Photoresponsive Polypeptides. Photomodulation of the Macromolecular Structure in Poly(N^{ϵ}((phenylazophenyl)sulfonyl)-L-lysine)[§]

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ABSTRACT: Poly(L-lysine) was reacted with *p*-phenylazobenzenesulfonyl chloride to give polypeptides containing azobenzene units linked to the Lys chains by means of sulfonamide functions. The azo-modified polymers were soluble in hexafluoro-2-propanol (HFP), in which they exhibited photochromism due to the trans/cis photoisomerization of the azobenzene units. The sulfonate azobenzene moieties were found to be thermally stable at room temperature, so that interconversions between the two isomers were achieved only photochemically, by irradiating at 340 and 417 nm, respectively. Previously reported poly(N^{ϵ}-(phenylazobenzoyl)-L-lysine) was found to adopt the α-helix structure in HFP and did not give photoinduced conformational changes. For poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine), by contrast, CD spectra showed that the macromolecules adopted a disordered structure in HFP, when azo units were either in trans or in cis configuration. However, when appropriate amounts of methanol or 1,2-dichloroethane were added to the HFP solutions, irradiation at 340 and 417 nm, alternately, produced reversible formation of a helical and random coil structure of the macromolecules. The photostimulated structural changes have been discussed on the basis of the interactions between HFP and azobenzenesulfonyl groups, which seem to be different depending on whether the azo units are in trans or in cis configuration.

Introduction

Photochromic compounds are able to exist in two different states, such as two isomeric structures, whose relative concentration depends on the wavelength of the incident light. For instance, in azobenzene derivatives the photochromism involves the photoisomerization between the trans and cis isomers;[1] in spiropyran compounds, it is due to the interconversion between the spiro form and the merocyanine form.[2] The occurrence of two different structures that can be reversibly interconverted by means of a light stimulus can be the basis for a so-called molecular switch. Moreover, since the two photoisomers are characterized by different geometries and different polarities, their interconversion can affect the structure of attached macromolecules and, in turn, the physical properties of polymeric materials.

In 1967 Lovrien reported the earliest observation of a light-induced conformational change in poly(methacrylate)s having azobenzene pendant groups; the photoisomerization of the azo units produced conformational variations of the macromolecules which were revealed by variations of the viscosity of the solutions.[3] After that pioneering work, a great deal of photoresponse effects, including photocontrol of polymer viscosity and solubility, photostimulated sol−gel transitions, photoresponsive membranes, and photomechanical effects, have been observed.[4,5] A rapidly expanding area concerns the application of photochromic polymers in optical data storage,[6−8] and photochromic liquid crystals.[9]

From the point of view of macromolecular structure, polypeptides are quite special polymers because they can exist in disordered or regularly folded structures typical of those existing in proteins, such as α-helix and *β*-structures. When polypeptides contain photochromic molecules attached to the side chains, their photoreactions can induce order−disorder structural changes of the macromolecular main chains, thus amplifying the primary photochemical event.[10,11] Moreover, since the various polypeptide structures are characterized by very different optical rotatory power values and circular dichroism (CD) spectra, the photoinduced structural changes are accompanied by large and reversible changes of chiroptical properties. For these reasons photochromic polypeptides seem to be good candidates as "chiroptical switches",[12] and suitable materials performing the functions needed to build devices that can be photomodulated.

Several photochromic polypeptides have been described in the literature. Most of them have been obtained by introducing azobenzene units into the side chains of various poly(α-amino acid)s such as poly(L-phenylalanine),[13] poly(L-aspartate)s,[14−16] poly(L-glutamate)s,[17−29] poly(L-lysine),[30,31] poly(L-ornithine),[32,33] and other poly(L-lysine) analogs.[34,35] A photoresponsive azo-modified elastin-like polypeptide,[36] a cyclic peptide including an azobenzene moiety able to switch the peptide conformation,[37] and an azobenzene-containing amphiphilic sequential polypeptide[38] have also been recently described. Other photochromic polypeptides have been obtained by introducing spiropyran units into the side chains of poly(L-glutamic acid),[39−43] poly(L-lysine),[44,45] and poly(N^{ϵ}-succinyl-L-lysine).[46]

As far as azo-modified poly(L-lysine) is concerned, the polymers previously reported[30,31] contained azobenzene units linked to the Lys side chains by means of an amide function (Chart 1, structure **I**). In this paper we describe azo-modified poly(L-lysine) in which the azobenzene units are linked to the Lys side chains by means of a sulfonamide function (Chart 1, structure **II**). The

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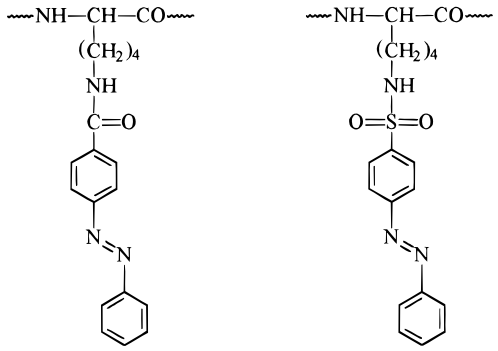
Chart 1



Structure I **Structure II**

new photochromic L-lysine polymers exhibit a photochromic and conformational behavior completely different from that shown by the polymers previously reported. These last polymers, in fact, were soluble in haxafluoro-2-propanol (HFP) where they adopted the α-helix structure, independently of the trans or cis configuration of the azobenzene units; so, no reversible photoinduced conformational changes were observed.[47] Differently from polymers having structure **I**, azo-modified poly(L-lysine) having structure **II** adopts a random coil conformation in pure HFP, not affected by the photoisomerization of the side chains. However, when appropriate amounts of cosolvents such as methanol or 1,2-dichloroethane are added to the HFP solution, the system responds to light, giving rise to reversible photoinduced random coil/α-helix conformational transitions, the extent of the photoresponse depending on solvent composition.

Experimental Section

Materials. Poly(L-lysine hydrobromide) was prepared as already described.[48] It showed a viscosity value $\eta_{sp}/c = 0.867$ ($c = 1$ g/dL) measured in 1 M NaCl, pH 3, 25 °C, corresponding to an average molecular weight, evaluated on the basis of the equation of Yaron and Berger,[49] $\bar{M}_v = 200\,000$. For the polymer modification, the polypeptide hydrobromide was dialyzed against 0.01 N HCl to replace the bromide with chloride counterions.

Commercial hexafluoro-2-propanol (HFP) was purified by catalytic hydrogenation, subjecting the solvent to 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.

The commercial azo reagent *p*-phenylazobenzenesulfonyl chloride was recrystallized from petroleum ether.

***N*-*n*-Butyl-*p*-phenylazobenzenesulfonamide (III).** *p*-Phenylazobenzenesulfonyl chloride (0.84 g, 3 mmol) dissolved in anhydrous dioxane (30 mL) was added to a solution containing *n*-butylamine (0.73 g, 10 mmol) in dioxane (30 mL), and the reaction mixture was stirred overnight at room temperature. The solvent was then evaporated, the residue was dissolved in ethyl acetate, and the organic phase was washed with saturated $NaHCO_3$, diluted HCl, and water. Finally, ethyl acetate was evaporated, and the product was crystallized from ethanol/water to give 0.84 g of the pure derivative **III** (87% yield). ^{1}H-NMR (200 MHz, $CDCl_3$), δ: 0.85 (t, 3H, CH_3), 1.2−1.5 (m, 4H, CH_2CH_2), 3.0 (m, 2H, CH_2N), 4.7 (m, 1H, NH), 7.5−8.0 ppm (m, 9H, aromatic). Absorption spectrum ($c = 0.032$ g/L, in HFP): $\lambda_{max} = 320$ nm, $\epsilon_{max} = 24\,500$ M^{-1} cm^{-1}.

***N*$^\epsilon$-((*p*-Phenylazophenyl)sulfonyl)-L-lysine (IV).** Basic $CuCO_3$ (5.5 g, 25 mmol) was added gradually to a boiling solution of L-lysine hydrochloride (9 g, 55 mmol) in 100 mL of water. After cooling, the mixture was filtered and to the blue solution of the copper complex of L-lysine was added dioxane (100 mL), MgO (4.43 g), and *p*-phenylazobenzenesulfonyl chloride (7.23 g, 26 mmol). After stirring for about 4 h, the reaction gave rise to a dark thick suspension which was treated with 200 mL of acidified boiling water (pH 2). The treatment produced the separation of a dark precipitate, leaving a red-orange solution which was filtered off. The precipitate was further washed with boiling water; all fractions were collected and kept overnight at 4 °C. The product precipitated as a red-orange crystalline material which was recrystallized from boiling water and finally dried to give 2.12 g (5 mmol) of *N*$^\epsilon$-((*p*-phenylazophenyl)sulfonyl)-L-lysine. ^{1}H-NMR (200 MHz, CF_3COOD), δ: 1.2−1.6 (m, 6H, $CH_2CH_2CH_2$), 3.1 (m, 2H, NCH_2), 4.7 (m, 1H, $C^\alpha H$), 7.5−8.5 ppm (m, 9H, aromatic). Absorption spectrum ($c = 0.0152$ g/L, in HFP): $\lambda_{max} = 320$ nm, $\epsilon_{max} = 23\,500$ M^{-1} cm^{-1}.

Modification of Poly(-lysine). Poly(Lys·HCl) (0.35 g, 2.13 mmol of Lys residues) was dissolved in a small amount of water (15 mL), and the solution was diluted with dimethylformamide (DMF) (80 mL). The apparent pH was adjusted to about pH 8 by adding triethylamine; then a second solution containing *p*-phenylazobenzenesulfonyl chloride (1.20 g, 4.5 mmol) in DMF (50 mL) was slowly added. The reaction mixture was kept under stirring at room temperature in the dark for 1 week, during which triethylamine was occasionally added in order to keep the apparent pH at a slightly alkaline value. At the end of the reaction, the polymer was recovered by precipitation with ether. Any unreacted azo reagents and water-soluble materials were removed by repeated dissolutions in DMF and precipitations with ethanol and water, alternately. Finally, the polymer was dried to give 0.45 g of poly(*N*$^\epsilon$-((*p*-phenylazophenyl)sulfonyl)-L-lysine) as a yellow material. Sulfur elemental analysis: calculated for 100% Lys modification, 8.6%; found, 8.7%. ^{1}H-NMR (300 MHz, CF_3COOD), δ: 1.7 (m, 2H, $C^\alpha CH_2$), 2.2 (m, 4H, CH_2CH_2), 3.2 (m, 2H, NCH_2), 4.4 (m, 1H, $C^\alpha H$), 7.4−8.8 ppm (m, 9H, aromatic). UV absorption spectrum ($c = 0.015\,22$ g/L, in HFP): $\lambda_{max} = 320$ nm; $\epsilon_{max} = 24\,000$ M^{-1} cm^{-1}.

All methods indicated a modification extent of about 100%. In another experiment, starting from poly(Lys·HCl) (0.5 g, 3 mmol of Lys residues) and *p*-phenylazobenzenesulfonyl chloride (0.85 g, 3 mmol), carried out with the same procedure, poly(L-lysine) containing 46 mol % azobenzene units was obtained.

HPLC Separation of the Cis and Trans Isomers. Compound **III** was dissolved in 20 mM Tris−Cl buffer pH 8 at a concentration of 3.3×10^{-4} M and kept in the dark at 25 °C. A 3.6 mL aliquot of this solution was irradiated at 340 nm for 15 min and injected onto a 4.5 × 250 mm Spherisorb ODS2 C_{18} column equilibrated in 0.1% trifluoroacetic acid (TFA) in water (v/v) (solvent A). Elution was carried out with 0.1% TFA in acetonitrile (v/v) (20%−70%, v/v) for 25 min in solvent A. The flow rate was 1 mL min^{-1}, and fractions of 0.5 mL were collected. The chromatogram was recorded at 254 nm. Two peaks were eluted at retention times of 22.1 min (peak 1) and 28.1 min (peak 2), respectively. After evaporation of the solvent, the spectra of the pooled fractions containing the two separate peaks were recorded, and peak 1 was identified as the cis isomer, the peak 2 as the trans isomer. The values of the molar extinction coefficient was $\epsilon_{320} = 24\,500$ for the trans isomer and $\epsilon_{270} = 7900$ M^{-1} cm^{-1} for the cis isomer, respectively.

Measurements. Solutions of photochromic compounds were prepared in red light and kept in the dark. The concentration used for spectroscopic measurements were in the range 1×10^{-2}−6×10^{-2} g/L. Molar concentrations of polymer solutions are expressed on the basis of the monomeric unit molecular weight.

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, based on the mean residue molecular weight.

Irradiations of the samples were carried out with a halogen lamp (150 W) filtered with narrow band interference filters from Balzer. Irradiation times of about 5 min were enough to achieve the photostationary state. No photoisomerization

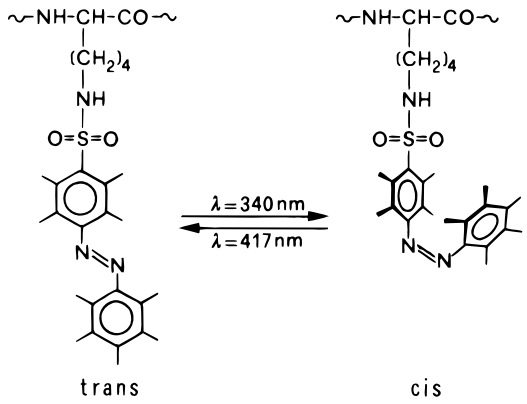
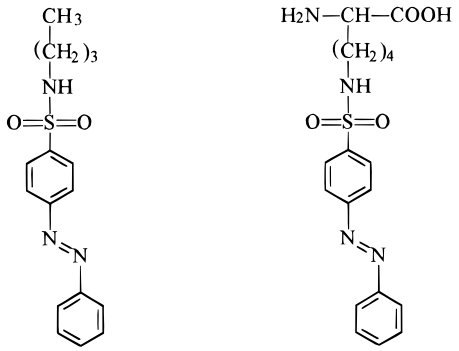


Figure 1. Structure and photochromic reactions of poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine).

Chart 2



III **IV**

was detected during recording of the absorption and CD spectra.

Results

Synthesis and Photochromic Behavior. Azobenzene units were linked to the side chains of poly(L-lysine) ($\bar{M}_v$ = 200 000) by reacting the poly(α-amino acid) with *p*-phenylazobenzenesulfonyl chloride (see Experimental Section). The modification extent was determined by ^{1}H-NMR, by sulfur elemental analysis, and by comparing the absorbance of the polymers with the molar extinction coefficient of the low molecular weight compounds *N*-*n*-butyl-*p*-phenylazobenzenesulfonamide (**III**) and N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine (**IV**). In an experiment the side chains of poly(L-lysine) were quantitatively modified and the azo lysine residues **II** were found to be substantially 100%. In another experiment, a sample containing 46 mol % azobenzene units was obtained. Both the samples were found to be soluble in hexafluoro-2-propanol (HFP).

In the above fluorinated solvent, the azo-modified polypeptides exhibit an intense photochromism associated with the trans ⇌ cis photoisomerization of the azobenzene units (Figure 1). As with other sulfonated azobenzene compounds,[50] azobenzenesulfonyl-modified polymers of L-lysine, as well as the low molecular weight model compounds **III** and **IV**, are very stable in the cis form, and no thermal decay is observed at room temperature during periods of times as long as several weeks. Interconversion between the two forms at room temperature can be only obtained by irradiation at appropriate wavelengths. This behavior prompted us to purify and to isolate by chromatography the trans form and the cis form of the model compound **III**, in order to measure the absorption spectra of the two pure photoisomers.

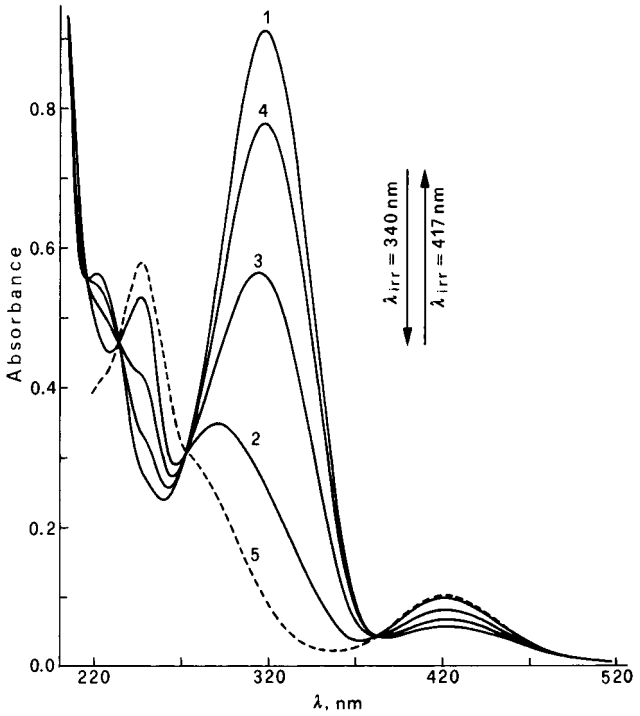


Figure 2. Absorption spectra in HFP of poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) and of the low molecular weight compound **III**. (**1**) Dark-adapted polymer sample (l = 1 cm, c = 6.4 × 10^{-5} mol/L); the spectrum is completely similar to that of the pure-trans isomer of **III** having the same molar concentration. (**2**) Photostationary state upon irradiation at 340 nm. (**3**) Intermediate spectrum before reaching the photostationary state. (**4**) Photostationary state upon irradiation at 417 nm. (**5**) Pure-cis isomer having the same molar concentration as the polymer solution.

The absorption spectra in HFP are reported in Figure 2. The pure trans form of the model compound **III** shows two main bands at 220 and 320 nm, respectively, which can be assigned to $\pi-\pi^*$ electronic transitions, and a weak band at 420 nm which corresponds to the n$-\pi^*$ electronic transition of the azo chromophore. In the pure cis form, the shortest-wavelength peak is shifted to 250 nm, the main band at 320 nm practically disappears and is seen as a shoulder at about 270 nm, whereas the n$-\pi^*$ band becomes more intense. The absorption spectra of the polymers are similar to those of the low molecular weight model compounds. Of course, the specific pattern of the spectra depends on the relative composition of the two geometric isomers which, in turn, depends on the wavelength of the incident light.

The trans/cis isomeric composition at the photostationary state can be easily determined on the basis of eq 1, where A_{st} is the absorbance of the sample at the

$$A_{st} = A_{trans}f_{trans} + A_{cis}f_{cis} \quad (1)$$

photostationary state, A_{trans} and A_{cis} are the absorbances of the pure-trans and the pure-cis isomers, respectively, and f_{trans} and f_{cis} are their molar fractions, so that

$$f_{trans} + f_{cis} = 1 \quad (2)$$

On the basis of eqs 1 and 2, the percentages of the trans and the cis isomers will be given by the expressions

$$\text{trans isomer, \%} = \frac{A_{st} - A_{cis}}{A_{trans} - A_{cis}} \times 100$$

$$\text{cis isomer, \%} = (1 - f_{\text{trans}}) \times 100$$

In HFP, irradiation at 340 nm gives rise to the maximum trans-to-cis photoconversion, the isomeric composition at the photostationary state containing 82% of the cis isomer. Irradiation at 417 nm produces the maximum yield for the opposite cis-to-trans back-reaction, the isomeric composition at the photostationary state containing 85% of the trans isomer. The "photochromic cycles" obtained by irradiating at 340 and 417 nm, alternately, are completely reversible, thus indicating the absence of side photochemical reactions. This is confirmed by the observation of four well-defined isosbestic points at 215, 235, 270, and 385 nm, respectively (Figure 2). The photoconversion extent also depends on the solvent: in a HFP/1,2-dichloroethane = 10/90 solvent mixture, irradiation at 340 nm produces markedly higher photoconversions and gives rise to the cis isomer in 95% yield.

It is worth pointing out that the cis isomer has a relatively small absorption in the region of the main absorption band of the trans isomer. For this reason the isomeric composition is commonly estimated by assuming that the contribution of the cis isomer, A_{cis}, to the optical density may be neglected. Estimation of isomeric composition on the basis of the above assumption leads to slightly higher values (of about 7−8%) of the photoconversion extent.

Photoregulation of Conformation. Poly(L-lysine) containing azosulfonyl groups in the side chains is a random coil in pure HFP, and the disordered conformation is not affected by the trans ⇌ cis photoisomerization of the azo side chains. However, when appropriate amounts of cosolvents, such as methanol or 1,2-dichloroethane (DCE), are added to the HFP solution, the system is able to respond to light, giving rise to reversible variations of the polypeptide conformation. Figure 3 shows the CD spectra of 100% azo-modified poly(L-lysine) in HFP/MeOH, at various solvent compositions. In pure HFP, the CD spectra are typical of random coil polypeptides either when the sample is irradiated at 340 nm (trans → cis isomerization) or when it is irradiated at 417 nm (cis → trans isomerization). At methanol concentrations higher than 20%, both samples exhibit the typical CD pattern of the α-helix, characterized by the two negative bands at 222 and 208 nm. The intensity of the 222 nm CD signal corresponds to the value measured in HFP solution for poly(N^{ϵ}-carbobenzoxy-L-lysine), ($[\Theta]_{222} = -28\,900$), that can be assumed as corresponding to 100% α-helix.[51] At methanol concentrations in the range between 2 and 15%, illumination at 340 and 417 nm, alternately, produces photoinduced variations of the helical content, the extent of the photoresponse depending on the solvent composition. Photoinduced variations of helical structure up to about 80% are observed at 8−10% methanol concentrations (Figure 3).

When the intensity of the CD band at 222 nm, which is also a parameter of the α-helix content, is plotted as a function of methanol concentration, one observes that addition of methanol induces a coil → α-helix transition of the macromolecular chains (Figure 4). However, the amount of methanol needed to induce the conformational transition is different for the sample irradiated

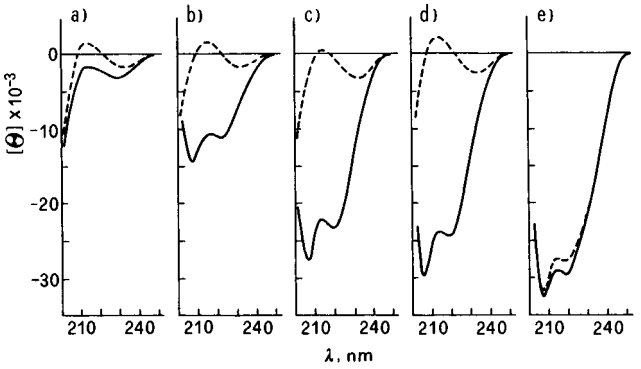


Figure 3. CD spectra of poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) in various HFP/MeOH solvent mixtures: (—) kept in the dark or irradiated at 417 nm; (- - -) irradiated at 340 nm. MeOH (v/v): (a) 0%; (b) 2%; (c) 5%; (d) 8%; (e) 15%.

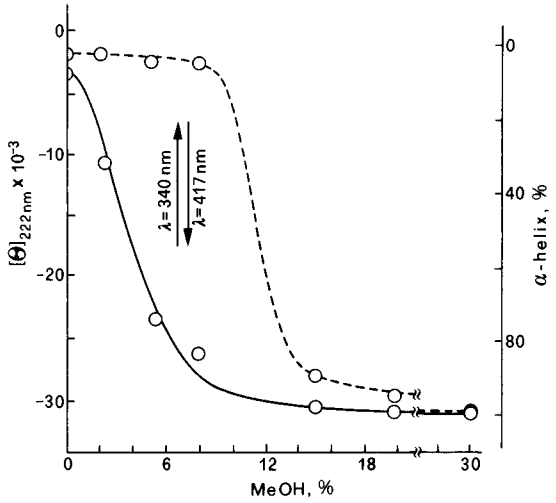


Figure 4. CD spectra of poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) in HFP/MeOH solvent mixtures: ellipticity at 222 nm and α-helix content percent, as a function of methanol concentration, for the samples irradiated at 417 (—) and 340 nm (- - -).

at 340 (trans-to-cis isomerization) and the sample irradiated at 417 nm (cis-to-trans isomerization). Therefore two separate curves are observed for the two samples. From Figure 4 it clearly appears that in pure HFP, both the "trans" and the "cis" samples are essentially random coils; in HFP/MeOH = 80/20, both the samples are essentially helical; at solvent compositions in the range between the two curves, irradiation at 340 and 417 nm, alternately, gives rise to the folding or unfolding of the macromolecular chains.

Analogous effects are observed in HFP/DCE solvent mixtures (Figure 5). As can be seen from the figure, however, there is a much larger range of HFP/DCE solvent compositions, with respect to HFP/MeOH solvent mixtures, suitable to obtain photoinduced variations of polypeptide conformation.

The conformational behavior of 46 mol % azo-modified poly(L-lysine) is similar to that above described for the 100% azo-modified poly(L-lysine), but the differences between the samples irradiated at 340 and 417 nm, respectively, are strongly reduced and the photoinduced conformational changes observed are rather small.

Even though poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) is not soluble in chlorinated solvents, addition of DCE to HFP solutions up to about 95% does not give rise to the polymer precipitation. Figure 6 shows the

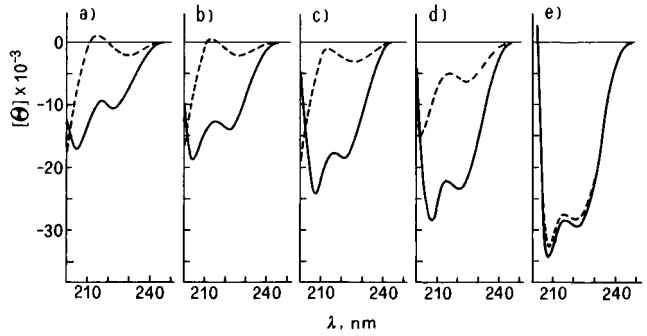


Figure 5. CD spectra of poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) in various HFP/DCE solvent mixtures: (—) kept in the dark or irradiated at 417 nm; (- - -) irradiated at 340 nm. DCE (v/v): (a) 5%; (b) 15%; (c) 25%; (d) 40%; (e) 62%.

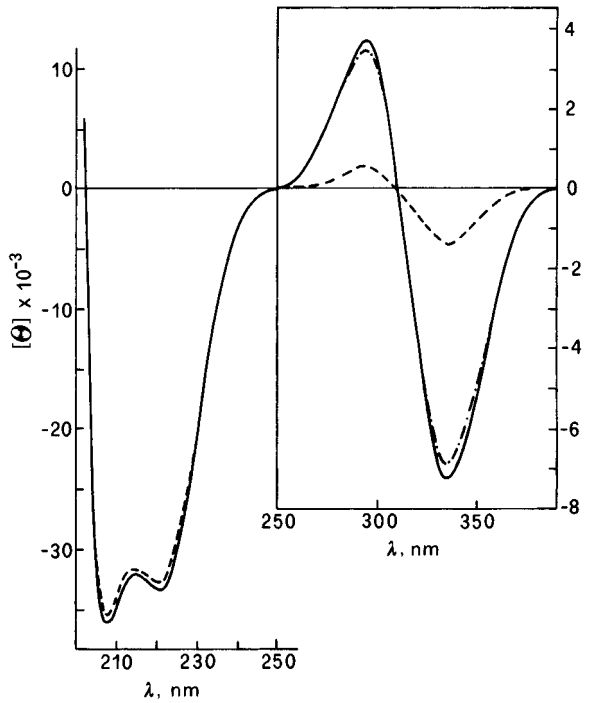


Figure 6. CD spectra of poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) in DCE/HFP = 90/10: (—) kept in the dark; (- - -) irradiated at 340 nm; (—·—) irradiated at 340 nm and irradiated again at 417 nm.

CD spectra recorded in DCE/HFP = 90/10. In the peptide region (λ < 250 nm), the polymer shows the typical CD pattern of the α-helix, the intensity of the bands corresponding to the value exhibited by 100% helical polypeptides in chlorinated solvents.[51] Above 250 nm, the sample kept in the dark shows a couplet of CD bands corresponding with the main absorption band of the azo chromophore. This couplet of bands can be assigned to the exciton splitting of the $\pi-\pi^*$ electronic transition at 320 nm, due to the dipole−dipole interactions between the side chain azobenzene units in trans configuration. Irradiation at 340 nm, and the consequent trans-to-cis photoisomerization, strongly reduces the intensity of the side chain CD bands (the residual ellipticity is likely to be due to the residual trans isomers at the photosteady state), while irradiation at 417 nm restores the original bands. However, irradiation does not affect at all the CD spectra in the peptide region, thus indicating that, in these conditions, the trans/cis photoisomerization of the side chains does not induce any variation of the helical main chains.

Discussion

As mentioned in the Introduction, azobenzene-containing polymers of L-lysine have been already described by us[30] and other authors.[31] In polymers previously reported, azobenzene units are linked to the Lys residues by an amide bond (Chart 1, structure **I**), whereas in polymers described here, azobenzene units are linked to the Lys residues by means of a sulfonamide bond (Chart 1, structure **II**).

The two classes of azo−poly(L-lysine), corresponding to structures **I** and **II**, respectively, exhibit a completely different conformational behavior. In fact, poly(L-lysine) containing phenylazobenzoyl units in the side chains (structure **I**) is α-helical in HFP and the backbone conformation is not affected by the photoisomerization of the side chains.[30] A decrease of the CD signal in the peptide region was observed by Yamamoto and Nishida[31] after long irradiation times up to 4 h with a 400 W mercury lamp at 360 nm, but the variation of the CD signal was not reversible; so it could not be considered as a probe for the occurrence of photoinduced structural variations of the macromolecules. By contrast, poly(L-lysine) containing azo sulfonyl units (structure **II**) is disordered in HFP solution and is able to give reversible random coil/α-helix changes upon combined action of light and solvent.

The different conformational behavior suggests that the monomeric units **II** may interact with HFP differently from units **I**. Actually, the strong protonating solvent HFP (pK_a = 9.30)[52] is known to form electrostatic complexes with various organic compounds, including amines and dimethyl sulfoxide.[53] On the other hand, sulfonamides are significantly protonated in acid media:[54] we may presume that protonation and formation of electrostatic complexes can occur for azo sulfonyl Lys residues, as well. In HFP therefore, polypeptides having structure **I** can adopt the ordered α-helix structure, while polypeptides having structure **II** should be forced by the electrostatic interactions above described to adopt a disordered conformation.

Of course, stability and formation of "HFP·azo sulfonyl−Lys" complexes should be less favored on going from pure HFP to HFP/MeOH or HFP/DCE solvent mixtures. In fact, at high concentrations of MeOH or DCE, azo polypeptides **II** adopt the α-helix conformation, independently of the trans or cis configuration of the azo side chains, similarly to azo polypeptides **I**. At appropriate and critical solvent compositions, formation of the electrostatic complexes above described might be favored or inhibited by the electronic situation of the azo moieties, which is different depending on whether they are in the apolar, conjugated trans form, or in the more polar, not conjugated cis form. In other words, the primary photochemical event is the trans/cis photoisomerization of the azo units, but the simple geometric variation of the azo side chains does not seem to be sufficient to induce appreciable variations of the backbone conformations (see Figure 6). In critical solvent conditions, the trans/cis photoisomerization of the azo sulfonyl units **II** should cause, as a consequence, the protonation/deprotonation of the sulfonamide functions, which should be the key factor responsible for photoregulation of polypeptide conformation.

Conclusions

Poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine) provides an additional example of photochromic polypeptides that respond to light giving reversible α-helix/

random coil cooperative transitions of the macromolecular main chain, thus working as amplifiers and transducers of the primary photochemical event occurring in the photosensitive side chains. The observed photoresponse can be defined as a *gated photoresponse*,[55] in the sense that it occurs only in a selected range of the environmental conditions.

The phenomenon is analogous to that already described for poly(spiropyran-L-lysine);[45] however, the present system based on photochromic azo sulfonamide units is characterized, as other sulfonated azobenzenes, by a much higher *fatigue* resistance and thermal stability: indeed, at room temperature, the system is so stable that interconversions between the α-helix and random coil structure can be obtained only photochemically, by irradiating at 340 nm (trans-to-cis) and 417 nm (cis-to-trans isomerization).

The reported properties suggest that poly(N^{ϵ}-((phenylazophenyl)sulfonyl)-L-lysine), as other photoresponsive polypeptides, may have future opportunities as a suitable material for designing sensors, optical and chiroptical switches, and devices that can be photomodulated.[5,11,12]

References and Notes

(1) Rau, H. In *Photochromism*; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; Chapter 4.
(2) Guglielmetti, R. In *Photochromism*; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; Chapter 8.
(3) Lovrien, R. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 236.
(4) Irie, M. *Adv. Polym. Sci.* **1990**, *94*, 27.
(5) Pieroni, O.; Ciardelli, F. *Trends Polym. Sci.* **1995**, *3*, 282.
(6) Brown, D.; Natansohn, A.; Rochon, P. *Macromolecules* **1995**, *28*, 6116.
(7) Natansohn, A.; Rochon, P.; Ho, M. S.; Barret, C. *Macromolecules* **1995**, *28*, 4179.
(8) Ho, M. S.; Natansohn, A.; Rochon, P. *Macromolecules* **1995**, *28*, 6124.
(9) Ikeda, T.; Tsutsumi, O. *Science* **1995**, *268*, 1873 and references therein.
(10) Pieroni, O.; Fissi, A.; Ciardelli, F. *React. Funct. Polym.* **1995**, *26*, 185.
(11) Cooper, T. M.; Natarajan, L. V.; Crane, R. L. *Trends Polym. Sci.* **1993**, *1*, 400.
(12) Feringa, B. L.; Jager, W. F.; De Lange, B. *Tetrahedron* **1993**, *49*, 8267.
(13) Goodman, M.; Falxa, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3863.
(14) Ueno, A.; Takahashi, K.; Anzai, J.; Osa, T. *Macromolecules* **1980**, *13*, 459.
(15) Ueno, A.; Takahashi, K.; Anzai, J.; Osa, T. *J. Am. Chem. Soc.* **1981**, *103*, 6410, and references therein.
(16) Ueno, A.; Adachi, K.; Nakamura, J.; Osa, T. *J. Polym. Sci., Part A: Polym. Chem.* **1990**, *28*, 1161.
(17) Pieroni, O.; Fissi, A.; Houben, J. L.; Ciardelli, F. *J. Am. Chem. Soc.* **1985**, *107*, 2990.
(18) Pieroni, O.; Fabbri, D.; Fissi, A.; Ciardelli, F. *Makromol. Chem. Rapid Commun.* **1988**, *9*, 637.
(19) Fissi, A.; Pieroni, O. *Macromolecules* **1989**, *22*, 1115.
(20) Higuchi, M.; Takizawa, A.; Kinoshita, T.; Tsujita, Y. *Macromolecules* **1987**, *20*, 2888.
(21) Sato, M.; Kinoshita, T.; Takizawa, A.; Tsujita, Y. *Macromolecules* **1988**, *21*, 1612.
(22) Aoyama, M.; Youda, A.; Watanabe, J.; Inoue, S. *Macromolecules* **1990**, *23*, 1458.
(23) Sisido, M.; Ishikawa, Y.; Itoh, K.; Tazuke, S. *Macromolecules* **1991**, *24*, 3993.
(24) Narisawa, H.; Kishi, R.; Sisido, M. *Macromol. Chem. Phys.* **1995**, *196*, 1419.
(25) Menzel, H.; Weichart, B.; Hallensleben, M. L. *Thin Solid Films* **1993**, *223*, 181.
(26) Menzel, H.; Hallensleben, M. L.; Schmidt, A.; Knoll, W.; Fischer, T.; Stumpe, J. *Macromolecules* **1993**, *26*, 3644.
(27) Menzel, H. *Macromol. Chem. Phys.* **1994**, *195*, 3747.
(28) Menzel, H.; Weichart, B.; Schmidt, A.; Paul, S.; Knoll, W.; Stumpe, J.; Fischer, T. *Langmuir* **1994**, *10*, 1926.
(29) Büchel, M.; Sekkat, Z.; Paul, S.; Weichart, B.; Menzel, H.; Knoll, W. *Langmuir* **1995**, *11*, 4460.
(30) Fissi, A.; Pieroni, O.; Ciardelli, F. *Biopolymers* **1987**, *26*, 1993.
(31) Yamamoto, H.; Nishida, A. *Macromolecules* **1986**, *19*, 943.
(32) Yamamoto, H.; Nishida, A.; Takimoto, T.; Nagai, A. *J. Polym. Sci., Polym. Chem.* **1990**, *28*, 67.
(33) Yamamoto, H.; Ikeda, K.; Nishida, A. *Polym. Int.* **1992**, *27*, 67.
(34) Yamamoto, H.; Nishida, A.; Kawaura, T. *Int. J. Biol. Macromol.* **1990**, *12*, 257.
(35) Yamamoto, H.; Nishida, A. *Polym. Int.* **1991**, *24*, 145.
(36) Strzegowski, L. A.; Martinez, M. B.; Gowda, D. C.; Urry, D. W.; Tirrell, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 813.
(37) Ulysse, L.; Cubillos, J.; Chmielewski, J. *J. Am. Chem. Soc.* **1995**, *117*, 8466.
(38) Higuchi, M.; Minoura, N.; Kinoshita, T. *Macromolecules* **1995**, *28*, 4981.
(39) Ciardelli, F.; Fabbri, D.; Pieroni, O.; Fissi, A. *J. Am. Chem. Soc.* **1989**, *111*, 3470.
(40) Fissi, A.; Pieroni, O.; Ciardelli, F.; Fabbri, D.; Ruggeri, G.; Umezawa, K. *Biopolymers* **1993**, *33*, 1505.
(41) Cooper, T. M.; Obermeyer, K. A.; Natarajan, L. V.; Crane, R. L. *Photochem. Photobiol.* **1992**, *55*, 1.
(42) Pachter, R.; Cooper, T. M.; Natarajan, L. V.; Obermeyer, K. A.; Crane, R. L.; Adams, W. W. *Biopolymers* **1992**, *32*, 1129.
(43) Cooper, T. M.; Tondiglia, V.; Natarajan, L. V.; Shapiro, M.; Obermeier, K.; Crane, R. L. *Appl. Opt.* **1993**, *32*, 674.
(44) Pieroni, O.; Fissi, A.; Viegi, A.; Fabbri, D.; Ciardelli, F. *J. Am. Chem. Soc.* **1992**, *114*, 2734.
(45) Fissi, A.; Pieroni, O.; Ruggeri, G.; Ciardelli, F. *Macromolecules* **1995**, *28*, 302.
(46) Cooper, T. M.; Stone, M. O.; Natarajan, L. V.; Crane, R. L. *Photochem. Photobiol.* **1995**, *62*, 258.
(47) A decrease of the CD signal in the peptide region was observed by Yamamoto and Nishida after long irradiation times up to 4 h with a 400 W mercury lamp at 360 nm, but the variation of the CD signal was not reversible [see ref 31].
(48) Fasman, G. D.; Idelson, M.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 709.
(49) Yaron, A.; Berger, A. *Biochem. Biophys. Acta* **1963**, *69*, 397.
(50) Inscoe, M. N.; Gould, J. H.; Brode, W. R. *J. Am. Chem. Soc.* **1959**, *81*, 5634.
(51) Woody, R. W. *J. Polym. Sci., Macromol. Rev.* **1977**, *12*, 181.
(52) Middleton, W. J.; Lindsey, R. V., Jr. *J. Am. Chem. Soc.* **1964**, *86*, 4948.
(53) Purcell, K. F.; Stickeleather, J. A.; Brunk, S. D. *J. Am. Chem. Soc.* **1969**, *91*, 4019.
(54) King, J. F. In *The Chemistry of sulfonic acids, esters and their derivatives*; Patai, S., Rappoport, Z., Eds.; Wiley: Chichester, U.K., 1991; pp 249−259.
(55) Irie, M.; Miyatake, O.; Ukida, K. *J. Am. Chem. Soc.* **1992**, *114*, 8715.

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