Photoresponsive Polymers. Photomodulation of the Macromolecular Structure in Poly(L-lysine) Containing Spiropyran Units

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ABSTRACT: Poly(L-lysine) was reacted with spiropyran reagents to give polypeptides containing 46 and 25 mol % spiropyran units in the side chains. The resulting modified polymers were soluble in hexafluoro-2-propanol (HFP), where they exhibited reverse photochromism. In pure HFP, the photochromic polymers were random coils and the structure was not affected by the photoisomerization of the side chains. When appropriate amounts of cosolvents such as triethylamine or methanol were added to the HFP solutions, exposure to visible light (500 nm) or to sunlight for a few seconds produced the coil → α-helix transitions of the macromolecular chains. The back-reaction in the dark was accompanied by the progressive decrease of the α-helix content and recovery of the original disordered conformation. CD measurements showed that the photoisomerization of the photochromic side chains was able to trigger the coil → helix transition of the macromolecules only in a very narrow range of HFP/NEt_3 or HFP/MeOH solvent composition; therefore, spiropyran-modified poly(L-lysine) can be considered a typical example of photoresponsive systems having a "gated photoresponse".

Introduction

Photochromic compounds exhibit reversible variations of their absorption spectra upon exposure to light having appropriate wavelength. The phenomenon is due to the ability of the photochromic molecules to exist in two different states, for instance, two isomeric structures, whose relative concentration depends on the wavelength of the incident light. The occurrence of two different states, which can be reversibly switched from one to another by means of an external light stimulus, can be the basis for molecular switches.

Incorporation of organic photochromic molecules in polymer matrices allow one to combine their intrinsic photochemical properties with polymer processability and stability, thus improving the properties of the photoactive material. Moreover, when photochromic molecules are bound to macromolecular chains, their photoisomerization can induce structural variations of the attached macromolecules. Therefore, photochromic polymers may be highly promising materials for application in optical technology as well as in development of devices which can be photomodulated.[1−3]

As far as structure and conformation is concerned, poly(α-amino acid)s with photochromic side chains are very special polymers: in fact, they can exist in disordered or regularly folded chains such as α-helix or β-structure. Moreover, the photoinduced order−disorder conformational changes of the main chain occur as cooperative transitions, thus amplifying the perturbation due to the photochemical reaction of the chromophores in the side chains.

The most extensively studied photochromic polypeptides are poly(α-amino acid)s such as poly(L-aspartate)s,[4,5] poly(L-glutamate)s,[6−15] poly(L-lysine),[16] and poly(L-ornitine)[17] containing azobenzene units bound to the amino acid side chains; recently, also a photoresponsive azo-modified elastin-like polypeptide has been described.[18] Photochromic polypeptides containing spiropyran units in the side chains have been studied much less than those containing azobenzene. Literature data are limited to poly(spiropyran-L-glutamate),[19−23] which was found to give large coil → helix conformational changes upon exposure to sunlight,[19,20] and preliminary results on poly(spiropyran-L-lysine).[24]

In this paper we report a detailed investigation of photochromic polymers of L-lysine containing spiropyran units in the side chains. The photochromic and conformational behavior is discussed on the basis of spiropyran content and solvent conditions. Differently from the analogous polymers of L-glutamic acid, in fact, the spiropyran-modified polymers of L-lysine did not give photoresponse effects in pure solvent such as hexafluoro-2-propanol (HFP). By contrast, when irradiation was carried out in a HFP/triethylamine or HFP/methanol solvent mixture, the system responded to light, giving reversible coil−helix transitions. The extent of the photoinduced conformational changes was dependent on solvent composition, so the photoresponse could be actually inhibited, amplified, or modulated by combined action of light and environment.

Experimental Section

Materials. Hexafluoro-2-propanol (HFP) was purified by catalytic hydrogenation under 70 atm hydrogen pressure in the presence of 10% palladium on charcoal at 80 °C for 48 h. At the end of the reaction the catalyst was filtered off and the solvent was distilled over calcium oxide.

Poly(L-lysine) was obtained by polymerization of N^{ϵ}-carbobenzoxy-L-lysine *N*-carboxyanhydride, followed by removing the side chain protecting groups with anhydrous HCl and HBr.[25] The polypeptide hydrobromide was dialyzed against 0.01 N HCl to replace the bromide with chloride counterions. Then it was subjected to ultrafiltration through a Spectra/Por Ultra Filter, cutoff 50K, to remove low molecular weight ($\bar{M}$

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< 50000) polymers. Finally, it was lyophilized to give poly(Lys·HCl) as a white material having $[\eta] = 0.87$ (1 M NaCl, pH 3) corresponding to $\bar{M}_V = 300000$.[25]

Preparation of 1-(β-Carboxyethyl)-3′,3′-dimethyl-6-nitrospiro(indoline-2′,2[2*H*-1]benzopyran) (Sp-COOH). 2,3,3-Trimethylindolenine (10.1 mL, 0.06 mol) and 3-iodopropanoic acid (12.6 g, 0.06 mol) were heated under nitrogen at 100 °C for 3 h. The resulting solid material was dissolved in water, and the solution was washed with chloroform. Evaporation of water gave 1-(β-carboxyethyl)-2,3,3-trimethylindolenine iodide (75% yield), which was used without further purification. ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.55 (s, 6 H, 2 CH_3), 2.90 (s, 3 H, NCH_3), 3.0 (t, 2 H, CH_2COO), 4.68 (t, 2 H, NCH_2), 7.61 (m, 2 H, aromatic protons), 7.83 (m, 1 H, aromatic proton), 8.0 (m, 1 H, aromatic proton).

The above iodide (14 g, 0.04 mol), 5-nitrosalicylaldehyde (6.5 g, 0.04 mol), and piperidine (3.8 mL, 0.04 mol) were dissolved in methyl ethyl ketone, and the red solution was refluxed for 3 h. On standing overnight, the product precipitated as a yellow powder. This was filtered and washed with methanol to give 11.4 g of Sp-COOH (78% yield). ^{1}H-NMR (200 MHz, DMF-d_7) δ 1.14, 1.23 (2 s, 6 H, 2 CH_3), 2.66 (m, 2 H, CH_2COO), 3.60 (m, 2 H, CH_2N), 6.10 (d, 1 H, aromatic proton), 6.74–7.04 (m, 3 H, aromatic protons), 7.15–7.33 (m, 3 H, aromatic protons), 8.08–8.26 (m, 2 H, olefinic protons).

Preparation of the Model Compound Sp-CONHBu^n. Sp-COOH (1.05 g, 2.76 mmol) was dissolved in anhydrous dimethylformamide (DMF), and the solution was cooled to −10 °C and added to *N*-methylmorpholine (0.68 mL, 6.2 mmol) and isobutyl chloroformate (0.4 mL, 3.12 mmol). After 15 min, *n*-butylamine (0.38 mL, 3.8 mmol) was added and the reaction mixture allowed to react for 2 h at −10 °C and at room temperature overnight. The solvent was evaporated, and the residue was taken up in ethyl acetate and washed with 10% citric acid, 5% sodium bicarbonate, and water. Evaporation of ethyl acetate gave a solid material which was dissolved in benzene and crystallized from benzene/petroleum ether to give 0.85 g of Sp-CONHBu^n (75% yield)). $R_f = 0.33$ (benzene/ethyl ether = 7/3). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.82 (t, 3 H, butyl CH_3), 1.06 and 1.22 (2 s, 6 H, 2 CH_3), 1.24 (m, 4 H, $-CH_2-CH_2$), 2.41 (m, 2 H, CH_2CO), 3.12 (q, 2 H, CH_2NH), 3.56 (m, 2 H, CH_2N), 5.4 (s, 1 H, NH), 5.56 (d, 1 H, aromatic), 6.58 (d, 1 H, aromatic), 6.5–7.5 (m, 5 H, aromatic), 7.95–8.02 (m, 2 H, olefinic protons).

Modification of Poly(L-lysine). (a) Mixed Anhydride Method. Sp-COOH (2.6 mmol) was reacted with pivaloyl chloride (2.6 mmol) and triethylamine (2.6 mmol) in anhydrous DMF (100 mL) at 0 °C. After 15 min the reaction mixture was added to a precooled solution of poly(Lys·HCl) ($\bar{M}_V$ = 300000) (2.6 mmol of Lys residues) in DMF/water = 10/1 (100 mL); the apparent pH was adjusted to pH 8 with NEt_3, and the solution was stirred at room temperature for 24 h in the dark. The polymer was precipitated in ethanol, and any unreacted reagents were removed by repeated dissolutions and precipitations. The polymer was soluble in HFP and contained 46 mol % spiropyran units, evaluated by comparing the absorption spectrum with that of the *n*-butylamide deriviative SpCONHBu^n (spiro form: λ_{max} = 355 nm; ϵ_{max} = 11200 M^{-1} cm^{-1} in HFP).

(b) Active Ester Method. The spiropyran compound Sp-COOH (3.0 mmol) was reacted with *N*-hydroxysuccinimide (3.0 mmol) in the presence of dicyclohexylcarbodiimide (3.0 mmol) and 4-pyrrolidinopyridine (3.0 mmol) in anhydrous DMF (60 mL) in the dark. After 24 h, the solvent was evaporated, the residue dissolved in ethyl acetate, and the organic phase washed with $NaHCO_3$ saturated solution and water. The active ester was finally crystallized from ethyl acetate/petroleum ether. The active ester (2.6 mmol) was added to poly(Lys·HCl) (2.6 mmol of Lys residues) in DMF/water = 5/1 (100 mL), the apparent pH was adjusted to pH 7–8 with NEt_3, and the solution was stirred for 24 h in the dark. The modified polymer, recovered as described above, was soluble in HFP and contained 25 mol % spiropyran units.

Measurements. Solutions of photochromic compounds were prepared in red light and kept in the dark. For the preparation of spiropyran salts, trifluoroacetic acid (TFA) was added to HFP solutions in the ratio HFP/TFA = 2/1 × 10^{-3}. The resulting solutions contained about 100 equiv of acid with respect to the lysine residues.

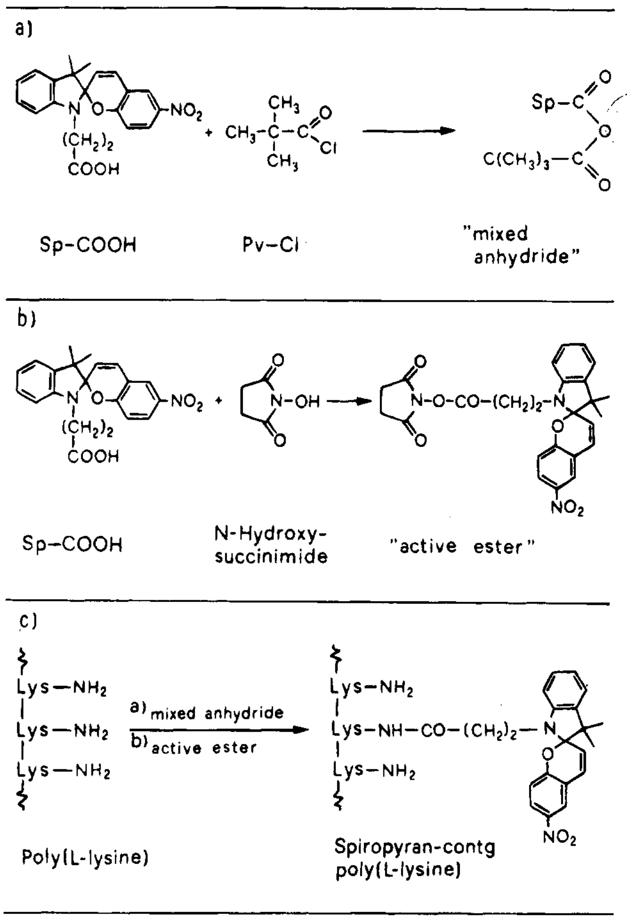


Figure 1. Modification reactions of poly(L-lysine) with spiropyran reagents.

Absorption spectra were recorded on a Jasco Uvidec 510 spectrophotometer and CD spectra with a Jasco J500A spectropolarimeter. CD data are expressed in terms of molar ellipticity [Θ], based on the mean residue molecular weight. Intrinsic viscosities [η] were determined with a Desreux-Bishoff dilution viscometer at 25 °C.

Irradiations of samples were carried out by exposing the sample to sunlight or by irradiating with a 100 W superpressure mercury lamp, employing an interference filter with bandpass 500–550 nm. Irradiation times of about 10 s were enough to produce the complete bleaching of the solutions. No photoisomerization was detected during recording of the absorption and CD spectra.

Results and Discussion

Modification of Poly(L-lysine). Introduction of spiropyran units into the side chains of poly(L-lysine) was performed by the mixed anhydride and the active ester methods. In the former procedure, the spiropyran reagent Sp-COOH was treated with pivaloyl chloride to give the intermediate mixed anhydride (Figure 1, reaction a). In the latter procedure, the spiropyran reagent Sp-COOH was converted to the *N*-hydroxysuccinimide ester (Figure 1, reaction b). The mixed anhydride and the active ester were then reacted with poly(L-lysine hydrochloride) (Figure 1, reaction c). Although anhydrous conditions must be maintained during the preparation of the ester and anhydride intermediates, the condensation reaction with the amino compound can be performed in aqueous media as well.[26] This was

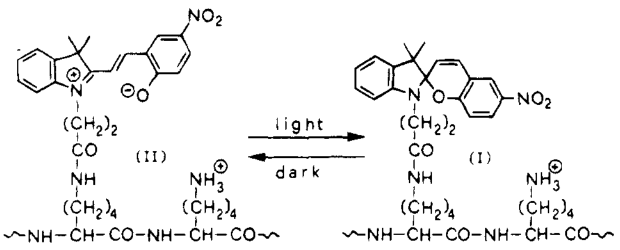
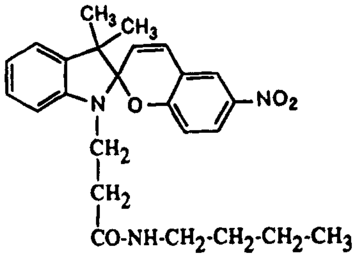


Figure 2. Photochromic behavior of spiropyran-modified poly(L-lysine) in hexafluoro-2-propanol.

actually necessary in this case, since poly(Lys•HCl) is insoluble in organic solvents.

The modification extent of the polymers was determined on the basis of the molar extinction coefficient of the n-butylamide derivative Sp-CONHBu^n, taken as the low molecular weight compound containing the same chromophore. The resulting spiropyran content was 46 and 25 mol % for poly(L-lysine) modified through the mixed anhydride and the active ester procedure, respectively. The determination of polymer composition by comparison of the integrated areas of the proton resonances of the NMR spectra led to different values depending on what set of signals were considered. The average discordance was about ±5% with respect to the values estimated from the absorption spectra.



Sp-CO-NH-Bu^n

Photochromism. All samples were found to be soluble in HFP where they showed reverse photochromic behavior analogous to that already described for spiropyran-modified poly(L-glutamates)[19,20] and illustrated in Figure 2. At room temperature in the dark, they give yellow-orange solutions due to the presence of the merocyanine species **II**. Irradiation with visible light or just exposure to sunlight produces the complete bleaching of the solutions due to formation of the colorless spiro form **I**. The back-reaction occurs in the dark and the original color is reversibly recovered.

Figure 3 shows the effect of light on the absorption spectra in HFP of poly(Lys) containing 25 mol % photochromic units. The colored solution kept in the dark exhibits two intense bands at 485 and 360 nm due to the merocyanine species **II**. Irradiation with incident light at 500–550 nm cancels the intense band in the visible region and produces the spectrum corresponding to the spiro form **I**, having absorption maxima at 355 and 270 nm. On dark adaptation, the original spectrum is progressively restored, the spectra monitored over time passing through an isosbestic point at 295 nm.

The spectra of the present polymers of L-lysine are very similar to the spectra of spiropyran-containing poly(L-glutamate)s already described,[20] even though the maximum in the visible region is observed at 500 nm for poly(L-glutamates) and 485 nm for poly(L-lysine). The blue shift is probably a result of the more polar environment provided by the amine side chains which are likely to be protonated by the acid solvent HFP (see Figure 2).

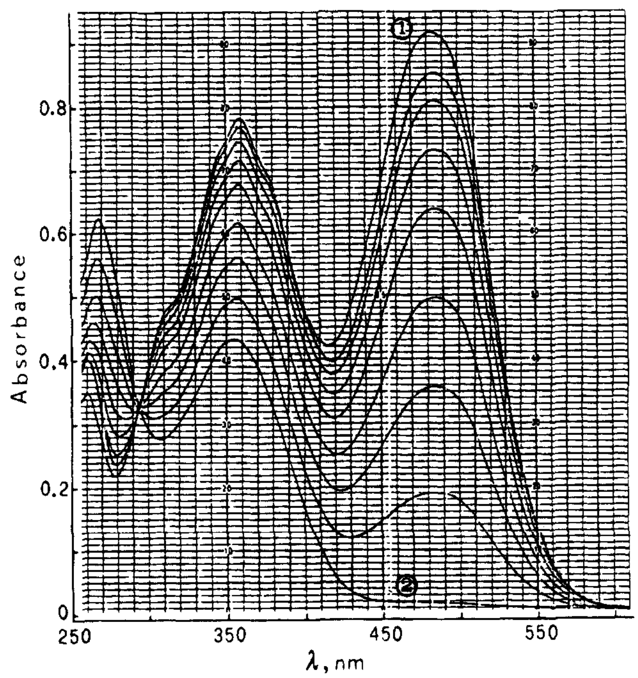


Figure 3. Absorption spectra in HFP of poly(L-lysine) containing 25 mol % spiropyran units: (1) sample kept in the dark; (2) sample exposed to sunlight; intermediate lines are the spectra recorded during decay in the dark at room temperature.

The absorption band at longest wavelength observed in the sample kept in the dark has been attributed to a $\pi-\pi^*$ electronic transition of the extended and conjugated merocyanine chromophore,[27] while the band at 360 nm has been assigned to a charge-transfer transition in the benzopyran ring.[28] The electronic transitions, however, cannot be assigned with confidence, since the relative intensities of the bands and the whole shape of the spectrum strongly depend on temperature and dye concentration. This is likely to be due to aggregation phenomena between the zwitterionic merocyanine species which have a strong propensity to give intra– and intermolecular stacking.[29–31] For the colorless indolinospiropyran species **I**, the absorption band at 355 nm has been assigned to a charge-transfer transition localized in the benzopyran half of the molecule, while the band at 270 nm has been assigned to a $\pi-\pi^*$ transition localized in the indoline half of the molecule.[32] The absorbance at 355 nm of the spiropyran species in the irradiated samples is a linear function of chromophore concentration, so it was used to estimate the spiropyran content in the modified polypeptides (see Experimental Section).

The photochemical reaction is very fast. Indeed, exposure to sunlight for a few seconds is enough to produce the full conversion of the merocyanine to the spiro form. The kinetics of the back-reaction in the dark is much slower. It has been followed by measuring the rate of appearance of the 485-nm band at 25 °C (Figure 3). For the low molecular weight compound Sp-CONHBu^n, the decay follows first-order kinetics with a rate constant of 5.7×10^{-3} min^{-1} and half-life time of 122 min. For the polymers, the kinetics slightly deviates from monoexponential decay. The time necessary to restore half of the original absorbance is about 180 min for poly(L-lysine) containing 46 mol % spiropyran units

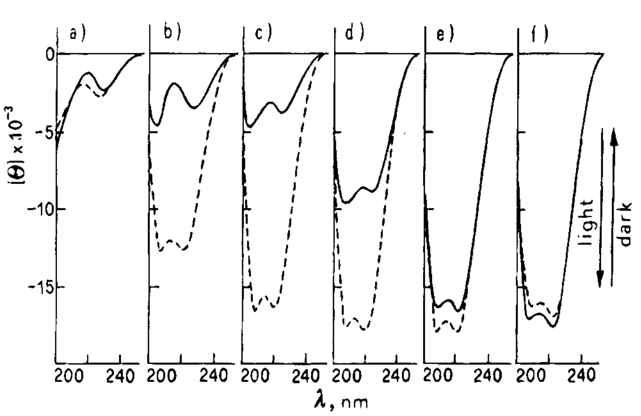


Figure 4. Poly(L-lysine) containing 46 mol % spiropyran units. Effect of irradiation on CD spectra in various HFP/NEt$_3$ solvent mixtures. NEt$_3$: (a) 3%; (b) 6%; (c) 8%; (d) 10%; (e) 13%; (f) 16%. (—) Dark adapted; (- - -) irradiated samples.

and about 150 min for the sample containing 25 mol % photochromic units. The behavior is similar to that observed for the analogous polymers of poly(L-glutamic acid).[20] In both cases, the more complex kinetics in the polymers with respect to the low molecular weight compounds may be a consequence of aggregation and electronic interactions between colored species in the side chains,[29-31] thus making the absorbance change nonlinearly with their concentration.

The photochromic cycles seem to be completely reversible. This can be probably achieved due to the reverse photochromism of the system in HFP. In fact, irradiation with low-energy visible light (reverse photochromism) instead of with high-energy UV light (normal photochromism) will limit severely side photochemical reactions and the consequent fatigue phenomenon.

Photoregulation of Conformation: Effect of Triethylamine on Photoresponse. Spiropyran-modified polymers of L-lysine are essentially random coils in HFP and the conformation is not affected at all by the photoisomerization of the side chains. However, when appropriate amounts of triethylamine are added to the HFP solutions, the system responds to light, giving coil → α-helix conformational changes. Figure 4 shows the effect of light on the CD spectra for poly(Lys) containing 46 mol % spiropyran side chains in various HFP/NEt$_3$ solvent mixtures. When the triethylamine concentration is lower than 3%, both the dark-adapted and the irradiated samples are essentially random coils. At NEt$_3$ concentrations higher than 16%, both samples exhibit the CD pattern of the α-helix, characterized by the two typical bands at 222 and 208 nm. At NEt$_3$ concentrations in the range between 3 and 16%, exposure to light or dark conditions, alternately, produces reversible variations of helix content, the extent of the photoresponse depending on the solvent composition. An analogous behavior is observed for poly(Lys) containing 25 mol % photochromic units (Figure 5). There is no CD contribution of the photochromic side chains, either when they are present as merocyanine (dark samples) or spiropyran form (irradiated samples).

For several polypeptides, all having α-helical conformation, the intensities of the bands were found to show significant variations when the CD spectra were measured in HFP. Assuming for 100% α-helix $[\Theta]_{222} = -28900$, measured for poly(carbobenzoxy-L-lysine) in HFP solution,[33,34] the intensities of the CD bands observed for spiropyran-modified poly(L-lysine) correspond to photoinduced variations of helical structure up to about 60%.

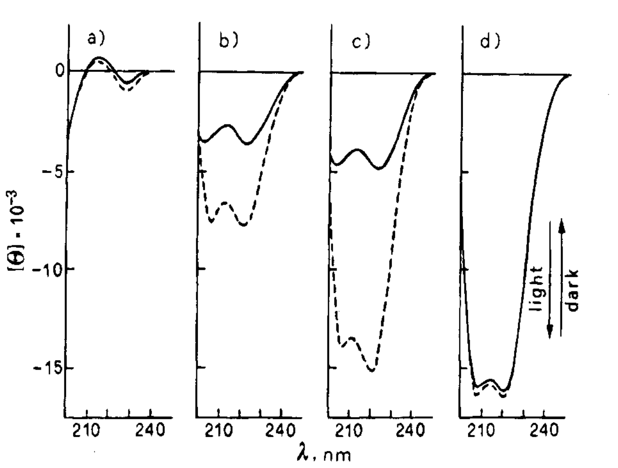


Figure 5. Poly(L-lysine) containing 25 mol % spiropyran units. Effect of irradiation on CD spectra in various HFP/NEt$_3$ solvent mixtures. NEt$_3$: (a) 0%; (b) 3%; (c) 5%; (d) 10%. (—) Dark adapted; (- - -) irradiated samples.

When the intensity of the 222-nm CD band, which is also a measure of the helix content, is plotted as a function of triethylamine concentration, one observes that triethylamine induces the coil → helix transition of the polypeptide chains (Figure 6a). The most remarkable aspect is that the amount of NEt$_3$ needed to induce the transition is different for the dark-adapted sample and the illuminated one; so at solvent compositions in the range between the two curves, exposure to light and dark conditions produces reversible photoinduced conformational changes. Poly(L-lysine) having a lower content of photochromic units (25 mol %) shows a similar behavior, but there is a narrower "window" of NEt$_3$ concentration suitable to obtain the photoresponse effect (Figure 6b).

Addition of triethylamine to the HFP solutions kept in the dark does not produce significant variations of the absorption spectra, thus excluding variations of the isomeric compositions. A possible effect of triethylamine could be due to its basicity. In pure HFP, in fact, the amino groups of the unmodified Lys side chain are likely to be protonated by the solvent acting as an acid ($pK_a = 9.30$)[35] (see Figure 2). Therefore the polypeptide is essentially a polycation and the macromolecules are forced to adopt an extended coil conformation independent of the isomerization of the photochromic units. Triethylamine could remove protons from the unmodified Lys side chains. In these conditions, the polypeptide conformation is controlled by the isomerization of the photochromic groups: when they are in the charged merocyanine form (dark conditions), the macromolecules are random coils; when they are isomerized to the apolar spiro form (light conditions), the macromolecules adopt the α-helix conformation. At NEt$_3$ contents above 16%, the high concentration of a NEt$_3$·HFP saline complex could exert a shielding effect between the merocyanine side chains, thus allowing the polypeptide to retain the α-helix conformation even with charged side chains.

Alternatively, the system might behave as other polypeptides which are random coils in a pure solvent but become helical in a mixed solvent. This was observed for poly[N-(ω-hydroxyalkyl)-L-glutamines] in formic acid/water mixtures[36] and for poly(L-tyrosine) in DMSO/dichloroacetic acid mixtures.[37] These effects were attributed to complex formation between the

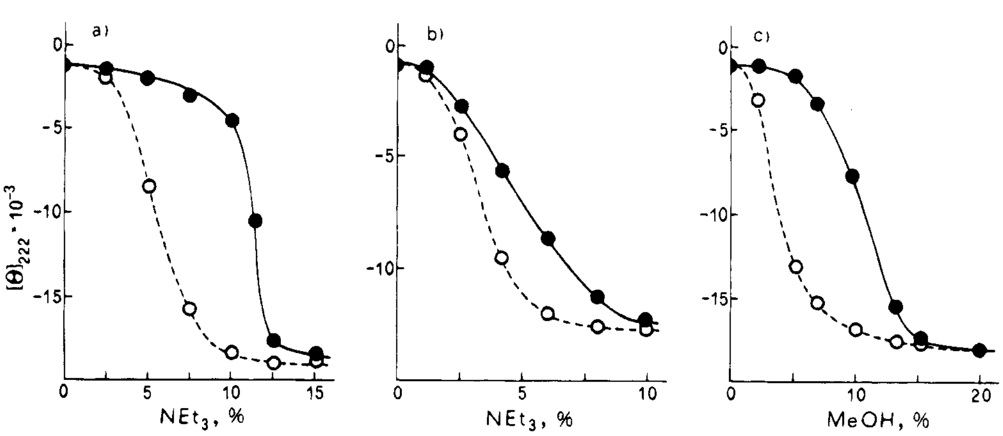


Figure 6. Coil/α-helix transitions for the dark-adapted (—) and the irradiated samples (- - -) observed in (a) 46 mol % spiropyran-modified poly(L-lysine) in HFP/NEt$_3$, (b) 25 mol % spiropyran-modified poly(L-lysine) in HFP/NEt$_3$, and (c) 46 mol % spiropyran-modified poly(L-lysine) in HFP/MeOH.

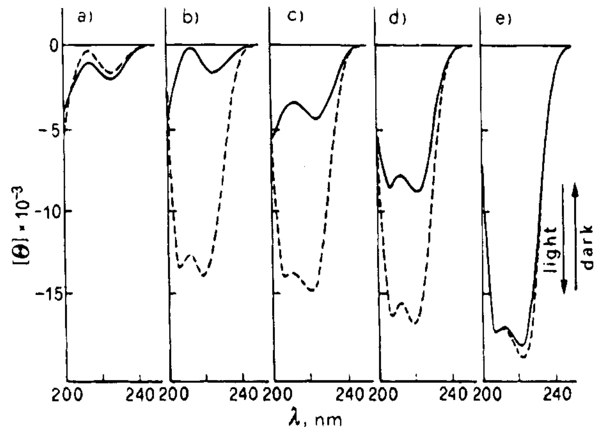


Figure 7. Poly(L-lysine) containing 46 mol % spiropyran units. Effect of irradiation on CD spectra in various HFP/MeOH solvent mixtures. MeOH: (a) 1%; (b) 6%; (c) 8%; (d) 10%; (e) 16%. (—) Dark adapted; (- - -) irradiated samples.

solvent components, which decreased their ability to solvate the polypeptide chain and therefore favored the coil–helix transition. For the present system, mixing of HFP and triethylamine is strongly exothermic, and definite evidence for formation of a HFP·NEt$_3$ saline complex is reported in the literature.[38] However, the concentration of saline complex, and therefore the amount of triethylamine needed to allow the α-helix structure should be different for the dark-adapted samples (NH$_3^+$ groups surrounded by polar merocyanine moieties) and for the irradiated ones (NH$_3^+$ groups surrounded by apolar spiro moieties), thus explaining the occurrence of two separate curves (Figure 6).

Effect of Methanol on Photoresponse. The effect of methanol seems to be analogous to that of triethylamine. In fact, its addition to the HFP solutions induces the coil → α-helix conformational transition of the polypeptide chains, but the transition occurs at different methanol concentrations for the sample kept in the dark and for the irradiated one (Figure 6c). Thus, also for HFP/MeOH solvent mixtures, exposure to light or dark conditions in the range between the two curves gives rise to folding or unfolding of the macromolecular chains. Figure 7 shows the effect of light on the CD spectra for 46% spiropyran-modified poly(L-lysine) at various HFP/MeOH solvent mixtures. As in the case of HFP/NEt$_3$, the extent of the photoresponse depends on solvent composition. Photoinduced variations up to 50% of helix content can be obtained in HFP/MeOH = 94/6 solvent mixture.

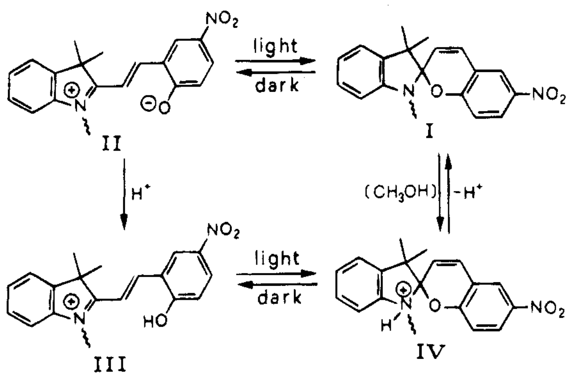


Figure 8. Photochromic reactions of spiropyran compounds in the absence and in the presence of acid.

Considering the polycationic nature of these polymers in HFP (see Figure 2), the effect of methanol might be analogous to that observed for poly(α-amino acid)s with charged side chains such as poly(L-lysine hydrochloride)[39] and poly(sodium L-glutamate),[40] which are random coils in water but become helical upon addition of methanol. The role of methanol in solvent-induced transitions of polyelectrolytes is correlated with its ability to favor "contact ion pairs" between the counterions and the polymer charges, thus stabilizing helical conformations.[40,41] For spiropyran-modified poly(L-lysine), the more remarkable aspect is the splitting of the methanol-induced transition which occurs at different methanol concentrations depending on whether the side chains are present as merocyanine species (dark conditions) or spiropyran species (light conditions).

Photochromic and Conformational Behavior in Acid Conditions. When spiropyrans are treated with acids, they are converted into spiropyran salts which exhibit the photochromic behavior illustrated in Figure 8. The acid protonates the phenoxy group of the merocyanine **II** and gives rise to the O-protonated species **III**. This open form is converted by light to the N protonated spiro structure **IV**. Since spiropyrans are fairly strong bases in the open form but are very weak bases in the closed spiro form, the charged species **IV** can lose a proton and the neutral structure **I** is actually formed.[42–44]

We have recently described large photoinduced conformational changes for poly(spiropyran-L-glutamate) in the presence of trifluoroacetic acid (TFA),[20] so we have performed the same experiment for poly(spiropyran-L-lysine). The photochromic polypeptide containing 46 mol % spiropyran units in the side chains was dissolved

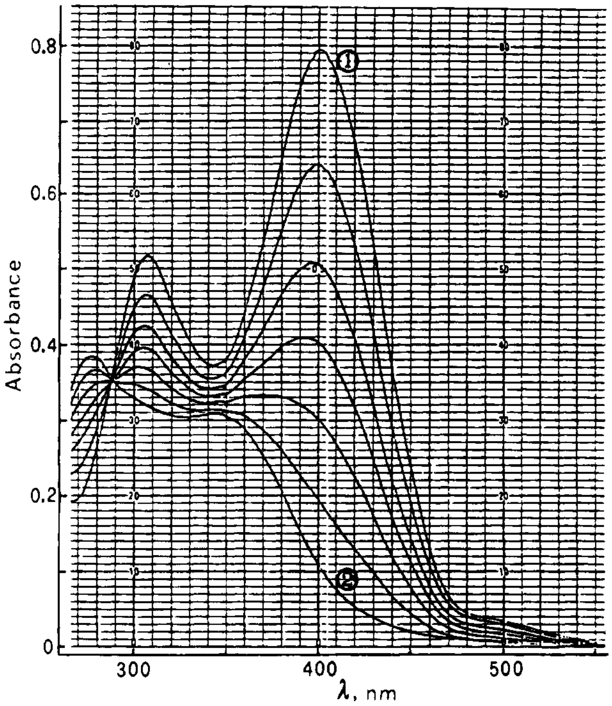


Figure 9. Absorption spectra of poly(L-lysine) containing 46 mol % spiropyran units in HFP in the presence of trifluoroacetic acid ($c = 5 \times 10^{-4}$ g/L): (1) sample kept in the dark; (2) irradiated in the presence of 10% MeOH; intermediate lines are the spectra recorded during decay in the dark at room temperature.

in HFP and added of a small amount of TFA. The concentration of the acid ($c = 5 \times 10^{-4}$ g/L) was in excess with respect to the molar content of photochromic groups, but absolutely negligible as far as denaturation of the polypeptide is concerned.

Protonation of the merocyanine was immediately revealed by the color change of the solution from orange to bright yellow and by the drastic variations of the absorption spectra (Figure 9), as observed for spiropyran-containing poly(L-glutamic acid). Moreover, for the present polymer of L-lysine even the unmodified amino side chains are converted into trifluoroacetate salts, so in acid conditions the polymer is really a polyelectrolyte.

The conformational behavior in the presence of TFA is shown in Figure 10. In HFP (Figure 10a), both the dark-adapted and the irradiated samples show the typical CD spectra of random coil polypeptides. Addition of methanol affects the two samples in a different way. In HFP/MeOH = 90/10 (Figure 10b), exposure to light produces the full conversion to the α-helix, while the sample kept in the dark becomes only partially helical. On increasing methanol concentration (Figure 10c,d), also the sample kept in the dark becomes fully spiralized.

The conformational behavior of spiropyran-modified poly(L-lysine trifluoroacetate) may be a confirmation that the role of methanol is analogous to that proposed for the above-mentioned poly(L-lysine hydrochloride), which is random coil in water but becomes helical upon addition of methanol.[39] Again, methanol affects the conformation in a different way, depending on whether the samples are kept in the dark (photochromic units present as polar protonated merocyanine species) or exposed to light (photochromic units present as apolar spiropyran species).

Relationship between α-Helix and Side Chain Isomeric Composition. The largest photoinduced conformational variations were obtained for poly(L-lysine) containing 46 mol % spiropyran units in HFP/NEt$_3$ = 93/7 and HFP/MeOH = 94/6 solvent mixtures. Samples of this photochromic polymer were irradiated in the above solvent conditions and then dark-adapted, and the decay in the dark was followed by monitoring over time both the rate of the helix-to-coil back-conversion and the rate of appearance of the longest wavelength absorption band. The α-helix content was then plotted as a function of time (Figure 11) and of spiropyran/merocyanine isomeric composition (Figure 12).

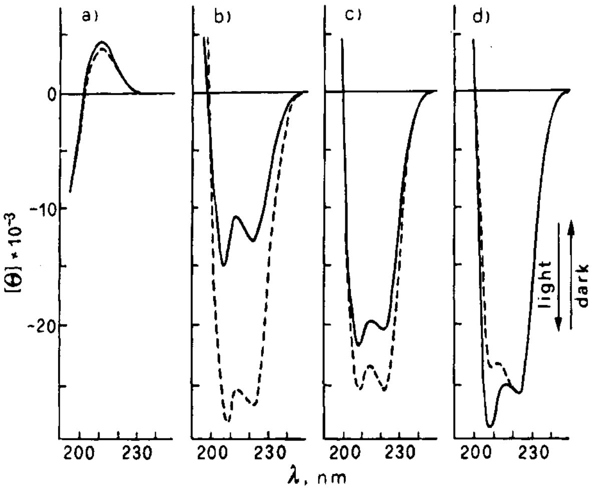


Figure 10. Effect of irradiation on CD spectra for poly(L-lysine) containing 46 mol % spiropyran units in various HFP/MeOH/TFA solvent mixtures. TFA = 1×10^{-3} mL in 2 mL of mixed solvent. MeOH (v/v): (a) 0%; (b) 10%; (c) 20%; (d) 40%. (—) Dark adapted; (- - -) irradiated samples.

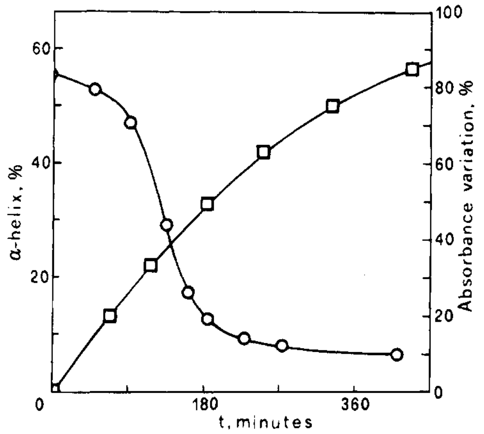


Figure 11. Poly(L-lysine) containing 46 mol % spiropyran units in HFP/NEt$_3$ = 93/7. α-Helix content (○) and maximum absorbance at 485 nm (□) as a function of time during decay in the dark.

It is worth comparing the variation of the absorbance with the variation of the helix content as a function of time (Figure 11). In fact, the absorbance increases asymptotically, even though the curve slightly deviates from a monoexponential function. By contrast, the helix−coil variation is described by a sigmoidal curve, indicating that the helix−coil variation takes place as a cooperative transition.

The variation of the α-helix as a function of the side chain isomeric composition is rather similar but not exactly the same in HFP/NEt$_3$ and in HFP/MeOH (Figure 12). In both cases, the helical structure is essentially destroyed when about 20% of the spiropyran units are converted to merocyanine species. However,

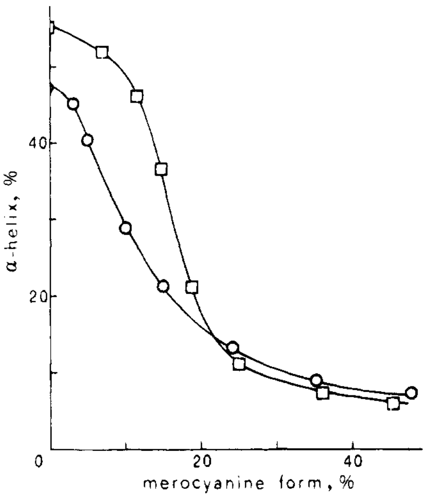


Figure 12. Poly(L-lysine) containing 46 mol % spiropyran units. α-Helix variation as a function of spiropyran/merocyanine isomeric composition of the side chains in HFP/NEt$_3$ = 93/7 (○) and in HFP/MeOH = 94/6 (□).

Table 1. Viscosity Values before and after Exposure to Sunlight for Polypeptides Containing Spiropyran Units

		η_{sp}/c	
polypeptide	spiropyran content, %	dark	irrad
poly(Glu)[a]	41	3.20	0.93
poly(Glu)[a]	85	3.63	1.40
poly(Lys)[b]	46	5.71	3.14
poly(Lys)[b]	25	5.40	3.10

[a] In HFP at 25 °C. [b] In HFP in the presence of 6% NEt$_3$ at 25 °C.

in the presence of triethylamine, the helical structure breaks up rapidly as soon as merocyanine species begin to be formed; in the presence of methanol, the curve is characterized by a more sigmoidal shape and the helical structure does not collapse until about 10% of the spiro groups are isomerized to the merocyanine form.

Photoinduced Variations of Viscosity. The photoinduced conformational variations of the macromolecules were found to be accompanied by large variations of viscosity of the solutions. Table 1 shows the viscosity values for various polypeptides containing spiropyran units before and after exposure to light. As already reported for the analogous polymers of L-glutamic acid, the solutions kept in the dark display high values of viscosity which strongly decrease upon exposure to light. The higher viscosity of the random coil with respect to the helical polypeptides is likely to be a consequence of their polyelectrolyte nature. In any case, no linear relation seems to exist between viscosity and conformation in poly(α-amino acid)s with charged side chains.[45] For poly(L-lysine hydrochloride), the viscosity corresponding to the α-helix was found to be higher or lower with respect to the random coil depending on temperature and solvent composition.[46]

Analogous photoinduced viscosity effects have been described by Irie et al. for poly(methacrylate)s and for poly(methacrylic acid) containing spiropyran units in the side chains.[47-49] Reversible variations of viscosity up to 50% have been achieved upon alternate irradiation with visible and ultraviolet light. The photoinduced variations of viscosity we have observed in spiropyran-modified polypeptides are much larger (about 250–300%) than those reported in the literature.[47-49] This may be due to the higher dye content in the side chains or may be a result of the polypeptide nature of the present polymers, which undergo conformational changes as cooperative transitions.

Conclusions

In pure HFP, polymers of L-lysine containing spiropyran units in the side chains adopt a random coil structure which is not affected by the photoisomerization of the side chains. Addition of cosolvents such as triethylamine or methanol to the HFP solutions induces the coil → α-helix transition; however, the amount of triethylamine or methanol needed to induce the transition is different for samples kept in the dark and irradiated ones. Therefore, at solvent compositions in the range between the two transition curves, exposure to light causes the α-helix formation of the polypeptide chains.

A remarkable aspect of some photochromic compounds is the so-called "gated photoresponse", which means that the system is able to respond to light only when an external stimulus is applied.[50,51] Spiropyran-modified poly(L-lysine) provides an example of a photochromic system characterized by such a gated response. In fact, the photoinduced structural changes were found to be activated or inhibited by acid/base conditions, and the photoisomerization of the photochromic units was able to trigger the coil → α-helix transition of the macromolecules only in a very narrow range of solvent composition.

Examination of the mechanisms and the driving forces responsible for the photostimulated conformational changes allows one to conclude that poly(α-amino acid)s bearing photochromic units are quite special photoresponsive materials in which the primary photochemical event occurring in the side chains is amplified and transduced by the structural variations of the macromolecular main chains. This suggests their potential application in devices that can be photomodulated. More specifically, the photoinduced structural variations are accompanied by large and reversible variations of optical activity (CD bands and optical rotatory power). Therefore spiropyran-modified poly(α-amino acid)s can be proposed as "chiroptical molecular switches" in optical data storage,[51,52] in which a record written by irradiation may be read by monitoring the optical rotation.

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