Nonlinear Optical Characterization of Chromophore-Modified Poly[L-glutamate] Thin Films

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The structural properties, molecular side-chain reorientation rates, and the magnitude of the second-order nonlinear tensor components were evaluated for poly[L-glutamate] modified with nonlinear optical chromophores. Circular dichroism showed that the α-helical backbone conformation of the polypeptides remained intact after modification of the side chains with the polar aromatic chromophores. The (100) *d* spacing (WAXS) of the polypeptide thin films was indexed to a hexagonal unit cell. The *d* spacing increased with the size of the side chain and the extent of modification. The onset temperature of side chain motion and chain libration, T_α, in these films increased with the size and polarity of the chromophore. The side-chain chromophores were aligned by electric field poling, and their reorientation was monitored by the decrease in second harmonic generation. A biexponential decay model was used to describe the first 2 h of the second harmonic decay process. We found that polypeptides with higher T_α values produced greater long-term side-chain alignment stability, as measured by the slower decay rate of the second harmonic signal. The second-order tensor component, d_{33}, was determined by the Maker Fringe technique to be ca. 7 pm/V at a fundamental of 1064 nm. Poly[L-glutamates]-based nonlinear optical materials have promise for electrooptical applications as they retain the nonlinear optical signal and α-helical secondary conformation.

Introduction

Synthetic polypeptides in the rigid α-helical conformation possess a noncentrosymmetric center of inversion for the entire polymer, a requirement for second-order nonlinear optical (NLO) processes. The chiral α-carbon of each L-enantiomeric amino acid produces a polar ordering of the backbone and induces the side chains to wrap around the helix main chain directed toward the N terminus.[1] A large side-chain NLO coefficient and a noncentrosymmetric bulk structure are expected to generate a large second harmonic (SH) optical response from these polypeptides. The natural tendency of adjacent polypeptides is to align in an antiparallel orientation and for the side chains on neighboring helices to interdigitate into parallel, overlapping pairs.[2] Orientation of the optically active side chains into a noncentrosymmetric, unidirectional manner requires an external electric field or a mechanical process. Directionally aligned polypeptides have been constructed by the polymerization of activated alanine and phenylalanine onto a special functionalized substrate.[3] The aligned backbone produces greater opportunity for noncentrosymmetric side-chain orientation and increased orientational stability. The rodlike, α-helical backbone conformation of polypeptides is ideal to restrain the orientation of the optically active groups to a greater extent than in comparably modified synthetic polymers, such as poly(methyl methacrylate) and poly(styrene). The random coil conformation of these latter polymers produces a two-stage relaxation mechanism in which side-chain reorientation precedes additional motion by the backbone. The relaxation mechanism of helical polypeptides and main-chain NLO polymers proceed predominately by a side-chain relaxation[4] mechanism. Additional methods to frustrate the reorientation of the aligned molecules include polymer densification (physical aging)[5] and cross-linking.[6]

Little information regarding polypeptides as a NLO material has been published to date. The SH efficiencies for L-valine,[7] L-valine-*p*-nitroanilide,[8] and dipeptides and short oligopeptides[8] have been examined. The NLO properties of the ordered polar amide groups along the helical backbone of poly(γ-benzyl-L-glutamate) (PBLG) have been determined in solution using an electric-field-induced second harmonic generation technique.[9] The second-order hyperpolarizability, β, of the α-helical

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backbone is 7.6×10^{-32} esu/residue,[9,10] with negligible contribution from the benzyl side chains. Incorporation of an electron-accepting nitro group to produce poly(γ-(*p*-nitrobenzyl)-L-glutamate) enhances the β value of the side-chain to -2.5×10^{-31} esu/residue.[10] Contact poling of lyotropic PBLG produces thick films (20 μm) of noncentrosymmetrically aligned backbones that generate a SH value of $0.1 \times I^{2\omega}_{\text{quartz}}$.[4]

In the present work we have attempted to explore the use of rigid α-helical polypeptides as sutiable matrices to incorporate NLO chromophores as side chains. The synthesis, characterization, and side chain reorientation kinetics of poly(γ-methyl-L-glutamate), poly(L-glutamic acid), and succinylated poly(L-lysine) functionalized with either disperse red 1, spiropyran (characterization), photolyzed spiropyran (merocyanine NLO relaxation properties), nitrobenzyl, or *N*-pentyl-4-nitroaniline are examined. The close packing nature of α-helical polypeptides and the inter- and intramolecular interaction between side chains constrained to inter-rod spaces is expected to constrict side-chain mobility so that when ordered by some director their naturally reduced mobility aids retention of order over time. We describe the role of the side chains to preserve the rigid-rod nature of the main-chain backbone, chromophore alignment, and second harmonic signal from decay over a period of several months.

Experimental Section

The four NLO chromophores investigated are disperse red 1 [DR1, (4-[*N*-ethyl-*N*-(hydroxyethyl)amino]-4′-nitroazobenzene), Sigma], photolyzed spiropyran [1′-(β-hydroxyethyl)-3,3-(dimethyl-6′-nitrospiro(indoline-2,2′[2H-1]benzopyran)), Chroma Chemicals], 4-nitrobenzyl alcohol [NBA, Aldrich], and *p*-nitro-*N*-(hydroxypentyl)aniline (NHPA) with corresponding β hyperpolarizability values of 32×10^{-30} esu,[11] 1×10^{-28} esu (merocyanine form of spiropyran),[12] 1.9×10^{-30} esu,[10] and 34×10^{-30} esu,[13] respectively. Illustrated below is the common polypeptide structure and the NLO side chains:

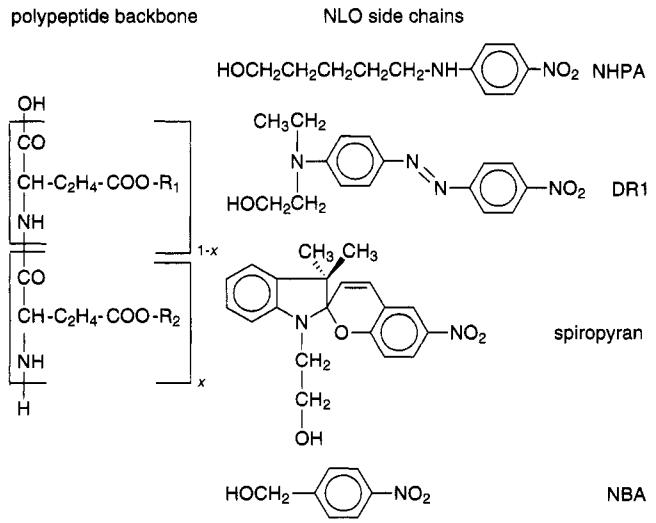

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Table 1. Side-Chain Modification, α-Transition Temperatures, and 100 Reflection

polypeptide	R_1	R_2	x^b	T_α, °C	*d* spacing, Å
I	CH_3	NHPA	5	25–30	11.75
II	CH_3	DR1	10	40–45	11.40
III	H	NHPA	10	35–40	14.52
IV	H	DR1	8	80–85	12.90
V	H	NBA	37	25–35	14.63
VI	H	spiropyran	9	20–30	amorphous
VII[a]	H	spiropyran	35	55–60	amorphous
PG-0	H	N/A	N/A	0–10	9.90
PG-1	CH_3	N/A	N/A	0[c]	11.00
PG-2	C_2H_5	N/A	N/A	0[c]	11.60
PG-5	CH_3	C_5H_{11}	100	−21[c]	14.00
PG-12	CH_3	$C_{12}H_{25}$	100		23.80
PG-12	CH_3	$C_{12}H_{25}$	100		20.30[d]

[a] Side chain: $\cdot C_4H_8 \cdot NH \cdot CO \cdot C_2H_4 \cdot COO \cdot R_1$ (or R_2). [b] *x* is the mole % substitution by R_2. [c] T_α values for PG-1 and PG-2 were obtained from ref 18, and for PG-5 from ref 37. [d] Measured at 75 °C.[18]

Table 1 gives the composition, onset temperature of side chain motion and chain libration, T_α, and (100) spacing of the equatorial Bragg reflection for each polypeptide.

Synthesis. *(1) p-Nitro-N-(hydroxypentyl)aniline (NHPA)*: A mixture of *p*-nitrofluorobenzene (7.4 g, 52.5 mmol, Aldrich), 5-amino-1-pentanol (5.4 g, 52.5 mmol, Aldrich), and potassium carbonate (14.5 g, 0.105 mol, Aldrich) was refluxed for 2 h, poured into water, and then extracted with dichloromethane. The concentrated organic layers were recrystallized in toluene: 89% yield and mp 69–71 °C. ^{1}H NMR ($CDCl_3$) δ = 1.52 (m), 3.12 (m), 3.56 (t), 6.43 (d), 7.95 ppm (d). IR (KBr) 3476 (OH), 3281 (NH), 3081 (Ar–H), 2934 (CH), 1603 (NH), 1475 and 1294 (NO_2), 838 cm^{-1} (*p*-Ar). Anal. Calcd for $C_{11}H_{16}N_2O_3$ (224.26): C, 58.86%; H, 7.13%; N, 12.49%. Found: C, 58.86%; H, 7.23%; N, 12.47%.

(2) Modified poly(γ-methyl-L-glutamate) (PG-1): A solution of PG-1 (0.5 g, 3.5 mmol, Sigma [DP > 700]), NHPA (7.81 g, 35 mmol), *p*-toluenesulfonic acid (3.23 g, 17 mmol, Aldrich), and 70 mL of dioxane/dimethylformamide (1:1) was heated for 5 days at 80 °C. The liberated methanol was removed by distillation to drive the reaction toward completion, the solution was then concentrated, and the polymer was precipitated in chilled methanol. DR1-modified PG-1 was prepared by a similar procedure.

(3) Modified poly(L-glutamic acid) (PG-0): A solution of PG-0 (0.5 g, 3.4 mmol, Sigma [DP > 575]), dicyclohexylcarbodiimide (0.82 g, 4.0 mmol, Aldrich), (*N,N′*-dimethylamino)pyridine (0.081 g, 0.66 mmol, Aldrich), NHPA (1.56 g, 7.0 mmol), and dioxane/dimethylformamide (80 mL, 10:1) was heated to 80 °C for 3 days. The precipitated *N,N′*-dicyclohexylurea was filtered from the reaction mixture, and the polymer was purified using cold ethanol and dimethylformamide as nonsolvent and solvent. DR1, NBA, and spiropyran-modified PG-0 were prepared by a similar procedure.

(4) Modified succinylated poly(L-lysine) (SPL): A solution of SPL (1 g, 4.38 mmol of monomer, Sigma [DP > 220]), spiropyran (1.54 g, 4.38 mmol), diisopropylcarbodiimide (0.686 mL, 4.38 mmol, Aldrich), (dimethylamino)pyridine (0.0535 g, 0.438 mmol) and dimethylformamide (70 mL) was stirred at room temperature for 48 h. Product purification was similar to the procedure described above using cold ethanol as the nonsolvent.

Material Characterization. UV–vis absorption spectra were obtained with a Perkin-Elmer Lambda 9 spectrophotometer. The extent of modification, shown in Table 1, was estimated by measuring the absorbance of a known weight concentration of polymer solution at 400 nm (ϵ = 20 500 cm^{-1} M^{-1}) for **I** and **III**, at 480 nm (ϵ = 32 300 cm^{-1} M^{-1}) for **II** and **IV**, at 286 nm (ϵ = 11 200 cm^{-1} M^{-1}) for **V**, and at 356 nm (ϵ = 11 200 cm^{-1} M^{-1}) for light-adapted **VI** and **VII**. Infrared spectra were obtained on a Perkin-Elmer FTIR-1725X spectrophotometer. A Jasco Model 720 CD spectropolarimeter was used to measure the circular dichroism (CD) spectra of polypeptides dissolved in hexafluoroisopropyl alcohol (HFIP). ^{1}H NMR spectra were recorded using a Hitachi R-1500 FT-NMR.

The onset temperature of side-chain motion and chain libration, α-transition temperature (T_α), was measured on a Perkin-Elmer Model DSC-2C thermal analysis data station using a heating rate of 10 °C/min. The existence of the lyotropic liquid-crystalline phase was determined prior to casting with a Nikon OptiPhot polarizing optical microscope (POM).

Flat-film X-ray photographs of thin films were taken at room temperature and under vacuum (Warhus camera) at a sample-to-film distance of 5 cm. The Ni-filtered, Rigaku rotating anode, Cu Kα source was passed through a 0.020 in. pinhole collimator. Sixteen hour exposures at 60 mA/180 kV were required, and the spatial measurements of the equatorial X-ray reflections on the film were measured to ±0.005 cm.

The equatorial Bragg reflections from PG-0 and several poly(n-alkyl-L-glutamates) (n is the number of alkyl carbons in the PG-n chain; n = 0, 1, 2, 5, 12) were tentatively indexed to the (100) spacing of a hexagonal unit cell and used to reference the Bragg reflections from the chromophore modified polypeptides to the interpolymer distances of poly(n-alkyl-L-glutamates), as described in the X-ray scattering section of the discussion. Synthesis of n-pentanol and n-dodecanol modified PG-1 was similar to the ester exchange reaction described above and PG-2 was obtained from Aldrich.

Second Harmonic Generation. All thin films were prepared on clean oven-dried ITO coated glass slides using a Solitec spin coater in a certified class 100 clean room. The polypeptides were dissolved in dichloromethane and then filtered through a 0.22 μm pore size nylon filter. Solution viscosities and spin speeds were adjusted to produce 1−2 μm thick films. The films were then annealed in vacuo at 60−70 °C for 10 days, and the film thickness was determined by a Sloan Dektak IIA surface profilometer. UV irradiation converted the spiropyran molecules into the NLO active merocyanine form.

A tungsten needle-to-plane geometry at a distance of 7 mm from the sample and a constant 1 μA poling current (−3000 to −5000 V) aligned the polypeptide side chains. The optimum poling conditions were determined by monitoring the SH as a function of temperature. Higher corona poling currents were not employed because the excessive surface charges resulted in the dielectric breakdown of the material[14] and damaged the films.

The p-polarized fundamental beam from a Molectron MY34-20 Nd:YAG laser (200−350 μJ, 15 ns pulses, 64 μm spot size) was incident on the sample at the nonlinear Brewster angle and the SH efficiency was monitored with time. A Hamamatsu R636 GaAs PMT with a SRS boxcar integrator measured the transmitted SH signal at 532 nm. An empirical calibration function referenced the SH from the sample to a Y-cut (010) quartz (d_{11} = 0.5 pm/V).[15]

Conversion of the free space fundamental wavelength, λ, from the Nd:YAG laser to its SH signal at 532 nm probed the NLO properties of the films. The SH wavelength occurred within the DR1 absorption band and resulted in resonance enhancement of the second order NLO coefficient, d_{ij}. Kleinman symmetry[16] ($d_{15} = d_{31}$) is invalid in this situation, so a Maker fringe method[17] to account for the absorption of the SH radiation and to evaluate the d_{33}, d_{15}, and d_{31} nonlinear tensor components was adopted from Hayden.[17] The SH power generated by the sample, $P_{2\omega}$, while propagating through an anisotropic polypeptide film of thickness, L, is given by

$$P_{2\omega}^{(b\to a)} = \frac{128\pi^3}{cA}\left(\frac{2\pi L}{\lambda}\right)^2 \frac{P_\omega^{\,2}}{\cos^2(\theta)} \frac{\sin^2(\Psi)+\sinh^2(\chi)}{\Psi^2+\chi^2} \times \exp(-\Phi)|\mathbf{F}^{(b\to a)}|^2 \quad (1)$$

where A is the beam cross section and c is the free space velocity of light. The phase difference factor, $\Psi = 2\pi L/\lambda(n_\omega \cos(\theta_\omega) - n_{2\omega}\cos(\theta_{2\omega}))$, and the absorption factors

$$\chi = \frac{L}{2}\left(\frac{\alpha_\omega}{\cos(\theta_\omega)} - \frac{\alpha_{2\omega}}{2\cos(\theta_{2\omega})}\right)$$

$$\Phi = L\left(\frac{\alpha_\omega}{\cos(\theta_\omega)} + \frac{\alpha_{2\omega}}{2\cos(\theta_{2\omega})}\right)$$

are functions of the angle of incidence of the fundamental in air in accordance to Snell's law [$\sin(\theta) = n_\omega \sin(\theta_\omega) = n_{2\omega}\sin(\theta_{2\omega})$]. The absorption coefficient α is measured at the fundamental or SH frequency. The angular projection factor, **F**, is dependent on the ($b \to a$) polarization configurations (p−p), (s−p), and (ϕ−s), and it is the product of the Fresnel transmittance factors and the effective d coefficient as defined by

$$\mathbf{F}(\text{p}-\text{p}) = \mathbf{t}_{2\omega\text{p}}\mathbf{t}_{\omega\text{p}}^{\,2}[2d_{15}\cos(\theta_\omega)\sin(\theta_\omega)\cos(\theta_{2\omega}) + (d_{31}\cos^2(\theta_\omega) + d_{33}\sin^2(\theta_\omega))\sin(\theta_{2\omega})] \quad (2a)$$

$$\mathbf{F}(\text{s}-\text{p}) = \mathbf{t}_{2\omega\text{p}}\mathbf{t}_{\omega\text{s}}^{\,2}d_{31}\sin(\theta_{2\omega}) \quad (2b)$$

$$\mathbf{F}(\phi-\text{s}) = \mathbf{t}_{2\omega\text{s}}\mathbf{t}_{\omega\text{s}}\mathbf{t}_{\omega\text{p}}d_{15}\sin(\theta_\omega)\sin(2\phi) \quad (2c)$$

where $\mathbf{t}_{jk}$ is the standard Fresnel transmission factor for j = ω or 2ω and k = s or p, and $\phi = 45°$ was used to maximize the signal for (ϕ−s) measurement. The SH power was referenced to the maximum quartz d_{11} measurement at normal incidence. The d_{33} coefficient calculated by this method is equal to 3 times the average of d_{15} and d_{31} and accounts for the absorption of the SH radiation. The SH intensity is proportional to the NLO coefficient in eq 1 through the Fresnel transmission factors in eq 2.

Results and Discussion

Modification of the polypeptide side chains by the NLO chromophores is presumed to be random along the polymer backbone. Table 1 shows that UV−vis spectroscopic measurements did not indicate more than 37% substitution, possibly because the attached chromophores pose steric and electrostatic interactions that hinder additional attachment onto the unreacted sites. These steric and electrostatic effects did not prevent the modification of PG-1 by flexible, n-alkyl moieties from going to completion (determined by ^{1}H NMR).

Circular Dichroism Spectroscopy. Circular dichroism (CD) spectra were taken to determine the secondary conformation of the modified polypeptides. The CD spectra of the modified polypeptides were compared to PG-5, a well-known α-helical polypeptide.[18] Figure 1 illustrates the CD spectra of **II** and **IV**; the spectrum for **II** is representative of **I**−**III**, **V**, and unphotolyzed **VI** and **VII**. Its two well-defined negative peaks at 208 and 220 nm and the positive band at 190 nm indicate an α-helical secondary conformation. However, absorption by the chromophore in the ultraviolet region contributed to the molar ellipticity values of the backbone and made the extent of α-helical content difficult to quantify. It is evident from this spectrum that the degree of side-chain modification did not disrupt the helical conformation of any polypeptide. Several studies that attached larger chromophores with short leader

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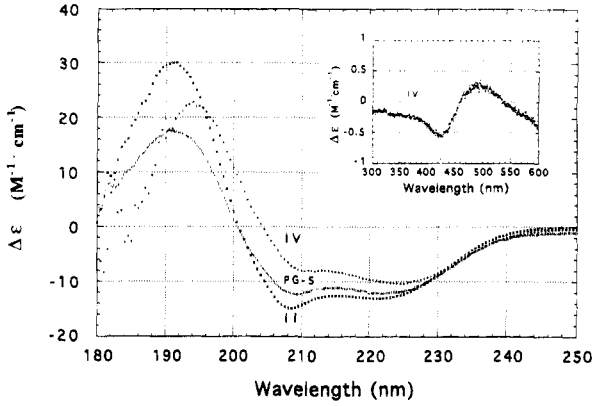

Figure 1. CD spectra of **II**, **IV**, and PG-5 in HFIP at room temperature. The induced dichroism for **IV** is shown in the insert.

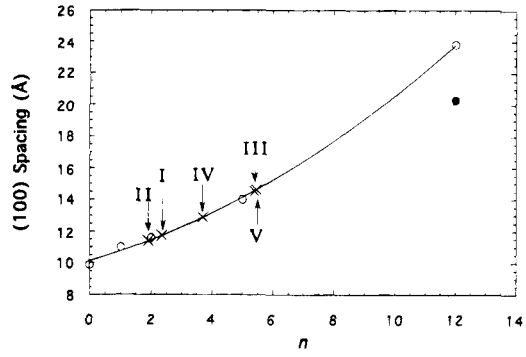

Figure 2. Variation in spacing from the first equatorial reflection (100) in a hexagonal unit cell as a function of *n*-alkyl and the equivalent *n*-alkyl spacing for the chromophoric side chains. (×) data from modified PG-0 and PG-1; (○) data from *n*-alkyl polymers; (●) denotes measurement at 75 °C for PG-12.[18]

groups[19,20] or that photochemically changed the side-chain structure,[21] as in photolyzed **VI**,[22] reported backbone conformation changes from α-helix to random coil in solution.

The two unique features for **IV** in Figure 1 are the red shift of the positive peak to 194 nm from 190 nm and the induced circular dichroism at 425 and 495 nm (see insert). The former is indicative of α-helical polypeptide aggregation while the latter two peaks indicate either transition dipole coupling or interchromophore coupling to the helical backbone conformation.[22] Hydrogen bonding between the unmodified carboxyl side chains and the nitro moiety of DR1 is believed to contribute to this effect. Increasing the length of the leader group separating the chromophore from the backbone can decouple these interactions. An induced dichroism was not observed with **I** and **III** (both possessing pentyl leader groups) nor with **VII** (attached to a succinylated lysine leader group).

Thermal Phase Transitions. Table 1 summarizes the α-transition temperatures of all the polypeptides investigated. Side-chain modification increased the T_α by at least 25 °C over the starting PG-0 and PG-1 polymer values. Hydrogen bonding between the nitro moiety of DR1 and the carboxyl group of the unmodified side chain produced a T_α of 80 °C in **IV**. Hydrogen bonding of the methyl ester, unmodified side chain with the nitro moiety of DR1 is not possible in **II**. As a result, the T_α for **II**, containing the same extent of DR1 modification as **IV**, was 40 °C. A DR1 side-chain melting endotherm was not observed for either compound, indicating a random distribution of the modified sites and a low degree of DR1 aggregation.

A combination of the leader group length, the extent of modification, the shape and polarity of the chromophore, and poor hydrogen bonding attributed to the low T_α's of **I**, **III**, **V**, and **VI**. The 13.3 Å extended conformation length of the leader group in **I** and **III** can decouple the chromophore-backbone intrapolymer interactions much more readily than the 8.5 Å leader length in **II** and **IV**. As a result, the less restricted motion of the backbones in **I** and **III** yielded lower T_α values (25–35 °C). The leader group length and the extent of modification for **IV** and **VI** are approximately equal, but the T_α for **VI** was only 20 °C. Spiropyran is less polar than DR1 and it is attached laterally to the end of the leader group. This makes interpolymer spiropyran interactions much weaker.

X-ray Scattering. The interpolypeptide distance is expected to increase with the type of chromophore, the extent of modification,[23] and the leader group length. The (100) Bragg reflection is proportional to the interhelical polypeptide separation, for a unit cell of hexagonal symmetry,[24] by a factor of 1/cos(30). The (100) spacings obtained from X-ray scattering measurements of the starting polymers were 9.9 Å for PG-0 and 11.0 Å for PG-1, shown in Figure 2 at $n = 0$ and $n = 1$, respectively. The curve in this figure illustrates the increase in the spacing as a function of *n*-alkyl units attached to poly(L-glutamate). Below $n = 8$, the curve follows the characteristic hexagonal packing behavior of poly(*n*-alkyl-L-glutamates).[18] At $n = 12$ and 75 °C (solid circle in Figure 2), the side chains behaved as a fluid phase and a smaller spacing was observed. Watanabe et al.[18] indicate that the side chains gather in between layers of polypeptide backbones without distorting of the α-helical conformation: a shape analogous to the columnar, comblike structures found in flexible main-chain polymers with side-chain crystallinity.[18,25] The spacing for PG-12 at 75 °C is the interhelical separation within such a layer.[18] This indicates that side-chain interactions influence the packing, the interhelical separation, and the amount of interpolymeric free volume[26] of the polypeptides.

The length of the NHPA side chain in **III** and the high degree of NBA modification in **V** produced the largest observed spacing of 14.5 Å. The low degree of NHPA modification in **I** marginally increased the 11.7 Å spacing. These polypeptides are expected to have a lower degree of interchromophore interaction than the DR1-modified polypeptides and to pack in the expected cylindrical molecular shape. The strong DR1 interac-

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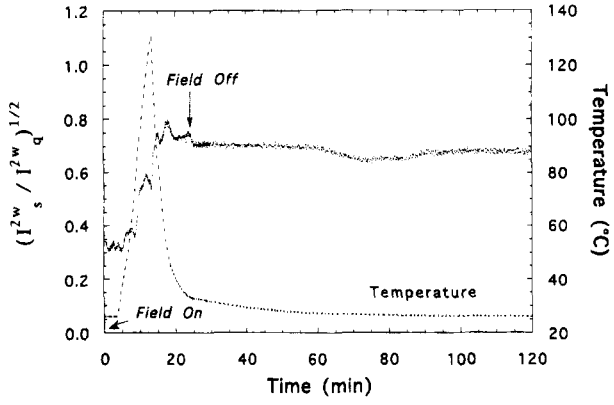

Figure 3. SH of corona-poled **IV** as a function of time and temperature. Signal is relative to Y-cut quartz.

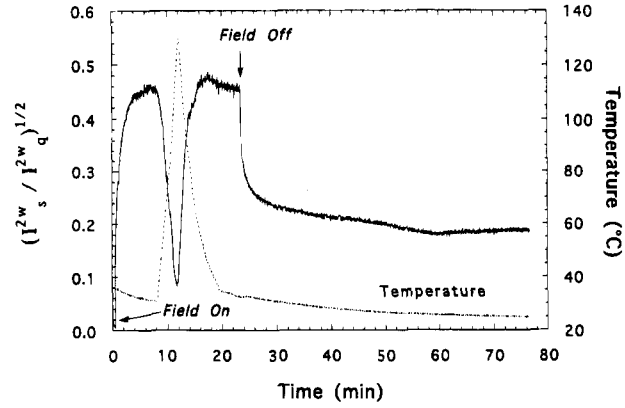

Figure 4. SH of corona-poled **II** as a function of time and temperature. Signal is relative to Y-cut quartz.

tions may produce a deformation of the molecular shape to encourage layer packing. This may explain the lower observed spacings for **II** and **IV**, but at present the evidence is inconclusive.

A comparison between doped and covalently attached polypeptides determined the location of the chromophore within the polypeptide material. The Bragg reflection of PG-0 or PG-1 thin films doped with 10 mol % NHPA or DR1 did not increase more than 3–5%. The (100) spacing was expected to increase significantly if the dopant molecules became entrapped between the individual polypeptides during solvent evaporation. The free chromophores must reside external to the polypeptide chains, possibly between the clusters of aggregated polypeptides[27] and not affecting the Bragg reflection. Chromophore confinement in between the polymer chains increased interpolymer distance in **I** through **V**. Polypeptides **VI** and **VII** did not produce a X-ray diffraction pattern because, unlike the other polypeptides, these materials were not liquid-crystalline and long-range macroscopic order was not observed.

Second Harmonic Generation. The primary contribution to the SH signal was attributed to the chromophore. The antiparallel orientation of adjacent polypeptides eliminates any amide backbone nonlinearities due to the centrosymmetry of the bulk medium. The magnitude of the SH signal is then a measure of the degree of orientation of the side chains. The initial rise in the SH signal during poling is the result of dc-induced SH generation and the alignment of the unhindered molecules in the large free-volume domains. The former is produced by the $\chi^{(3)}$ contribution to the nonlinear polarization.[28] Subsequent movement and collisions continue the unidirectional alignment of the remaining chromophores and increase the magnitude of the SH signal.

Figures 3 and 4 illustrate the SH signal originating from DR1 as a function of time and the effect of temperature during corona poling of polypeptides **IV** and **II**, respectively. At the onset of poling, the SH intensity, $(I_s^{2\omega}/I_q^{2\omega})^{1/2}$, for **IV** reached a value of 0.3 at room temperature, as shown in Figure 3. At higher temperatures, the DR1 molecules are expected to overcome rotational energy barriers, high interpolymer viscosity, and hydrogen bonding interactions, enabling them to align with the poling field. The $(I_s^{2\omega}/I_q^{2\omega})^{1/2}$ value increased to 0.75 upon cooling to a temperature slightly higher than the T_α at 80 °C. After the film was cooled to room temperature and the poling field removed, the rotational barriers reformed and constrained most of the aligned DR1 molecules in the unidirectional oriented state for the next 100 min.

Figure 4 illustrates the SH intensity behavior for **II** poled under similar conditions. After the initial rise in $(I_s^{2\omega}/I_q^{2\omega})^{1/2}$ to 0.46 at room temperature, the signal deteriorated to a value of 0.08 as the film was heated above 100 °C. This large decrease in SH intensity occurred at a temperature 60 °C higher than the T_α of the material. At the elevated temperature, the Boltzmann effect ($k_BT \geq \mu E$) causes the material to behave according to an oriented gas model[29] that predicts a decrease in the SH signal with increasing temperature. The librational and translational motions of the α-helical backbone increase significantly above the T_α of the polypeptide, making side-chain alignment difficult to maintain. The 40 °C lower T_α of **II** resulted in the decline of the SH intensity at a lower poling temperature and to a greater extent than the signal for **IV**. Long-term, high-temperature stability is desirable because current electrooptic device concepts require retaining 80% nonlinear activity at 70 °C for 5 years.[26] The relaxation process was predominately due to thermal considerations. The 1–2% decrease in the electrical resistance of the film at the higher temperature could not have produced the large change in the SH intensity. This phenomenon was prevalent in polypeptides with low T_α values.

The SH signal for **II** recovered to a value of 0.46 as the film cooled to room temperature. The SH signal declined by 40% within the first 2 min, after removing the poling field. The rotational barriers that constrained the DR1 molecules in the aligned state at room temperature in **IV** were much weaker in **II**. Hydrogen bonding played a more significant role in frustrating the relaxation mechanism and increasing the T_α in **IV** than in the other polypeptides.

A comparison between the covalently attached and the polymer dispersed unattached NLO chromophores exemplifies the importance of covalent anchoring. The SH intensity from PG-0 and PG-1 films doped with NHPA or DR1 to a concentration of 10 mol % reached

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Table 2. Temporal Stability Parameters[a] for Corona-Poled Polymers

polypeptide	$(I_s/I_q)_{t=0}^{1/2}$	A	τ_1, h	τ_2, h	R
I	0.361	0.6	0.002	0.12	0.979
II	0.45	0.6	0.01	1.3	0.962
III	0.89	0.5	0.01	0.11	0.998
IV	0.72	0.04	0.2	40.4	0.651
V	0.058	0.3	0.01	0.42	0.993
VI	0.23	0.1	0.24	18.5	0.927
VII	0.13	0.2	0.024	6.6	0.960
PG-0/NHPA	0.07	0.7	0.001	0.28	0.95
PG-1/NHPA	0.1	1	0.0004	N/A	0.96
PG-1/DR1	1.4	0.1	0.00005	0.005	0.99

[a] Data were fit to eq 3 after the thin films were cooled to room temperature and the poling field removed; the last three polypeptides contained 10 mol % dopant. The parameters represent the second harmonic decay process during the first 2 h of decay.

their maximum SH intensity within several seconds of corona poling. The rapid SH intensity rise in these films indicates that the doped chromophores have a greater degree of rotation freedom than their covalently attached counterparts. Heating the doped films above 65 °C produced a sharp decline in SH intensity similar to that observed with **II** in Figure 4. The SH regained its previous value by cooling through the T_α of the polypeptide. After removing the poling field at room temperature, the instantaneous decline in the SH magnitude to lower levels indicates that a large portion of the signal came from those chromophores with unrestricted motion, in the larger free-volume regions of the polymer matrix.

Second Harmonic Signal Decay. Equation 3 gives the biexponential model[30] that describes the decay in the SH intensity:

$$d_{eff}(t)/d_{eff}(t=0) = \mathbf{A}\exp(-t/\tau_1) + (1-\mathbf{A})\exp(-t/\tau_2) \quad (3)$$

where the decay time constants, τ_1 and τ_2, are a measure of the resistance to change in the SH signal in the slow and fast decay regimes. The first term in eq 3 represents the fast decay component attributed to the initial decay of field-induced SH and relaxation of those molecules that are free of any restricting molecular electrostatic interactions.[31] The **A** coefficient represents the volume fraction of the side chains that are in this fast decay regime.[31] The second term represents a slower relaxation mechanism that becomes dominant later in the SH decay process. Stable materials should have low **A** and high τ_1 and τ_2 values. Table 2 summarizes these constants calculated for the first 2 h of reorientation, and Figure 5 illustrates the long-term decay of the four most stable polypeptides.

Intermolecular side-chain interaction was the predominate factor that influenced the rate of side-chain reorientation and the T_α of the polypeptide. Hydrogen bonding in **IV** resulted in the highest T_α, τ_1, and τ_2 values and in the lowest **A** coefficient of all the chromophore-modified polypeptides. The SH signal remained constant for several hours after the poling field was removed (Figure 3), and more than 60% of d_{eff} was retained over a 6 month period of time (Figure 5). The extent of DR1 modification was comparable in **II**, but the absence of hydrogen bonding resulted in lower decay time constant values and a faster decline of d_{eff} over the 6 month period shown in Figure 5. Hydrogen bonding produced a higher τ_1 time constant value in **III** than in **I**, but the flexible, pentyl leader group effectively increased the overall rate of nitroaniline reorientation into a centrosymmetric configuration. A shorter leader group is expected to increase its rigidity, produce a stronger association between the small, polar chromophores of neighboring polypeptides, increase the T_α, and slow the SH decay rate of these polymers. Figure 6 illustrates the influence of T_α on the τ_1 and τ_2 time constant values. A further increase in the T_α of the polypeptide is expected to produce a constant d_{eff} value over a longer period of time.

The SH signal of **V** decreased faster than most of the other polymers but slower than the two polypeptides just described. The shorter leader group of the nitrobenzyl side chain contributed to the slow decay regime through the higher τ_2 time constant value. Elimination of the ester should produce a higher T_α and more stable SH values, but obtaining complete alignment would be more difficult because of the increased rigidity.

The high τ_2 time constant of the slow decay regime dominated merocyanine reorientation in **VI** and **VII**, retaining the high d_{eff} values over the 6 month period shown in Figure 5. The lower T_α value of **VI** increased the conversion of the nonlinear optically active mero-

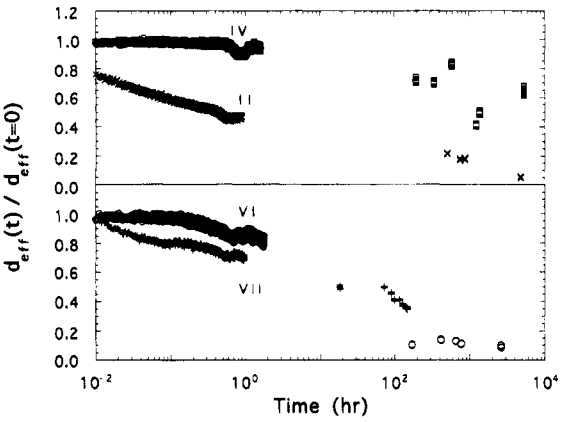

Figure 5. Long term d_{eff} decay of DR1-modified polypeptides, **II** (×) and **IV** (□), top figure; and spiropyran modified polypeptides, **VI** (○) and **VII** (+), bottom figure.

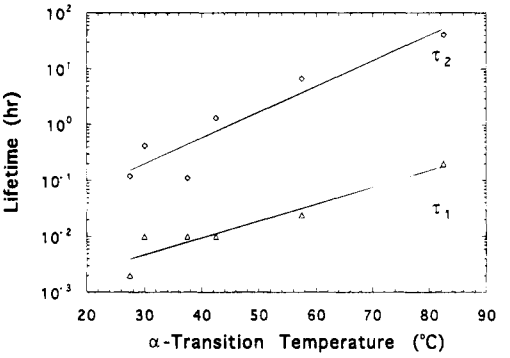

Figure 6. Second harmonic lifetime of the fast (τ_1) and slow (τ_2) decay time constants as a function of the α-transition temperature (°C) of the chromophore-modified polypeptides.

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Table 3. Nonzero Second-Order Tensor Elements

polypeptide	d_{33} (pm/V)	d_{31} (pm/V)	d_{15} (pm/V)	d_{31}/d_{33}	time[b] (h)
IV	1.8	0.8	0.7	0.46	100
V	0.7	0.2[a]	0.2	0.31	1
VI	7	2[a]	2	0.32	2600
VII	9	3[a]	3	0.31	25

[a] Assumed Kleinman conjecture ($d_{31} \approx d_{15}$) to obtain the best calculated fit of d_{33} from the p−p experimental data. [b] Time after poling for the evaluation of the d component by the Maker Fringe technique.

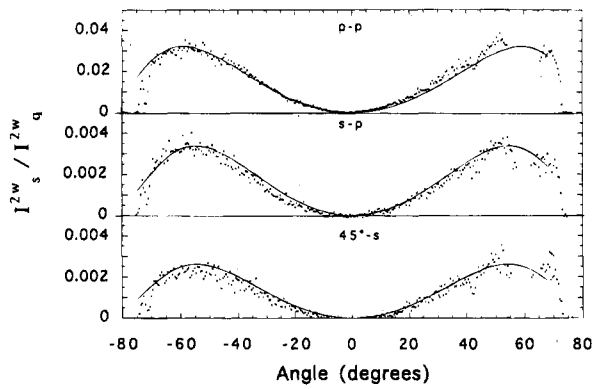

Figure 7. (p−p), (s−p), and (45°-s) polarized incident-detected relative SH intensity for **IV**. The solid curve is the SH power calculated from eq 1 with the corresponding projection factors at polarization configurations (p−p), (s−p), and (ϕ−s).

cyanine form back to the inactive spiropyran and, after several hundred hours, the d_{eff} value fell below that of **II**.

Nonlinear Tensor Components. The extent of chromophore alignment, its β value, and its number density in the material affected the magnitude of the SH signal. The second-order nonzero tensor elements for a material with ∞mm symmetry[32] (d_{33}, $d_{15} = d_{24}$, and $d_{31} = d_{32}$) were calculated using eq 1 and are tabulated in Table 3. Figure 7 illustrates the curve fitting method matching the calculated $I_s^{2\omega}/I_q^{2\omega}$ values with the experimental data for **IV** at the different polarization configurations. The SH wavelength fell within the absorption band of DR1 and produced a d_{15}/d_{31} ratio of 0.88. Kleinman conjecture predicts this value to be unity far from resonance while Hayden et al.[17] reported values ranging from 1.07 to 1.17 for DR1-PMMA films, measured at the SH wavelength of 532 nm. The variance of d_{15}/d_{31} from unity stems from the nonzero off-diagonal β coefficients that are equal to zero in one-dimensional molecules[33] and may be greater than β_{zzz} near resonance.[34]

The d_{15}/d_{33} value is related to the orientation average of the poled chromophore by the first and third Langevin functions[35] such that this ratio equals $^1/_2[L_1(p)/L_3(p) - 1]$. During corona poling p ($=\mu E_{eff}/k_BT$) is large, and the standard assumption used to determine d_{15}/d_{33} is not completely valid. The contributions from the off-diagonal tensor elements and the large poling fields may be responsible for the d_{15}/d_{33} value of 0.39 in **IV**. The one-dimensional molecule thermodynamic model was assumed to apply for **V** because resonance enhancement was negligible and for **VI** and **VII** because merocyanine faded back to the NLO-inactive spiropyran form during prolonged d_{33} measurements. The d_{33} values for the DR1 and merocyanine-modified polypeptides were comparable to those reported in the literature: 7 pm/V for DR1-PMMA[17] and 1 pm/V for merocyanine polyacrylate.[36]

Further investigation into the near-resonance values of the second-order tensor components is needed to understand the orientation effects and the dispersion of the β for multidimensional molecules. Cross-linking the polypeptide chains to enhance the temporal stability and $I_s^{2\omega}/I_q^{2\omega}$ values can aid in this process.

Conclusion

We have demonstrated that poly(L-glutamates) modified with NLO chromophores form high optical quality thin films and exhibit d_{33} values that are comparable to those reported for synthetic polymer systems, making them suitable matrices for further NLO studies. The α-helical secondary conformation of the polypeptide backbone was retained, but the spin-casting process produced amorphous films that did not take advantage of the lyotropic liquid-crystalline phase of these materials. The close packing nature of this phase is expected to increase the alignment stability of oriented NLO-active side chains. The large hydrodynamic frictional forces parallel to the α-helix would be expected to make relaxation of the aligned side chains more difficult in polypeptides than in comparable random-coil type polymers. The polypeptides with the highest α-transition temperatures maintained some degree of second harmonic activity for 6 months. A combination of hydrogen bonding and the large size of the DR1 or spiropyran chromophores enhanced the retention of the SH signal and the T_α values. The off-diagonal β components and near-resonance enhancement of the SH signal for DR1 contributed to d_{eff} values. Future work focusing on SH stabilization through cross-linking and alignment of the liquid-crystalline phase will assist in evaluating polypeptides as materials for electrooptical devices.

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