Influence of Polymer Conformation on the Shear Modulus and Morphology of Polyallylamine and Poly(α-L-lysine) Hydrogels

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ABSTRACT: Two polyamines with different conformational properties, poly(α-L-lysine) and polyallylamine, were chemically cross-linked to evaluate the effects of polypeptide secondary structures on gel properties. Both cationic gels are highly swollen at low pH and shrink as it increases primarily due to the reduction in of the concentration of associated counterions in the gel as its ionization decreases. Network shear moduli were determined under uniaxial compression. For the polyallylamine gels, the shear modulus (G) scales as $\phi_2^{0.31}$, where ϕ_2 denotes the polymer volume fraction, over the entire pH range. This result is in favorable agreement with the $G \sim \phi_2^{1/3}$ relationship predicted for networks of flexible chains. Poly(α-L-lysine) gels swollen below pH 11.0 obey the same scaling behavior. At higher pH levels, however, organized secondary structures are believed to develop, and the composition dependence of the modulus at high pH becomes consistent with the scaling relationship predicted for rigid-rod chain networks connected by flexible junctions ($G \sim \phi_2^{3/2}$). Further evidence of the existence of more organized microstructures in the poly(α-L-lysine) gels at high pH is provided from complementary thermoelasticity and morphological analyses.

Introduction

Hydrogels are water-swellable polymer networks used in products whose sales run into billions of dollars annually.[1] Examples include superabsorbents, chemical separations media, drug delivery systems, and biomedical devices like soft contact lenses, catheter coatings, and tissue engineering matrices.[1−3] Their versatility resides in the fact that their swelling degree (ratio of wet volume to dry volume) is widely adjustable, and consequently their permeabilities and swelling kinetics are as well. Unfortunately, mechanical performance of hydrogels declines with increasing water content, and this limits their utility in many applications. Although diverse conventional polymer synthesis, biopolymer modification, and cross-linking techniques have enabled the preparation of numerous classes of hydrogels for these applications, the next generation of advanced materials will rely more upon design concepts from biological systems, such as tissue, bone, and silk. These materials are characterized by multiple levels of structural organization and well-defined molecular architectures and biological recognition sites.[2] Emulation of these natural systems requires specification of intermolecular interactions between materials components, which in turn requires more precise control of molecular structure than is possible with conventional material synthesis.

Protein-based polymers can be synthesized biologically with virtually complete uniformity of molecular architecture using recombinant *E. coli* bacteria.[2] By combining knowledge of protein structure with fundamental concepts of polymer materials science, nonnatural protein-based materials can be designed that are capable of self-assembly into unique and uniform two- or three-dimensional topologies. Ultimately, it may become possible to design biopolymers capable of forming hierarchically ordered systems with multiple levels of interactive structure. Such materials have particular potential for biomedical use in areas such as drug delivery,[1] tissue regeneration,[2,3] environmentally responsive biomaterials,[1,2] and others.[4] For instance, tissues or organs can be potentially engineered by combining the cells of a patient with polymeric structural scaffolds.[3] In this scenario, cells are isolated from a tissue sample of the patient and, after being expanded and harvested in vitro, incorporated into polymeric scaffolds that, after its transplantation, will act as their counterpart matrices found naturally in tissues.[3] The choice of polypeptides as precursor materials in biomedical applications is appealing since the materials can be synthesized to mimic natural proteins to exhibit similar biocompatibility, degradation, and mechanical properties.[2−4] The possibility of controlled cellular interactions and degradation of these systems through judicious choice of key amino acid sequences affords a potential advantage of these materials in tissue engineering applications.[3] The elastic properties of these systems are another critical issue for biomedical applications since the polymer network must have sufficient mechanical integrity to allow cell tissue growth. Moreover, cell adhesion behavior and gene expression properties can be strongly influenced by the mechanical properties of these polymer matrices.[3] Therefore, a good understanding of how the molecular conformations (e.g.,

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secondary structures) of these materials influences mechanical performance is key to the development of materials possessing suitable characteristics for such emerging applications.[3,4] Because of the clear potential of such bioengineered polypeptides, their development has been rapidly expanding in recent years.[2−4] However, the potential influence of secondary structure on mechanical properties of such hydrogels has not received much attention.

Therefore, the objectives of the present work were (i) to examine the effects of polypeptide secondary structures on the swelling, mechanical, and morphological behavior of networks derived from amino acids and (ii) to demonstrate that such networks presenting secondary structures other than flexible coils can display properties that differ from those of gels which do not, such as those based on vinyl polymers. For this purpose, a polymer of a single amino acid—poly(α-L-lysine)—with known pH-dependent chain conformations was chosen as the model polypeptide. It was converted into a stable gel network by chemical cross-linking. Then its swelling and mechanical behaviors, as well as morphological characteristics, were systematically contrasted with those of a vinyl polymer gel—polyallylamine—with similar side groups but without potential for displaying multiple secondary structures.

Background

Stress−Strain Isotherms. From the classical theory of rubberlike elastic networks with Gaussian chain extension distributions, the relationship between stress and strain for uniaxial compression and elongation experiments is represented by

$$\sigma = f/A_0 = G(\alpha - \alpha^{-2}) \tag{1}$$

where σ is the applied engineering or nominal stress defined by the ratio of the applied force, f, to the undeformed cross-sectional area of the network, A_0; G is the shear modulus; and α is the ratio of the deformed length, l, to the undeformed length of the network, l_0.[5] More recent theories that take into account the effects of non-Gaussian distributions of chain length extensions, entanglements, inhomogeneities, or the presence of stiff chains generally produce eq 1 as a limiting case, especially at small deformations.[5−7] Isothermal stress−strain measurements of unswollen elastomers, principally for elongation tests, often deviate from eq 1 in that the modulus is not independent of the deformation, α. In these cases, stress−strain data are generally analyzed in terms of the semiempirical Mooney−Rivlin equation:[5,8]

$$\sigma/(\alpha - \alpha^{-2}) = 2C_1' + 2C_2'\alpha^{-1} \tag{2}$$

Here $2C_1'$ and $2C_2'$ are unspecified constants independent of the elongation. Comparison of eqs 1 and 2 shows that $2C_2'$ is a direct measure of the departure of the observed data from that predicted by the classical molecular theories.

Mechanical Properties of Polymer Networks. *Networks of Flexible Polymer Chains.* Historically, two different fundamental theories, known as the affine and phantom models, have been used to describe the limiting cases for the behavior of rubberlike material. They both represent the network as a system of ideal chains with no interactions, entanglements, and knots but make different assumptions about the behavior of the cross-link junctions. The affine theory assumes that the cross-links move affinely: that components of each chain position vector transform linearly with macroscopic deformation. The phantom theory assumes that while a small number of junctions are fixed at the surface of the network, the remaining ones are free to fluctuate in position over time.[5,7] The influence of network structure on the mechanical behavior is reflected principally through the macroscopic elastic modulus. On the basis of the affine and phantom Gaussian elasticity theories, the shear modulus is described as follows:[5,7]

$$G = \begin{cases} \rho_x R T \phi_2^{1/3} \phi_{2_o}^{2/3} & \text{(affine)} \quad (3a) \\ \left(1 - \frac{2}{f_x}\right) \rho_x R T \phi_2^{1/3} \phi_{2_o}^{2/3} & \text{(phantom)} \quad (3b) \end{cases}$$

where ϕ_2 is the polymer volume fraction in the gel; ϕ_{2_o} is the polymer volume fraction in the unperturbed state; R is the universal gas constant; T denotes the absolute temperature; ρ_x is the effective cross-link density in the polymer network; and f_x is the cross-link functionality. It has been reported, as suggested by eq 3, that the modulus of polymer networks decrease with increased swelling.[5,8] Many real networks display behavior that lies between the affine and phantom model limits. However, swollen networks ($\phi_2 < 0.2$) far from the limit of their chain extensibility tend to approach the phantom limit.[5] Thus, eq 3b will be applied to the systems studied in this work.

The dependence of the modulus with the polymer concentration ϕ_2 in neutral networks has been investigated by Obukhov et al.[9] Employing scaling concepts, they have determined that eq 3 will hold for networks expanded in a Θ solvent, whereas in a good solvent a larger power-law exponent (7/12) is proposed. This theory has been used to explain results obtained for polyurethane networks prepared in xylene[10] and those determined for polystyrene gels in benzene.[11] In addition, Dubrovskii and Rakova[12] observed agreement with the 7/12 theory for neutral polyacrylamide gels (at monomer concentrations < 10 wt %) under compression in water and water−acetone mixtures. At higher monomer concentration, however, classical elastic behavior of eq 3 is again observed. Thus, the model of Obukhov et al.[9] appears to be valid only at low polymer concentrations when relatively long network strands are present.[12]

Ionic Networks. Electrostatic interactions can dramatically modify the behavior of polymer gels, and it has been proposed[13−17] that such interactions can affect the magnitude of the elastic modulus. Unfortunately, no complete theory encompassing such effects, including counterion condensation, has been proposed to date. To circumvent the complexity inherent in ionic gel systems, a scaling theory has been proposed by Rubinstein et al.[15] to represent different physical situations involving polyelectrolyte gels. The theory predicts that the gel modulus will depend on the elongation of the network chains in a particular state and therefore on the swelling degree of the gel. Three different stretching regimes (representing weakly, intermediately, and highly swollen samples equilibrated in a monovalent salt solution) have been proposed. Additionally, the scaling exponent likewise depends on the salt concentration of the system. Exponents varying from $^1/_6$ to $^5/_6$ are expected, depending on the degree to which the gel is

swollen and the salt concentration in the system. The theory also predicts that the modulus may increase as the salt concentration in the system is raised and vary with the quality of the solvent for highly swollen networks.[15]

The prediction of an increase in modulus as salt is added to an ionic gel system has been observed experimentally, but the measured dependence of the modulus on charge concentration is weaker than that predicted theoretically, possibly due to counterion condensation effects.[15] Regarding the dependence of the modulus on the polymer concentration in the limit of low swelling, experimental studies in gels with relatively high cross-link densities and prepared at high monomer concentration (>15 wt %) have yielded results that are more consistent, within experimental accuracy, with the prediction from classical Gaussian theory, eq 3.[12,17,18] However, for poly(acrylamide) gels prepared at low monomer concentration (<15 wt %) and containing relatively long polymer chains, Dubrovskii and Rakova[12] observed the agreement between the experimental data and the theoretical predictions for weakly swollen networks at low salt concentration. In summary, both theoretical and observed exponents of ionic networks of flexible chains are of similar magnitude to that of the classic theories of eqs 3a and 3b.

Networks of Rigid Polymer Chains. Networks composed of rigid chains constitute another important class of systems relevant to this work. Two different cases have been described in the literature.[7,19] The first case is such that cross-links between rigid chains are also rigid—these are classified as "completely rigid networks". In the second case, the network is made out of rigid chain segments, but the cross-links between them are flexible rather than rigid—these have been classified as "flexibly hinged rigid networks".[7,19] As with flexible networks, the parameter that embodies the modulus and the deformation response of rigid networks is the free energy. However, these properties cannot be deduced from single chain arguments that are applicable to flexible networks. Instead, they must be determined from complex field models[6,7] or estimated on the basis of scaling arguments.[19] It is interesting to note, though, that completely rigid networks deform enthalpically, while flexibly hinged ones still deform entropically, as assumed in classical rubber elasticity theories.[6,7,19]

For ideal chains with no interactions, entanglements, or knots, calculations based on the field methods are possible for flexibly hinged rigid networks, if the rods are randomly oriented and do not form a liquid crystal phase. With these restrictions in mind, two limiting cases can be explicitly considered on the basis of the extent of deformation. At very small deformations, the phantom model, eq 3b, is recovered, and despite the very different physical scenario, the network has an entropy comparable to the entropy of a Gaussian network due to the flexibility of the cross-links.[6,7] At very large deformations, the rigid-rod behavior dominates the mechanical response, and the free energy of the system increases exponentially with increasing deformation. Additionally, flexibly hinged networks are predicted to have a modulus much lower than the modulus of a completely rigid network and on the same order of magnitude as those exhibited by Gaussian networks.[6,7,19]

Completely rigid and flexibly hinged networks have been addressed by Jones and Marques on the basis of scaling arguments.[19] An important distinction between the two types of networks is in the dependence of the modulus with respect to the polymer volume fraction in the network, viz.

$$G \sim \phi_2^{[(3\nu+1)/(3\nu-1)]} \quad \text{(rigid cross-links)} \qquad (4a)$$

$$G \sim \phi_2^{3\nu/(3\nu-1)} \quad \text{(flexible cross-links)} \qquad (4b)$$

where $1/\nu$ corresponds to the fractal dimension of the network chains. Of particular interest in the present study are networks made of rodlike chains, for which $1/\nu = 1$. In this case, eq 4 simplifies to $G \sim \phi_2^2$ (rigid cross-links) and $G \sim \phi_2^{3/2}$ (flexible cross-links).[19] It is important to recognize that these relationships significantly differ from that observed and predicted for the various types of flexible networks reviewed earlier, all of which display scaling exponents less than the cases wherein the quality of solvent is varied and the cross-linking density (dry basis) of the network remains constant.[8]

The validity of eq 4a has been confirmed for a variety of gel types,[19−22] including κ-carrageenan gels ($1/\nu = 1$, $G \sim \phi_2^{2\pm0.01}$),[19] silica gel systems ($1/\nu \cong 2$, $G \sim \phi_2^{5\pm0.5}$),[20,21] and thermoreversible physical poly(vinyl chloride) gels in organic solvents ($1/\nu = {}^3/_2$, $G \sim \phi_2^3$).[22] A system that obeys eq 4b is described by Aharoni and Edwards,[23] who prepared rigid gels of a polyamide ($1/\nu = {}^1/_2$) with flexible cross-links and determined that $G \sim \phi_2^{3.14}$.

Degree of Cross-Linking Effect. The scaling relationships provided in the previous section for networks of flexible polymer chains refer to the dependence of the modulus on polymer volume fraction for cases where the cross-link density or concentration of network chains is not altered; i.e., the gel volume is varied by changing the solvent quality and degree of ionization or partially drying the specimens. However, the volume and thus modulus of polymer networks in equilibrium with a given solution at constant temperature and pressure can also be varied by changing the cross-link density used at synthesis. Scaling relationships between the modulus and polymer concentration, which take into account changes in the degree of cross-linking, also have been proposed for flexible networks.[8] The power law exponent depends on the quality of the solvent. It is nearly 2.3 in a good solvent and 3.0 in a Θ solvent and increases infinitely in poor solvents.[8] Note that in all such cases the exponent is always much greater than the cases reviewed earlier, except for the completely rigid systems, whose volumes and moduli are expected to be less influenced by changes in cross-link density. [6,7,19]

Thermoelasticity. Equation 1 is a valid representation of stress−strain behavior of flexible and rigid networks under small deformations.[5,7] It is of importance for the analysis of force−temperature measurements, which allows determination of the ratio of the energetic contribution, f_e, to the total elastic force, f, which is the sum of the energetic (f_e) and entropic (f_s) contributions. In the case of uniaxial deformation (elongation or compression), f_e is usually expressed in terms of the fraction of the total force that is of energetic origin, f_e/f.[5,24] On the basis of eq 1, Shen and Blatz proposed the following expression to evaluate f_e/f:[24]

$$\frac{f_e}{f} = 1 - T\frac{\mathrm{d}\ln G}{\mathrm{d}T} - \frac{\beta T}{3} \qquad (5)$$

where $\beta = (\partial \ln V/\partial T)_p$, denoting the thermal expansion

coefficient of the sample. Combination of eqs 3 and 5 yields a very important relationship for flexible networks, namely

$$f_e/f = T\, \mathrm{d} \ln \overline{r_0^2}/\mathrm{d}T \qquad (6)$$

where $\overline{r_0^2}$ is the mean-square end-to-end distance of an unperturbed free polymer chain, also called the unperturbed dimension.[5,24] Equation 6 establishes a direct connection between the thermodynamic quantity f_e/f and its molecular counterpart $\overline{r_0^2}$ and indicates that the energy associated with the elasticity of polymer networks is directly affected by the intrachain interaction energies of the network chains.

Experimental Section

Materials. Poly(allylamine hydrochloride) (PAlAm) with average number molecular weight of 70 000 Da (Aldrich) and poly(α-L-lysine hydrobromide) (PLL) with average mass molecular weight of 99 500 Da and polydispersity of 1.2 (Sigma) were used as received. The analytical grade reagents *N*,*N*′-methylenebisacrylamide (Aldrich), *N*,*N*,*N*-triethylamine (Aldrich), 1.0 N hydrochloric acid (Fisher), 1.0 N sodium hydroxide (Fisher), and double distilled deionized water (Fisher) were used without further purification. Sodium *p*-phenolsulfonate dihydrated (Acros) was purified according to the method of Sarger et al.[25] to produce the anhydrous compound with a yield about 40% as reported. Sodium chloride (Merck) was purified as described elsewhere.[26]

Methods. *Solution Preparation.* Anhydrous sodium *p*-phenolsulfonate was used to prepare buffer solutions (ranging from 5.5 to 11.4) at a constant ionic strength of 0.05 M according to the procedures of Long.[26] Sodium chloride solutions with ionic strength of 0.05 M and pH ranging from 2.0 to 11.9 were also prepared by dissolving appropriate amounts of either standard 1.0 N hydrochloric acid or 1.0 N sodium hydroxide.

Gel Synthesis. Poly(allylamine hydrochloride) and poly(α-L-lysine hydrobromide) were cross-linked with *N*,*N*-methylenebisacrylamide (MBA) by a Michael-type addition reaction. This novel cross-linking procedure, which generated well-defined cross-links (in contrast to cross-linking with glutaraldehyde or by irradiation), was developed by adapting the polymerization methods reported by Hulse[27] and Ferruti and co-workers[28] as well as by Sela et al.[29] in the preparation of poly(α-L-lysine). A 20% w/v polymer solution containing a predetermined amount of MBA to yield a molar ratio of cross-link agent to amino side group of either 0.05 or 0.10 was prepared by first dissolving the cross-linker in deionized water (flushed with nitrogen for 5 min) and then adding the polymer. *N*,*N*,*N*-Triethylamine, the cross-linking catalyst, was added to the solution and mixed thoroughly. After its complete mixing, the nitrogen flux was ceased. Gel disks were produced by molding using a silicon rubber gasket (McMaster-Carr) measuring 8 cm long, 8 cm wide, 1.6 or 3.2 mm thick with circular holes (~6 mm in diameter) that served as the sample molds. This gasket was glued to a Teflon sheet bonded to a glass plate. When the precursor solution became noticeably viscous (about 15−20 min before gelation), it was transferred by micropipet into the small circular holes in the gasket, which was subsequently covered with a second Teflon sheet also fixed to a glass plate. The assembly was clamped and held at ambient temperature to 1 h and then cooled to ca. 3 °C and held there for an additional 24 h. After this time, hydrogel disks appearing smooth with parallel flat surfaces and measuring either 1.6 or 3.2 mm thick were carefully removed from the molds. Table 1 summarizes the cross-linking conditions employed to prepare the gel networks investigated here. Two series of poly(α-L-lysine) gels (designated PLL-1x and PLL-2x) were produced with cross-link concentrations differing by a factor of 2.

Table 1. Synthesis Conditions of Polymer Networks

polymer[a]	molar ratio[b] NH_3/catalyst	molar ratio[b] NH_3/MBA	cross-linker/polymer mass ratio	gel designation	gelation time, min
PA1Am	5	20	0.070	PA1Am	~55
PLL	5	20	0.034	PLL-1x	~75
PLL	5	10	0.069	PLL-2x	~55

[a] Concentration of precursor solution: 0.2 g/mL. [b] Amine side groups/*N*,*N*,*N*-triethylamine and amine side groups/MBA molar ratios.

Potentiometric Titration. Ionic contents of linear and cross-linked polyallylamine and poly(α-L-lysine) were determined by potentiometric titration according to an experimental procedure described elsewhere.[30,31]

Swelling Behavior. Once removed from the mold, polyallylamine and poly(α-L-lysine) gel samples were immersed into 0.05 M sodium chloride solution or 0.05 M sodium *p*-phenolsulfonate maintained at approximately 2 °C for 1 week (solution changed every 24 h) to leach away any unreacted species. Before measuring gel volumes or shear moduli, the gel samples were equilibrated in a specified buffer or sodium chloride solution for at least 1 week at 2 °C, with the solutions renewed every 12 h. Gel disks were stored in solutions at ambient temperature overnight prior to analysis. The volume of gel specimens at different pH levels was determined by measuring their thickness and diameter using a microscope (Olympus) equipped with a digital Filar eyepiece (Lasico). Six measurements were collected from each gel at a given pH. All volume changes induced by pH changes occurred isotropically. Averaged over 10 different specimens, the gel volume at synthesis conditions was determined to be 0.0499 ± 0.0003 cm^3.

Mechanical Behavior. Stress−strain isotherms were determined from stress−relaxation measurements of the gel disks under uniaxial compression using an apparatus described elsewhere.[32] The force and deformation were measured within an accuracy of ±2.5 mN and ±1 μm, respectively. No noticeable distortion of the samples due to plate-induced friction (the "barreling effect"[5]) was evident upon visual inspection during the tests. Uniaxial compression was performed in both thermodynamically open and closed systems. In open system experiments, which are most commonly used in the study of hydrogels,[8] the gel specimen was kept in a sufficient quantity of solution to prevent the swollen network from drying. In closed analyses, which are typically applied to elastomeric materials,[5] a nonsolvent (mineral oil) was employed to prevent evaporation of the aqueous solvent from the gel. Stress−strain measurements were acquired in the deformation range $0.8 < \alpha < 1.0$ in open tests and $0.7 < \alpha < 1.0$ in closed tests. The sample volume before and after each experiment performed was ascertained, and no significant change in swelling was observed in any case.[33]

The dependence of stress−compression isotherms on polymer concentration or temperature (thermoelasticity) was determined. In tests where composition was varied, the temperature was held constant at 23.0 ± 0.05 °C by placing the equipment in a thermostatically controlled water bath (Neslab). The sample temperature was continuously monitored with a thermocouple in close proximity to the sample. To vary the polymer composition of the gels (ϕ_2) in systematic fashion, consistent with eqs 3b and 4b, the specimens were either placed in solutions of different pH or allowed to dry partially by exposing their upper surface to air. In the latter case, the gel was carefully surrounded by mineral oil to avoid breaking during drying. Partially dehydrated samples were then fully immersed in mineral oil for at least 24 h before mechanical analysis to redistribute any accumulated stresses and concentration gradients.

In force−temperature measurements, gel composition was held constant and stress−strain behavior was investigated at different temperatures. Polyallylamine gel equilibrated at pH 6.5 and poly(α-L-lysine) gels swollen at pH 6.5 and 11.4 were

Table 2. Experimental Conditions for Thermoelastic Measurements

gel	temp range, °C	L_0, mm	A_0, cm^2	ϕ_2
PA1Am (pH = 6.5)	25−65	3.28	0.250	0.207
PLL-1x (pH = 6.5)	5−55	3.40	0.249	0.198
PLL-1x (pH = 11.4)	5−55	2.67	0.206	0.305

partially dried to equalize composition, as described above. Gravimetric analysis conducted before and after the thermoelasticity measurements consistently revealed mass losses under 1%, confirming constant gel composition. Each gel specimen was covered with a thin layer of mineral oil, and its length and cross-sectional area were determined prior to full immersion in mineral oil and subsequent mechanical compression. Following Shen and Blatz,[24] values of the shear modulus were derived as a function of temperature from eq 1 by fitting a straight line with zero intercept to the data. Initial experiments were performed with increasing temperature and were followed by a reduction in temperature to check for hysteresis and confirm reproducibility. After each test, the gel was allowed to recover freely for at least 12 h. The dimensions and compositions of the gel specimens, together with the temperature range explored, are provided in Table 2. The thermal expansion coefficients of the specimens were estimated by pyncometry.

Morphological Analysis. The morphologies of the gels prepared in the present work were elucidated by field-emission scanning electron microscopy (FESEM), which uses a narrow spot size and bright source to yield high signal-to-noise images. To retain the structural characteristics of environmentally sensitive hydrogels as they exist in their native hydrated state, it was necessary to prepare the gels in such a way to preserve the form and shape of their inherent network.[34] Wet gel specimens were cut with a clean scalpel into squares measuring approximately 1−2 mm on a side. The squares were immediately blotted onto filter paper to remove excess water and then hand-plunged into a small reservoir of liquid ethane (LE) to minimize water crystallization (and potential network rupture). The LE was generated from ethane gas that was condensed into a 1 cm diameter brass cylinder cooled by liquid nitrogen (LN2). Upon quenching, each sample was held by forceps in the LE for about 3−4 s and then quickly removed to a reservoir of LN2. Once several samples of the same gel had accumulated in the LN2 reservoir, they were then placed into a plastic vial filled and kept cool with LN2 for transport to a vacuum-drying unit. Samples were dried using a lyophilizer (Virtis model R1381), which applied a continuous vacuum at low temperature. To keep the samples cold during drying, they were immersed in a tub of dry ice. Samples were freeze-dried in batches overnight on an as-needed basis. Dried samples were subsequently mounted using double-sided carbon tape on stubs measuring ∼2 cm in diameter and coated with a thin (∼15 nm) layer of a conductive 60−40 Au−Pd alloy in a sputter-coater (Anatech Hummer V) to minimize charge buildup in the FESEM. The stubs were slightly tilted to one side and then the other to achieve uniform coating, especially around edges, and the coating was pulsed to avoid sample heating. Samples were imaged with a JEOL-6400 FESEM at an accelerating voltage of 5.0 kV.

Results and Discussion

Ionic Analysis. Titration of a linear or cross-linked polymer can be used to ascertain its ionic content and apparent pK value.[30,31,33] As shown in Table 3, the measured charge content of the linear and cross-linked polyelectrolytes examined here are close to the theoretical values as calculated from the polymer content, verifying the cationic character of the two polymers. Table 3 also provides apparent pK values of the linear and cross-linked polyelectrolytes (expressed as pK_0 and p$K_{1/2}$). Values of these parameters for polyallylamine are in good agreement with those reported by Ochiai et al.[31]

Table 3. Titration Results for the Linear and Cross-Linked Polyamines

	charge contents, mequiv/g		apparent dissociation constant	
system	theor	measd	pK_0[a]	p$K_{1/2}$[b]
PA1Am solution	10.7	9.7	9.3 (9.41)[c]	8.5 (8.57)[c]
PA1Am gel	8.6	8.7	9.6	8.6
PLL solution	6.1	5.7	9.8	9.7
PLL-1x gel	5.5	5.8	10.0	9.9

[a] Extrapolated value of apparent pK to $i = 1$. [b] Value of apparent pK at $i = 0.5$. [c] Values from ref 31.

(in 0.05 M sodium chloride solution). Values for poly(α-L-lysine) indicate that the side groups in this polypeptide are more strongly basic than the ones in the vinyl polymer. For both polymers, the pK values for the polymer and gel match within experimental error, though the values in the gel are consistently slightly higher than in the solution.

Electrostatic counterion condensation and Donnan exclusion effects (for the networks) are usually considered as the principal factors responsible for deviation of polyelectrolyte apparent pK values from their "true" values.[35] Donnan exclusion effects, for instance, can increase the pH inside cationic gels beyond that of the external solution, sometimes by more than one pH unit.[35] Because of these effects, the potentiometric titration of polybase gels may yield apparent dissociation constants that are lower than expected (the ϵ-amino groups of L-lysine, for example, are known to possess a pK_0 of 10.44).[26,30] Counterion condensation effects can also promote significant deviation of the degree of dissociation (i), obtained in the potentiometric titration, from the effective fraction of neutral or uncharged side groups in the polymer or, likewise, the degree of neutralization or protonation ($1 - i$) from the real extent of charged groups.[35] Also, the apparent pK values are dependent on ionic strength and the nature of the added salt. For instance, Ochiai et al. have reported that the pK of polyallylamine increases with increasing anion size in sodium halides.[31] Moreover, hydrophobic counterions, such as ethylbenzenesulfonate, can strongly interact with the polymer by ion-pair formation and even induce precipitation.[36]

Swelling Behavior. Figure 1 displays the variation of gel volume between pH 2 and 12 for polyallylamine in isotonic buffers (I = 0.05 M), primarily sodium p-phenolsulfonate buffers. The ordinate represents the volume swelling degree, q, the ratio of the swollen gel volume to the dry gel volume. Two swelling curves are presented in Figure 1 showing the volume change upon both increasing or decreasing pH (and constructed using different gel samples). Despite these differences, the data lie essentially on the same curve, which demonstrates (i) the reproducibility of the experiments, (ii) the reversibility of the gel swelling behavior (no hysteresis), and (iii) stability of gel samples upon repeated testing.

The polyallylamine gels swell to a relatively large extent at low pH and continuously shrink as the pH increases, collapsing by a factor of about 9 as the pH is increased from 2.0 to 11.9. The swelling behavior of polyallylamine gel is typical of polycationic gels and contrasts with the swelling of polyampholyte gels, which display considerable hysteresis and a strong dependence on initial test conditions.[37] Thus, this figure is consistent with the titration data in Table 3 that the gel is cationic. The gel displays a volume transition in sodium p-

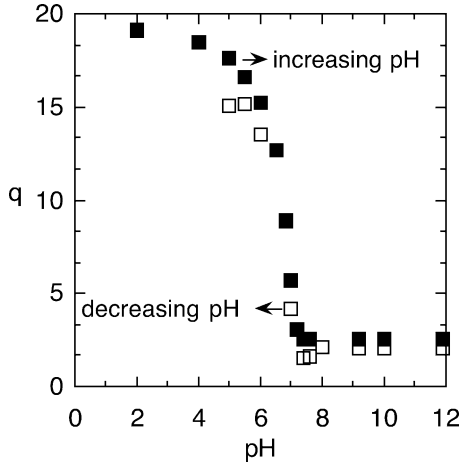

Figure 1. Volumetric swelling degree, q, of polyallylamine gels in isotonic (I = 0.05 M) *p*-phenolsulfonic acid/sodium hydroxide buffer ($5 \leq pH \leq 11.4$) and sodium chloride solutions ($pH < 5.0$; $pH > 11.4$). Filled symbols represent data collected upon increasing pH, 7 days after sample preparation, and the open squares represent data determined upon decreasing pH, 30 days after sample preparation.

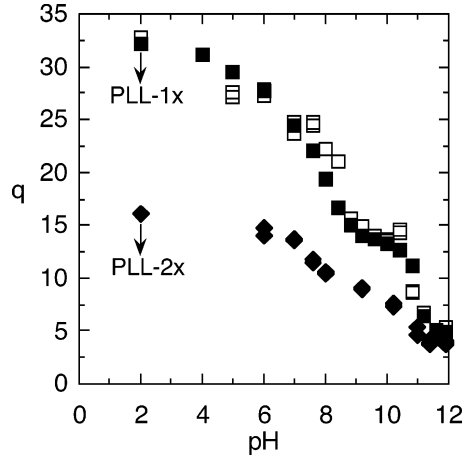

Figure 2. Volumetric swelling degree, q, of poly(α-L-lysine) gels in isotonic (I = 0.05 M) *p*-phenolsulfonic acid/sodium hydroxide buffer ($5 \leq pH \leq 11.4$) and sodium chloride solutions ($pH < 5.0$; $pH > 11.4$). Filled squares represent data collected for PLL-1x series upon increasing pH, 7 days after sample preparation, and the open squares represent data determined upon decreasing pH, 30 days after sample preparation. Data in PLL-2x series (filled lozenges) have been acquired only upon increasing pH.

phenolsulfonate buffer at a pH lower than expected based on the pK values obtained in 0.05 M aqueous sodium chloride (see Table 3). The collapse of the gels at a lower pH in the presence of aromatic anions can be attributed to the high polarizability of these ions, which can enhance ion-pair complex formation (different associative complexes between the polymer and the anions have been considered[35,38]) and therefore decrease osmotic pressure and favor gel collapse. Electrostatic interactions between the polymer and the aromatic sulfonates may likewise contribute to gel collapse. This mechanism has been proposed by Itaya et al. for polyallylamine in sodium *p*-ethylbenzenesulfonate solutions as well as by Rao et al. on the basis of the collapse of glutaraldehyde-cross-linked polyallylamine gels equilibrated in sodium *p*-styrenesulfonate and small-angle scattering of such gels swollen in the presence of aromatic anions (e.g., benzenesulfonate and *p*-stryrenesulfonate).[35,38]

The variation of gel volume between pH 2 and 12 for poly(α-L-lysine) gels prepared with two different levels of cross-linking in isotonic buffers (I = 0.05 M), primarily sodium *p*-phenolsulfonate buffers, is presented in Figure 2. These data are obtained similarly to that presented in Figure 1. Since the data lie on virtually the same curve, the expansion and contraction of the gel are deemed reversible, with no hysteresis. As with the polyallylamine gels in Figure 1, poly(α-L-lysine) gels are highly swollen at low pH and continuously contract (by a factor of 6 for the PLL-1x series or four in the case of the PLL-2x series) as the pH is increased from 2.0 to 11.9. In the swollen, low-pH state, gels in the 1x series swell about twice as much as the gels in the 2x series—consistent with the difference in cross-linking (see Table 1). In contrast to the polyallylamine gel, the polypeptide hydrogel does not collapse around neutral pH conditions. Rather, two transition regimes are evident in Figure 2: one in the range $6.0 < pH < 8.0$ and the other in the range $10.0 < pH < 11.0$.

The interpretation of this double transition requires recognition of the fact the buffering ions in the swelling solutions, the *p*-phenolsulfonate anions, are monovalent at low pH but divalent at high pH. The concentration of divalent species is very low (less than 0.001 M) in the isotonic buffer solutions at pH 7.0 but increases to about 0.02 M at pH 11.0.[25,33] Thus, monovalent counterions inside the highly charged network are increasingly replaced by divalent anions as the pH rises. Since only half of these ions are necessary to balance the charge on the network, the ionic osmotic pressure inside the gel decreases. This causes the gel to absorb less solvent, as predicted by the theory of Donnan ion exclusion as applied to gels.[35,39] A similar double transition in the swelling vs pH curve for anionic pH-responsive hydrogels in solutions composed of univalent anions and divalent cations was observed and quantified by Ricka and Tanaka.[39] The analogous interpretation applies here, considering only the fact the opposite dependence of swelling upon pH exists for a cationic network as opposed to an anionic one. This double transition is not observed in the case of polyallylamine gel (Figure 1), simply because the collapse of that gel occurs in the pH range where the buffer ions are in the monovalent form; once the gel is nonionic and in the collapsed state, it is no longer affected by further increases in pH or changes in salt valence.

Further evidence that ionic effects are primarily responsible for the two swelling transitions of poly(α-L-lysine) gels is provided in Figure 3, which presents gel swelling as a function of pH in isotonic sodium chloride solutions (I = 0.05 M), as opposed to the buffered solutions of Figure 2. The change from a mono/divalent salt to a monovalent salt solution eliminates the transition in pH 8−10 range, as expected. The single transition curves seen in Figure 3 are similar to that reported by Noguchi[40] for poly(α-L-lysine) cross-linked with formaldehyde and swollen in sodium bromide solution (though the swelling hysteresis observed by Moroi and Kokufuta[41] for poly(α-L-lysine) gels cross-linked by glutaraldehyde is not observed). The swelling transition in the 10−12 pH range for the gels in Figures 2 and 3 coincides with the polypeptide secondary structure transition for poly(α-L-lysine), which occurs between pH 10.0 and 11.0. At room temperature in this pH range, the linear polypeptide exhibits a coil-to-β-sheet transition if the polymer concentration in the

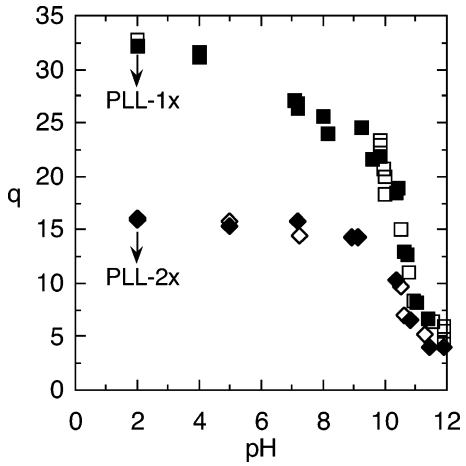

Figure 3. Volumetric swelling degree, q, of poly(α-L-lysine) gels in isotonic ($I = 0.05$ M) sodium chloride/sodium hydroxide or hydrochloric acid solutions. Filled and open symbols (PLL-1x, squares; PLL-2x, lozenges) represent data determined upon increasing and decreasing pH, respectively.

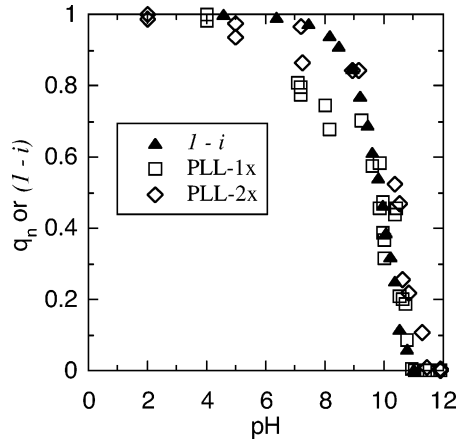

Figure 4. Reduced volumetric swelling degree, q_n (open symbols), and degree of neutralization, $1 - i$ (filled symbols), presented as functions of pH for poly(α-L-lysine) gels in sodium chloride solutions ($I = 0.05$).

solution is higher than ~10 wt % and a coil-to-α-helix transition at concentrations below this.[42] It must be recognized, however, that on the basis of the pK data in Table 3, the gel is still charged at the volume transition pH: $(1 - i) \approx 10\%$ at pH 10.5. This is probably sufficient for ion osmotic pressure to dominate hydrogel swelling behavior since even a few percent of charged groups can transform networks of polymers with limited water solubility into swollen hydrogels in aqueous media.[2,35] The thermodynamic origin of this phenomenon relates to the osmotic pressure accompanying the presence of mobile ions in the network, which can be sufficiently large to dominate other effects, such as polymer−solvent mixing.[1,13,35]

The importance of osmotic contributions to the swelling of poly(α-L-lysine) gels is further demonstrated in Figure 4. In this figure, the network's degree of neutralization $1 - i$ and the normalized volumetric degree of swelling q_n are presented as functions of pH for samples equilibrated in sodium chloride solutions ($I =$ 0.05 M). Values of q_n have been determined from

$$q_n = [(q - q_{min})/(q_{max} - q_{min})] \qquad (7)$$

where q_{max} and q_{min} denote the maximum and minimum degrees of swelling, respectively, across the full range of experimental data. In Figure 4, values of q_n for the 1x series have been determined over the range $4.0 \leq$ pH ≤ 11.0, which corresponds to the same pH range of the titration curve. In the 2x series, the pH range is slightly larger: $2.0 \leq$ pH ≤ 11.9. Since the data are scattered around the same curve, it follows that the amount of solvent absorbed by the gel is directly proportional to the fraction of charged groups in the network. That is, the swelling of these cationic gels reflects the osmotic pressure generated due to the difference in the number of charged species residing inside and outside the gel. It is possible to quantify such pH-responsive behavior; however, the main significance of these results in the context of this work is the observation that poly(α-L-lysine) gels have conventional pH-dependent swelling behavior, qualitatively similar to polyallylamine gels, despite the expectation that PLL polymer chains should undergo conformational transitions at high pH.

Table 4. Values of the Mooney−Rivlin Constants $2C_1'$ and $2C_2'$ and the Shear Modulus G for the Hydrogels under Uniaxial Compression

gel	swelling degree, q	C_1', kPa[a]	$2C_2'$, kPa[a]	shear modulus G, kPa[a]
PA1Am	19.6	46 ± 3	−1 ± 3	45 ± 1
	2.6	95 ± 1	−7 ± 1	82 ± 1
PLL-1x	17.0	26 ± 1	−1 ± 1	25 ± 1
	4.9	61 ± 3	0 ± 2	61 ± 2
PLL-2x	9.6	38 ± 1	4 ± 1	43 ± 1
	6.9	52 ± 7	0 ± 5	53 ± 1

[a] Uncertainties correspond to the intercept and slope errors of eq 1 or eq 2.

Stress−Strain Isotherms. The relationship between stress and deformation has been evaluated for each hydrogel on the basis of the Mooney−Rivlin equation, eq 2, and some representative values of $2C_1'$ and $2C_2'$ are listed for completeness in Table 4. The values of $2C_2'$ are essentially zero within the experimental error, indicating that these hydrogels display ideal stress−strain behavior. This observation is consistent with previously reported results, suggesting that $2C_2'$ does not generally play an important role in unidirectional compression for swollen networks ($\phi_2 < 0.2$).[5,8] Since $2C_2'$ is negligible, $2C_1'$ constitutes a direct measure of the shear modulus G, for which the corresponding values are included in Table 4. Since virtually all of the compression curves (σ plotted as a function of $|\alpha - \alpha^{-2}|$) for gels tested in this work are linear (correlation coefficients and probabilities of linear relationships greater than 0.99), the stress−strain data reported here can be accurately represented by eq 1.[33,43]

Dependence of Modulus on Composition. The scaling relationship between the shear modulus, G, and polymer volume fraction, ϕ_2, can be used to infer the conformation adopted by the network chains. Figure 5 shows the composition dependence of G for polyallylamine gels swollen to various extents by p-phenolsulfonic acid buffer solutions differing in pH. Over the composition range examined, the experimental points follow the same straight line on double-logarithmic coordinates, in which case the dependence of G on composition can be expressed as

$$G \sim \phi_2^{0.31\pm0.01} \qquad (8)$$

This relationship is in remarkably good agreement with

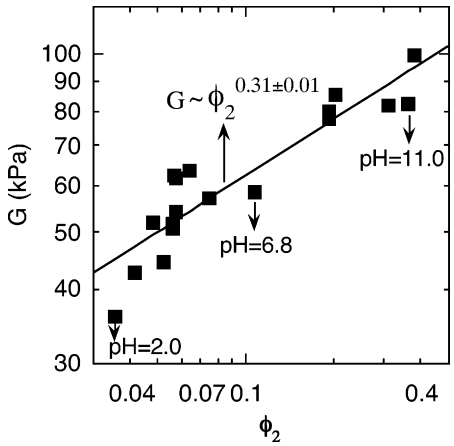

Figure 5. Dependence of the shear modulus, G, on polymer volume fraction, ϕ_2, in polyallylamine gels at 23.0 °C. Points are measured at various pH values, as shown in Figure 1. For reference, data points corresponding to different pH levels: 2.0, 6.8, and 11.0 are specifically indicated. The slope of the line shown matches the theoretical prediction of $^1/_3$.

theoretical predictions of an exponent of $^1/_3$ for networks composed of flexible polymer chains (eqs 3). This result likewise agrees with the findings of Candau and coworkers for poly(acrylic acid) gels and of Dubrovskii and Rakova for weakly ionized polyacrylamide gels prepared with monomer concentrations above 15 wt %.[12,17,18] The dependence of G on composition obeys the classical theory of elasticity for Gaussian chains (eq 3) and more closely resembles the behavior of neutral gels[8] than that predicted for polyelectrolyte gels by Rubinstein et al.[15] This difference is likely due to the fact that the measurements were made at a relatively high ionic strength ($I = 0.05$ M), which will tend to screen out the electrostatic interactions between the chains. Also, for most of the data, the buffer ions are divalent, rather than monovalent (as considered by this theory), and divalent ions shield the charges in the gel more effectively, again allowing the gel to behave more like a neutral network.[36,38] Another consideration is that the polyallylamine chains comprising the present networks are not very long.[33] Dubrovskii and Rakova have found that agreement with the theory proposed by Rubinstein et al. is more likely when ionic gels consist of very long polymer strands.

Figure 6a,b shows the dependence of G on polymer composition for the poly(α-L-lysine) gels; Figure 6a displays data collected of PLL-1x gel series while Figure 6b presents data obtained from PLL-2x. In these figures, open symbols represent gels swollen in solutions with pH < 11.0, whereas closed symbols identify gels swollen at pH ≥ 11.0. Since the linear correlation coefficients used to determine the following scaling relationships exceed 0.90, the probability that these data can be represented by the derived equations is greater than 99%.[33,43] As with the polyallylamine networks, poly(α-L-lysine) gels exhibit the scaling behavior expected for flexible networks when the specimens are swollen in solutions with pH < 11.0. Under these conditions, the scaling relationships of the protonated and cationic gels in the two poly(α-L-lysine) gel series are given by

$$G \sim \phi_2^{0.33\pm0.02} \quad \text{(PLL-1x)} \tag{9a}$$

$$G \sim \phi_2^{0.34\pm0.04} \quad \text{(PLL-2x)} \tag{9b}$$

In marked contrast, gels swollen in solutions with pH

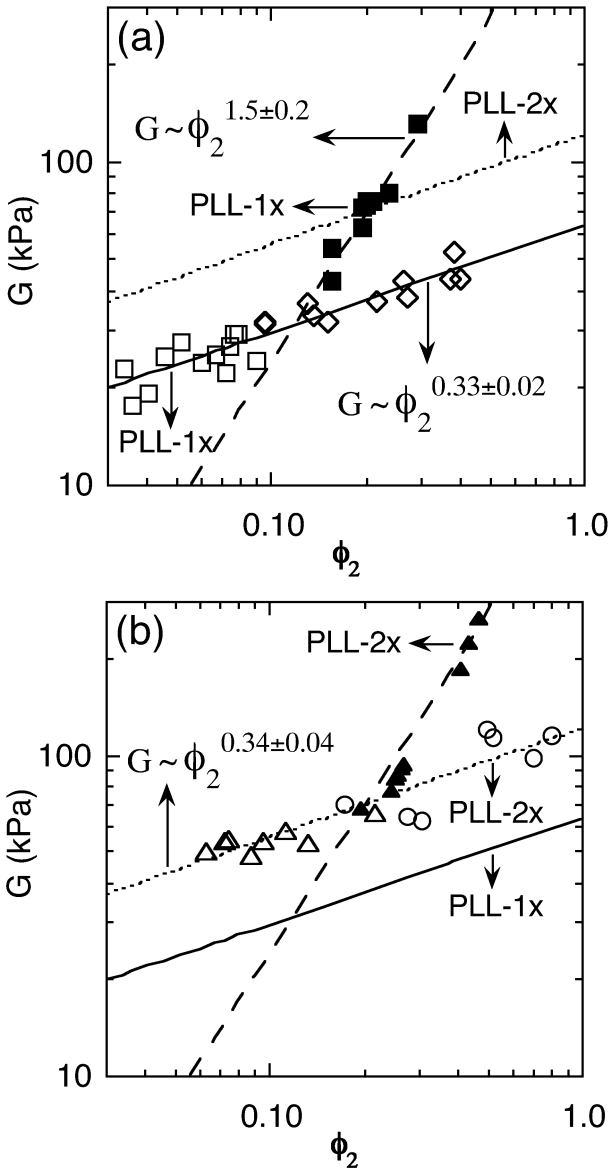

Figure 6. Dependence of the shear modulus, G, on the polymer volume fraction, ϕ_2, in poly(α-L-lysine) gels at 23.0 °C. Data of PLL-1x series (square and lozenge points) are shown in (a). Data of PLL-2x series (triangle and circle points) are displayed in (b). Open symbols correspond to samples swollen in solutions of pH < 11.0 and closed symbols samples swollen in systems of pH ≥ 11.0. Open square and triangle points correspond to samples swollen in the pH range 2.0 ≤ pH < 11.0. Open lozenge and circle data were obtained from samples swollen at pH 6.5 and partially dehydrated. The shear modulus depends on the polymer volume fraction of poly(α-L-lysine) gels, as varied by changes in pH or water content. The slope matches theory for networks of flexible chains below pH 11.0 (PLL-1x, solid line in (a) and (b); PLL-2x, dotted line in (a) and (b)) and theory for networks of rigid rods with flexible cross-links above this pH (broken line in (a) and (b)).

≥ 11.0 are deprotonated and nonionic, and the corresponding composition-dependent moduli for the two series can be written as

$$G \sim \phi_2^{1.5\pm0.2} \quad \text{(PLL-1x)} \tag{10a}$$

$$G \sim \phi_2^{1.69\pm0.09} \quad \text{(PLL-2x)} \tag{10b}$$

This behavior agrees quite well with predictions from scaling theories for networks composed of rigid-rod

polymer chains connected by flexible junctions, for which the predicted scaling exponent is $^3/_2$. Note that this abrupt change in mechanical behavior coincides with the pH where poly(α-L-lysine) undergoes its secondary structure transition.[30,42] Moreover, the exponents in eqs 10a and 10b are much smaller than those expected (≥2.3) from effects related to an increase in the number of connection points between the network chains.[8] Thus, the alteration in the mechanical behavior is apparently due to a significant change in the conformations of the network chains, from flexible to stiff. If the chains had remained flexible and an increase in cross-linking due to a physical process caused the change in mechanical behavior, the modulus should scale with an exponent of 2.3 or higher, as predicted by the corresponding scaling law theories of elasticity for flexible networks.[8] Finally, it is apparent that the variation of cross-link density has relatively less influence on the *G* values of these rigid networks than it has on *G* of the flexible hydrogels (pH < 11.0), as the data for both networks in the rigid chain regime lie on essentially the same line.

If the secondary structure of the polypeptide chains is the driving force responsible for rigid network behavior at high pH and not simply polymer concentration, gels swollen at low pH and later partially dehydrated at constant pH ought to retain their random-coil conformation and exhibit classical elastic behavior.[30,42] This hypothesis is confirmed in Figure 6a,b. Experimental values of *G* measured as a function of polymer volume fraction for partially dehydrated specimens previously equilibrated at pH 6.5 (open triangles and circles for PLL-1x and PLL-2x, respectively) fit the same straight lines as the samples whose polymer fraction was varied by changing pH below 11.0 (open squares and lozenges for PLL-1x and PLL-2x, respectively). Another important feature of Figure 6a,b is that the moduli of specimens swollen at high pH are several times larger in magnitude than for gels swollen at low pH and dehydrated to the same polymer volume fraction; in short, gels at pH > 11 are much stiffer than those below pH 11. Though notably higher, the moduli of the high-pH gels are still of the same order of magnitude as those at lower-pH values. This is in accord with predictions of contemporary elastic theories that expect that changes in modulus with changes in chain and cross-linker conformation exceeding an order of magnitude are expected only for completely rigid networks (rigid chains and cross-links).[7,19] Nonetheless, the ability to increase shear modulus at constant water content by altering chain conformation may have practical significance, considering that poor mechanical properties are one of the major drawbacks of hydrogels in many applications.

The conformational properties of soluble, linear poly(α-L-lysine) and the nature of its secondary structure transition have been the subject of a number of previous studies.[30,42,44] The conformation of poly(α-L-lysine) below pH 11 is found to be random coil. Hence, gels made from this polymer would be expected to behave as flexible networks in this pH range and follow the scaling relationships observed in eqs 9a and 9b. However, if the pH of a solution of poly(α-L-lysine) is increased above 11.0, it converts to a new secondary structure, either an α-helix or *β*-sheet depending upon the polypeptide molecular weight and concentration, ionic strength, pressure, temperature, and the type of salts or organic compounds present.[30,42,44] It is possible that at a specific

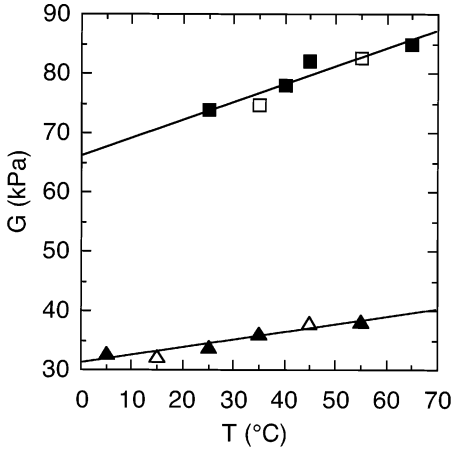

Figure 7. Temperature dependence of the shear modulus, *G*, of polyallylamine (squares) and poly(α-L-lysine) (triangles) gels at pH 6.5. Filled symbols represent data collected upon heating cycle and open symbols data determined upon cooling cycle.

Table 5. Thermoelastic Properties of Polyallylamine and Poly(α-L-lysine) Hydrogels

gel[a]	(d ln *G*/d*T*) × 10^{-3} [b]	β^b × 10^{-3}	f_e/f	(d ln $\overline{r_0^2}$/d*T*)[b] × 10^{-3} [b]
PA1Am	3.8 ± 0.6[c]	0.3	−0.1 ± 0.2	−0.5 ± 0.6
PLL-1x (pH = 6.5)	3.7 ± 0.6	0.3	−0.1 ± 0.2	−0.5 ± 0.6
PLL-1x (pH = 11.4)	5.6 ± 0.9	0.2	−0.7 ± 0.3[d]	

[a] Reference temperature: 20 °C. [b] In °C^{-1}. [c] Uncertainty corresponds to the slope error of the linear regression. [d] Assuming that the stress−strain behavior can be described by eq 1.[7,19]

set of these system variables all three conformations may coexist, though an antiparallel *β*-sheet conformation is favored at high pH, polymer concentration, and temperature.[30,42,44] Continuing work on this material now underway using dynamic mechanical analysis indicates that the conformation is most likely α-helix at room temperature, which converts to *β*-sheet above 45 °C.[45] However, for the analysis undertaken in this paper, the key point is that, upon increasing pH above 11, the modulus increase of poly(α-L-lysine) gels at constant water content is consistent with the assumption that the network chains rearrange from a random coil to a stiffer secondary structure, whether α-helix or *β*-sheet.

Thermoelasticity Analysis. The influence of polypeptide secondary structure on gel properties was further probed through thermoelasticity analysis. Following the method outlined above, values of *G* are deduced from the initial slope of stress−strain responses at different temperatures while holding the polymer concentration constant. By presenting the resultant moduli as a function of temperature (Figure 7), the corresponding thermal coefficient of the shear modulus (d ln *G*/d*T*) can be obtained by fitting a linear relationship to the data. Values of this coefficient, the gel thermal coefficient *β*, the fraction of force that is energetic in origin f_e/f (as determined from eq 5), and its molecular counterpart d ln $\overline{r_0^2}$/d*T* are provided in Table 5.[24] Theoretical and experimental values of f_e/f for systems related to the hydrogels studied in this work are included for comparison in Table 6. Although the uncertainties in the values of f_e/f listed in Table 5 are substantial, they are sufficiently accurate to permit the comparisons intended here, since the energetic contribution to the force

Table 6. Thermoelastic Properties of Selected Polymer Networks

polymer network	f_e/f
poly(2-hydroxypropyl acrylate)[a]	−0.30
poly(isobutyl acrylate)[a]	−0.20
poly(vinyl alcohol)[b]	−0.36
poly(α-L-alanine)[c]	−0.70[d] (−0.76)[e]
poly(α-L-valine)[c]	−0.80[d] (−1.07)[e]

[a] From ref 24. Reference temperature: 30 °C. [b] From ref 5. Reference temperature: 20 °C. [c] From ref 45. Reference temperature: 20 °C. [d] Theoretical value: local hydrogen bonding between residues was taken into account.[45] [e] Theoretical value: no hydrogen bonding between residues was taken into account.[45]

resulting from stiff secondary structures should be substantially greater than other factors normally encountered in networks of random-coil chains.[5,19] This contribution relates the part of the force to deform a polymer network that is due to changes in energy, associated with modifications in chain configurations and dimensions. Different chain configurations generally have different conformational energies, which is why the unperturbed chain dimensions are temperature-dependent.[5,24]

As listed in Table 5, polyallylamine and poly(α-L-lysine) gels (swollen at pH 6.5) have negative values of f_e/f, meaning that, in these chains, the higher energy conformations are less extended than those of lower energy. Consequently, the unperturbed dimension of the polymer chains decreases as the temperature is raised. In the case of poly(α-L-lysine), expanded at pH 6.5, and polyallylamine gels, about 10−30% of the total force comes from the changes in conformational energies of the polymer chains. These results are in qualitative agreement with reported values of f_e/f for some vinyl polymers (Table 6) and indicate that the origin of the elastic force in these networks is mostly entropic, which is in accordance with the previous findings that the gels, under such conditions, behave as flexible networks. It is likely that the extended trans conformation represents the lowest energy state for polyallylamine and coiled poly(α-L-lysine) since it would allow the greatest separation of the side groups (some possibly charged). At higher temperatures, some states of higher energy (possibly gauche) are permitted to occur favoring the presence of more compact conformations.[5]

The measured value of f_e/f for the poly(α-L-lysine) gel swollen at pH 11.4 is also negative. Note, however, that it is several times greater than the value determined for the gel at nearly neutral pH. This result confirms that the fraction of the total force due to energetic contributions is considerably higher for the gel at conditions associated with the existence of rigid secondary structures. Furthermore, the value of f_e/f is in quite good agreement with the theoretical estimates listed in Table 6 for poly(α-L-alanine) and poly(α-L-valine) homopolymers found from calculations of $d \ln \overline{r_0^2}/dT$ by DeBolt and Mark.[46] These predictions, generated from rotational isomeric state models that take into account (or disregard) the existence of local hydrogen bonds between residues, imply that a large negative f_e/f reflects the chemical regularity of the polypeptide chains. Such regularity usually contributes to the presence of conformational sequences capable of forming uniform and relatively large dimensional structures, such as crystal sheets. An increase in temperature promotes a reduction in the regular sequences, which, in turn, results in a decrease in unperturbed dimensions.[5,46] On the basis of thermoelastic measurements in different polymer networks, it is reported[5] that f_e/f is essentially independent of the network degree of cross-linking, whether chemical or physical, the network degree of swelling, the network synthesis conditions, the solvent used to swell the polymer, and the degree of crystallinity of the network.[5,24] This is consistent with its intramolecular origin.[5,24] This is also a strong indication that the change in the mechanical behavior of poly(α-L-lysine) gels results from transformations in the polypeptide chain conformations, rather than an increase of the effective cross-link density in the gel. This corroborates the explanation that the change in the modulus−composition scaling relationships—eqs 9 and 10—are primarily related to the formation of stiff secondary structures by poly(α-L-lysine) chains.

Despite the comparatively high-energy contribution to the elastic force, poly(α-L-lysine) gels do not behave as completely enthalpic or rigid networks due to entropic contributions that provide the mechanical behavior of a rigid network with flexible junctions. The dependence of f_e/f on external conditions, such as pH, constitutes a highly unusual thermoelasticity signature. Most reported values of f_e/f, for instance, are independent of solvent.[5,24] This distinguishing feature indicates that the polypeptide chains possess pH-sensitive conformations with different elastic characteristics. The results presented thus far indicate that (i) poly(α-L-lysine) has pH-dependent conformations with different degrees of rigidity, (ii) the more rigid conformation exists in the present gels at high pH, either α-helix or β-structures, and (iii) these stiff secondary structures are capable of strongly influencing gel mechanical properties.

Morphological Analysis. The influence of secondary structure on the mechanical properties of polypeptide networks motivates examination of the morphologies of these polyelectrolyte gels at different pH values. Figure 8a,b shows FESEM (secondary electron) images of polyallylamine gel in *p*-phenolsulfonic acid buffer at pH 6.5. They display a microporous morphology in which the struts appear as short, highly connected fibrils exhibiting variable thickness. The pores appear open and larger than the struts and exhibit no discernible pattern. Although quantitation of void space from SEM images is problematic, the voids in Figure 8a seem to account for more than 50% of the total gel volume (which includes polymer and the buffer removed during freeze-drying). This observation assumes negligible dimensional change during specimen preparation for FESEM. Micrographs of this gel are qualitatively similar, though more dense, at pH 7.6, where the gel is in the collapsed state.[47] Figure 8b displays the FESEM image collected from the 1x series of poly(α-L-lysine) gel swollen in *p*-phenolsulfonic acid buffer at the same pH as the polyallylamine gel (6.5). They likewise exhibit a microporous morphology closely resembling the ones in Figure 8a. In Figure 8b, the struts are relatively short and nonuniform in size. It is interesting to note that the morphologies of both polymer gels at nearly neutral pH appear quite similar to those observed in many other swollen polymeric networks, such as microporous hydroxypropylcellulose hydrogels.[34] The unoccupied (void) spaces are randomly arranged and again seem to account for more of the total gel volume than the polymer.

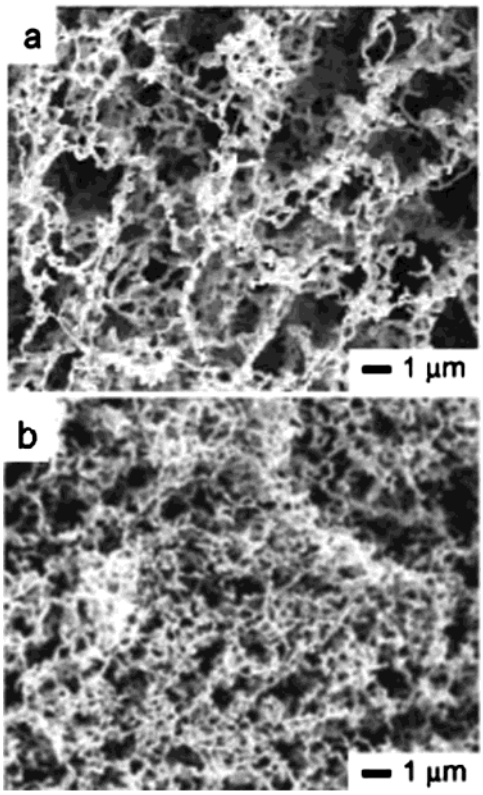

Figure 8. FESEM images of polyallylamine (a) and poly(α-L-lysine) (b) gels in *p*-phenolsulfonic acid buffer at pH 6.5. The image in (b) was taken from a PLL-1x sample.

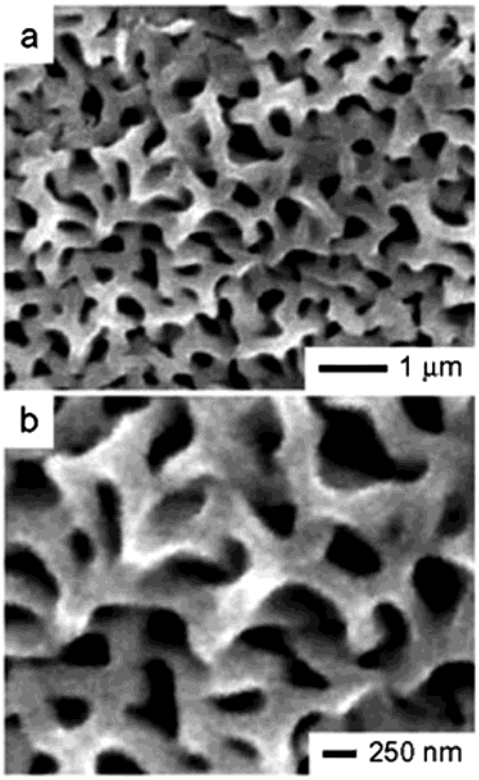

Figure 9. FESEM image of the same poly(α-L-lysine) gel displayed in Figure 8, but at pH 11.4, which is above the pH where the conformational transition from random coil to an organized secondary structure is expected. The image in (b) is an enlargement of (a) to permit closer examination of the gel morphology.

The images presented in Figure 9 have been obtained from the same poly(α-L-lysine) hydrogel series as shown in Figure 8b, but at a higher pH (11.4) that is associated with the rigid chain conformation (whether α-helix or *β*-sheet) hypothesized to exist under these conditions. This gel possesses a morphology that appears unique in the study of hydrogels. The struts are substantially larger than those in gels prepared at lower pH and appear more highly concentrated and interconnected. They are relatively uniform in size and shape and appear short, smooth, and uniformly positioned—almost patterned. In addition, they also seem to occupy more volume than the voids, in contrast to Figure 8a,b. This hydrogel is clearly more solidlike in appearance than any other hydrogel examined here. The formation of such a quasi-ordered morphology is consistent with the existence of an organized secondary structure due to a pH-induced conformational change of the network chains, as indicated by modulus studies. Comparison of mechanical property characteristics and predicted scaling behavior suggests that the poly(α-L-lysine) gel displayed in Figure 9 can be described in terms of a network made of flexibly connected rodlike chains. Because of the elastic scaling law behavior of the gels in high-pH solutions and the presence of water molecules in these networks, the rods should be randomly connected so that they do not form a nematic mesophase.[33] The morphological characteristics evident in Figure 9 are consistent with the description derived from the theory of elasticity for rigid polymer chains.[6,7,19] Further studies, however, are still necessary to determine whether the observed texture is a complete reflection of the molecular-level arrangement of the polymer chains that are responsible for the mechanical properties observed.

Conclusions

Linear polyallylamine and poly(α-L-lysine) were successfully cross-linked into gels through amine side groups by a Michael-type addition reaction with *N*,*N*′-methylenebisacrylamide. As cationic gels, both networks swell at low pH and contract as the pH is increased. The ϵ-amino groups of the lysyl residues in poly(α-L-lysine) are more basic than the amino side groups of the vinyl polyallylamine, so the transition occurs at about 7.4 for polyallylamine but pH 11 for poly(α-L-lysine). The polyvinyl gel drastically collapses in a single transition in sodium *p*-phenolsulfonate buffer solutions, whereas the polypeptide gel displays two transitions with increasing pH. In contrast, a single, reversible transition is observed during the expansion and contraction of poly(α-L-lysine) gel in sodium chloride solutions. This behavior is explained by the conversion of the buffer ion from a monovalent to divalent form between pH 8 and 10, thus reducing osmotic swelling pressure in the gel. As the polyallylamine gel is nonionic in this range, it is unaffected by this behavior. In all respects with regard to pH-sensitive swelling, both the polyvinyl and polypeptide gels behave similarly, in accord with well-established theory for polyelectrolyte gels.

The isothermal shear modulus of polyallylamine gels exhibits a single scaling relationship with respect to polymer volume fraction over the entire pH range examined. The scaling exponent (0.31 ± 0.01) is in excellent quantitative agreement with predictions of a scaling exponent of $^1/_3$ for networks composed of flexible chains. This behavior is similar to that of other vinyl

hydrogels such as polyacrylamide, poly(acrylic acid), poly(vinyl alcohol), and many others. Poly(α-L-lysine) hydrogels obey the same scaling relationship, with a scaling exponent of 0.33 ± 0.02, when the samples are swollen below pH 11.0. However, at pH levels above 11, the scaling exponent for poly(α-L-lysine) increases substantially to 1.5 ± 0.2, in agreement with theoretical predictions of $^3/_2$ for networks consisting of rigid-rod chains connected by flexible junctions. However, additional studies such as circular dichroism and FTIR are still necessary to determine whether α-helix or β-sheet is the principal secondary structure formed in the gels above pH 11. Thermoelasticity analyses also indicate the existence of rigid structures that affect the mechanical properties of the polypeptide gel at high pH. In addition, the energy contribution to the total elastic force is several times higher for the network swollen at pH > 11.0 relative to the same gel at nearly neutral pH or the polyvinyl gel at any pH in the range explored here. The morphology of the polypeptide network is also affected by the development of secondary structure. Relatively random structures exist for polyallylamine in both swollen and collapsed states, and at low pH, poly(α-L-lysine) gel is qualitatively similar. But at high pH poly(α-L-lysine) gel has an unusual, relatively ordered morphology. Morphology is therefore consistent with the fact that the polyvinyl gels behave as flexible networks at all pH values and that the poly(α-L-lysine) changes from flexible coil behavior at low pH to that of a network of rigid rods connected by flexible cross-links at high pH values.

Therefore, the results reported here show that the mechanical behavior and morphology of cross-linked polypeptides depend significantly on the pH, with clear evidence that this is due to the ability of the polypeptide chains to adopt different secondary conformations even in the cross-linked state. More importantly, these results provide evidence that responsive polypeptide hydrogels can be designed to perform differently from conventional synthetic gels and that the mechanical properties and morphology of these gels may be tailored in controlled fashion for emerging applications such as tissue engineering where gels of polypeptides such as biosynthetic elastin show great promise. Typically, the compliance of the implant material must be closely matched to that of the original tissue and vary in a controlled way as the scaffold degrades and the tissue regenerates. Thus, the increase in strength that stiff secondary structures can impart to poly(α-L-lysine) gels could prove a useful means of enhancing material performance and its dependence upon environment as a route for tuning behavior. Simply recognizing that such changes may be induced by changes in the material's environment, as may develop during its degradation, could become important in the development of various biomedical devices. Combination of stiff and flexible structures in a single material may also provide interesting new systems.

Finally, this reversible pH-responsive behavior is of distinctly different nature—variable chain conformation—than that observed with other pH-triggered hydrogel responses, which are typically osmotic in origin. This behavior is likely to be observable in other biopolymer gels, especially those of proteins and polypeptides, since these often have pH-responsive secondary structures. In contrast, gels made from synthetic polymers are unlikely to show this behavior, since such polymers rarely exhibit analogous transitions in chain conformation. Since polypeptide chain conformation also depends on other variables such as temperature, these stimuli likely can be used to trigger comparable transitions to those shown here using pH.

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