Fig. 3. Reciprocal of specific viscosity η_{sp} vs. time for 0.1% collagen solutions at pH 2.2 (A is an arbitrary constant for convenience in plotting).

isoionic point.[8] Since our solutions were far on the acid side of the isoionic point, the molecule should have a net positive charge, and the charge repulsion will cause molecular extension. The analysis should be reasonable, then, for both collagen and gelatin.

The equation for the rate of chain scission in solution becomes

$$1/\eta_{sp} - 1/(\eta_{sp})_0 = k't \tag{7}$$

where $(\eta_{sp})_0$ = specific viscosity at $t = 0$; k' = rate constant, sec^{-1}; and t = time, min. A plot of the reciprocal of the specific viscosity against time should yield a straight line whose slope is the rate constant (Fig. 3). The plots resulted in straight lines except for some initial curvature in the experiments run at 35°, 32.5°, and 30°C. This curvature is due to the collagen–gelatin transition, which occurs almost instantaneously above 35°C and not at all below 25°C. Once the transition is completed, however, the reaction proceeds as expected.

Activation Energies from Viscosity Data

An Arrhenius plot for the temperature dependence of the rate constant from viscosity–decay data is presented in Figure 4. The curve obtained is similar to that in Figure 2 for the hydrolysis of gels. The activation energy above 40°C is 20 kcal/mole, which is lower than that obtained for the gel. This could be due in part to experimental error, but the major effect is probably a stabilizing influence by the crosslinks in the gel. This value is also lower than the literature value of 25 kcal/mole, but since this is telopeptide-poor collagen, it is probably not as stable as native collagen. Between 40° and 25°C, the rate drops appreciably, which is again attributable to the collagen–gelatin transition. Below 25°C, the curve seems to straighten out again, and the activation energy for this region is the same as before. This sudden drop in rate for both the solution and gel between 40° and 25°C is due mainly to a change in the preexponential factor in the Arrhenius equation.

This change in the preexponential factor can be explained easily if one uses an expression for the rate constant from transition state theory:[9]

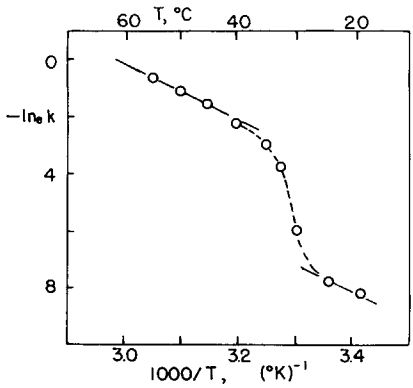

Fig. 4. Temperature dependence of rate constant k derived from plots like Fig. 3 (0.1% collagen, pH 2.2). Energy of activation derived from straight-line portions is 20 kcal/mole.

$$k = \left(\frac{ekT}{h}\right) \exp\left(\frac{E_a}{RT}\right) \exp\left(\frac{\Delta S^{0\ddagger}}{R}\right) \tag{8}$$

where e, k, and h = universal constants; T = absolute temperature; E_a = activation energy; R = gas constant; and $\Delta S^{0\ddagger}$ = standard entropy of activation. The standard entropy of activation is the difference between the entropy of the activated complex and the entropy of the reactants. Any change in this standard entropy will result in a change in the intercept on an Arrhenius plot. Heating a collagen solution above 25°C induces a conformational change to gelatin, which will cause a great change in entropy of the molecule. Furthermore, a scission in the gelatin molecule will create a greater entropy change than in the collagen molecule, since the product would be free to split apart in the gelatin, whereas the helical structure of the collagen could retain a great amount of order. This would explain the sudden drop in the rate constant for this reaction.

Modulus Jump and Optical Rotation

Another interesting effect of heating the gels is the initial increase in modulus of the gels. The ratio of the maximum modulus at each temperature to the original modulus at 4°C is plotted against temperature in Figure 5. The specific rotation of the 0.5% gel was also measured as a function of temperature, and this is also plotted in Figure 5.

The maximum modulus ratio remained constant until the temperature reached 27.5°C. The modulus then jumped upon heating to a value which increased until the temperature reached 37.5°C. At this point, the maximum modulus ratio leveled off at 4.0. This modulus jump was attributed to the uncoiling of the triple helix of collagen. Collagen has a specific angular rotation around 400°, while that for gelatin is near 180°.[10] The specific rotation, then, will be an indication of the state of denaturation of the molecule. It is shown in Figure 5 that the collagen to gelatin transition occurs between 25° and 40°C. This is the same temperature region for the change in maximum modulus ratio, and for the shift in the preexponential factor in the Arrhenius

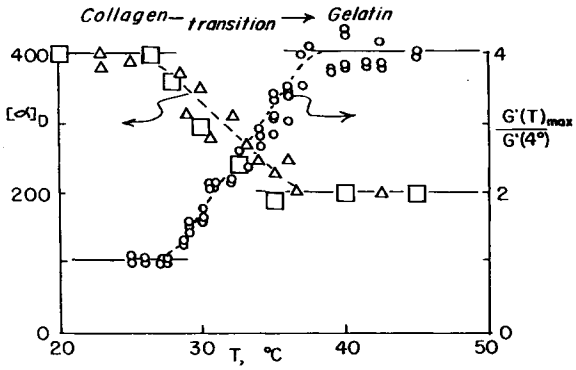

Fig. 5. Specific rotation $[\alpha]_D$ and maximum modulus ratio at increasing temperatures. For samples designated by squares, rotation was measured only at one temperature. For the triangles, the temperature was raised sequentially for the same sample.

plots for the hydrolysis reaction. It is only reasonable, then, to attribute all these effects to the helix–coil transition.

It is somewhat surprising that the denaturation of the protein produces an increase in the modulus of the gel. The viscosity of a collagen solution decreases tremendously after denaturation, as can be seen in the specific viscosity data. This is to be expected since the uncoiling of the triple helix produces three nonrigid "random coils," which greatly lowers the viscosity of the solution. In the gel, however, the protein molecule is crosslinked to other protein molecules, and possibly intramolecularly crosslinked. The individual chains of the helix, then, are not able to move into a random coil, but do move enough to release some strain in the gel system and to possibly form hydrogen bonds with other chains. These effects combine to allow the gel to store more energy and, hence, to increase its modulus.

The time dependence of the denaturation of collagen was also investigated by following the optical rotation of 0.5% gels. This was found not to occur at all for days for temperatures below 25°C, and to occur completely within a few minutes for temperatures above 35°C. For temperatures between 25° and 35°C, the partial denaturation occurred within 20 min, and then no further reaction occurred. Since the modulus jump occurs immediately after heating the gel, this phenomenon is consistent with the time dependence for the denaturation. The chain-scission reaction, however, occurs at long times relative to the uncoiling of the helix and hence cannot be attributed to it.

CONCLUSIONS

For the collagen gel to be useful as a replacement part for the body, a more stable gel at physiological temperatures is required. Even if the gel is not completely stable, it may find applications as a drug-release medium in the body. A drug could be bound to the collagen, and then diffuse out slowly before the gel melted. This study has shown that UV-induced crosslinks are at least as stable as the peptide linkages in the collagen itself. It would seem that production of stronger, more stable crosslinks is not as important as stabilization of the collagen molecule itself. Efforts to increase gel modulus and

stability may include modification of the protein itself, co-crosslinking with synthetic polymers, and changing the pH of the solution. Investigation of these and other effects may produce a more stable collagen gel.

References

1. G. Hamed and F. Rodriguez, *J. Appl. Polym. Sci.*, **19,** 3299 (1975).
2. T. Miyata, T. Sohde, A. L. Rubin, and K. H. Stenzel, *Biochim. Biophys. Acta,* **229,** 672 (1971).
3. K. H. Stenzel, M. W. Dunn, A. L. Rubin, and T. Miyata, *Science,* **164,** 1282 (1969).
4. J. D. Ferry, *Viscoelastic Properties of Polymers,* Wiley, New York, 1970.
5. E. S. Weaver, Ph.D. Thesis, Cornell University, Ithaca, New York, 1959 (Professor P. J. Flory, Advisor).
6. G. Scatchard, J. L. Oncley, J. W. Williams, and A. Brown, *J. Amer. Chem. Soc.*, **66,** 1980 (1944).
7. N. Grassie, *The Chemistry of High Polymer Degradation Processes,* Interscience, New York, 1956.
8. G. Stainsby, *Nature,* **169,** 662 (1952).
9. I. Amdur and G. Hammes, *Chemical Kinetics,* McGraw-Hill, New York, 1966.
10. G. N. Ramachandran, *Treatise on Collagen,* Vol. 1, Academic Press, New York, 1967.

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