vergence, $\langle(\Delta r)^2\rangle$ for a two-tiered tetrafunctional tree is 0.340, for a three-tiered tree it is 0.363, and for an infinite tree it is 0.375.

Appendix B

If the results given in Appendix A are substituted into eq 12, the scattering law for f functional networks with strands of equal length is obtained

$$S(\mathbf{q}_\parallel) = \frac{1}{(\nu\alpha\xi)^{1/2}}\left(\frac{2\alpha\xi+1}{\alpha\xi}\right)\left\{F_1\left(\frac{\nu}{4\alpha\xi}\right)^{1/2} - \exp[-\nu(\alpha\xi+1)]F_1\left(\left(\frac{\nu}{4\alpha\xi}\right)^{1/2}(2\alpha\xi+1)\right)\right\} + \frac{1}{\nu\alpha\xi}\{\exp[-\nu(\alpha\xi+1)]-1\} \quad \text{(B1)}$$

where $\zeta = (f-2)/f$ and $\mathbf{q}$ is the scattering vector parallel to the principal strain axis. The other symbols are defined after eq 16. A similar expression is found when $\mathbf{q}$ is perpendicular to the strain axis.

$$S(\mathbf{q}_\perp) = \frac{1}{(\nu\beta\xi)^{1/2}}\left(\frac{2\beta\xi-1}{\beta\xi}\right) \times \left\{\exp[-\nu(1-\beta\xi)]F_2\left(\left(\frac{\nu}{4\beta\xi}\right)^{1/2}(1-2\beta\xi)\right) - F_2\left(\frac{\nu}{4\beta\xi}\right)^{1/2}\right\} - \frac{1}{\nu\beta\xi}\{\exp[-\nu(1-\beta\xi)]-1\} \quad \text{(B2)}$$

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(13) The matrix Γ as defined in eq A3 has dimensions $\mu \times \mu$. To conform with $\mathbf{R}$ it should be expanded to $3\mu \times 3\mu$ by direct multiplication with the 3×3 identity matrix. Furthermore Γ will not be positive definite unless one of the fixed points is chosen as an internal coordinate. When this is done the dimensionality of $\mathbf{R}$ is reduced to $3(\mu-1)$ and Γ to $(\mu-1)\times(\mu-1)$. See B. E. Eichinger, *Macromolecules*, **4**, 496 (1972).
(14) The number of free junctions in a symmetric tree built up from a central strand is $\mu_J = 2[(f-1)^t - 1]/(f-2)$ where f is the functionality of the junctions and t is the number of tiers in the tree.

Interactions between Poly(L-lysine) and Chondroitin 6-Sulfate. Quasi-Elastic Light Scattering Studies

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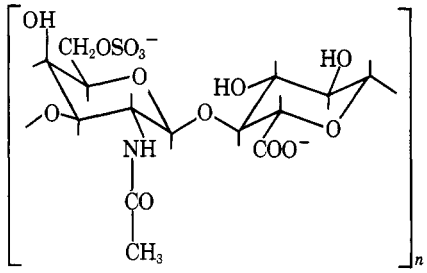
ABSTRACT: Quasi-elastic laser light scattering studies have shown that large multimolecular aggregates are formed on mixing dilute aqueous solutions of poly(L-lysine) and chondroitin 6-sulfate. For the two components in equimolar residue proportions at a total concentration of 0.178 mg/mL, the aggregates have hydrodynamic radii of 1200 Å. Circular dichroism spectroscopy indicates that the polypeptide conformation changes from a coil to the α-helix on cooling the solution. This change can be reversed by increasing the temperature; the midpoint for the transition is 47 °C. Throughout these changes the size of the aggregates remains approximately constant, and thus the conformational transition detected by circular dichroism occurs within the aggregates, which otherwise remain intact. In addition, changes in ratio of the two components, pH, and ionic strength, affect the size of the aggregates.

Our studies of the interactions between oppositely charged polyelectrolytes originate from interest in the structure and composition of the extensive extracellular regions of connective tissue. These regions consist primarily of protein fibers surrounded by a hydrated matrix, comprised mainly of proteoglycans; the latter are branched macromolecules consisting of a protein core to which numerous polyanionic glycosaminoglycan chains are covalently attached as side chains. It has been suggested that an electrostatic attraction occurs between positively charged lysine and arginine residues of collagen and the negatively charged glycosaminoglycans.[1] Previous work in this laboratory has investigated the interactions in model systems comprised of individual glycosaminoglycans (GAGs) and poly(L-lysine) (PLL) or poly(L-arginine) (PLA) in dilute aqueous solution.[2-6] Circular dichroism (CD) spectroscopy indicates that a conformational change is induced in the polypeptides in the presence of the polysaccharides. Maximum interaction between the polypeptides and the seven common GAGs, as judged by the maximum change in ellipticity at 222 nm (reflecting α-helix content), occurs at amino acid:disaccharide ratios characteristic of each system. In addition, the α-helix-directing effects break down as the temperature is increased; each system exhibits a "melting effect", with a characteristic melting temperature, above which the polypeptide reverts to a nonhelical form.

A conformational change for the polypeptide is almost all that can be determined from the CD spectra, and we learn little else about the molecular nature of the interaction. However, some of the CD spectra show scattering distortions indicative of large aggregates in the interacting mixtures, which in extreme cases are visibly turbid. We have used laser homodyne light scattering to investigate the dimension of the aggregates in the interacting mixtures. Initially we have concentrated on the interactions between poly(L-lysine) and chondroitin 6-sulfate. The polysaccharide approximates to an alternating copolymer of β(1,4)-linked D-glucuronic acid and β(1,3)-linked 2-deoxy-2-acetoamido-D-galactosamine 6-sulfate, as shown below.



The laser homodyne scattering technique analyzes the frequency broadening of Rayleigh scattered light from concentration (or refractive index) fluctuations caused by particles undergoing Brownian motion. The relaxation of the concentration fluctuations ($\delta C(\bar{r},t)$) at any position $\bar{r}$ in a dilute solution is described by Fick's second law:[7]

$$\frac{\delta}{\delta t}[\delta C(\bar{r},t)] = D_T \nabla^2 [\delta C(\bar{r},t)] \quad (1)$$

where D_T is the translational diffusion coefficient. Light scattered at an angle θ due to these fluctuations also exhibits a temporal fluctuation behavior with relaxation time Γ_T^{-1} given by

$$\Gamma_T = D_T \kappa^2 = D_T \left[\frac{4\pi n}{\lambda_0} \sin \theta/2\right]^2 \quad (2)$$

where n is the index of refraction of the medium, λ_0 is the wavelength of incident light, θ is the angle between the transmitted and scattered beam, and κ is the scattering vector. The power spectrum ($S_i(\nu)$) of the photocurrent produced by the scattered light is a single Lorentzian curve if the solute is monodisperse:

$$S_i(\nu) \propto \frac{2\Gamma_T/2\pi}{\nu^2 + (2\Gamma_T/2\pi)^2} \quad (3)$$

where Γ_T/π is the half-width and ν is the frequency (in hertz) of scattered radiation. For very dilute solutions of large molecules, the hydrodynamic size of the particles may be calculated using the Stokes–Einstein relation:[8]

$$R_h = kT/6\pi\eta D_T \quad (4)$$

where R_h is the effective hydrodynamic radius, k is the Boltzmann constant, T is the temperature in degrees Kelvin, and η is the viscosity of the solvent.

Experimental Section

Chondroitin 6-sulfate with a (specified) molecular weight of 40–80 000 that had been isolated from shark cartilage was obtained from Miles Chemical Co., Inc., Lot 4703. Two samples of poly(L-lysine) were used: New England Nuclear Corp., Lot L-112, molecular weight 100 000, and Sigma Chemical Co., Lot 16C-5017, molecular weight 68 000. Mixtures of the two components were prepared by adding a predetermined volume of 0.0005 M polypeptide solution to a measured volume of 0.0005 M glycosaminoglycan solution with the molarities expressed in terms of amino acid or disaccharide residues. For a 1:1 mixture (one amino acid residue/disaccharide residue) the total final concentration was 0.178 mg/mL. All solutions were in 0.1 M NaCl and were filtered prior to mixing through Millipore filters of pore size 0.2 μm. After combining the two components, the mixtures were filtered directly into the scattering cell with a filter of pore size 8 μm. The mixtures containing different ratios were then diluted with 0.1 M NaCl (filtered directly into the scattering cell) to a constant overall macromolecular concentration of 0.1 mg/mL. A similar set of solutions were also prepared by mixing 0.0001 M stock solutions and diluting to a macromolecular concentration of 0.02 mg/mL. For the studies of the effect of ionic strength and pH, the 1:1 mixture at 0.178 mg/mL macromolecular concentration was used. The ionic strength was changed by filtering a volume of NaCl at twice the final desired concentration into an equal volume of 1:1 mixture in the scattering cell. The pH was changed by adding drops of diluted hydrochloric acid to 1:1 mixtures in the scattering cell. The pH was checked after the individual spectra were recorded to avoid contamination by the pH electrode. All spectra for the samples were recorded within 10–15 min of mixing or changing the conditions, unless indicated otherwise. For studies of the dependence on angle, salt, pH, and the component ratio, the mixtures were cooled to 10 °C first to induce maximum α-helical content. CD spectra were recorded for the same specimens as used for the light scattering studies.

The laser light scattering instrument consisted of a Coherent Radiation Model 52B argon ion laser, EMI 9656 KR phototube, Keithley 244 high voltage supply, Keithley 104 wide band amplifier, and Saicor SA1-52 real time 400 point spectrum analyzer and digital integrator. Spectra were recorded in the homodyne configuration. The angle between the scattered and transmitted beam was calibrated using globular bovine serum albumin, as described elsewhere.[9] Spectra were recorded at angles of 38.2° unless otherwise indicated. Voltage spectra rather than power spectra were recorded, since the spectrum analyzer processes the former information more rapidly. The power spectra were obtained from the difference between the square of the voltage and the squared shot noise voltage at 40 equally spaced points. These data formed the input for a Lorentzian curve fitting program, from which the half-width was determined.[9]

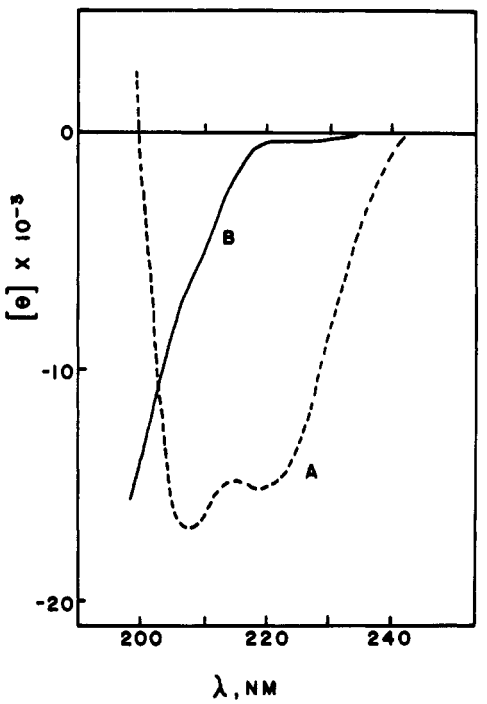


Figure 1. Circular dichroism spectra of 1:1 mixtures of poly(L-lysine) and chondroitin 6-sulfate: (A) after formation of α-helix; (B) before formation of α-helix. The ellipticity as calculated based on an average molecular weight of the amino acid and disaccharide residues.

Results

Figure 1, curve A, shows the CD spectrum for a 1:1 mixture of poly(L-lysine) and chondroitin 6-sulfate. The data indicate at least 80% α-helicity for the polypeptide. The Rayleigh spectrum for the same mixture obtained under identical conditions is shown in Figure 2; the points are the calculated power spectrum and the solid line is the best least-squares Lorentzian curve to fit the data as described in the Experimental Section. The excellence of the fit for this and most of the other mixtures suggests a relatively narrow dispersity of particle sizes.[10] When the half-width of the Lorentzian spectrum is a linear function of $\sin^2 \theta/2$ as in eq 2, the frequency distribution is caused solely by translational diffusion. At larger scattering angles, other molecular dynamic modes of the system may affect the spectrum.[11,12] Figure 3 shows that the data at angles up to 60° describe the translational diffusion of the particle. Deviations from linearity do occur at higher angles, and we will discuss the interpretation of these data in a future paper.

The half-widths of spectra such as that shown in Figure 2

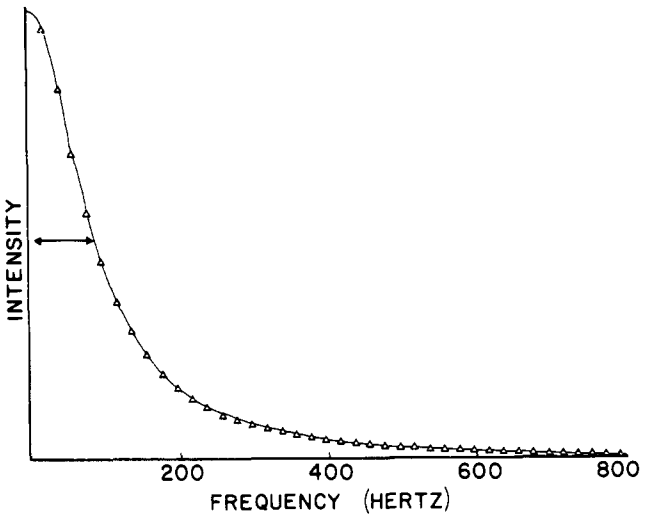


Figure 2. Power spectrum of scattered light from a 1:1 mixture of poly(L-lysine) and chondroitin 6-sulfate at a scattering angle of 38.2°: half-width = 90 Hz, hydrodynamic radius = 1140 Å.

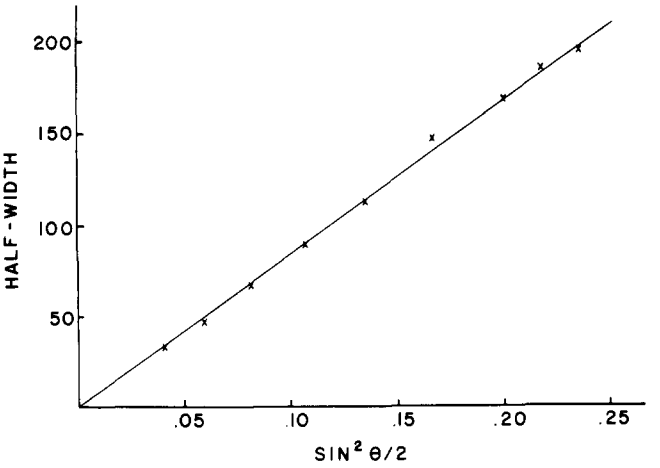


Figure 3. Angular dependence of the half-width for a 1:1 mixture of poly(L-lysine) and chondroitin 6-sulfate: plot of half-width in hertz against $\sin^2 \theta/2$.

indicate the presence of relatively large aggregates in solution. From a series of independent measurements of a number of identical 1:1 mixtures of poly(L-lysine) and chondroitin 6-sulfate, the aggregates had average radii in the range 1100–1300 Å. The translational diffusion coefficient, D_T, is calculated to be 2.2×10^{-8} cm²/s from the slope of the plot of half-width against $\sin^2 \theta/2$, eq 2. Application of eq 4 is rigorous only at infinite dilution. Some authors have reported a concentration dependence of D_T for certain macromolecular solutions.[13-15] However, in the present work, the values of D_T determined for successive dilutions of a 1:1 mixture were independent of concentration, and thus extrapolation to infinite dilution was not necessary. (If PLL and C6S solutions were separately diluted and mixed in a 1:1 ratio, there was a decrease in radius with decreasing concentration. Details of this work will also be published later.)

Maximum induced α-helical content in a 1:1 mixture for this system occurs only after the mixture is cooled below 10 °C. If the components are mixed at room temperature, the CD spectrum is that shown as curve B in Figure 1. However, the half-width of the Rayleigh spectrum for the solution prior to the conformational change reveals the presence of particles of the same size (R = 1200 Å). Cooling to 10 °C induces the α-helical conformation which is then stable at room temperature, but undergoes a melting transition and reverts to the

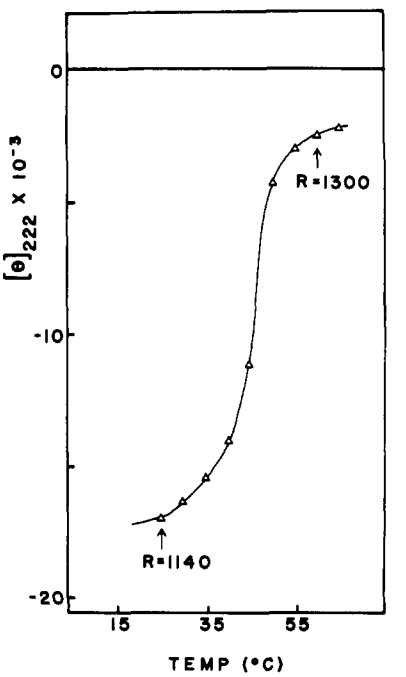


Figure 4. Plot of ellipticity at 222 nm against temperature for a 1:1 mixture of poly(L-lysine) and chondroitin 6-sulfate, showing the calculated hydrodynamic radii (in Å).

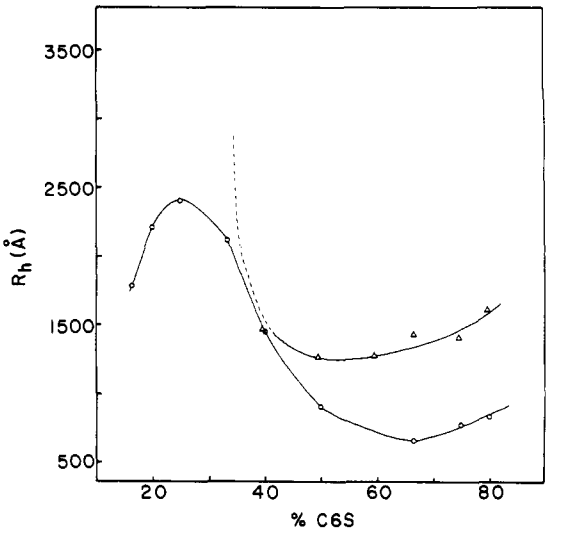


Figure 5. Effect of mixing on the size of the aggregates: a plot of mole-residue percentage chondroitin 6-sulfate versus hydrodynamic radius: (○) radii for mixtures prepared from 0.0001 M stock solutions, diluted to a total concentration of 0.02 mg/mL; (△) radii for mixtures prepared from 0.0005 M stock solutions, diluted to a total concentration of 0.1 mg/mL.

random form as the temperature is increased. The radii of the aggregates determined by the Stokes–Einstein equation are constant as the temperature increases. Figure 4 is a plot of ellipticity at 222 nm, $[\theta]_{222}$, vs. temperature, indicating that the α-helix breaks down at 47 °C whereas the radii remain approximately constant up to at least 60 °C.

In general, the light scattering results for specimens which had been cooled were indistinguishable from those for specimens kept at room temperature. Similarly, the two samples of PLL with different molecular weights gave identical results.

The effect of changing the ratio of the two components is illustrated in Figure 5, which is a plot of hydrodynamic radius against mole-residue percentage of chondroitin 6-sulfate; 50% C6S corresponds to the 1:1 mixture and the maximum induced

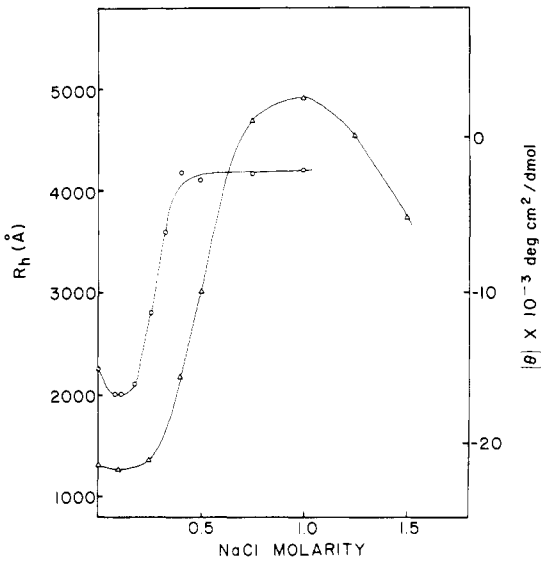


Figure 6. Comparison of the circular dichroism and light scattering data for the effect of sodium chloride concentration on 1:1 mixtures of poly(L-lysine) and chondroitin 6-sulfate: (○) ellipticity at 222 nm as a function of NaCl concentration; (△) hydrodynamic radius as a function of NaCl concentration.

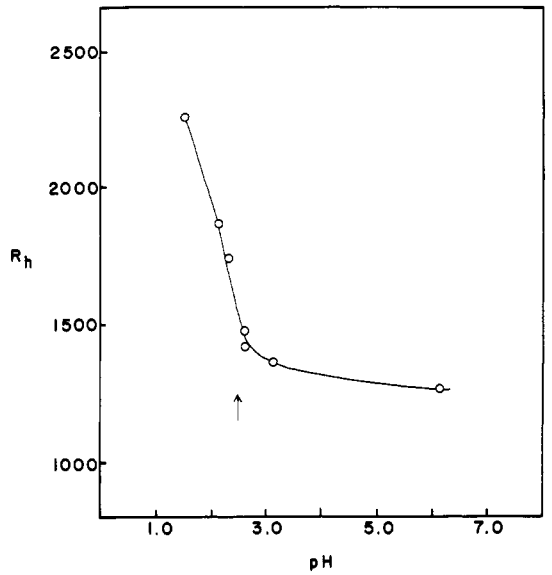


Figure 7. Plot of hydrodynamic radii vs. pH for 1:1 mixtures of poly(L-lysine) and chondroitin 6-sulfate. The arrow indicates the midpoint of the equivalent CD transition.

conformational change. Two sets of data are included here: a series for mixtures prepared from 0.0005 M stock solutions with dilution to a total macromolecular concentration of 0.1 mg/mL and a series from 0.0001 M stock solutions with dilutions to a total of 0.02 mg/mL. Mixtures containing excess C6S show only minor variations in particle size. However, there is a large increase in size occurring between 1:1 and 2:1 (50 and 33.3%, respectively), which is most apparent with the 0.0005 M series. However, in the latter case, the 2:1 and 3:1 samples were extremely turbid and aggregated rapidly with time, leading to precipitation within 1 h. The maximum initial radius for these mixtures was >5000 Å. In the 0.0001 M series, the maximum initial radius of 2400 Å occurred around 25% C6S. At excess polypeptide, the radius for the dilute series was also time dependent. This was not as rapid as for the concentrated series, but the 2:1 and 3:1 dilute mixtures precipitated within 24 h. This time dependence of the radius was apparent only for mixtures containing excess polypeptide. At mole-residue proportions of C6S greater than 50%, the radii in both series were approximately constant over a 24 h period. At very low proportions of C6S (20% as in a 4:1 mixture), the radius began to decrease again.

The α-helical directing effect is also disrupted upon increasing the ionic strength or decreasing the pH. The effect of concentration of added sodium chloride on the CD spectra of 1:1 mixtures of PLL-C6S is shown in Figure 6 as a plot of $[\theta]_{222}$ vs. molarity of NaCl. Between zero and 0.1 M NaCl, there is a high α-helical content, as judged by the ellipticity at 222 nm. (The ellipticities are based on an average residue molecular weight of the amino acid and disaccharide. Assuming the spectral component for the polysaccharide does not change, we can subtract out the C6S contribution and obtain the ellipiticity for PLL alone, which for a 1:1 mixture at zero salt concentration is ~30 000 deg cm^2/dmol at 222 nm and indicates helicity approaching 100%.) At salt concentrations higher than 0.1 M, there is a loss of α-helical content, and at 0.4 M NaCl the α-helix is completely disrupted. The transition is sigmoidal with midpoint at 0.27 M NaCl. Figure 6 shows the equivalent light scattering data; the hydrodynamic radius is approximately constant for NaCl concentrations up to 0.3 M, whereupon a large increase occurs, which is accompanied by visible turbidity. Each point is an average of at least three different measurements. A maximum radius of 5000 Å occurs around 0.8 M NaCl after which the aggregate size begins to decrease. Mixtures at ionic strengths in the range 0.5–1.0 M precipitated out after several hours, indicating further aggregation with time.

A similar change in particle size is seen with 1:1 mixtures (in 0.1 M NaCl) of varying pH. Figure 7 shows the increase in aggregate radius with decreasing pH, with the arrow pointing to the pH at which the α-helix is disrupted (i.e. the midpoint of the transition detected by CD). The open circles show data taken from spectra recorded within 15 min of changing the pH. At low pH there is a strong time dependence of the size. After a further 20 min or so, the radii of particles in mixtures below pH 3.5 almost doubled, and precipitation eventually occurred. However, mixtures prepared at higher pH showed no increase in size over a period of 24 h.

Discussion

The most significant conclusion from this light scattering study is that interacting mixtures of PLL and C6S in dilute solution form large multimolecular aggregates within which the conformation of the polypeptide can change from coil to α-helix, and vice versa. Inasmuch as we can see that the PLL and C6S molecules are not isolated chains in solution, we can now say something about the nature of the mechanism of interaction. The first step appears to be the formation of the aggregate, which, under the conditions selected here, is approximately 1200 Å in radius; at this point the poly(L-lysine) is in a nonhelical conformation (probably a mixture of charged coil and disordered structure). If this mixture is cooled to approximately 10 °C, the PLL undergoes the conformational change to the α-helix. Increases in temperature do not disrupt the aggregate, which when heated to 60 °C slightly increased in radius (from 1140 to 1300 Å). In addition, although the α-helix-directing effect breaks down on increasing the ionic strength or decreasing the pH, these conditions produce large increases in particle size. We cannot be certain whether these effects are due to swelling or further aggregation of the particles, and both processes may well occur. Increasing the NaCl concentration could shield charges on both polyions and effectively destroy any charge repulsion between aggregates already formed in the primary interaction. This neutrality would permit the particles to coalesce. Loss of possible specific interactions may permit solvation of the charged residues, leading to swelling. The breakdown in the α-helix occurs im-

mediately prior to the increase in radius. However, this is probably not responsible for the change in radius, since similar increases in radius are obtained by increasing the salt concentration for a non-α-helical aggregate (i.e., a 1:1 mixture prepared at room temperature).

The ratio of mixing of the components also controls the size of the aggregates. As Figure 5 shows, a maximum particle radius occurs around 25% chondroitin 6-sulfate, which corresponds to a mixing ratio of PL/C6S at 3:1. These data are for radii measured within 15 min of mixing. The mixtures containing excess C6S are clear, and the radii do not change with time. In contrast, the mixtures with excess poly(L-lysine) are turbid; the particles increase in size gradually with time, and the mixtures precipitate out completely within 24 h. Other workers studying interactions between oppositely charged polyelectrolytes[16-18] show changes in turbidity by classical light scattering techniques, which reflect size changes with the mixing ratio of components, although they do not report any time dependence of aggregation. In some cases, maximum turbidity is obtained at exact neutrality,[16,17] although this is frequently not the case,[17,18] and the maximum appears to depend on the chemistry of the two polyions. For PLL and C6S, charge neutrality occurs at a ratio of 2:1, and maximum particle size occurs significantly below that at approximately 3:1, which implicates such factors as differences in polymer–solvent interactions, charge density, and chain conformation and flexibility. However, there is a large increase in size on going from 1:1 to exact neutrality at 2:1. This probably is the major reason for the increase in size at low pH in the 1:1 mixture when the carboxyls are protonated, i.e., the charges are now balanced. Decrease in the solubility of the polysaccharide due to protonation may also be a factor.

There is no evidence for polydispersity of the particles despite the heterogeneity of molecular weights of the polysaccharide (40–80 000) and polypeptide (68 000 and 100 000). Frederick et al.[10] have performed calculations on the effect of polydispersity in molecular weight on the Rayleigh spectra and predict slight deviations from Lorentzian behavior. Specifically, there is a slight decrease in amplitude at low frequencies and an increase at higher frequencies, as compared to the predictions for the monodisperse system. However, as Figure 2 shows, we obtain an excellent Lorentzian fit to squared points in the power spectrum for a 1:1 mixture and hence any polydispersity is not extensive. Nevertheless, often in the case of larger particles at higher ionic strengths, some deviations from Lorentzian behavior do occur, suggesting greater polydispersity under these conditions.

Much of the evidence presented here can be explained within the framework of the theoretical model developed by Veis[19-23] to describe the phase separation behavior of solutions of oppositely charged polyelectrolytes. For the admittedly highly simplified system of two flexible polyions P^+ and Q^-, symmetric in molecular weight and total charge density, the model proposes first the formation of neutral aggregates

$$H^+ + OH^- + P^+(aq) + Q^-(aq) \rightarrow (PQ)_{agg} + H_2O$$

in which minimization of the electrostatic free energy occurs. Subsequently, formation of a complex-coacervate which represents a concentrated phase in which the polyions are in an independent random coil conformation. The overall driving force for phase separation is the increase in entropy due to the depletion of the interpolyion aggregates in the dilute phase (I).

$$(PQ)_{agg} \rightarrow (PQ)^{I}_{agg} + [P^+ + Q^-]^{II}_{coac.\ random}$$

In the coacervate (II), the polyions can have increased configurational and external degrees of freedom.

Accordingly, we propose that the first stage of the interaction is the formation of large intermacroion aggregates of poly(L-lysine) and chondroitin 6-sulfate, driven by interactions between pairs of opposite charges. The poly(L-lysine) chains in these aggregates are thermodynamically in a metastable state because of the possible specific charge-pairing interactions, and when the thermal molecular vibrations are reduced by cooling, it is possible to nucleate and form the α-helical conformation. In this process the aggregate must accumulate excess negative charges because of the specific (1:1) peptide–disaccharide pairing. Since these aggregates do not change size, either the poly(L-lysine) chains are highly kinked or charge pairing is no longer required after formation of the α-helix. Since the polyions are quite different in average molecular weight and molecular weight distribution (i.e., the system is unsymmetrical), substantial variation in aggregate size may occur, although this does not appear to be the case for 1:1 mixtures at low ionic strength and neutral pH. Again, as a consequence of the unsymmetrical character of our system, and also because of the very low concentrations, phase separation is less favored.

As the ionic strength of the solution is increased (Figure 6), disruption of α-helix occurs (0.25–0.4 NaCl), followed by a dramatic increase in aggregate size (0.4–0.7 M NaCl). These large aggregates are more prone to coalescence because of the loss of specific interactions and decrease in the interaggregate repulsions (due to excess negative charge) at higher ionic strengths. Complex-coacervation behavior can then ensue and phase spearation indeed occurs on long standing. At even higher ionic strengths, however, aggregate size decreases because of salt suppression, i.e., further reduction in polyion electrostatic attraction. The changes in aggregate size with pH and mixing ratio are also in accordance with the analysis due to Veis. As the pH is decreased, increasing numbers of the polysaccharide carboxyl groups become uncharged. This could result in a loss of the specific charge pairing interactions which are perhaps necessary to maintain the α-helix because of the constraints of molecular geometry. Since, however, the system is now brought into a state of electrical equivalence, large aggregates are again formed and complex-coacervation behavior ensues. On varying the mixing ratio, a similar behavior is observed; as the proportion of poly(L-lysine) increases toward the point of electrical equivalence, the aggregate size increases and time-dependent demixing occurs, depending on the total polymer concentration. This again indicates either complex coacervation or coprecipitation behavior.

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Notes

The "$\Delta\chi$ Effect" and Polystyrene–Poly(vinyl methyl ether) Compatibility in Solution

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It is well-known that most pairs of polymers (components 2 and 3) are incompatible corresponding to a positive value of the χ_{23} interaction[2] larger than 2 for the simple case of equal molecular weights.[3] Since χ_{23}, defined[2] by Flory, is proportional to molecular weights, the value of 2 corresponds, for high polymers, to a very small repulsive interaction. If a common solvent (1) is added to an incompatible pair, the resulting solution is phase-separated but becomes homogeneous for a solvent volume fraction (φ_1) greater than a certain critical value, φ_{1c}. According to Flory–Huggins solution thermodynamics, as applied to such ternary systems by Scott[4] and Tompa,[5] phase equilibrium does not depend on the solvent but only on χ_{23} related to φ_{1c} through

$$\chi_{23} = 2(1 - \varphi_{1c})^{-1} \quad (1)$$

According to eq 1, solution-phase separation, i.e., φ_{1c} between 0 and 1, indicates $\chi_{23} > 2$ and hence incompatibility in the absence of the solvent. However, eq 1 was derived assuming that $\chi_{13} = \chi_{12}$. Recent treatments[6,7] relax this restriction and predict that phase equilibrium in ternary systems containing two polymers and a solvent depends not only on χ_{23} but also on any difference in strengths of the polymer–solvent interactions, i.e., on

$$|\Delta\chi| = |\chi_{12} - \chi_{13}| \quad (2)$$

Due to this "$|\Delta\chi|$ effect", phase separation is predicted[6,7] even when the polymers are compatible ($\chi_{23} < 2$ or negative) provided that $|\Delta\chi|$ is sufficiently large. The phase diagram shows a closed region in which phase separation occurs, such as is seen in Figures 1 or 2. For zero and small φ_1 the system is homogeneous, then becomes cloudy in an intermediate range of φ_1. Koningsveld and collaborators have studied two systems showing this behavior: benzene/(polyisobutylene + ethylene–propylene–diene terpolymer)[8] and diphenyl ether/linear polyethylene + atactic polypropylene.[9] Unfortunately, it is not obvious that $|\Delta\chi|$ is large in these cases. However, the present work shows that the behavior in certain solvents of the compatible pair polystyrene (PS) + poly(vinyl methyl ether) (PVME) provides definite evidence of the $|\Delta\chi|$ effect.

Recent work[10] shows PS–PVME to be a compatible (2–3) pair at ordinary temperatures, and consistent with this, a GLC study[11] indicates a very small positive χ_{23} value. Thies and collaborators[12] found that the pair PS ($\bar{M}_n \sim 104\,000$–$150\,000$) and PVME ($\bar{M}_n \sim 524\,000$) gave clear solutions when mixed with benzene, toluene, and tetrachloroethene but cloudy solutions with chloroform, dichloromethane, and trichloroethene. Table I shows the χ_{12} and χ_{13} values for the solvents as found by the GLC method at 40 °C. It is apparent that the solutions are either clear or cloudy depending on whether the solvents give small or large values of $|\Delta\chi|$. We have calculated the spinodals for these systems, using $\chi_{23} = 0$ and with the χ_{12} and χ_{13} values found in the table. When chloroform, dichloromethane, and trichloroethene are the solvents, one predicts very large closed two-phase regions, so that although the polymers are compatible, the addition of a few percent of these solvents brings about incompatibility. On the other hand, when toluene and tetrachloroethene are the solvents, compatibility is predicted for these polymers in solution. For benzene, where the $|\Delta\chi|$ value is larger, one predicts a small closed two-phase region, which is not found experimentally and which vanished when χ_{12} was made slightly negative. It is evident that the theory, together with the GLC interaction parameters, gives good qualitative results. One aspect, however, of the observations of Thies and collaborators is inconsistent with this explanation. Whereas clear films are cast from the homogeneous solutions, the cloudy solutions give films which show a microscopic phase separation. The latter systems should show two-phase regions closed at low φ_1. Thus, for solvent evaporation under quasiequilibrium conditions, the solution should become homogeneous at low φ_1 followed by the formation of a clear film. Presumably the viscosity of these high molecular weight systems at low φ_1 is too high for the solution to clear and the film remains in a metastable phase-separated state, as suggested by Nishi and Kwei.[10]

In the present work, low molecular weights of PS and PVME were taken, respectively 2100 and 11 000. Solutions of different concentrations were made up and the tempera-

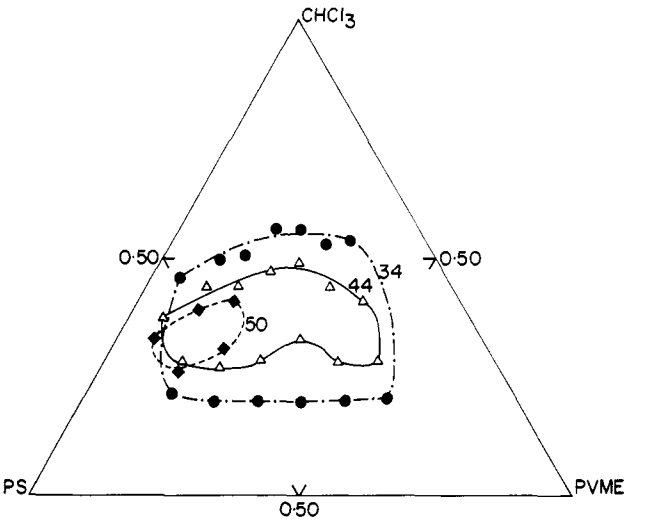

Figure 1. Experimental closed regions of incomplete miscibility for the chloroform/[PS(2100) + PVME(1100)] system at various temperatures in °C. The system is turbid inside the region and clear outside. Solution compositions are in volume fractions.