Notes

Viscoelastic Distinction between Helical and Coiled Macromolecules

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Collagen solutions are considerably more viscous than gelatin solutions. During the helix–coil transition, occurring in dilute solution, the intrinsic viscosity of the rigid, rodlike tropocollagen molecules, which have dimensions 2800 × 15 Å, drops from about 11.5 dl/g to the considerably lower level, about 0.5 dl/g, characteristic of the flexible gelatin chains.[1]

The question poses itself whether an equally clear distinction between the helical and coiled forms of this protein in the solid state can be made by measurements of the tensile modulus and, if so, whether the indisputably higher stiffness of isolated tropocollagen molecules possibly imparts to a solid-state assembly of such helical molecules a higher modulus relative to the equivalent assembly of gelatin chains. Measurements recently conducted in our laboratory show that a distinction between helices and coils in the solid state can indeed be clearly drawn on the basis of mechanical measurements. On the other hand, we observe that gelatin films have considerably higher moduli than collagen films.

Solutions of tropocollagen in 0.05 *M* acetic acid were prepared by extraction from rat tail tendon with dilute acetic acid, following a procedure which has been described in detail.[2] The nativity of collagen in these solutions was verified by measurement of the specific optical rotation, [α], and of the intrinsic viscosity, [η], the results of such measurements agreeing closely with published values.[1,3] Gelatin solutions in distilled water were prepared from a grade of bone gelatin, the properties of which have been described elsewhere.[4] Removal of the aqueous solvent by atmospheric evaporation at room temperature leads to formation of films of collagen or gelatin which incorporate about 10–15 wt % water.

The viscoelastic behavior of polymers can be conveniently determined in general by measuring the isochronous modulus over a 150° range of the temperature.[5] Such a procedure is, however, impractical with hydrated gelatin and collagen films because they readily lose a substantial[6] fraction of their water upon heating close to 100° and undergo thereby significant changes in their mechanical behavior. It has, however, been shown that cumulative isothermal incorporation of diluent can cause a polymer to adopt successively behavior which spans the entire viscoelastic spectrum.[7] In this work we have relied on the latter method to elicit distinctions between the viscoelastic behavior of collagen and gelatin films at room temperature. Since hydrated films exchange water with the environment at inconveniently rapid rates, anhydrous glycerol was used as diluent in this work. The procedure for preparation of gelatin–glycerol films containing only traces of moisture has been described[4] and was also used for preparation of collagen–glycerol films. The 10-sec tensile stress relaxation modulus was measured at 25° with an Instron tester, using procedures which have been described elsewhere in detail[8] and employing precautionary measures for the exclusion of moisture uptake by the specimens, either before or during testing, which have been also described.[4]

Since the goal of this research was to distinguish mechanically between specimens composed of helical molecules and diluent, on one hand, and randomly coiled chains and diluent, on the other, considerable attention was paid to the structural analysis of the specimens. Gelatin films were cast at 65° ("hot-cast" gelatin) and wide-angle X-ray photographs of such films confirmed the absence[9] of any intense reflections, while measurement of the specific optical rotation, [α], of these films at 365 mμ showed that they possessed rotatory power identical with that observed with solutions of randomly coiled gelatin chains[3] (Figure 1). In the diluent concentration range 50–90 wt %, gelatin films formed rubbery gels which were allowed to stand for several hours at 25° prior to testing until the modulus reached a limiting value. After casting in the presence of glycerol, collagen films were redissolved in 0.05 *M* acetic acid at 25° and the intrinsic viscosity as well as the specific optical rotation were once more determined and found to be unchanged from the values determined with aqueous solutions of tropocollagen freshly extracted. In addition, wide-angle X-ray diffraction photographs obtained with the beam normal as well as parallel to the plane of the collagen films showed several Debye–Scherrer rings or arc reflections corresponding to spacings which have been attributed to the triple helical structure of collagen.[10] These X-ray diagrams leave no doubt that the helical marcomolecules in the collagen films are randomly disposed in the plane of the film. The specific optical rotation of collagen films incorporating glycerol was measured at 365 mμ and found to be about −3200°, almost identical with values of [α] obtained with films which had never been exposed to glycerol. The value of [α] observed with collagen films at 365 mμ can be compared with a value of about −1700° observed with gelatin films cast at 23° ("cold-cast" gelatin); such comparison provides additional independent evidence that the collagen triple helix[11,12] was conserved during casting of films from the solutions of tropocollagen[13] which we had prepared and, in particular, that the helix was unaffected by incorporation of glycerol over the entire range of dilution covered in this study (Figure 1). We also wish to mention that the level of dehydration achieved with all films used in this study was in the vicinity of 1 wt % water and never sufficiently low to cause covalent cross-linking which occurs upon dehydration of gela-

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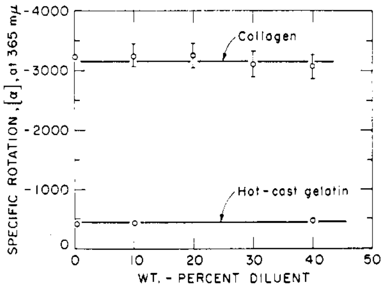

Figure 1. Independence of the specific optical rotation, measured at 365 mμ, from effects due to incorporation of diluent (glycerol).

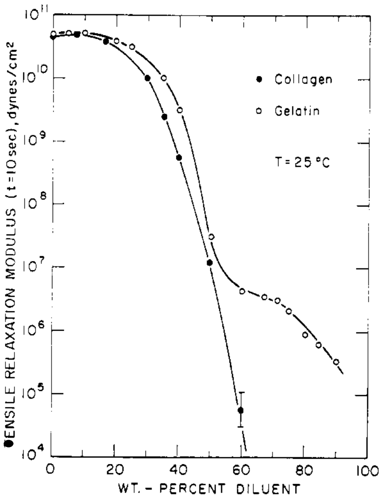

Figure 2. The 10-sec tensile relaxation modulus of collagen and gelatin films, plotted as function of the diluent content.

tin[14] and collagen[15] to moisture levels below *ca.* 0.5 wt % water. Flat-plate X-ray photographs were taken with a Norelco camera using Ni-filtered Cu Kα radiation and an exposure time of 1 hr. Measurements of optical rotation of films were made with a Cary 60 CD spectropolarimeter precautions having been taken to prevent the development of birefringence in films during casting.

The results in Figure 2 show that the 10-sec tensile relaxation modulus of gelatin films measured at small strains is almost ten times higher than that of collagen films in the diluent concentration range 30–50 wt %. In this range of relatively low diluent content, gelatin films showed their well-known ability of elongating up to at least 100%, with delayed but complete recovery following removal of the load. By contrast, collagen films in the same diluent range (30–50 wt %) deformed permanently. At higher diluent concentration levels, between 50 and 80 wt %, the disparity in properties between collagen and gelatin specimens became even sharper (Figure 2). In this concentration range, gelatin forms tough gels which behave as rubbers,[16] possessing small-strain moduli in the region 10^6–10^7 dyn/cm^2 and capable of extending to *ca.* 700% with complete and immediate recovery up to fracture; by comparison, we found that collagen preparations were not capable of forming gels at all, even after prolonged residence at temperatures as low as 4°. Instead, collagen specimens were very weak solids at about 60 wt % diluent and became viscous liquids somewhat above this level of diluent content.

The observation of substantially lower moduli and of plastic deformation with collagen films is consistent with a simple model composed of stiff helical macromolecules, not bonded covalently to each other and immersed in a viscous Newtonian fluid (glycerol). In gelatin specimens, the diluent swells a network of entangled chains which are equipped with a profusion of evenly spaced "hinges" at the chain backbone (glycine residues) around which relatively unrestricted rotation can occur. The high intrinsic flexibility of the gelatin chains exhibits itself clearly macroscopically only when individual chains are sufficiently separated from each other by incorporation of diluent. When, however, these flexible polypeptide chains are locked in the orderly arrangement of the collagen helix, the resulting triple-stranded macromolecules behave as rods which fail to entangle due to their apparent stiffness. In the presence of diluent, these assemblies of collagen molecules behave as highly viscous suspensions which, even though concentrated enough to exhibit a sizable tensile modulus, are incapable of any mechanical recovery.

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Ideal Network Behavior of Anionically Prepared Polystyrene Gels

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Recently Johnson and Mark[1] communicated the results of a series of experiments to settle a still remaining controversy in the molecular theory of rubber elasticity. The controversy centers on the existence of a logarithmic form in the free energy of network deformation and on the value of the coefficient, B, in front of it

$$\Delta F_{\text{net}}/RT = (\nu/2)(\lambda_x^2 + \lambda_y^2 + \lambda_z^2 - 3) - B\nu \ln \lambda_x\lambda_y\lambda_z \quad (1)$$

In eq 1 λ_x, λ_y, and λ_z are the deformation ratios with respect to an isotropic reference state in which the polymer chains between cross-links are unstrained; ν is the number of elastically effective chains in the network; and B is a numerical factor about which no certainty exists.

Previous work by a variety of authors—summarized in a review paper in 1969[2]—had already established that the logarithmic term exists. Theoretical as well as experimental arguments were presented to support the Flory–Wall value of $B = 2/f = {}^1/_2$ for a tetrafunctionally cross-linked network ($f = 4$). Unequivocal proof for the precise value of B has proven to be elusive because it requires a precise knowledge of ν. No networks have been prepared to date for which the chemistry and topology allow the determination of ν with absolute certainty. Johnson and Mark's experiments neatly eliminated the necessity of a knowledge of ν by comparing the elastic stress in two types of networks, both swollen to the same degree in pure diluent, but cross-linked in solution and in bulk, respectively. This analysis required the assumption that the chain dimensions do not alter during the cross-linking process.

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