Photoresponsive Polypeptides[⊥]

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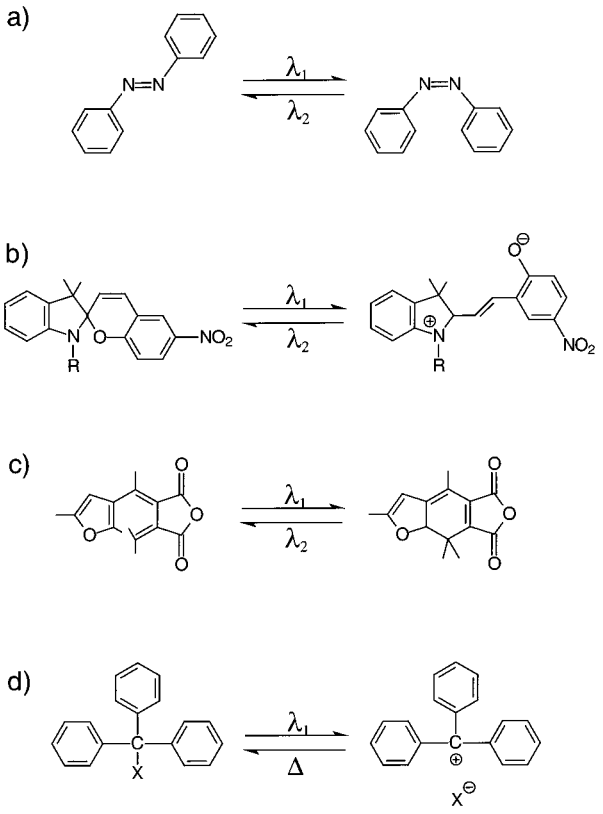
ABSTRACT

Polypeptides containing azobenzene or spiropyran units attached to the macromolecules respond to light or dark conditions giving reversible variations of their structure. In this Account we provide a short overview of current research in the field and describe the most significant experimental examples of photoresponse effects. They include photoinduced random coil/α-helix transitions, helix-sense reversal, photostimulated aggregation/disaggregation processes, and photomechanical effects. These fascinating properties suggest that photoresponsive polypeptides may become suitable materials for designing sensors and devices that can be photomodulated. Findings also demonstrate that it is possible to synthesize model systems which respond to light similarly to naturally occurring photoreceptors.

Introduction

Photochromic compounds are able to exhibit variations of their color, or more generally of their absorption spectra, when exposed to light of an appropriate wavelength; they reversibly regain their original color when placed in the dark or upon exposure to light of a different wavelength. The photochromic behavior is due to the ability of these compounds to exist in two different states, whose relative concentration depends on the wavelength of the incident light. The photoreactions for some of the most extensively investigated photochromic systems are shown in Scheme 1. In azobenzene derivatives, the photochromism involves the photoisomerization between the trans and the cis isomers; in spiropyran compounds, the photochromic behavior is due to an interconversion between the colorless closed spiro structure and the colored open merocyanine form.

Scheme 1. Photochemical Reactions Responsible for the Photochromic Behavior in (a) Azobenzene Derivatives, (b) Spiropyran Compounds (R = Alkyl Groups), (c) Fulgides, and (d) Triphenylmethane Derivatives (X = −OH, −CN)



In recent years, research in the area of photochromism has become increasingly important in connection with phenomena other than merely color change. In fact, the occurrence of two different structures which can be reversibly interconverted by means of an external light stimulus can be the basis for a molecular switch triggered by light; thus, photochromic compounds may be highly promising materials for application in photooptical technology as well as in the design of devices which can be photomodulated.[1−5]

In nature, photochromic molecules represent the basic molecular triggers for many important biological photoreceptors, which recognize the quantity and the quality

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[⊥] Dedicated to Prof. Erseo Polacco, Department of Physics, University of Pisa, on the occasion of his 70th birthday.

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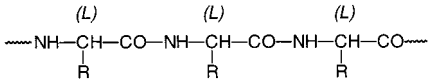
Scheme 2. Chemical Structure of Polypeptides (I)



I

of light in the environment. Among these biological photodetectors, absorbing from the blue to the red region of the spectrum, some of the more extensively studied are retinal proteins (from rhodopsins in higher organisms[6] to sensory rhodopsins in halobacteria[7]), open-chain-tetrapyrrole proteins,[7] and the xanthopsins,[7] e.g., the photoactive yellow protein, PYP, found in eubacteria such as *Ecothiorhodospira halophila*, which contain a deprotonated 4-hydroxycinnamoyl moiety.

These naturally occurring photosensors are characterized by the following salient common features: (a) they contain a photochromic molecule attached to a macromolecular protein matrix; (b) on irradiation, the photochromic moiety undergoes reversible stereochemical rearrangements between two or more isomeric forms, the direction being determined by the wavelength of the incident light; (c) this primary photochemical reaction induces a conformational change in the attached protein matrix, the "photosignaling state", which finally leads to the physiological response.

Like biological systems, synthetic polymers containing photochromic moieties have been found to undergo reversible variations of their structure and conformation upon exposure to light or dark conditions. Remarkable effects have been observed in polypeptides (**I**, Scheme 2).[8,9]

From the point of view of molecular structure, polypeptides are quite special polymers because they can exist in disordered or regularly folded structures, typical of those existing in proteins, such as the α-helix and β-structure. When photochromic molecules, such as azobenzene or spiropyran units, are attached to the macromolecular chains, polypeptides may respond to light, giving large photoinduced structural changes.

This Account provides a short overview of current research in the field of photoresponsive polypeptides, and herein we describe the most significant experimental examples of photoresponse effects. They include photoinduced random coil/α-helix transitions, helix-sense reversals, photostimulated aggregation/disaggregation processes, and photomechanical effects in polypeptide monolayers.

Photoinduced Random Coil/α-Helix Transitions in Poly(L-Glutamic Acid) Containing Azobenzene Units

Polypeptides having the structure **II** (Scheme 3) have been obtained by introducing various contents of azobenzene units into the side chains of high-molecular-weight poly(L-glutamic acid).[10−12] At room temperature in the dark, all azo groups are in the trans configuration, which is planar and fully conjugated. Irradiation at 350 nm produces the isomerization to the cis form which is characterized by a nonplanar, twisted geometry. The back reaction to the trans form is obtained by irradiation with 450-nm light or dark adaptation.

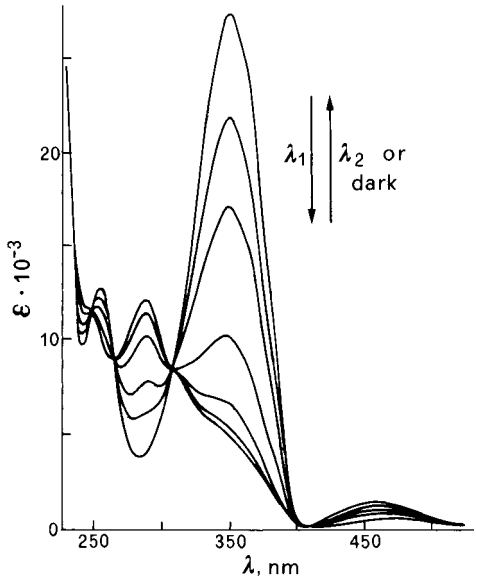
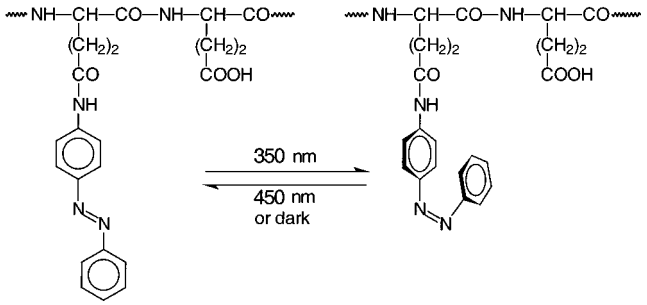


FIGURE 1. Reversible light-induced variations of the absorption spectra in azo-modified poly(L-glutamic acid) (**II**).

Scheme 3. Chemical Structure and Photochromic Reactions of Poly(L-glutamic acid) Containing Azobenzene Units (II)



II *trans* **II** *cis*

As a consequence of the different electronic situations in the two isomers, the photoisomerization is accompanied by strong variations of the absorption spectra (Figure 1). Using a lamp of 200 W, irradiation for 1 or 2 min is enough to achieve the photostationary state. The thermal decay in the dark is much slower. At room temperature, it takes more than 200 h to restore the full-trans isomeric composition. The photochromic cycles are completely reversible and can be repeated at will, without any apparent *fatigue*.

The macromolecular conformation can be easily detected by circular dichroism (CD) spectroscopy, since the various polypeptide structures—random coil, α-helix, and β-structure—are characterized by standard CD spectra. In organic solvents, such as trifluoroethanol or trimethyl phosphate, the azo-modified polymers show the CD pattern of the α-helix structure. Irradiation at 350 nm produces the trans-to-cis isomerization of the azo side chains but does not modify at all the CD spectrum of the α-helix. This indicates that, in these solvents, light does

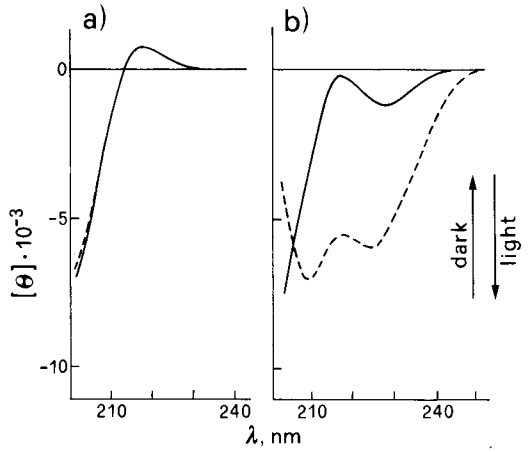


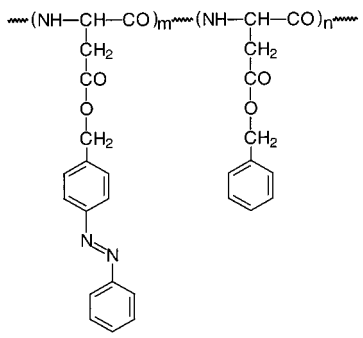
FIGURE 2. Poly(L-glutamic acid) containing 20 mol % azobenzene units (**II**). Circular dichroism (CD) spectra at pH 7.6 (a) in the absence of dodecylammonium chloride (DAC) and (b) in the presence of DAC at its critical micelle concentration. In organic solvents such as trimethyl phosphate or 1,2-dichloroethane, the CD spectra of dark-adapted samples also show exciton couplets centered at 350 nm, in correspondence of the main absorption band of the azo chromophore. These CD bands, which disappear upon exposure to light, are not observed in protonating solvents such as trifluoroethanol, hexafluoropropanol, and aqueous solutions.

not induce any variation in the main-chain structure. The same result has been also reported for polypeptides containing phenylazo-phenylalanine residues.[13]

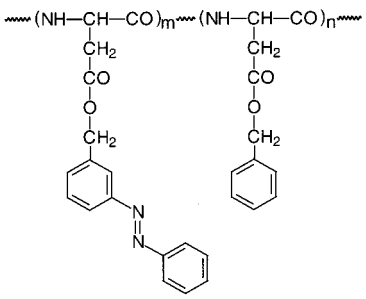
The behavior is quite different in water solution, at critical pH values,[12] or in the presence of surfactants.[14] A 20% azo-modified poly(L-glutamic acid) at pH 7.6, in the absence of surfactants, shows the CD pattern of the random coil structure, and the spectrum is not affected at all by irradiation (Figure 2a). In the presence of the surfactant dodecylammonium chloride (DAC) at its critical micelle concentration, the sample kept in the dark is a random coil, but the sample exposed to 350-nm light shows the spectrum of the α-helix structure, characterized by the two typical negative bands at 222 and 208 nm (Figure 2b). In other words, irradiation at 350 nm (trans-to-cis isomerization) induces an evident coil-to-helix transition, even though the content of helical structure is not over 30%. The variation is completely reversed when the sample is dark-adapted or irradiated at 450 nm (cis-to-trans isomerization). Thus, in the presence of DAC micelles, the polypeptide conformation can be photomodulated by an alternating exposure to light or dark, or irradiation at two different appropriate wavelengths.

The mechanism of this photoresponse can be explained as follows. When azo units are in the planar, apolar, trans form, they dissolve into the hydrophobic core of the micelles, forcing the polypeptide chains on the surface of the micelles to assume a coil conformation. Isomerization of the azo units to the skewed, polar, cis form inhibits hydrophobic interactions and causes the azo units to get out of the micelles, thus allowing the polypeptide chains to adopt the α-helix structure. In other words, the primary photochemical event is the trans/cis isomerization of the azobenzene units, but the driving force of the coil/helix transition should be the different location of the macromolecules relative to the micelles.

Scheme 4. Chemical Structure of Poly(*β*-L-aspartate)s Having Various Contents of *p*- (III) and *m*-Phenylazobenzyl (IV) Units in the Side Chains



III



IV

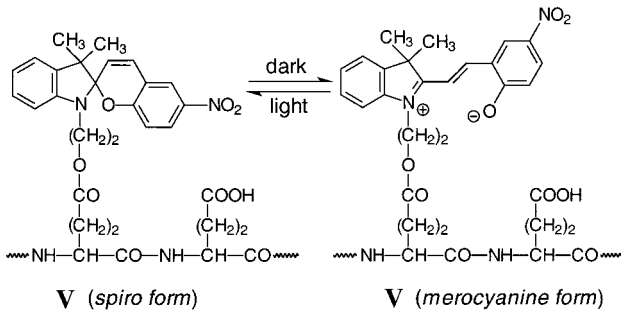
An interesting and analogous photoresponse effect has been reported for a partially esterified poly(L-glutamate) containing 13 mol % azobenzene units in the side chains.[15] The polypeptide was incorporated into a bilayer membrane of vesicles composed of distearyl dimethylammonium chloride. UV light irradiation of the vesicles, and the consequent trans-to-cis isomerization of the azo units, caused a transfer of the polypeptide molecules from the hydrophobic interior to the hydrophilic surface of the bilayer membrane. This synthetic system mimics some biological photoreceptors, such as photopigments of frog retinal membranes, which change their location toward the aqueous surface or the hydrocarbon core of the membrane, depending on whether the photopigment is irradiated or kept in the dark.[16]

Photoinduced Helix-Sense Reversal in Azobenzene-Containing Poly(L-aspartate)s

Poly(L-aspartate)s are quite special polypeptides, because they are able to adopt helical structures of both left-handed and right-handed screw senses, the stability of the two helices depending on the chemical structure of the ester group in the side chains. On the basis of this observation, Ueno et al. prepared a series of poly(L-aspartate)s containing azobenzene units in the side chains (Scheme 4) and investigated the effect of trans/cis isomerization of azo side chains on macromolecular structure.[17−19]

In 1,2-dichloroethane, polypeptides containing 59 and 81 mol % of *p*-phenylazo-L-aspartyl residues (Scheme 4, structure **III**) exhibited CD spectra characterized by a

Scheme 5. Structure and Reverse Photochromic Reactions in Hexafluoro-2-propanol of Poly(L-glutamic acid) Containing Spiropyran Units in the Side Chains (V)



positive CD band at about 220 nm, indicative of a left-handed helical structure. The CD band became negative after irradiation. The change in sign provided evidence for the reversal of the helix sense induced by the trans/cis photoisomerization of the azo units.[17]

Large photoresponse effects were observed in solvent mixtures, provided the irradiation was carried out at appropriate solvent compositions. Two polymers containing respectively 8 and 10 mol % of *m*-phenylazo-L-aspartyl residues (Scheme 4, structure **IV**) were found to be left-handed helices in pure 1,2-dichloroethane, while they were right-handed helices in pure trimethyl phosphate, independently of light or dark conditions. In a mixed solvent containing 25−30% trimethyl phosphate, the polypeptides were left-handed before irradiation, but they became right-handed helices after irradiation, indicating that, under these solvent conditions, a photoinduced reversal of the helix sense occurred even for polypeptides containing small amounts of photochromic units.[18]

Reversible Random Coil/α-Helix Transitions Induced by Sunlight in Spiropyran-Containing Polypeptides

As mentioned in the Introduction, photochromism of spiropyran compounds involves two photoisomers, the neutral spiro form and the zwitterionic merocyanine form, which are characterized by large differences in geometry and polarity (see Scheme 1); therefore, their interconversion may strongly affect the structure of the attached macromolecules. Moreover, in polypeptides containing azobenzene units, generation of cis and trans isomers, and hence photoregulation of conformation, requires an artificial source of UV radiation. Spiropyran compounds, by contrast, respond to visible light, so their introduction into polypeptide macromolecules leads to photoresponsive polypeptides whose macromolecular structure can be modulated upon exposure to sunlight.[20−22]

Poly(L-glutamate)s containing spiropyran units in the side chains (Scheme 5) are soluble in hexafluoro-2-propanol (HFP), where they exhibit an intense reverse photochromism, i.e., a photochromic behavior opposite to that usually observed in most common organic solvents. At room temperature in the dark, they yield colored solutions owing to the presence of the merocyanine form; irradiation with visible light or just exposure to sunlight causes the complete bleaching of the solutions as a result of the formation of the colorless spiro form. The back reaction occurs in the dark, and the original color is reversibly recovered.

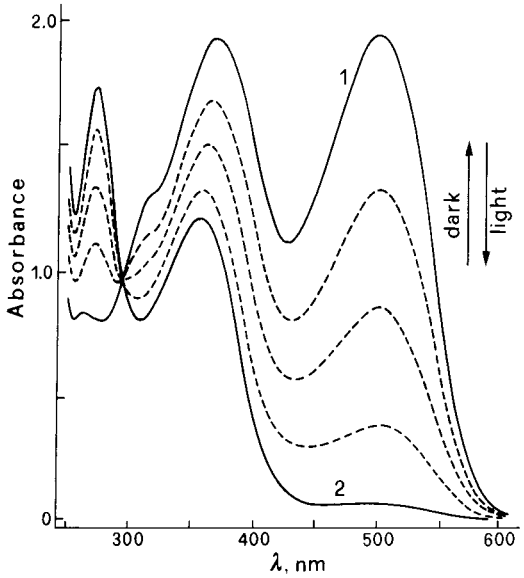


FIGURE 3. Absorption spectra of poly(L-glutamic acid) containing 85% spiropyran units (**V**), in HFP: (1) sample kept in the dark and (2) exposed to sunlight. Dashed lines: intermediate spectra during decay in the dark.

Figure 3 shows the effect of light on the absorption spectra of a poly(L-glutamate) containing 85 mol % photochromic units in the side chains. The spectrum of the colored solution kept in the dark exhibits two intense bands at 500 and 370 nm due to the presence of the merocyanine species. Irradiation with visible light (500−550 nm) cancels the intense band in the visible region and produces the spectrum corresponding to the spiro form, characterized by absorption maxima at 355 and 272 nm. On dark adaptation, the original spectrum is progressively restored. 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 back reaction is much slower: it takes about 150−250 min for the sample kept in the dark to regain half of the original absorbance.

The macromolecular conformation of spiropyran-modified poly(L-glutamate)s is strongly affected by light or dark conditions, as revealed by CD spectra (Figure 4). Before irradiation, the polypeptides adopt a random coil conformation; after exposure to sunlight, they display the typical CD pattern of the α-helix; on dark adaptation, the helix content progressively decreases, and the original disordered conformation is restored.

Absorption and fluorescence measurements provided evidence[23] that the driving forces responsible for the photoinduced conformational change are the interactions between the photochromic side chains, as schematically illustrated in Figure 5. In the dark, the merocyanine units have a strong tendency to form dimeric species; as a result, the macromolecules are forced to adopt a disordered structure. When the side chains are photoisomerized to

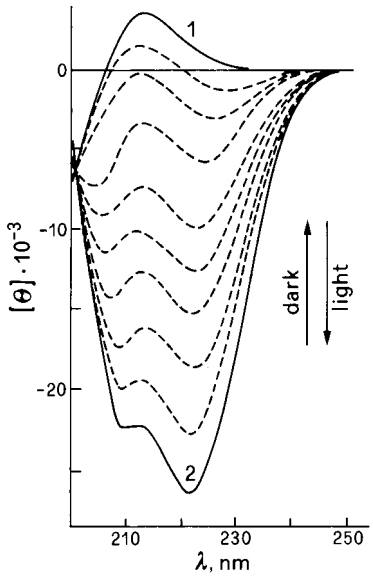


FIGURE 4. Effect of irradiation and dark adaptation on CD spectra of poly(L-glutamic acid) containing 85 mol % spiropyran units (**V**), in HFP: (1) sample kept in the dark and (2) exposed to sunlight. Dashed lines: intermediate spectra during decay in the dark over 8 h. Very weak CD bands, due to the contribution of the photochromic side chains, are also observed at lower energy in the range 300−500 nm. These CD bands have not been reported in the figure.

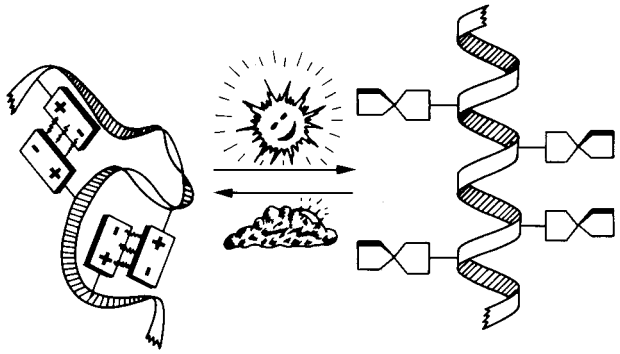


FIGURE 5. Schematic illustration of the coil/α-helix transition occurring for spiropyran-modified poly(L-glutamic acid) in hexafluoro-2-propanol. In the dark, the merocyanine units ["(+ −) boxed" groups] have a strong tendency to give dimeric species; as a result, the macromolecules are forced to adopt a disordered structure. When the side chains are photoisomerized to the spiro form ["neutral boxed" groups], such dimers are destroyed, and the macromolecules assume the helical structure.

the spiro form, such dimers are destroyed, and the macromolecules assume the helical structure.[23]

Modulation of Photoresponse by Environment and Solvent Conditions

A quite interesting photoresponsive behavior is observed when spiropyran-modified poly(L-glutamate)s (**V**) are dissolved in HFP and a small amount of trifluoroacetic acid (TFA) is added to the solution.[24] In the presence of acid, the photoisomerization of the photochromic side chains does not result in any conformational variation of the macromolecular main chains, and the macromolecules are random coils both in the dark and after light exposure. However, when appropriate amounts of methanol are added as a cosolvent, the system again responds to light, leading to random coil → α-helix transitions.

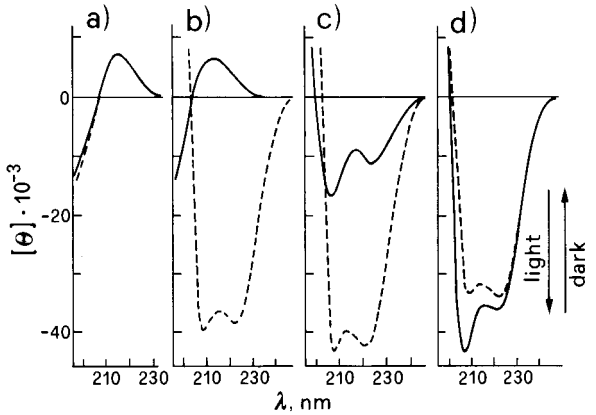


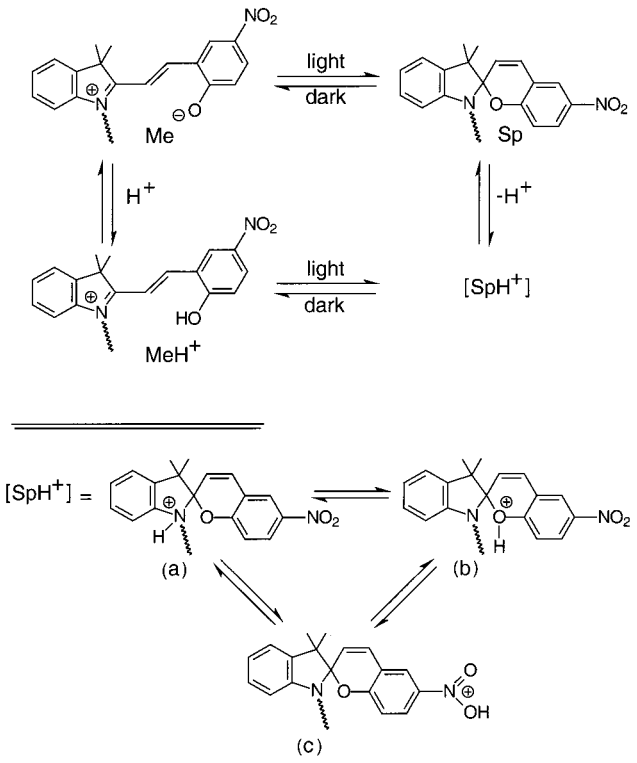
FIGURE 6. Poly(L-glutamic acid) containing 85 mol % spiropyran units in the side chains (**V**). Effect of irradiation on CD spectra in various HFP/MeOH solvent mixtures in the presence of trifluoroacetic acid (TFA, $c = 5 \times 10^{-4}$ g/mL). MeOH: (a) 0−5%; (b) 10%; (c) 20%; (d) 40%. Continuous line, dark adapted; dashed line, irradiated samples.

The effect of light on CD spectra is shown in Figure 6. When methanol concentration is below 5%, both the dark-adapted and the irradiated samples show the typical CD pattern of disordered polypeptides. In HFP/MeOH = 90/10, the sample kept in the dark is a random coil, whereas the sample exposed to light displays the standard CD pattern of the α-helix. The intensity of the bands indicates that, under these conditions, light causes the full conversion from random coil to 100% α-helix. Upon an increase in the methanol concentration, the dark-adapted sample becomes also partially helical, and finally when the methanol concentration is higher than 40%, both the dark and the irradiated samples are fully helical. This shows clearly that the photoinduced structural changes depend on solvent composition, so the photoresponse can be modulated by a combined action of light and chemical environment.

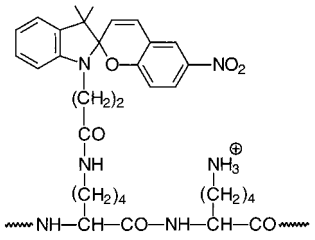
The mechanism of photoresponse can be rationalized on the basis of the chemical reactions reported in Scheme 6. In HFP acidified by addition of TFA, spiropyran compounds are present as protonated merocyanine MeH^+. Exposure to light converts the species MeH^+ into the ring-closed spiro species SpH^+. In the presence of acid, therefore, the photochromic side chains are present as cationic species either in the dark or in the light. In both cases, the repulsive electrostatic interactions among the charged side chains force the macromolecules to adopt an extended random coil structure, and no photoinduced conformational change is observed.

When appropriate amounts of methanol are added to the HFP solution, the protonated species MeH^+ present in the dark are not altered, but the equilibrium between protonated and unprotonated spiro units present in the irradiated solution is shifted toward the neutral form. Under these conditions, the photochromic species in the side chains are charged in the dark but are neutral in the light, so irradiation induces formation of α-helix as it does

Scheme 6. Photochromic Reactions of Spiropyrans under Acid Conditions



Scheme 7. Chemical Structure of Poly(L-lysine) Containing Spiropyran Units in the Side Chains (VI)



VI

in HFP without acid. At high methanol concentration, formation of α-helix even in the dark-adapted samples may be due to the same effect observed for other poly(α-amino acid)s with ionic side chains, such as poly(sodium L-glutamate) and poly(L-lysine hydrochloride), which are random coils in water but become helical upon addition of excess methanol.[25]

An analogous conformational and photoresponsive behavior has been observed in poly(L-lysine) containing spiropyran units[26,27] (**VI**, Scheme 7) or azosulfonyl units[28] in the side chains (**VII**, Scheme 8).

Figure 7 shows the CD spectra of polymer **VII** measured under various solvent conditions. In pure HFP, the CD spectra are typical of random coil polypeptides either when the sample is irradiated at 340 nm (azo units in cis configuration) or when it is irradiated at 417 nm (azo units in trans configuration). At methanol concentrations higher than 15%, both samples exibit the CD pattern of the α-helix. At methanol concentrations in the range between 2 and 15%, the trans/cis photoisomerization of the azo

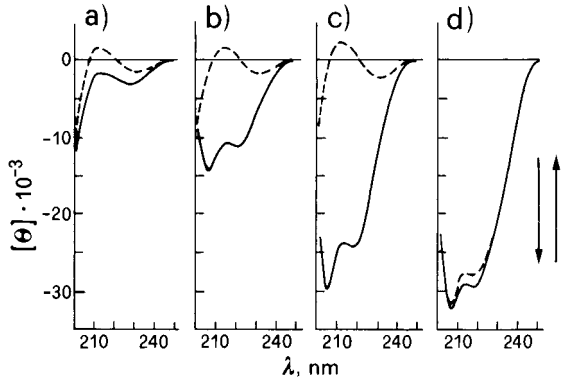


FIGURE 7. CD spectra of poly(azobenzensulfonyl-L-lysine) (**VII**) in various HFP/MeOH solvent mixtures (v/v): (a) 0%; (b) 2%; (c) 8%; (d) 15%. Continuous line, kept in the dark or irradiated at 417 nm; dashed line, irradiated at 340 nm.

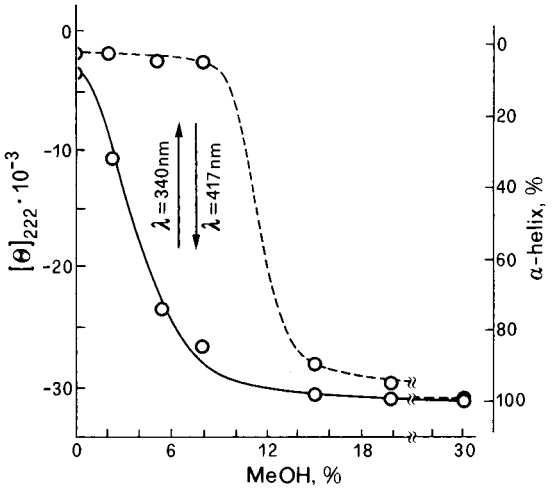
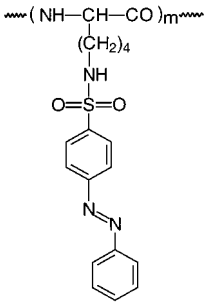


FIGURE 8. Poly(azobenzensulfonyl-L-lysine) (**VII**) in HFP/MeOH solvent mixtures: intensity of the CD band at 222 nm and α-helix percent, as a function of methanol concentration, for the samples irradiated at 417 (continuous line) and 340 nm (dashed line).

Scheme 8. Chemical Structure of Poly(azobenzensulfonyl-L-lysine) (VII)

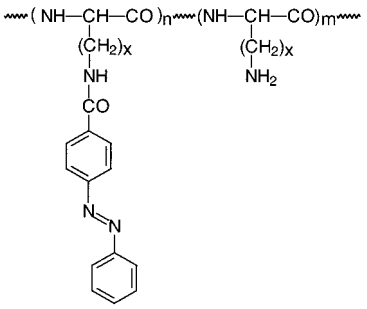


VII

side chains produces photoinduced variations of the helical content, the extent of the photorespose depending on solvent composition.

When the intensity of the CD band at 222 nm, which can be considered 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 (Figure 8). However, the amount of

Scheme 9. Chemical Structure of Azo-Modified Polypeptide Analogues of Poly(L-lysine) VIII (x = 1, 2, 3, and 4)



VIII (x = 1, 2, 3 and 4)

methanol needed to induce the transition is different for the sample irradiated at 340 (cis units) and the sample irradiated at 417 nm (trans units). Therefore, two separate curves are observed: at solvent compositions in the range between the two curves, an alternating irradiation at 340 and 417 nm gives rise to the folding or unfolding of the macromolecular chains. The described systems can be considered examples of photoresponsive systems with a *gated photoresponse*,[29] in the sense that the photoisomerization of the side chains is able to trigger the coil → α-helix transition of the macromolecules only in a narrow "window" of environmental conditions.

Photostimulated Aggregation−Disaggregation Processes and Photocontrol of Solubility

Azo-modified polypeptides undergo reversible aggregation−disaggregation processes upon exposure to light or dark conditions, as revealed by the variations of their CD spectra and light-scattering intensity.[30] In particular, for poly(L-glutamic acid), having more than 80% azobenzene units in the side chains (**II**), the photoinduced aggregation−disaggregation processes result in large and reversible variations of polymer solubility.[31] The polypeptide stored in the dark is soluble in HFP, where it assumes the α-helix structure; addition of a small amount of water (15% by vol) to the HFP solution produces formation of aggregates followed by the total and quantitative precipitation of the polymer. Irradiation of the suspension for a few seconds at 350 nm brings about the complete dissolution of the polymer, while irradiation of the solution at 450 nm drives the polymer precipitation. In this solvent mixture, therefore, the "precipitation−dissolution" cycles can be controlled by irradiation at the two different wavelengths. Similar photosolubility effects have been observed for azo-modified poly(L-ornithine) (**VIII**, $x = 3$)[32] and poly(L-α,β-diaminopropanoic acid) (**VIII**, $x = 1$) (Scheme 9).[33]

The photoinduced variations of solubility observed in HFP/water could, in principle, be a consequence of the higher polarity of the cis isomer with respect to the trans isomer of azobenzene groups. Actually, in azobenzene, the dipole moment was reported to be 3.0−3.1 D for the cis isomer and 0.0−0.5 D for the trans isomer.[34] If the higher polarity of the cis isomer were the deciding factor which causes the dissolution, polymer solubility should gradually increase with increasing cis content. However, the variation of solubility as a function of the trans/cis isomeric composition was described by a sharp sigmoidal curve typical of a cooperative phase transition.[31] Therefore, the photosolubility effect was interpreted on the basis of an association among macromolecules, through hydrophobic interactions and stacking of azobenzene side chains, as schematically illustrated in Figure 9.

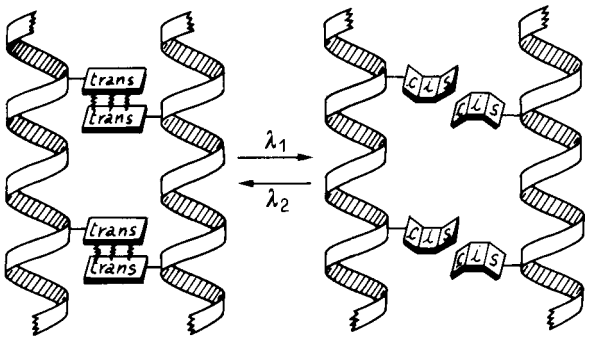


FIGURE 9. Schematic illustration of reversible "aggregation−disaggregation" effects among helical macromolecules, as a photoresponse to the trans/cis photoisomerization of azobenzene units. When azobenzene moieties are in the planar trans configuration, hydrophobic interactions and stacking between the azo groups are favored, so aggregation and precipitation occur. When the azo moieties are photoisomerized to the skewed cis configuration, interactions and stacking between azo groups are inhibited, so disaggregation of the macromolecules takes place, and polymer dissolution occurs.

The photostimulated precipitation and dissolution processes have suggested the possible application of these systems in photoresist technology.[35] They may be also relevant to some molecular mechanisms responsible for photoregulated processes in biology. It is interesting to compare the above-described photostimulated aggregation changes with the photobehavior of phytochrome, the photosensitive regulatory system of plants. This photochromic protein exists in two forms, the biologically inactive P_r (red-absorbing phytochrome) and the biologically active P_{fr} (far-red-absorbing phytochrome), which are interconvertible by light. In unirradiated tissues, phytochrome present as the P_r form is uniformly distributed throughout the cytoplasm, and the pigment is soluble upon extraction in aqueous buffers. Photoconversion to the P_{fr} form (irradiation at 660 nm) leads to a rapid association of the previously soluble pigment and formation of pellets localized on the membrane. Reconversion to the P_r form (irradiation at 730 nm) results in disaggregation and redissolution of the pigment molecules.[36,37]

$$\left\{\begin{matrix}\text{soluble}\\ \text{in}\\ \text{cytoplasm}\end{matrix}\right\} P_r \underset{730\ \text{nm}}{\overset{660\ \text{nm}}{\rightleftharpoons}} P_{fr} \left\{\begin{matrix}\text{insoluble and}\\ \text{localized on}\\ \text{membrane}\end{matrix}\right\}$$

Photomechanical Effects in Monolayers

Poly(L-lysine), containing about 40 mol % of *p*-phenylazobenzoyl units (**VIII**, $x = 4$),[38] was reported to form a stable monolayer at the water/air interface.[39] When the

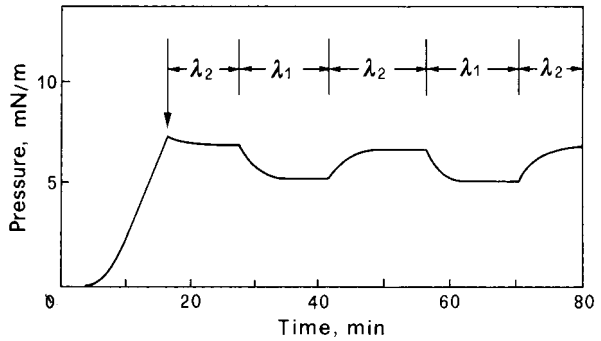
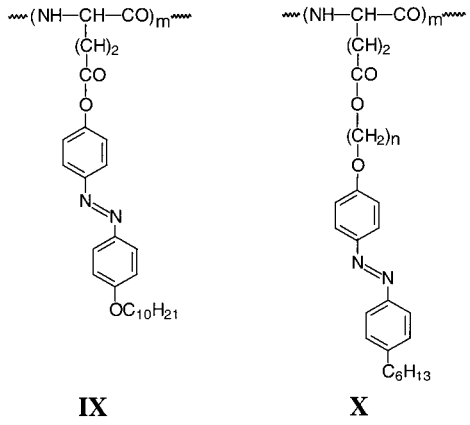


FIGURE 10. Reversible surface pressure changes in a monolayer of poly(L-lysine) containing 43 mol % azobenzene units (**VIII**, $x = 4$). The monolayer at the water−air interface was first compressed to 7 mN m^{-1} and then was kept at constant area and illuminated alternately with 450 (λ_2) and 365 nm (λ_1) radiation.

Scheme 10. Chemical Structure of Poly(L-glutamate)s IX and X Used To Obtain Photoresponsive Monolayers



IX **X**

area of the monolayer was kept constant, alternating irradiation with 365- and 450-nm light produced reversible changes of the surface pressure (Figure 10). When the pressure was kept constant, irradiation at the two different wavelengths produced reversible contraction and expansion of the surface area of the monolayer.

Monolayers prepared from azobenzene-containing poly(L-glutamate)s having the structures **IX** and **X** ($n = 2$) (Scheme 10) showed photomechanical effects which were opposite with respect to those described above for azo-modified poly(L-lysine). In fact, the monolayers expanded when exposed to UV radiation (trans-to-cis isomerization) and shrank when exposed to visible light (cis-to-trans isomerization).[40]

Investigations of photoresponsive systems in the monolayer state are quite fascinating and of increasing interest, because they may be regarded as energy conversion media from light to mechanical work.

Concluding Remarks and Future Prospects

Photochromic compounds such as azobenzene and spiropyran derivatives can exist in two different states and can be reversibly switched from one state to the other by means of a light stimulus having appropriate wavelength. When such photochromic molecules are incorporated into macromolecular matrixes, the interconversion between the two photoisomers can induce structural changes in the attached macromolecules. Thus, the photochromic units actually work as photochemical molecular switches, and photochromic polymers may provide the basis to construct light-driven switching systems.

The results reported in this Account deal to a considerable extent with our own research. Several other photoresponsive peptides have been recently reported, in which the photocontrol of a specific structure is the essential feature for the design of devices that can be photoregulated.[41−44] Investigations, of course, are not limited to photoresponsive polypeptides but involve other synthetic polymers and chemical systems. Much effort has been directed toward the development of photoregulable membranes,[45,46] biological photosensors,[4,5,47,48] and several applications in the area of optical memories and optical switches. Excellent and extensive review articles on the last topic have been very recently published in a special thematic issue of *Chemical Reviews* edited by Irie;[49] we refer the reader to that journal.

A crucial point that must be addressed concerns the "amplification" of the primary light signal, associated with the photoisomerization of the photochromic moiety, which is usually a weak effect. The greater the amplification factor, the greater is the sensitivity of the system. Substantial amplification can be achieved when the primary photochemical reaction is coupled to a subsequent event that occurs after absorption of light.

From this point of view, polypeptides containing photochromic units in the side chains are quite special polymers. In fact, they can exist in ordered or disordered conformations, and the photoisomerization of the photochromic side chains can produce "order−disorder" conformational changes. These photostimulated structural variations, such as random coil/α-helix, occur as highly cooperative transitions; therefore, photochromic polypeptides actually work as amplifiers and transducers of the primary photochemical event in the photosensitive side chains.

Even though photoresponsive polypeptides still require further research work to determine possible applications, they are likely to become quite promising compounds in the future. Moreover, fundamental research in the field of photoresponsive biopolymers can lead to a better understanding of the molecular mechanisms operating in natural photoreceptors. A knowledge of these mechanisms is very important for developing photoresponsive artificial devices characterized by a high efficiency and reversibility that are typical of biological systems.

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