Forschung, Wissenschaft

Chiral Synthetic Macromolecules as Models for Biopolymers*

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Abstract

Chiroptical properties of chromophoric systems chemically attached to the side chains of polypeptides are described, in view of conformational analysis of biopolymers.
Advantages arising from the use of inherently chiral chromophores and photochromic systems, as suitable conformational probes, are discussed.
Use of photochromic macromolecules, as synthetic models for photoregulated biological polymers, is also pointed out.

Introduction

It is well known [1] that the three standard conformations α-helix, β-sheet, and random coil, on which the conformational analysis of polypeptides and proteins is based, are characterized by markedly different chiroptical properties.
Circular dichroism (CD) spectra of α-helical polypeptides show two negative bands at 222 nm ($[\theta] -$ 35,000) and 208–210 nm ($[\theta] - 30{,}000$), and a positive band at 192 nm ($[\theta] + 50{,}000$). Although small variations of maximum ellipticity and wavelength are observed depending on polymer sample and conditions, the described spectrum is highly characteristic of simple α-helical polypeptides.
The CD features of the standard β-structure are a negative band at 217 nm ($[\theta] - 16{,}000$) and a positive band at 195 nm ($[\theta] + 21{,}000$), which are more sensitive to polymer sample and external conditions than the α-helix bands.
CD spectra of random coil polypeptides reveal a weak positive band at 218 nm ($[\theta] + 2{,}000$) and a strong negative band at 195 nm ($[\theta] - 30{,}000$).
These reference spectra are usually obtained from model systems such as synthetic polypeptides, for which a more detailed characterization can be carried out, because of their regular primary and secondary structure. The use of the model CD spectra makes it possible to analyse the more complex CD spectrum of proteins in terms of the three parameters α-helix, β-sheet and random coil [2].
The major interfering factor in this analysis is given by prosthetic groups [3] and aromatic side chains [4–6] which can give CD bands in the spectral region of the peptide chromophore. The problem is well pointed out by the investigation of polypeptides containing aromatic residues and optically active hydrocarbon polymers with aromatic side chains.
The former show very complex CD spectra which do not allow direct identification of the secondary structure [5]. More troublesome study of CD spectra of copolymer series has been necessary to derive the conformation of these polypeptides with non-transparent side chains [6]. It has thus been possible to show that also polymers from L-phenylalanine [7], L-tyrosine [8] and L-tryptophan [9] assume, in suitable solvents, α-helical conformations, which are however characterized by CD spectra markedly different from α-helical polypeptides with transparent side chains. The interference of the aromatic group transitions with peptide electronic transition has been also demonstrated by calculation of chiroptical properties [10].
Evidence that aromatic chromophores included in an ordered macromolecular chain give CD bands of similar ellipticity and in the same region of the peptide chromophore is given also by a quite different approach. Coisotactic copolymers of optically active α-olefins with styrene, where the aromatic group is the only absorbing moiety over 180 nm, show strong CD bands between 270 and 190 nm; they have been associated with the electronic transitions of benzene groups disposed along one screw sense helical chain [11]. Fig. 1 shows the CD curves of one of such co-

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polymers and of poly(γ-methyl-L-glutamate) in α-helical conformation. It can be easily understood that the CD spectrum of an α-helical polypeptide with aromatic side chains will result from the combination of the two spectra, thus making direct conformational analysis impossible.

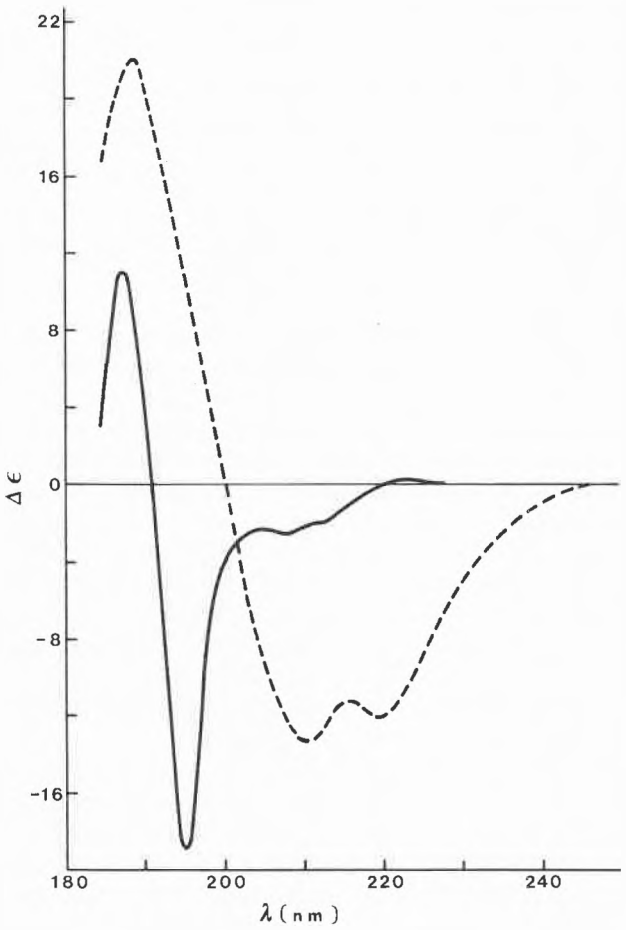


Fig. 1: CD spectra of a styrene/(R)-3,7-dimethyl-1-octene 20/80 coisotactic copolymer (full line) and poly(γ-methyl-L-glutamate) (dashed line) both in a righthanded helical conformation (after ref. [4]).

However, as extrinsic CD bands are related to chain conformation, the aromatic chromophore may be used for investigating secondary structure under conditions masking the peptide absorption bands, thus acting as a "conformational probe".

Suitable conformational probes should absorb at markedly higher wavelength than the peptide group and give rise to strong CD bands, to allow their use in relatively small amount.

In the present review paper we will first mention synthetic polypeptides containing nitrobenzyl and naphthyl groups. However larger emphasis will be given to the use of inherently chiral groups and photochromic azo groups, as they display the peculiar properties requested for conformational probes.

Polypeptides with nitrobenzyl and naphthyl groups in the side chains

The introduction of a nitrobenzyl chromophore into the side chains of poly(L-aspartate) and poly(L-glutamate) makes it possible to study the influence of side chains on the secondary structure [12–13]. The nitrobenzene group was chosen because: a) it presents three absorption bands in the 300–350 nm region, well away from the peptide region. b) These bands can be optically active and then it is possible to study, simultaneously, intrinsic and extrinsic CD bands.

Poly(β-benzyl-L-aspartate) assumes left-handed helical conformation in $CHCl_3$, but the introduction on the benzene ring of a nitro group induces a reversal of the helix sense to a right-handed form. This change takes place at about 20% nitro content when the nitro group is in the *para* position, and at about 50% when the nitro group is in the *ortho* position. The transition can be followed by measuring the ellipticity of the CD band of the nitrobenzyl chromophore at 330 nm [12].

In the case of poly(*o*-, *m*- and *p*-nitrobenzyl-L-glutamates), CD investigation showed that the symmetrical nitrobenzyl chromophore is optically active both in helicogenic (dichloroethane) and nonhelicogenic (dichloroacetic acid) solvents. From the dichroic band related to the $n-\pi^*$ transition of the nitrobenzyl chromophore (330 nm) the helix-coil transition curve induced by addition of dichloroacetic acid was determined [13].

Conformational studies in aqueous solution have been carried out also on copolymers of L-glutamic acid and *o*-nitrobenzyl-L-glutamate [14]. The copolymers show a *p*H dependence of the extrinsic 340 nm CD band, related to the helix-coil transition. For these polymers, however, the variation of $[\theta]_{340}$ does not parallel exactly the variation of $[\theta]_{222}$ and some discrepancies were observed between the *p*K values obtained either plotting $[\theta]_{340}$ or $[\theta]_{222}$ [14].

The naphthyl group has been also studied as potential conformational probe when attached to polypeptide chains. Poly(γ-1-naphthylmethyl-L-glutamate) [15–16] shows two negative CD bands at 215–220 nm and 230 nm, respectively. Another weak band seems to be present at 365 nm [16]. Poly(β-1-naphthylmethyl-L-aspartate) [17] shows a positive band at 220 nm, a negative band at 230 nm and a weak positive band at 280 nm.

These spectra are markedly different from those of standard polypeptide structures. The complex behaviour arises from strong interactions among the naphthalene side chains and between side chains and the backbone, as confirmed by excimer fluorescence.

Poly(L-naphthylalanine) and copolymers of naphthylalanine and γ-benzyl-L-glutamate have also been reported [18]. The CD spectrum of the copolymer containing a low naphthylalanine content (9.7%) is practically that of a pure α-helix, but the spectra become

more and more complex with increasing content of aromatic residues, due to dipole-dipole interactions among the naphthalene chromophores. Extrinsic CD bands at 280 nm, on the other side, are present only in copolymers with high naphthalene contents.

Polypeptides with inherently chiral chromophores in the side chains

Unsaturated peptides containing two dehydrophenylalanine residues, bis(dehydro-Phe), and a C-terminal L-amino acid show strong CD bands in the 280–300 nm region [19–20]. These bands are consistent with the existence in solution of an inherently chiral chromophore generated from the dissymmetric disposition of the two dehydro-Phe residues in a rigidly fixed conformation (Fig. 2).

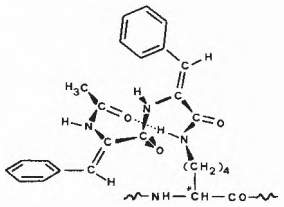


Fig. 2: Chiral disposition of the aromatic groups in the bis(dehydro-Phe) moiety attached to the ε-amino group of L-lysine residues (after ref. [20]).

The above chromophoric system can be easily introduced into the side chains of polypeptides or proteins containing lysine residues, carrying out the reaction at room temperature, in water/acetone solution (Scheme 1). Accordingly, a copolymer poly($Glu^{92}Lys^{8}$) was modified with 2.8 mole % of bis(dehydro-Phe) groups attached to the ε-amino functions of the lysine residues [21].

The modified polypeptide displays appreciable CD bands in the near-UV region, the shape of the curves depending on *p*H. At *p*H 5.0, where the polymer assumes an α-helix conformation, the spectrum shows an extrinsic negative band at 280–300 nm. At *p*H 8.5, where the polymer assumes coil conformation, the CD band at 300 nm has comparable intensity, but positive sign (Fig. 3).

Low molecular weight compounds containing the same chromophoric system also show CD spectra with bands in the near-UV region, which however do not show any *p*H dependence. Therefore the extrinsic CD bands observed in the polymer must be attributed to the perturbation of the inherently chiral bis(dehydro-Phe) group by the conformation of the macromolecular chain.

These results suggest that thebis(dehydro-Phe)chromophore can be a very suitable conformational probe, both for the easy attachment to polymers, as well as for its spectral properties. In fact: a) the modification

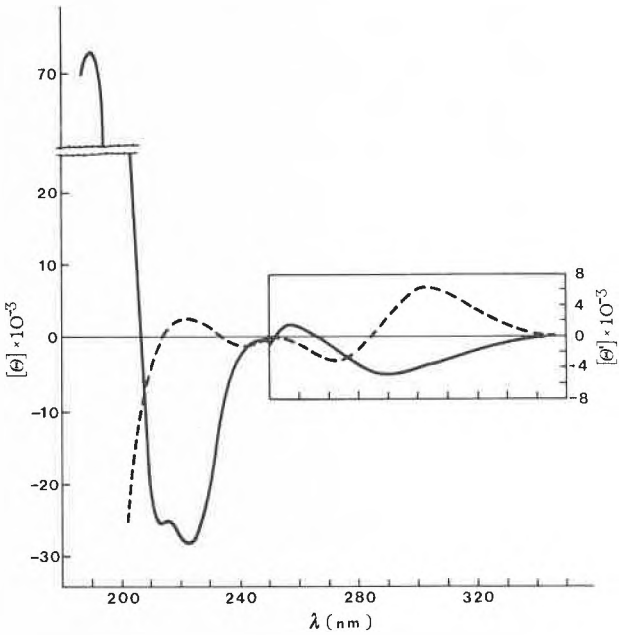


Fig. 3: CD spectra of poly($Glu^{92}Lys^{8}$) containing 2.8 mole % of bis(dehydro-Phe) chromophore; ——— *p*H 5.0; ----- *p*H 8.5 (after ref. [21]).

reaction proceeds under mild conditions which do not damage sensitive polymers such as proteins. b) The induced CD bands are in an accessible spectral region far away from the peptide region. c) The inherently chiral probe supply high intensity CD bands. d) Small amount of chromophores (less than 3 %) suffices for conformational investigation.

The validity of these conclusions is proved by the determination of the *p*K of the helix-coil transition of the polypeptide($Glu^{92}Lys^{8}$)$_n$ by recording the CD variations induced in the probe at various *p*H values. The plotting of the ellipticity at 305 nm vs *p*H shows a sharp variation at *p*H 6, which is in good agreement with values determined with independent techniques [21, 22].

Polypeptides with photochromic azo groups in the side chains

a) Characteristics of the azobenzene chromophore

The conformational analysis based on the optical properties of extrinsic chromophores attached to macromolecules can be greatly improved by using photosensitive molecules such as photochromic azo-dyes [23–28]. In fact, azo-aromatic molecules undergo reversible *trans* ⇄ *cis* isomerization upon exposure to light and dark. The two isomers differ markedly both in stereochemistry and electronic transitions. Thus, their introduction into polypeptide chains [29–30] provides very useful parameters to relate the induced optical properties with the conformation of both side chains and backbone.

Reaction of poly(L-glutamic acid) with p. amino-azobenzene in dimethylformamide, in the presence of di-

cyclohexylcarbodiimide (DCCI) as condensating agent, gives polymers soluble in chloroform and trifluoroethanol (TFE) [31]. When the reaction is carried out in the presence of DCCI and one equivalent of N-hydroxybenzotriazol, polymers soluble in trimethylphosphate (TMP) and water are obtained [32].

The absorption spectra of a typical azo-polypeptide are shown in the Fig. 4. The dark-adapted sample exhibits an intense band at about 350 nm which strongly decreases and shifts towards 330 nm upon irradiation. This band is associated with a $\pi - \pi^*$ transition, while the weak band centered at 450 nm is associated with the $n - \pi^*$ transition of the azo chromophore.

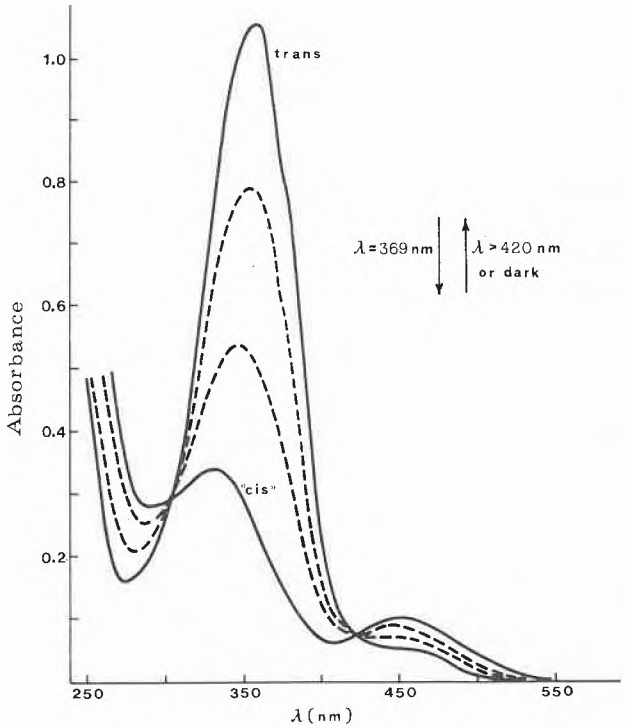
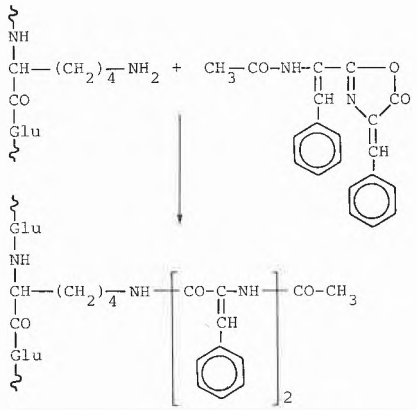


Fig. 4: UV and visible absorption spectra of poly(L-glutamic acid) containing 14% mole of azo groups in the side chains ($c = 0.0424$ g/l in $CHCl_3$; $l = 1$ cm) (after ref. [31]).

At room temperature in the dark, azobenzene groups are in the stable *trans* configuration. Irradiation gives rise to photoisomerization to the metastable *cis* isomer, the position of the photochemical stationary state being determined by the absorbance of each isomer at the wavelength of the incident light (Scheme 2). Irradiation at 369 nm produces rather complete *trans*-to-*cis* photoconversions (90% yield) in TMP, $CHCl_3$ and TFE, but markedly lower photoconversions (30–35%) in water. The opposite *cis*-to-*trans* isomerization occurs by irradiating at wavelengths longer than 420 nm or by thermal decay in the dark.

Scheme 1



b) CD spectra and conformation in different solvents

Azo-polypeptides soluble in chloroform and TFE display, in these solvents, the typical CD spectrum of α-helix, independent of irradiation and the azo configuration. In chloroform solution, intense bands are present in the 300–500 nm region, strongly depending on irradiation of the sample (Fig. 5). The couplet of bands observed at about 350 nm in the dark-adapted sample (all-*trans* chromophores) has been associated with a splitting of the $\pi - \pi^*$ transition originated by dipole-dipole interactions among azo side chains [31].

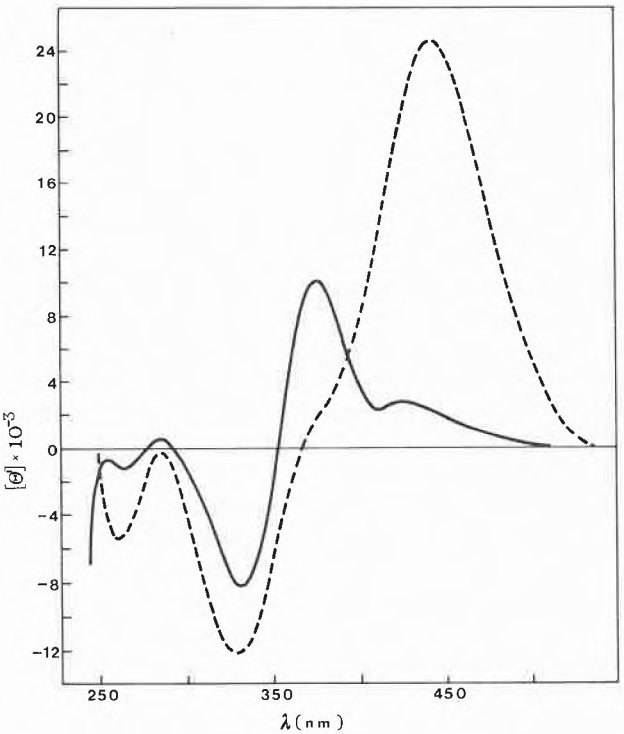
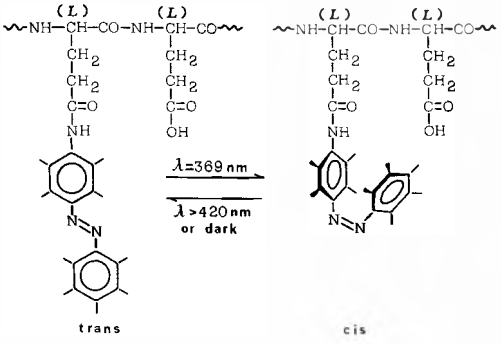


Fig. 5: CD spectra in $CHCl_3$ of poly(L-glutamic acid) containing 8% mole of azo groups in the side chains, before (——) and after (-----) irradiation at 369 nm (after ref. [31]).

The strong CD band at 450 nm observed in the irradiated sample (90% *cis* chromophores) has been associated with the inherent dissymmetry of azobenzene moieties, with a prevalence of one skew sense. In TFE solutions, only weak extrinsic bands were observed in the 300–500 nm region, clearly indicating that α-helix cannot be the only factor responsible for

the high optical activity of the side chains in $CHCl_3$. A "super-helix" [33] stabilized by hydrogen bonds between side chains has been suggested [31]. This super-helix is probably destroyed in TFE, which is a strong hydrogen-bonding acidic compound, but it is likely to survive in chloroform. Anyway, CD measurements provide evidence of the existence in solution of ordered chiral structures involving side chains up to the azo chromophore rather far from the backbone (Scheme 2).

Scheme 2



The CD spectra of an azo-polypeptide soluble in TMP are shown in Fig. 6. The dark-adapted sample displays a spectrum analogous to that in chloroform (Fig. 5); after irradiation (*trans*-to-*cis* isomerization), the spectrum does not change in the peptide absorption region, but it does not longer show dichroic bands around 450 nm.

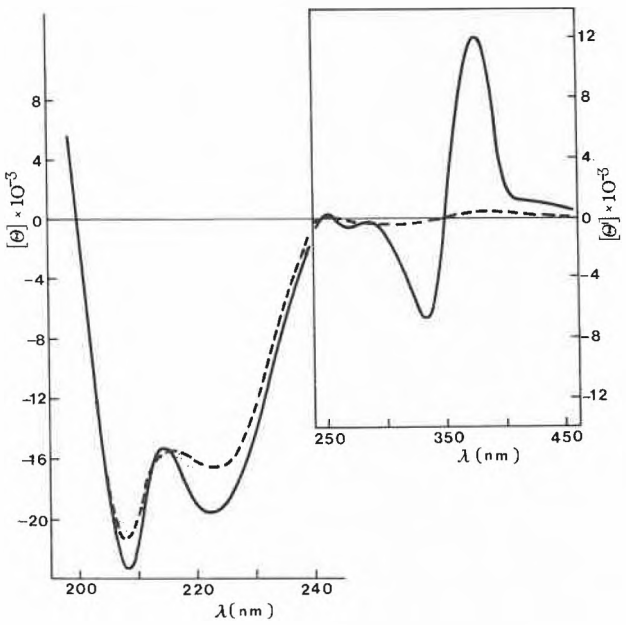


Fig. 6: CD spectra in TMP of poly(L-glutamic acid) containing 36% mole of azo groups, before (——) and after (-----) irradiation at 369 nm [32].

In water solution, the «*trans* polymer» CD spectrum is markedly affected by *p*H in both peptide and azo regions (Fig. 7). In the former region, at alkaline *p*H, the typical curve of the random coil conformation is observed, changing into that of a β-type ordered structure with decreasing *p*H to 5.5. In the latter region, a negative couplet is present, the amplitude of which increases with increasing content of the ordered structure. This clearly demonstrates that the couplet is associated with the regular conformation of the polypeptide chain, the opposite sign observed in the α-helix with respect to the β-structure being understandable considering the different relative geometries of the azo groups in the two conformations. The couplet in the irradiated sample does not disappear but it is only reduced (to about 50%) at any *p*H because of the low ($\sim$ 30%) photoconversion in water.

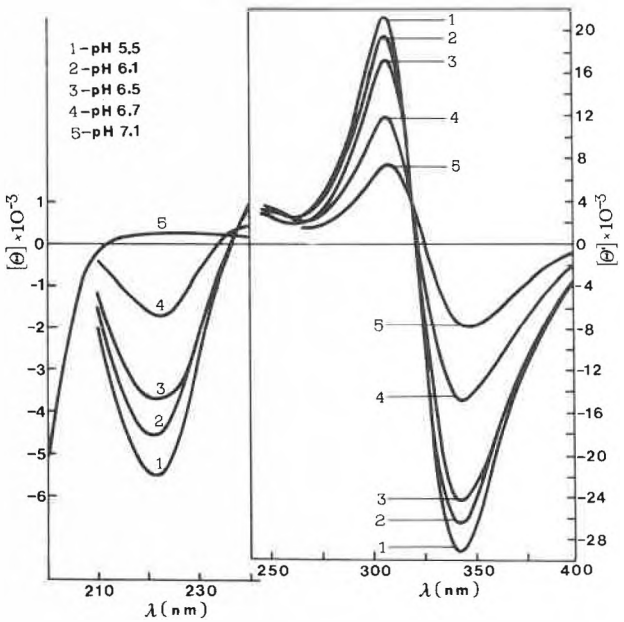


Fig. 7: CD spectra of dark-adapted poly(L-glutamic acid) containing 36% mole of azo groups, in aqueous solution at different *p*H values [32].

c) Light-induced conformational changes

The plotting of $[\theta]_{220}$ versus *p*H indicates (Fig. 8) that the order-disorder conformational transition in water occurs with different *p*K values for the dark-adapted sample (*p*K = 6.8) and the irradiated one (*p*K = 6.3). This means that the β-structure has different stability when the azo groups are either all *trans* or 70% *trans* and 30% *cis*. Accordingly, irradiation in the critical range of *p*H between the two *p*K values produces a conformational change of the macromolecular chain, as recorded at *p*H 4.8 and *p*H 6.5, respectively (Figs. 9 and 10). Indeed at *p*H 4.8, where the β-structure is very stable and not affected by azo side chains photoisomerization, the 30% *trans* → *cis* photoconversion gives a strong decrease of the ellipticity of the dichroic bands

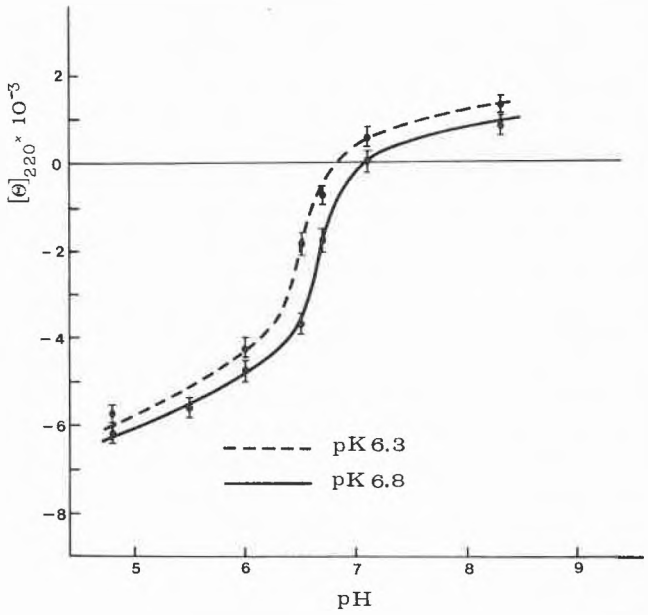


Fig. 8: Order-disorder conformational transition in dark-adapted (——) and irradiated (-----) samples of poly(L-glutamic acid) containing 36% mole of azo groups [32].

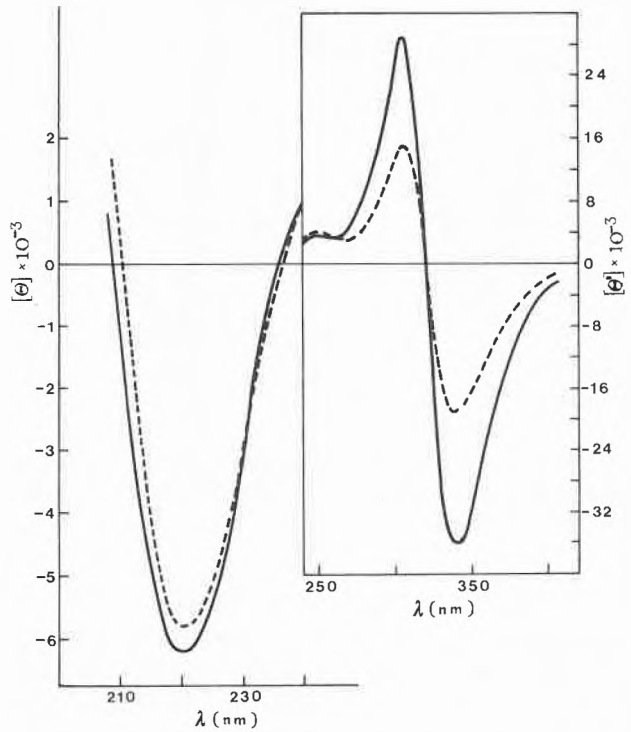


Fig. 9: CD spectra of poly(L-glutamic acid) containing 36% mole of azo groups, before (——) and after (-----) irradiation, in aqueous solution at *p*H 4.8 [32].

in the azo $\pi - \pi^*$ absorption region (350 nm), but no effect in the peptide region (Fig. 9). When the irradiation is carried out at *p*H 6.5, intermediate between the two above *p*K values, an anologous variation of the CD spectrum is observed in the azobenzene main absorption region. Moreover a contemporary remarkable decrease of the ellipticity of the 220 nm band, associated with the β-structure, can be observed (Fig. 10). The lack of variation in this region by irradiating at *p*H 4.8 excludes possible contributions of the azobenzene groups. The conformational change induced by light is completely reversible, exposition of the polypeptide alternately to light and dark conditions reproducing exactly the two expected CD spectra. Analogous variations of the optical properties, associated with *trans* → *cis* photoisomerization of azo groups, have been also observed [34–35] in polypeptides containing phenylazobenzyl-L-aspartate residues.

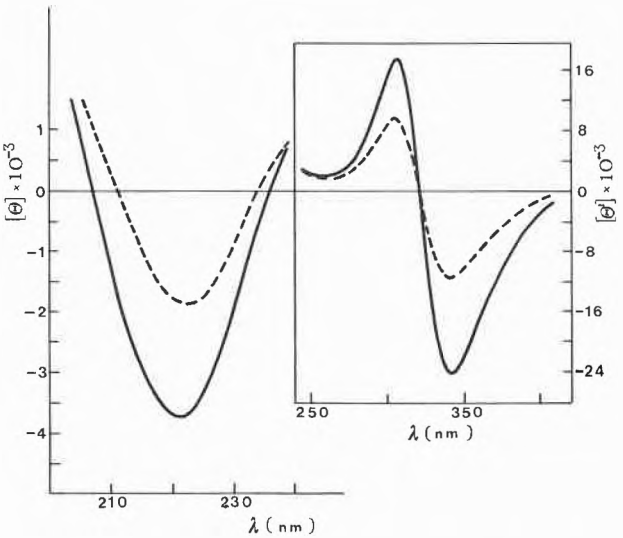


Fig. 10: CD spectra of poly(L-glutamic acid) containing 36% mole of azo groups, before (——) and after (-----) irradiation, in aqueous solution at *p*H 6.5 [32].

However, the spectral behaviour was rather complicated, and photoinduced conformational changes were observed only for homopolymers or copolymers containing rather high contents of azo chromophores.

Final remarks

The examples considered in this review indicate that a properly selected chromophoric group can be conveniently used to study by means of chiroptical techniques, polypeptides and proteins secondary structure in solution. In this connection, the fundamental properties for the potential use of a group as "conformational probe" are:

1. easy attachement to polypeptide side chains under mild conditions and according to a well defined specific chemical reaction.
2. Presence of electronic transitions centered at a wavelength well separated from the peptide group and at relatively low energy, that is in an easily accessible spectral region.
3. Structure capable of originating strong CD bands, thus allowing its use in very small amounts. Indeed

a large amount of extrinsic chromophores may modify the polymer chain conformation. Highly polarizable and/or inherently chiral systems seem to be very suitable.

Additional and useful probes can be found in peculiar molecules which can undergo structural modifications without altering the primary structure of the macromolecule. An example of this type is offered by photochromic azobenzene groups, the shape and steric hindrance of which can be reversibly modified by light irradiation, thus allowing to affect chain conformation by light, as occurs in photoregulated biological processes [36].

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