Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Application of CD Spectroscopy for Monitoring Photoinduced Molecular Structural Changes in Photochromic Polypeptides

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **79** (1985), n.5, p. 133–139.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1985_8_79_5_133_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1985.

SEZIONE II

(Fisica, chimica, geologia, paleontologia e mineralogia)

Chimica. — *Application of* CD *Spectroscopy for Monitoring Photo-induced Molecular Structural Changes in Photochromic Polypeptides.* Nota di OSVALDO PIERONI (*) (**), ADRIANO FISSI (*) e FRANCESCO CIARDELLI (**) (***), presentata (****) dal Corrisp. P. PINO.

RIASSUNTO. — Vengono discussi gli spettri di dicroismo circolare (CD) di polimeri di acido L–glutammico, contenenti quantità variabili di gruppi azobenzene nelle catene laterali. Le proprietà chiroottiche forniscono valide indicazioni sulle variazioni reversibili prodotte dalla fotoisomerizzazione del cromoforo azo nei polipeptidi. In particolare, viene presentata la possibilità di fotocontrollare la conformazione, lo stato di aggregazione e la solubilità dei polimeri.

Reversible variations of conformation and aggregation of biological macromolecules induced by light seem to be the primary molecular events occurring in biological photoregulated processes, such as vision in animals and phototropism in plants [1].

In previous papers [2, 4, 5], we reported that poly(L–glutamic acid) containing azobenzene groups in the side chains can undergo photoresponsive effects, as a consequence of the trans $\rightleftarrows$ cis photoisomerization of azobenzene groups (Scheme). High trans-to-cis photoconversions can be obtained by irradiating in the region of the long-axis polarized $\pi - \pi^*$ electronic transition, centred at 355 nm in organic non-protonating solvents and 340 nm in alcohol or aqueous solvents [3]. The opposite cis-to-trans isomerization is obtained by irradiating in the region of the $n - \pi^*$ transition centred at 450 nm, or by dark adaptation.

Under particular conditions of solvent, pH and azo-content, the photoisomerization is accompanied by reversible variations of polypeptide conformation [2, 4]. In organic/aqueous solvent mixture, the above azo-polypeptides undergo aggregation phenomena, which are favoured when azo-groups are in the trans configuration (irradiated at 450 nm, or dark adapted samples), whereas they are inhibited when azo-groups are in cis configuration (samples irradiated at 370 nm). So macromolecules can udergo reversible aggregation changes, depending on dark or light conditions [5].

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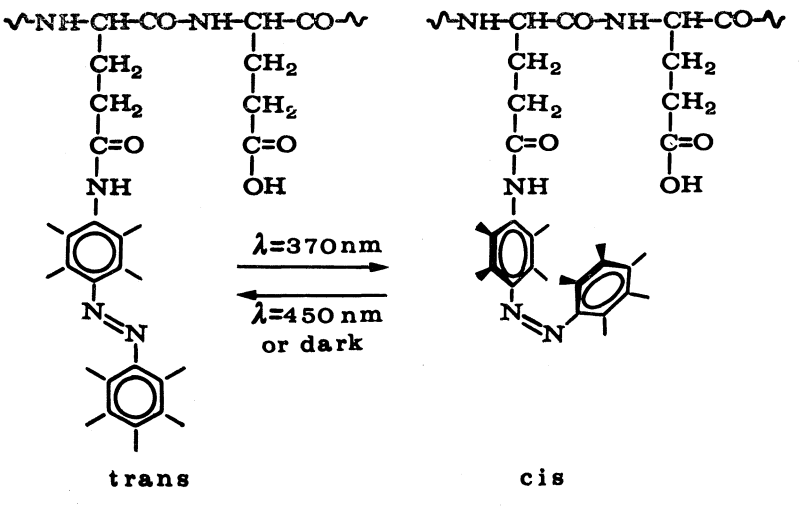
(****) Nella seduta del 22 novembre 1985.

In order to obtain a better understanding of the above processes at molecular level, CD spectra of poly(L–glutamic acid) containing variable amounts of azobenzene groups and in various solvents have been reviewed and compared with new data.

SCHEME



Results and Discussion

a) CD *in organic solvents : effect of azobenzene content.*

When the azobenzene is in the trans planar configuration, the CD spectrum of azo-modified poly(L–glutamic acid) is characterized by a couplet in the region of the A-band of the azo chromophore [3]. In the region of the peptide absorption bands, no influence of the aromatic groups seems to exist and the spectra are similar to those of polypeptides with transparent side chains [6]. CD data for samples containing 16 to 84% mol of trans-azobenzene groups in helicogenic non-protonating solvents, such as trimethylphosphate (TMP) and dimethylformamide (DMF), are reported in Table I.

The spectra between 400 and 300 nm show a positive couplet which seems to be connected to dipole-dipole electronic interactions between the side chains disposed along the polypeptide chain [3]. This last, as the CD data in the peptide region clearly indicate, is substantially in the α-helix conformation. The content of helix is dependent on chemical composition, reaching the minimum value for the polymer containing 36% azo groups and increasing again for higher contents. The azobenzene couplet, on the other hand, increases with the increasing of the azo-content, as expected assuming the exciton ori-

TABLE I.

Dependence of CD *Features* [a] *of Azo-Modified Poly*(L-*glutamic acid*) *on Trans-Azobenzene Content, in Organic Solvents.*

Trans-Azo mol. %	Solvent	Azo Absorp. Region λ_{max}, nm	Azo Absorp. Region $[\theta'] \times 10^{-3}$	Peptide Absorp. Region λ_{max}, nm	Peptide Absorp. Region $[\theta] \times 10^{-3}$
16	TMP	370	+ 3.0	222	— 30.0
		330	— 1.0	208	— 32.0
21	TMP	370	+ 7.5	222	— 22.5
		330	— 3.5	208	— 25.5
36	TMP	370	+ 12.0	222	— 19.5
		330	— 7.0	208	— 23.0
52	TMP	370	+ 45.0	222	— 27.0
		330	— 35.0	208	— 25.0
56	TMP	370	+ 72.0	222	— 33.0
		330	— 44.0	208	— 30.0
84	DMF	370	+ 27.5	nd.	nd.
		330	— 15.0	nd.	nd.
84	HFP	355	+ 7.5	222	— 25.0
		315	— 12.0	208	— 29.0
				190	+ 50.0

(*a*) The values are limited to the maxima and typical inflexion points.

gin [3]. In the above solvents, the trans-to-cis photoisomerization does not affect the backbone conformation, while the CD couplet of the azo group completely disappears.

These data clearly indicate that in these non-protonating solvents, the helix is stable enough to be unaffected by the trans-cis photoisomerization. The increased polarity of the cis form with respect to the trans one seems to be unimportant because of the medium. On the other hand, steric effects connected with the change of geometry going from the planar trans form to the skewed cis one probably do not play any role, because of the distance of the photochromic groups from the backbone. (Scheme).

b) CD *in water : hydrophobic effects.*

Only the polypeptides with less than 40% azo groups are soluble in water. In this case the couplet of the azo chromophore is shifted to shorter wavelengths and becomes negative, both when the α-helix is still the predominant secondary

structure (16% azo-polymer at pH 4.7) and when the β-structure seems to be the only ordered structure (36% azo-polymer at pH 5.5). In the latter case the azobenzene shows the strongest negative couplet, at least among the water soluble samples (Table II).

TABLE II.

Dependence of CD *Features of Azo-Modified Poly*(L-*glutamic acid*) *on Trans-Azobenzene Content, in Water Solution.*

Trans-Azo mol. %	Solvent	Azo Abs. Region λ_{max}, nm $[\theta'] \times 10^{-3}$		Peptide Abs. Region λ_{max}, nm $[\theta] \times 10^{-3}$	
16	H_2O (pH 4.7)	340	— 3	222	— 22.5
		310	+ 3.5	208	— 21.5
21	H_2O (pH 5.1)	340	— 5.0	222	— 9.0
		310	+ 6.0	208	— 9.0
36	H_2O (pH 5.5)	340	— 29.0	220	— 6.0
		310	+ 20.5		

More evident indications come from the 21% azo-containing polypeptide. This has a very weak positive couplet and a quite high helix content in TMP/H_2O = 1/1. On storage, the couplet shifts to shorter wavelengths and becomes negative, the ellipticity increasing with time. It may be due to formation of aggregates of helical polypeptide chains which are accompanied by variations of CD spectra in the peptide region [5]. In this case the exciton splitting should be originated from the stacking and hydrophobic interactions between trans-azobenzene moieties belonging to different macromolecular chains. It is remarkable that the amplitude of the negative couplet reaches a limiting value, after 72 h, very close to that observed for the 36% azo-polypeptide in H_2O, where a β-structure is present (Table II and III). The β-structure is the most suitable for the stacking of planar aromatic groups with formation of chiral ordered aggregates of chromophores.

c) CD *in aggregates : photocontrol of polymer solubility.*

The strong influence of azo-content on the conformation in solution and on photoresponsive effects [4, 5] induced us to prepare a polypeptide having a high content of azobenzene groups, by reacting poly(L-glutamic acid) with p.amino-azobenzene in the presence of 100% excess of the azo-reagent and with long reaction times (10 days). The resulting polypeptide contained 84% mol of azo-modified units and was soluble in hexafluoro-2-propanol (HFP) and DMF.

TABLE III.

Dependence of CD *Features of Azo-Modified Poly*(L-*glutamic acid*) *on Storage Time in the Dark, in Organic/Aqueous Solvent Mixture.*

Trans-Azo mol. %	Solvent	Storage time h	Azobenzene Abs. Reg. λmax, nm $[\theta'] \times 10^{-3}$		Peptide Abs. Region λmax, nm $[\theta] \times 10^{-3}$	
21	TMP/H_2O (1/1)	0	370	+ 1.0	222	— 24.5
			330	— 1.0	208	— 24.0
					192	+ 59.0
21	TMP/H_2O (1/1)	24	340	— 8.0	225	— 19.0
			310	— 6.0	208	— 12.0
					192	+ 28.0
21	TMP/H_2O (1/1)	48	340	— 22.0	225	— 17.00
			310	+ 13.0	208	— 8.0
					192	+ 24.0
21	TMP/H_2O (1/1)	72	340	— 24.5	nd.	nd.
			310	+ 19.0		
84	HFP/H_2O (17/3)	0	365	— 75.0	222	— 16.8
			320	+ 40.0	208	— 8.0
					192	+ 50.0
84	HFP/H_2O (17/3)	4	COMPLETE PRECIPITATION			

In the former solvent, the CD spectrum shows the typical dichroic bands of the α-helix, and the usual positive couplet in the azobenzene absorption region (fig. 1). Tihs couplet however, has a lower ellipticity than in less polar DMF and it is less intense than the analogous couplets observed for the 36% and 52% azo-containing polymers in TMP (Table I). This result may be due to the relative polarity of HFP. Addition of only 15% water causes an immediate inversion of the couplet sign with drastic variations of the spectrum in the peptide region, as a consequence of aggregation phenomena (fig. 1). Storage of this last solution in the dark produces the complete precipitation of the polymer in 4 hours. Irradiation at 338 nm makes the polymer dissolve again, with complete recovery of the CD spectrum in the peptide region (fig. 1, dashed line). The irradiated polymer displays only very weak bands in the azo absorption region, as a consequence of the non-quantitative photoconversion of the chromophore from the trans to the cis form. Therefore, poly(L-glutamates) with high contents of azobenzene groups are insoluble in aqueous solution; irradiation at 340-350 nm produces the dissolution of the polymer whereas dark adaptation or irradiation at 450 nm again gives precipitation. The conformation and aggregation changes, as well as the dissolution-precipitation

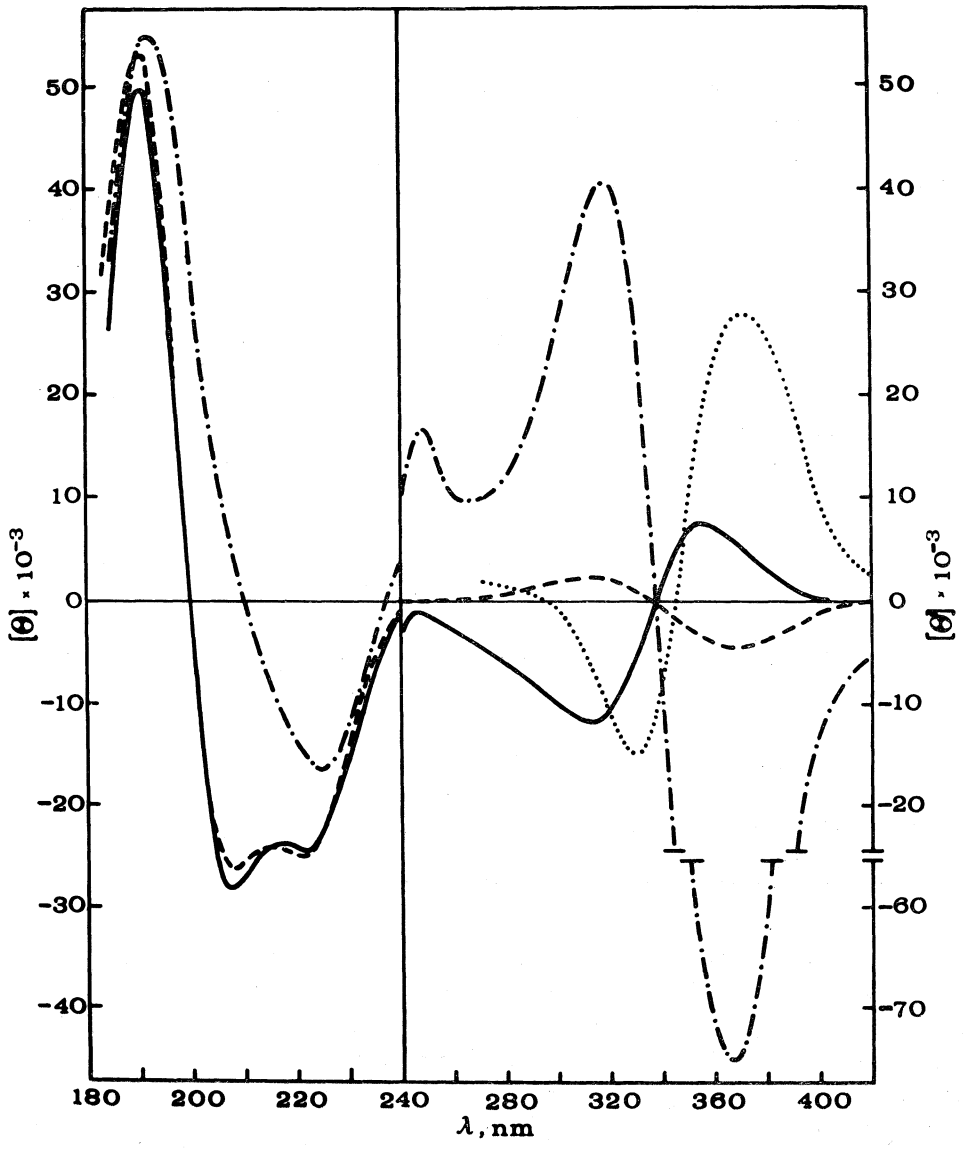


Fig. 1. - CD spectra of poly (L-glutamic acid) containing 84% mol azobenzene groups:
———— in HFP, before irradiation;
..... in DMF, before irradiation;
-.-.-.- in HFP/H_2O = 85/15, before irradiation;
------ in HFP/H_2O = 85/15, after irradiation at 338 nm.

cycles can be reversibly repeated several times, without apparent fatigue, allowing the photoregulation of conformation and aggregation, and the photocontrol of polymer solubility.

CONCLUSIONS

It is likely that the different polarity and geometry of the trans and the cis forms of the azo moieties provide the driving force for the photoresponsive effects. Aggregation may be formed between azobenzene groups in the presence of water, through hydrophobic interactions and ordered stacking of azo groups. These interactions are favoured in dark adapted samples, as trans-azobenzene moieties are essentially planar and very hydrophobic. Light induces the disaggregation process, as the cis form is not planar and much more polar, thus inhibiting the associative conditions and enhancing the polymer solubility.

The results provide a convincing indication that the photoresponsive effects observed in the described photochromic polypeptides can be well monitored by CD spectroscopy. Calculation of CD parameters in the azobenzene absorption region, in due course in cooperation with the Chemistry Department—University of California—Berkeley (Prof. I. Tinoco Jr.), are expected to provide further support and a better quantitative picture.

EXPERIMENTAL PART

Azo modification of poly(L–glutamic acid), irradiation and CD measurements have been performed as described in previous papers [3, 4].

Below 250 nm, CD data are expressed in terms of molar ellipticity [θ] based on the mean residue weight. Above 250 nm, the molar ellipticity [θ′] is based on the azo-Glu residue.

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