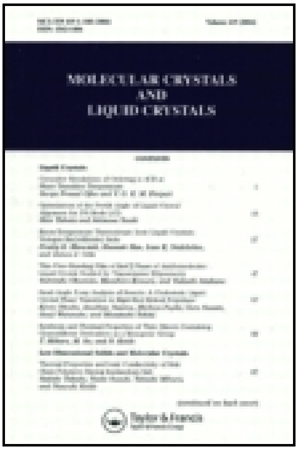

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Thermotropic Photochromic Polypeptides: Polyornithine and Polylysine Bearing Azobenzene Units in the Side Chains

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Thermotropic Photochromic Polypeptides: Polyornithine and Polylysine Bearing Azobenzene Units in the Side Chains

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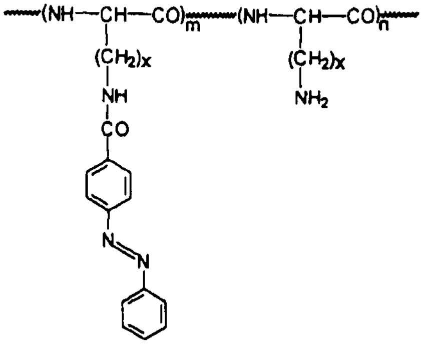
The structure of poly(L-ornithine)s and poly(L-lysine)s containing between 19% and 100% of azobenzene units in the side chains has been studied, by X-ray diffraction, between room temperature and 250°C. Polymers were found to exhibit mesomorphic structures of the smectic A type deriving from the β-structure of polypeptides. The influence of the nature of the amino acid is discussed.

Keywords: thermotropic polypeptides; poly(L-ornithine); poly(L-lysine); azobenzene; smectic structures

INTRODUCTION

Since the discovery of lyotropic properties of Poly(γ-benzyl-L-glutamate) in 1956 [1], a lot of studies showed that in the mesophases the polypepdide main chains exhibit α helical conformation [2-4]. More recently, a series of poly(γ-alkyl-L-glutamates) were synthesized.and the study of their behaviour, as a function of temperature, confirmed the high tendency of poly(L-glutamate) polypeptide chains to adopt the α-helix conformation [5-8].On the contrary poly(L-lysine)s can adopt both the α-helix and β-structure. We have recently sinthesized poly(L-lysine) containing various mole % of azobenzene in the side chains, described their liquid-crystalline behaviour and reported the first observation of a lamellar structure of the smectic A1 type with the polypeptide chains in a β-conformation [9,10]. In the present paper the investigation is extended to poly(L-ornithine)s (x=3) containing azobenzene units in the side chains: their liquid-crystalline structures are described and compared with those of the azobenzene modified polylysines (x=4) in order to analyse the influence of the nature of the amino acid.



EXPERIMENTAL

Synthesis of the polymers

Poly(L-Ornithine). It was obtained by polymerization of N^{δ}-carbobenzoxy-L-ornithine N-carboxy-anhydride, followed by removal of the side chain protecting groups with anhydrous HCl and HBr, according to the procedure reported for poly(L)lysine) [11]. The polypeptide hydrobromide was dialysed against 0.01 N HCl to replace the bromide with chloride counterions. Then it was subjected to ultrafiltration through a Ultra Filter, cutoff 50K, to remove low molecular weight ($M< 50{,}000$) polymers. Finally it was lyophilized to give poly(L-ornithine hydrochloride) as a white material having $[\eta]=0.54$ (1M NaCl, pH 3, 25°C), corresponding to $Mv=115{,}000$ [12].

Azo-modification of Poly(L-ornithine). Pivaloyl chloride (0.41 ml, 3.32 mmol) was added to a cooled solution of p-phenylazobenzoic acid (0.746 g, 3.32 mmol) and triethylamine (0.44 ml, 3.32 mmol) in anhydrous DMF (100 ml). After 15 min the solution was treated with a second precooled solution of poly(ornithine hydrochloride) (0.25 g; 1.66 mmol O residues) and thiethylamine (0.36 ml, 2.52 mmol) in 20 ml of water and 100 ml of DMF. The reaction mixture was left under stirring at 0°C for 1h, and at room temperature for 70h. The solvent was then partially evaporated and the polymer recovered by precipitation with ether. Any unreacted azo-reagent was removed by repeated dissolutions in DMF and precipitations with ether. The azo content, estimated on the basis of molar extinction coefficient of p-phenylazobenzoyl-glicine methyl ester (λ_{max}=324,000, ε_{max}=24,000 in 1,1,1,3,3,3-hexafluoro-2-propanol) was 31 mol%. ^{1}H-NMR (300 MHz, CF_3COOD), d ppm:1.6 (m, 2H, C^a-CH_2), 2.2 (m, 4H, CH_2-CH_2), 3.3 (m, 2H, N-CH_2), 4.4 (m, 1H, $C^{\alpha}H$), 7.5-8.5 (m, aromatic).

X-Ray Diffraction

X-ray diffraction experiments were performed on powder samples with two types of camera: a Guinier-type camera and a pinhole camera [10]. Several exposures were made in order to measure the strongest and the weakest reflections. Intensities of the different diffraction orders were measured with a laboratory made densitometer specially designed and built for that purpose. Experimental amplitudes of diffraction of the different orders of reflections from the smectic layers were corrected for the Lorentz and polarization factors [13] and normalized so that the strongest reflection has an amplitude of one. For instance in the case of Azo-Orn-31 the following corrected amplitudes were: $\mathbf{a}_1$=1 and $\mathbf{a}_2$=0.53

Infra red Spectroscopy

Infra-red measurements were performed with a FTIR spectrometer Nicolet 20SX using KBr samples

RESULTS

Synthesis of the Polymers

Poly(L-ornithine) was reacted with p-phenylazobenzoic acid according to the mixed anhydrides method (see Experimental Section). The modification reactions gave partially modified L-ornithine polymers containing respectively 31 mole % (**Azo-Orn-31**) and 19 mole % (**Azo-Orn-19**) azobenzene units in the side chains. The modification extent was determined spectroscopically and by NMR as in the case of modified polylysines [10]. Sequence distribution of azo units is not known, but it may be assumed to be substantially random.

Liquid-Crystalline Structures

The azobenzene-containing polyornithines were studied by X-ray diffraction between room temperature and 240°C, since a beginning of decomposition was observed at 250°C.

The X-ray diagrams exhibited 3 sharp reflections and a diffuse band (Fig.1).

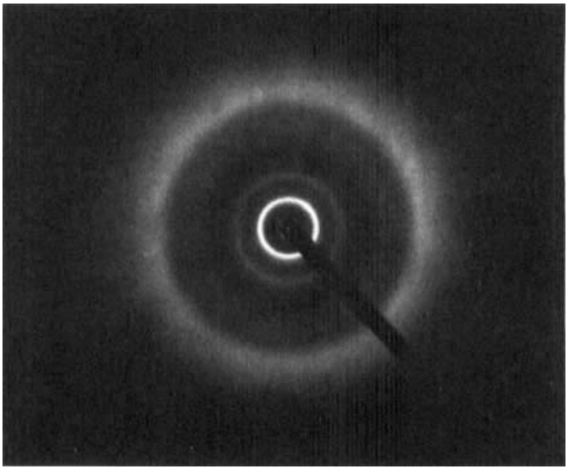


FIGURE 1 Example of pin-hole camera powder X-ray diagram of the azo-substituted poly(L-Ornithine) Azo-Orn-31 containing 31 mole % Azobenzene, showing the 3 sharp reflections and the diffuse band.

The 2 sharp reflections observed at the lowest angles can be indexed as the 00l reflections of a lamellar structure of thickness d. The sharp reflection observed at higher angles corresponds to a repeating distance b of 7.1A. The wide angle band corresponding to a distance a= 5.1A is similar to the distance between mesogenic groups in disordered smectic structures of side chain liquid crystal polymers [14,15].

Such X-ray diagrams suggest a lamellar structure of the Smectic A type deriving from the typical β-pleated-sheet structure of polypeptides and proteins [16]. The antiparallel β-structure is characterized by polypeptide chains having opposite direction linked by a pattern of hydrogen bonds, thus generating sheets. The side chains are perpendicular to the plane of the sheet and point alternatively above and below the sheet. The distance between two consecutive side chains along the same polypeptide chain is reported to be 7.00A [17], while the average distance between two parallel side chains belonging to adjacent macromolecules is about 5A (it varies between 4.5 and 5.5 depending if they point above or below the plane).

The diffuse band observed at a= 5.1 A is in agreement with the average distance between side chains belonging to adjacent molecules. The sharp reflection at

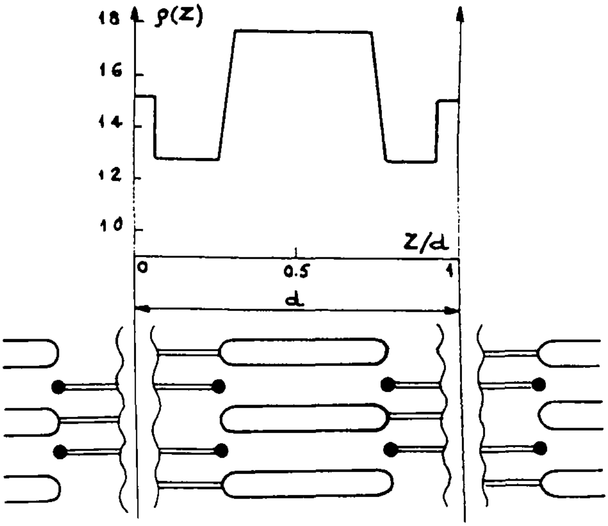


FIGURE 2 Schematic representations of the Smectic structure of the polymers containing 31 and 19 mole % azobenzene

7.1A is well in agreement with the repeat distance of pleated polypeptide chains. Infra Red spectroscopy measurements performed on the powder samples used for X-ray studies showed the presence of Amide A, Amide I and Amide II bands at 3279, 1629 and 1540 cm^{-1}, respectively, thus confirming the presence of an antiparallel β-structure. [17].

TABLE 1 L-Ornithine and L-Lysine polymers having various azobenzene contents in the side chains: Geometrical parame-ters of the liquid-crystalline structures at room temperature

POLYMER	Azo units,mol %	L_1(Å)	d(Å)	b(Å)	a(Å)
Azo-Orn-31	31	21	25.3	7.1	5.1
Azo-Orn-19	20	21	25.3	7.1	5.1
AzoSLys-100	100	24	25.0	7.3	5.1
Azo-Lys-44	44	23	32.0	7.6	5.0
Azo-Lys-41	41	23	31.8	7.5	5.0
Azo-Lys-31	31	23	33.3	7.4	4.8

The comparison of the intersheet spacing d with the length of the azobenzoyl-ornithine units (L_1=21A) and the length of the unmodified ornithine side chains (l=4A) measured on space filling CPK models (Table 1) showed that d is nearly equal to L_1+l (Table 1) and the lamellar structure is of the smectic A type with the side chains perpendicular to the smectic layers, as shematically illustrated in figure 2. Adjacent polypeptide chains are separated by an average distance a=5.1A. The reflection observed at 7.1A corresponds to the periodicity b of the pleats.

Supplementary informations about the structure can be obtained by deducing, from the intensities of the 001 reflections, the electron density profiles, along the z axis perpendicular to the smectic layers. For simmetry reasons ρ(z) can be expressed as a Fourier series containing only the cosine terms [10,18]:

$$\rho(z) = \Sigma\, a_m \cos(m2\pi z/d)$$

Experimentally we measure the intensity I_m of the orders of reflections, so we loose the phase of a_m. But as a_m is real the phase problem reduces to choising the right combinations of sign for a_m (m=1, 2...). For instance $\rho^{-+}(z)$ will correspond to the combination where a_1 is chosen negative while a_2 is chosen positive. As we observe 2 orders of diffraction, we obtain 4 combinations of sign for a_m that is to say 4 electron density profiles $\rho(z)$ that are illustrated in the case of polymer Azo-Orn-31 in Fig.3.

In order to chose between the 4 profiles the physically acceptable one, we have calculated the electron density of the different parts of the repeating unit of the polymer by dividing their number of electrons by their lengths measured on CPK models. We found: 7.8 e^-/A for the skeletton, 6.4e^-/A for the paraffinic spacer and 8.4 e^-/A for the mesogenic azobenzene groups.

In the case of an ortogonal SA smectic structure one must observe a central maximum corresponding to the interdigitated mesogenic groups surrounded by 2 minimums corresponding to the methylene spacers and 2 secondary maximums corresponding to the polymeric skeletton (Fig.2).

The 4 electron density profiles of the disordered smectic A structure of the polymer Azo-Orn-31 are represented on Figure 3(a)-(d). The 2 electron density profiles (c) and (d) must be rejected as they exhibit minimums at the position of the mesogenic cores. The electron density profile (a) must be rejected as it exhibits minimums at the position of the polypeptide main chains. On the contrary the electron density profile (b) corresponding to the combination of signs -+ exhibits a central maximum corresponding to the mesogenic azobenzene cores, 2 minimums corresponding to the spacers and 2 secondary maximums corresponding to the polypeptide chains in agreement with the physically acceptable one (Fig.2).

Therefore the mesomorphic structure of the azobenzene modified ornithine polymers is of the smectic A type and derives from the arrangement of the polypeptide chains in a β-pleated-sheet structure (Fig.2).

Comparison with modified polylysine

Recently we showed that azobenzene modified poly(L-lysine)s exhibit smectic A structures deriving from the β -structure of polypeptides, but the organization of the molecules in the smectic planes depends upon their degree of substitution [10]. For polymers containing free lysine side chains, each smectic layer results

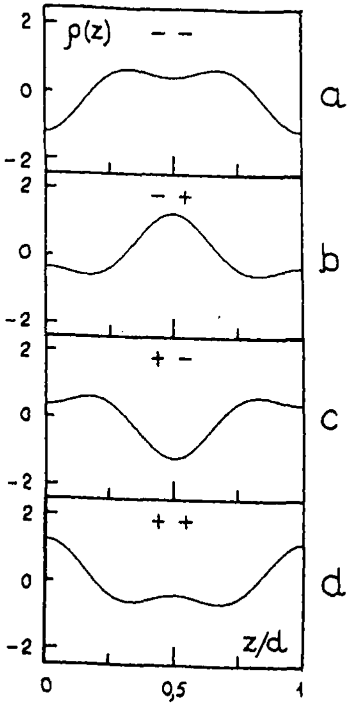


FIGURE 3 Projections of the electron density profiles corresponding to the different signs combinations of a_m for the polymer Azo-Orn-31 containing 31 mole % of azobenzene.

from the superposition of two layers: one contains the free lysine side chains, the other contains the azobenzene-modified lysine side chains and the polypeptide main chains (Fig7 in Ref.10). For polymers where all the lysines have been substituted by azobenzene (Fig.5 in Ref.10), there are no hydrophilic layers. On the contrary, for partly modified polyornithines there is no phase separation between azobenzane modified ornithine side chains and free ornithine side chains (Fig.2). Possibly the shorter length of the methylene spacer does not allow the phase separation. Two other differences are observed between lysine and ornithine deriving polymers: 1) Smectic A mesophases are obtained for lower azobenzene content in ornithine polymers (smaller than 20%) than in lysine polymers (higher than 20%) [10]; 2) if the two types of polymers exhibit only one mesophase (Smectic A) as a function of temperature and the thickness of the smectic layers increases with temperature until it reaches the thickness corresponding to a maximum of interactions between the azobenzene mesogens, the variation of the layer thickness with temperature is much smaller in the case of ornithine (4%) than in the case of lysine [10].

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