One-Dimensional Aromatic Crystals in Solution. 4. Ground- and Excited-State Interactions of Poly(L-1-pyrenylalanine) Studied by Chiroptical Spectroscopy Including Circularly Polarized Fluorescence and Fluorescence-Detected Circular Dichroism

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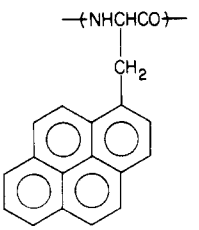
ABSTRACT: A novel aromatic poly(α-amino acid), poly(L-1-pyrenylalanine), was synthesized in the form of a block copolymer with poly(γ-benzyl DL-glutamate) as a solubilizing unit. The polymer solution in trimethyl phosphate and dimethylformamide was subjected to spectroscopic measurements including such chiroptical methods as circular dichroism (CD), circularly polarized fluorescence (CPF), and fluorescence-detected circular dichroism (FDCD). A significant hypochromicity in the absorption spectrum and strong CD Cotton bands indicated the ground-state interaction between pyrenyl groups which are kept in a helical arrangement along the polypeptide chain. The fluorescence spectra showed monomer and excimer emissions. The excimer/monomer intensity ratio of the L polymer was significantly lower than that of the corresponding random copolymer of D- and L-pyrenylalanines. The CPF spectra of the L polymer showed no dissymmetry in the monomer fluorescence but showed negative and positive dissymmetries in the excimer region, indicating the presence of two kinds of excimer species. One component with the negative CPF dissymmetry around 460 nm increased its contribution at higher temperatures, whereas the other with the positive dissymmetry at longer wavelengths than 500 nm was significant at lower temperatures. The FDCD spectrum of the L polymer monitored at $\lambda_{em} > 520$ nm was more intense than that monitored at 420–480 nm. It was therefore concluded that the excimer at the shorter wavelength originates from the excited pyrenyl groups situated in a randomly coiled or partially unfolded part of the polypeptide chain and the excimer fluorescing at longer wavelengths originates from a slightly perturbed region of the helix. The CPF spectrum of the L polymer was compared with those of intermolecular excimers of *N*-acetyl-L-1-pyrenylalanine methyl ester in concentrated solutions of dimethylformamide and toluene. The results suggested that the excimer of the polymer at shorter wavelength is nonpolar and has a definite geometry, whereas that at longer wavelength is polar and favored in dimethylformamide solution. The fluorescence rise and decay analysis supported the above assignments.

Introduction

Molecular electronic devices have been attracting extensive interest as the constituents of molecular computers or as an artificial medium of electron transport for biomedical use.[1] The essential part of the molecular devices consists of a regular array of chromophores in one- or two-dimensional order through which electrons can move.[2,3] Recently, we have shown that some aromatic poly(α-amino acids) are promising candidates for one-dimensional molecular conductors.[4,5] In the case of poly(L-1-naphthylalanine)[4] (poly(L-1-NapAla)) the circular dichroism (CD) showed that the naphthyl groups are helically arranged along the polypeptide chain and their ^1B_b excited state is an exciton state which resulted in an intense couplet in the CD spectrum. The regularity and the rigidity of the chromophoric array were demonstrated by the absence of excimer fluorescence as evidenced by circularly polarized fluorescence (CPF) spectroscopy.[6] There are, however, some disadvantages for naphthyl chromophores. The excitation energy may be too high to accept energy from energy donors. Similarly the electron affinity may be too low to accept electrons from electron donors. Furthermore, the interchromophore interaction may be too weak, because of the small π-system and little transition dipole moment of the fluorescent (^1L_b) state.

In order to obtain a chromophoric poly(α-amino acid) which has aromatic groups of higher electron affinity, we have undertaken a synthesis of poly(L-1-pyrenylalanine) (poly(L-1-PyrAla), I). The spatially extended π-system of the pyrenyl chromophores, when they are arranged helically along the polypeptide chain, will overlap significantly and will be a better candidate for the molecular conductor. There have been a few reports on the synthesis and properties of polymers carrying pyrenyl chromophores,[7,8] but the synthesis of neither poly(L-1-PyrAla) nor the DL copolymer has been reported yet. One of the difficulties in dealing with the aromatic poly(α-amino acids) is their limited solubility in organic solvents. In this study a block copolymer of poly(L- or DL-PyrAla) with poly(γ-benzyl DL-glutamate) was prepared to solubilize the former portion. The poly(γ-benzyl DL-glutamate) unit does not affect the fluorescence spectra and the chiroptical spectra of poly(L-1-PyrAla), since the pyrenyl chromophore absorbs and fluoresces at much longer wavelengths than the benzyl chromophore and the benzyl group of the DL polypeptide does not show any chiroptical properties. Hence, the block copolymer will be referred to simply as poly(L- or DL-PyrAla) hereafter.



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The use of the stereoregular polypeptide chain as a framework for the chromophoric array is of great advantage in that chiroptical spectroscopy is available to investigate the ground- and excited-state conformations and interactions of the chromophoric assembly. Besides the conventional (absorption) CD, CPF spectroscopy was used in this study, which measures the circular dissymmetry of fluorescence emission and affords information on the ge-

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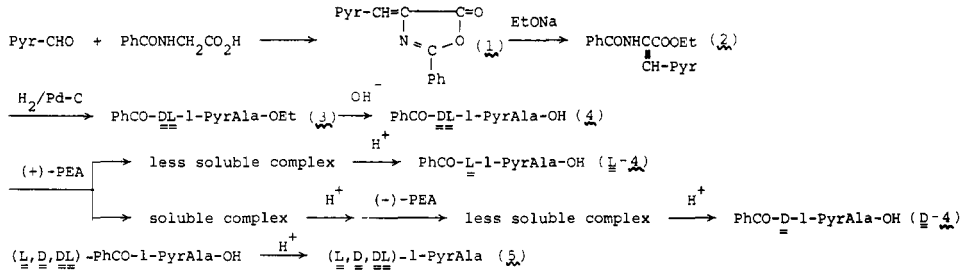
Scheme I



ometry of the excited state of chiral fluorophores.[9,10] Furthermore, fluorescence-detected circular dichroism (FDCD) was employed in this investigation, which measures the dissymmetry in the fluorescence-excitation spectrum.[11] The FDCD spectrum should coincide with the (absorption) CD spectrum when a single fluorophore is present in a dilute and fluid solution.[12-14] However, in the polymeric assemblies of chromophores, the FDCD may be different from CD for the following reasons: (1) The polymeric system usually consists of species with a variety of configurations and conformations, each having a particular fluorescence quantum yield and fluorescence wavelength. (2) Energy migration along the polymer chain is often so frequent that the fluorescence-emitting species is different from the original photoexcited species. In other words, the FDCD spectroscopy provides information concerning these points. In the present study three kinds of chiroptical spectroscopy were complementarily applied to the solution of poly(L-1-PyrAla) at different temperatures and the nature of the ground and excited states of the chromophoric polypeptide was investigated.

A preliminary report on the synthesis and CD and CPF spectra of poly(L-1-PyrAla) has been published.[15]

Experimental Section

Synthesis of Optically Active 1-Pyrenylalanine and Its Derivatives. 1-Pyrenylalanine was synthesized through an oxazolone derivative of pyrene, 2-phenyl-4-(1-pyrenylmethylene)-5-oxazolone (1),[16] as illustrated in Scheme I.[17] After many unsuccessful trials, two different procedures, i.e., enzymatic resolution and diastereomeric complex formation, were found to resolve the optical isomers of derivatives of pyrenylalanine. An enzymatic deacetylation of *N*-acetyl-DL-1-pyrenylalanine (Ac-DL-1-PyrAla) with acylase was useful for the resolution of a very small amount of the substrate. The amino acid obtained by the selective deacetylation was assumed to be an L isomer and the sign of optical rotation of the derivatives of the latter was used as a criterion of L isomers.

Chemical resolution by the formation of diastereomeric complex with chiral amines such as 1-phenylethylamine, brucine, quinine, and quinidine was also attempted. It was found that the combination of *N*-benzoyl-DL-1-pyrenylalanine with (+)- or (−)-1-phenylethylamine in ethanol was successful.

2-Phenyl-4-(1-pyrenylmethylene)-5-oxazolone (1).[16] A mixture of 1-pyrenecarboxaldehyde (5 g), *N*-benzoylglycine (4.4 g), and sodium acetate (2.8 g) was finely powdered and dissolved in acetic anhydride (100 mL). The mixture was heated at 120 °C for 3 h under stirring. After the mixture was cooled, the solvent was evaporated and the residue was poured into a large amount of water. The red precipitate was collected, washed with water, and dried. The yield of crude product was 9 g, which was used for the next step without purification.

Ethyl 3-(1-Pyrenyl)-2-benzamidopropenoate (2). Sodium (2 g) was put into anhydrous ethanol (300 mL) and benzene (100 mL) was added to the solution. The crude crystal of 1 (9 g) was gradually added to the solution with stirring. The solid was dissolved after the reaction was finished (1 h). The mixture was neutralized with acetic acid and the yellow crystal precipitated was collected. The filtrate was evaporated and the residue was dissolved in benzene. Insoluble inorganic materials were removed and the solution was cooled to obtain more of the yellow crystal: yield 6 g; mp 185–187 °C (lit.[16] mp 189–190 °C).

***N*-Benzoyl-DL-1-pyrenylalanine Ethyl Ester (3).** The yellow crystalline 2 (6 g) was dissolved in ethanol/ethyl acetate (1/1 v/v) mixture and hydrogenated with palladium carbon (2 g) at 50 °C for 2 days. The catalyst was removed and the solvent was evaporated. The residue was dissolved in chloroform and the insoluble part was filtered off. The solid obtained after the evaporation of chloroform was recrystallized from ethanol: yield 5 g; mp 168–170 °C. Anal. Calcd for $C_{28}H_{23}NO_3$: C, 79.79; H, 5.50; N, 3.32. Found: C, 79.68; H, 5.57; N, 3.23.

Optical Resolution of 3. *N*-benzoyl-DL-1-pyrenylalanine ethyl ester (PhCO-DL-1-PyrAla-OEt), 3 (4 g), was dissolved in ethanol (200 mL) and 20 mL of aqueous NaOH solution (2 M) was added. The mixture was heated at 50–60 °C for 1 h with stirring. Neutralization of the solution with hydrochloric acid yielded a crystalline *N*-benzoyl-DL-1-pyrenylalanine, 4: yield 3.6 g; mp 212–213 °C. The latter (3.6 g) was then suspended in ethanol (300 mL) and 1.1 g of (+)-1-phenylethylamine was dissolved in the mixture with heating. A crystal of diastereomeric complex, which appeared by a gradual cooling, was collected and recrystallized from ethanol, yield 1.7 g. Filtrates were combined together and acidified with hydrochloric acid to recover the *N*-benzoyl derivative (2 g). The D-rich *N*-benzoyl amino acid was again suspended in ethanol (200 mL) and the above procedure was repeated by using 0.7 g of (−)-1-phenylethylamine. The yield of the diastereomeric complex with (−)-1-phenylethylamine was 1.5 g. Each diastereomeric complex was recrystallized twice from ethanol. The complex (1 g) was dissolved in ethanol (100 mL), and 200 mL of 1 M hydrochloric acid was added to precipitate the free *N*-benzoylpyrenylalanine. Comparison of the optical rotation of the *N*-benzoyl amino acid with that obtained by the enzymatic resolution followed by *N*-benzoylation indicated that the (+)-1-phenylethylamine forms a complex with *N*-benzoyl-L-1-pyrenylalanine and the (−)-amine with the D isomer. L-4: $[\alpha]_D$ −232.5° in dimethylformamide (DMF) (*c* 5.0 mg/mL); mp 230–234 °C (dec). D-4: $[\alpha]_D$ +233° in DMF (*c* 5.0 mg/mL); mp 230–234 °C (dec).

L, D-, and DL-Pyrenylalanines (PyrAla's, 5). The corresponding *N*-benzoyl amino acid (1 g) was suspended in a mixture of acetic acid (100 mL) and concentrated hydrochloric acid (25 mL) and refluxed for 12 h. The deblocked amino acid was precipitated by neutralization with aqueous NaOH solution, washed with water, and dried under vacuum: yield 0.5–0.6 g; mp 240–250 °C (dec) for L, D, and DL isomers. [lit.[16] mp 255–258 °C (dec) for DL isomer].

L- and D-Pyrenylalanine Methyl Ester (6). L- or D-pyrenylalanine was dissolved in methanol saturated with HCl and the mixture was refluxed for 2 h. The amino acid ester hydrochloride was obtained after the evaporation of the solution and recrystallized from methanol. The hydrochloride was then suspended in aqueous $NaHCO_3$ solution and the free amino acid ester was extracted with chloroform. The extract was dried with Na_2SO_4, evaporated, and recrystallized from methanol: yield 30 mg; mp 79.5–80.5 °C (for both L and D isomers).

Optical Purity of 6. Proton NMR spectra of 6 in the presence of 0.6 equiv of tris[3-((trifluoromethyl)hydroxymethylene)-*d*-camphorato]europium(III) in $CDCl_3$ were measured. Figure 1 shows the NMR spectra in the O–CH_3 proton region of L- and D-PyrAla-OCH_3, and a mixture of L (0.6) and D (0.4) isomers. Under these conditions, the mixture showed two O–CH_3 peaks, whereas each enantiomeric ester showed a single peak with a

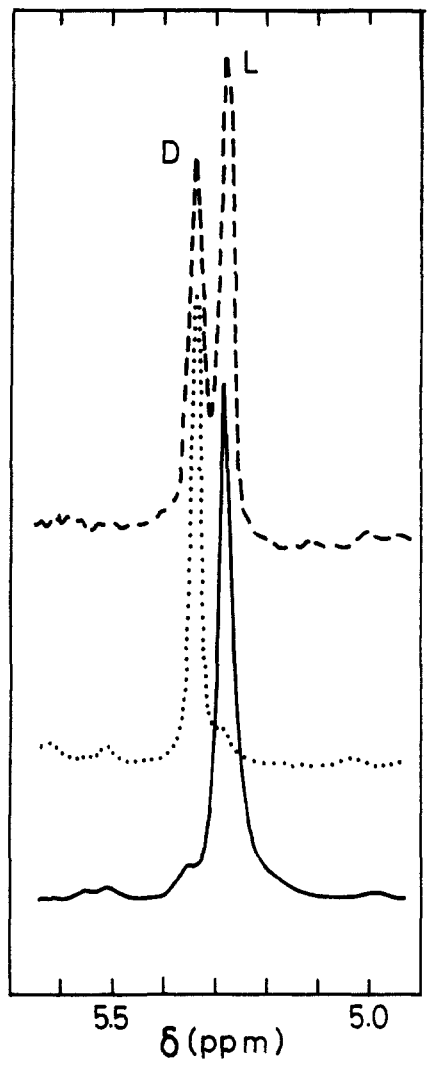


Figure 1. ^{1}H NMR spectra of 1-pyrenylalanine methyl ester in the presence of 0.6 mol of tris[3-((trifluoromethyl)hydroxymethylene)-*d*-camphorato]europium(III) in deuteriochloroform: (—) L isomer, (···) D isomer, (- - -) mixture of L (0.6) and D (0.4) isomers.

shoulder due to the optical antipode. The optical purity calculated from the spectra was 97–98% for each enantiomer.

***N*-Acetyl-L- and D-pyrenylalanine Methyl Esters (Ac-PyrAla-OMe, 7).** L- or D-pyrenylalanine methyl ester (20 mg) was dissolved in ethyl acetate, and 0.1 mL of acetic anhydride and 0.5 mL of pyridine were added. After standing overnight, the mixture was washed with dilute hydrochloric acid, dilute aqueous $NaHCO_3$ solution, and water and dried with Na_2SO_4. Recrystallization from methanol gave the acetyl derivative: mp 193–195 °C (dec) for L isomer, mp 194–196 °C (dec) for D isomer. Anal. Calcd for $C_{22}H_{19}NO_3$: C, 76.49; H, 5.54; N, 4.06. Found for L isomer: C, 76.19; H, 5.54; N, 4.03.

L- and DL-1-Pyrenylalanine *N*-Carboxy Anhydride (PyrAlaNCA, 8). Trichloromethyl chloroformate (0.5 mL) was added to freshly distilled tetrahydrofuran (50 mL) and warmed at 60 °C for 1 h to generate phosgene in the solution. To the phosgene solution, 100 mg of L or DL amino acid was added and stirred at 50 °C for 3 h, until the amino acid was dissolved. The solvent was evaporated and the residue was poured into a large amount of hexane. The precipitate was collected, washed with hexane, and dried under vacuum. The crude NCA was recrystallized from anhydrous ethyl acetate: yield 30 mg; mp 199–204 °C (dec) for DL isomer, mp 216–220 °C (dec) for L isomer. Anal. Calcd for $C_{20}H_{13}NO_3$: C, 76.18; H, 4.16; N, 4.44. Found for L isomer: C, 75.90; H, 4.25; N, 4.30. Found for DL isomer: C, 76.05; H, 4.45; N, 4.25.

Preparation and Characterization of Poly(L- and DL-pyrenylalanines). Since the homopolymer of L-1-pyrenylalanine was found to be insoluble in any solvents, a block copolymer with γ-benzyl DL-glutamate was prepared in DMF. The polymer solution was subjected to spectroscopic measurements without isolating the block copolymers.

An equimolar mixture of γ-benzyl L- and D-glutamate *N*-carboxy anhydride (each 400 mg) was dissolved in DMF (4 mL) and *n*-hexylamine (1/150 mol equiv of NCA) was added to initiate the polymerization. After the completion of polymerization (24 h), a portion of the polymer solution was used to initiate the polymerization of L- or DL-1-PyrAlaNCA (30 mg) in DMF. The latter polymerization was completed within 48 h, as confirmed by the disappearance of the IR peaks characteristic of the NCA. Since no precipitation was observed during the polymerization, the formation of homopolymers of PyrAla should be ruled out. The number-average degree of polymerization of the polymers prepared by the NCA polymerization is given by the molar ratio of NCA to amine.[18] The ratio was 150 for the poly(γ-benzyl DL-glutamate) unit, 8 and 20 for the poly(L-1-PyrAla) unit, and 20 for the poly(DL-1-PyrAla) unit. The ratio will be indicated by subscripts hereafter.

The block copolymers were subjected to gel-permeation chromatography (column, Shodex A803 equilibrated with DMF at 60 °C; detector, refractometer). The three polymers showed similar bimodal chromatograms, one peak being at the elution limit (molecular weight ≅ 70000 based on polystyrene), the other peak at molecular weight ≅ 8000. The latter fraction contained no poly(PyrAla) unit and, therefore, was ascribed to dead poly(γ-benzyl DL-glutamate), which could not initiate the polymerization of PyrAlaNCA. Since the amount of the latter component was small, nonfractionated samples were used for the following spectroscopic study.

Measurements. Circular Dichroism and Absorption Spectra. CD spectra were recorded on a Jasco J-20 spectropolarimeter. UV–visible absorption spectra were taken on a Hitachi EPS-3T instrument. A mother solution of polymer in DMF was diluted with DMF or trimethyl phosphate (TMP) until the concentration of pyrenyl groups became 5×10^{-4}–6×10^{-5} M. Spectra were examined over the temperature range of 3–60 °C in DMF. The variations of the refractive index and the volume of DMF with temperature were corrected by assuming that the absorption coefficient of the pyrenyl group at 346 nm is insensitive to temperature.

Fluorescence Spectra and Fluorescence Rise and Decay Curves. A Hitachi MPF-4 instrument was used. For the variable-temperature measurement, the sample solution was placed in a cylindrical quartz tube with a 5-mm diameter, and the tube was immersed in methanol which was maintained at constant temperatures. The sample solution was deoxygenated by passing nitrogen gas for 30 min before each measurement.

Fluorescence rise and decay curves were measured on a Hitachi time-resolved fluorometer. The analog output was digitized and processed by a microcomputer taking the width of the exciting pulse into account.

Circularly Polarized Fluorescence and Fluorescence-Detected Circular Dichroism. CPF spectra were measured as described before with a Jasco FCD-1F instrument.[6] A deoxygenated DMF solution (6×10^{-5} M with respect to the pyrenyl group) in a 1-cm cuvette was thermostated over the range of 3–60 °C. For the measurement of CPF spectra of the intermolecular excimer of the monomeric model compound, a 5×10^{-3} M solution was placed in a 0.1-cm cuvette. The CPF spectra were represented with Kuhn's emission dissymmetry factor g_{em}

$$g_{em} = 2(I_L^{em} - I_R^{em})/(I_L^{em} + I_R^{em}) \quad (1)$$

where I_L^{em} and I_R^{em} are fluorescence intensities which are left- and right-circularly polarized, respectively.

FDCD was measured by setting an appropriate filter (e.g., a saturated aqueous solution of $NaNO_2$ of 1-cm thickness combined with commercial glass filters) and a photomultiplier behind the sample cell of the J-20 CD spectrometer. The parallel arrangement has the advantage that an artifact due to the residual linear polarization in the exciting circularly polarized light can be eliminated.[14] However, care should be taken to eliminate the exciting light in this case. A saturated solution of $NaNO_2$ was found to fulfill this requirement for lower excitation wavelengths than 360 nm. The output of the photomultiplier was connected

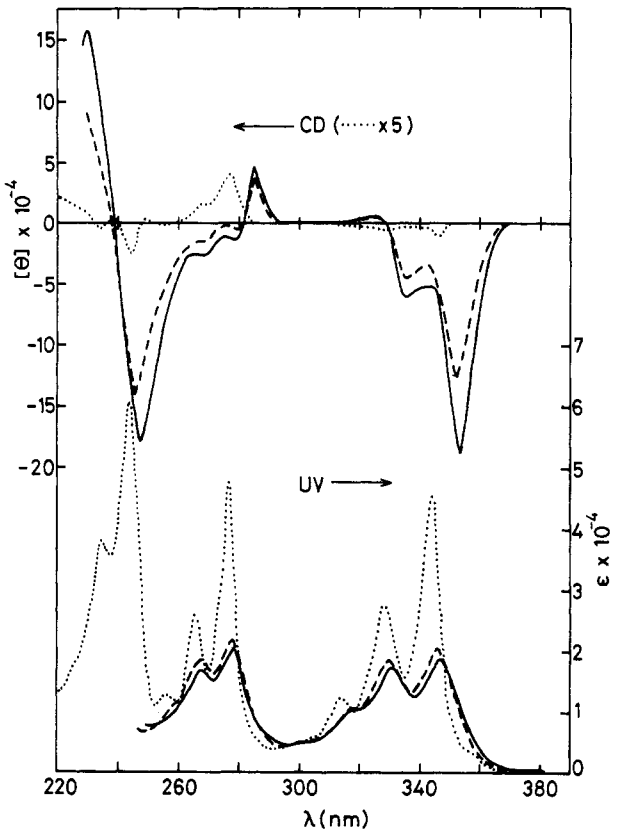


Figure 2. Circular dichroism (top) and absorption (bottom) spectra of Ac-L-1-PyrAla-OMe (···) in trimethyl phosphate, poly(L-1-PyrAla)$_8$ (- - -), and poly(L-1-PyrAla)$_{20}$ (—) in trimethyl phosphate containing 1% of dimethylformamide. [Pyr] = 5 × 10^{-5} M.

to the electronic circuit of the CD instrument. The recorder output of the FDCD, together with the CD and absorption data, was treated according to the method reported by Tinoco and Turner,[11] and the excitation dissymmetry factor, g_{ex}, was calculated as a function of the excitation wavelength

$$g_{ex} = 2(I_L^{ex} - I_R^{ex})/(I_L^{ex} + I_R^{ex}) \quad (2)$$

where I_L^{ex} and I_R^{ex} are fluorescence intensities when the sample is excited by left- and right-circularly polarized light, respectively.

For a single, noninteracting fluorescence in a fluid solvent, the excitation dissymmetry factor should coincide with the absorption dissymmetry factor which is defined by eq 3. The absorption

$$g_{ab} = 2(\epsilon_L - \epsilon_R)/(\epsilon_L + \epsilon_R) \quad (3)$$

dissymmetry factor was calculated by dividing the CD intensity by the absorbance at the same wavelength.

The interpretation of the CPF and the FDCD data becomes complex when the fluorescence is not depolarized.[12] The linear polarization of poly(L-1-PyrAla)$_8$ and poly(L-1-PyrAla)$_{20}$ was measured at different temperatures. The fluorescence was virtually depolarized ($P < 0.02$) within the instrumental accuracy in all cases.

Results and Discussion

Absorption and CD Spectra and Their Temperature Dependence. Figure 2 compares absorption and CD spectra of Ac-L-1-PyrAla-OMe, poly(L-1-PyrAla)$_8$, and poly(L-1-PyrAla)$_{20}$ in TMP (containing 1% of DMF in the case of the polymer solutions). The molar ellipticities observed are $[\theta]_{345.5} = -3.0 \times 10^3$, $[\theta]_{278} = 9.6 \times 10^3$, $[\theta]_{245} = -6.5 \times 10^3$ for Ac-L-1-PyrAla-OMe, $[\theta]_{352.5} = -1.25 \times 10^5$, $[\theta]_{285} = 3.6 \times 10^4$, $[\theta]_{246} = -1.42 \times 10^5$ for poly(L-1-PyrAla)$_8$, and $[\theta]_{353} = -1.9 \times 10^5$, $[\theta]_{285} = 4.6 \times 10^4$, $[\theta]_{247.5} = -1.8 \times 10^5$ for poly(L-1-PyrAla)$_{20}$. The polymers showed much larger ellipicities than the monomeric model compound by more than 1 order of magnitude, and in the case of poly(L-1-PyrAla)$_{20}$ a strong exciton splitting of the ^1B_a absorption band is observed: $[\theta]_{247.5} = -1.8 \times 10^5$, $[\theta]_{230} = 1.6 \times 10^5$. Therefore, it is indicated that the pyrenyl groups are arranged helically along a polypeptide chain and interact with each other in the excited state or, more exactly, in the Franck–Condon state.

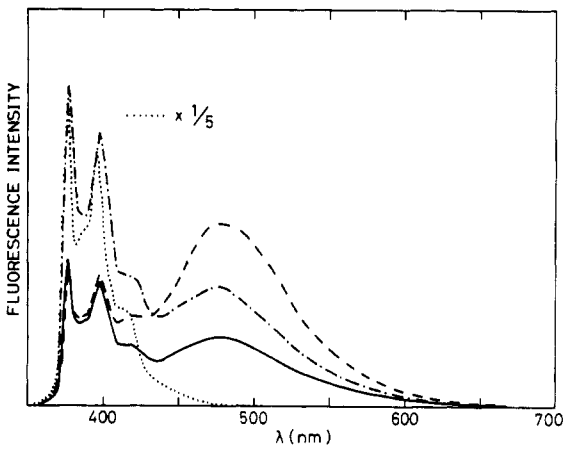


Figure 3. Fluorescence spectra of Ac-L-1-PyrAla-OMe (···), poly(DL-1-PyrAla)$_{20}$ (- - -), poly(L-1-PyrAla)$_8$ (- · -), and poly(L-1-PyrAla)$_{20}$ (—) in dimethylformamide; [Pyr] = 1 × 10^{-5} M, λ_{ex} = 346 nm.

The hypochromicity observed in the polymer spectra also suggests the interchromophoric interaction. Furthermore, the peak positions in the CD spectra of the polymers are substantially shifted to longer wavelengths than those in the absorption spectra, whereas those in the CD and absorption spectra are almost the same in the case of the model compound.

No appreciable change in the CD pattern was observed with the two poly(L-1-PyrAla's) having different chain lengths, except for a small decrease in the CD intensity of the shorter polymer. It is supposed that the helical conformation of poly(L-1-PyrAla) is not seriously affected by the decrease of the chain length.

The temperature dependence of CD spectra of poly(L-1-PyrAla)$_8$ and poly(L-1-PyrAla)$_{20}$ in DMF was studied. In both cases, the molar ellipticity increased by an approximate factor of 1.4, with decreasing temperature from 60 to 3 °C, probably because of decreased thermal fluctuation of the helical array of the pyrenyl chromophores. However, no change was observed in the spectral pattern, suggesting, again, the stability of the helix conformation. The molar ellipticities in DMF were smaller than those in TMP, but no appreciable difference was detected in the spectral pattern. Thus, the helical conformation in TMP should be retained also in DMF.

Fluorescence Spectra and Their Temperature Dependence. Fluorescence spectra of Ac-L-1-PyrAla-OMe, poly(L-1-PyrAla)$_{8,20}$, and poly(DL-1-PyrAla)$_{20}$ in DMF are compared in Figure 3. Spectra of the polymers consist of monomer and excimer fluorescences. Peak positions of the monomer fluorescence of the polymers shifted to wavelengths longer by 2–3 nm than those of the monomeric model. Quantum yields for the monomer and the excimer emissions are listed in Table I. The excimer quantum yield decreased in the following order: poly(DL-1-PyrAla)$_{20}$ > poly(L-1-PyrAla)$_8$ > poly(L-1-PyrAla)$_{20}$. On the other hand, the excimer/monomer intensity ratio decreased in the following order: poly(DL-1-PyrAla)$_{20}$ > poly(L-1-PyrAla)$_{20}$ > poly(L-1-PyrAla)$_8$. As can be seen in Figure 3 and Table I, the larger I_D/I_M ratio of poly(L-1-PyrAla)$_{20}$ than that of poly(L-1-PyrAla)$_8$ resulted from a more marked

Table I
Fluorescence Quantum Yields of Poly(1-PyrAla's) and Ac-L-1-PyrAla-OMe in Dimethylformamide at Room Temperature

	monomer	excimer	total
Ac-L-1-PyrAla-OMe	0.487		0.487
poly(DL-1-PyrAla)$_{20}$	0.053	0.198	0.252
poly(L-1-PyrAla)$_8$	0.108	0.126	0.234
poly(L-1-PyrAla)$_{20}$	0.047	0.073	0.120

decrease of monomer fluorescence than the decrease of excimer fluorescence.

The smaller amount of excimers in the L polymers than in the DL polymer may indicate that the regular arrangement of the pyrenyl chromophores in the L polymer suppressed the formation of the excimer. However, care must be taken for the interpretation of the excimer formation in chromophoric polymers, since an excitation energy can migrate along the polymer chain. If the regular array of the chromophore facilitates the energy migration very effectively, the regularity will not necessarily decrease the excimer fluorescence. This point will be discussed later together with the results of CPF and FDCD spectroscopy.

Incidentally, the excimer formation has been absolutely prohibited in poly(L-1-NapAla) in TMP.[4] The absence has been interpreted by the immobility of naphthyl chromophores which are helically arranged along the polypeptide chain.

Figure 4 shows the temperature dependence of the intensity ratios of excimer/monomer fluorescences for the three polymers. The ratio decreased with decreasing temperature, but the peak wavelengths for the monomer and excimer fluorescences were unchanged over the temperature range examined.

Fluorescence-Detected Circular Dichroism. FDCD is a technique for the selective measurement of the CD spectrum of a chromophore which absorbs light energy and leads to a particular fluorescence emission to be monitored. Figure 5 shows FDCD spectra of poly(L-1-PyrAla)$_{20}$ in DMF. The FDCD spectra are represented by the excitation dissymmetry factor g_{ex} and compared with the CD spectra represented by the absorption dissymmetry factor g_{ab}. The FDCD monitored at the longest wavelength (λ_{em} > 520 nm) is only slightly smaller than the CD at the ^1L_a absorption band (λ_{ex} = 330–360 nm), indicating that the electronic excitation energies absorbed by the pyrenyl groups in both the helical and somewhat disordered parts of poly(L-1-PyrAla) contribute to the excimer which fluoresces at λ_{em} > 520 nm. On the other hand, the g_{ex} value monitored at 420–480 nm is considerably smaller than the g_{ab} value and than the g_{ex} value monitored at λ_{em} > 520 nm. As will be described in the next section, poly(L-1-PyrAla) shows two excimer fluorescences, one being around 460 nm and the other at >500 nm. The smaller g_{ex} value monitored at λ_{em} = 420–480 nm indicates that the excimer which fluoresces at around 460 nm receives more excitation energy from the disordered or randomly coiled part of poly(L-1-PyrAla) than from the helical part.

Both the g_{ex} values monitored at λ_{em} > 520 nm and at 420–480 nm are substantially smaller than the g_{ab} value at the ^1B_b absorption band (λ_{ex} = 285 nm). This may suggest that the excitation energy absorbed at the ^1B_b band of pyrenyl chromophores, which are in a helical part of poly(L-1-PyrAla), is deactivated preferentially by a nonradiative process. The ^1B_b excited state, which shows a fully allowed transition and has a strong transition dipole moment, may form an exciton state in the helical part and, therefore, the excitation energy may migrate effectively along the helix. The enhanced energy migration will facilitate the nonradiative deactivation by some impurity sites.

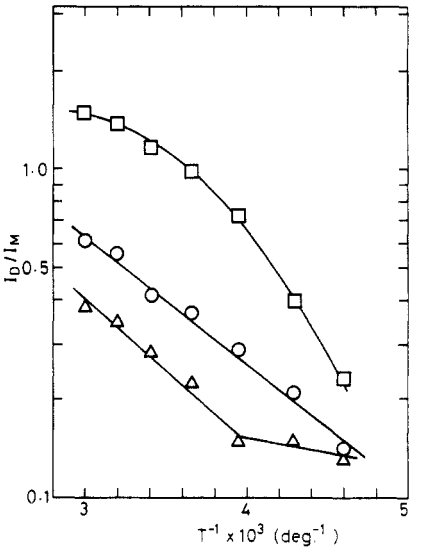


Figure 4. Temperature dependence of excimer (480 nm) to monomer (378 nm) intensity ratios I_D/I_M, for poly(DL-1-PyrAla)$_{20}$ (□), poly(L-1-PyrAla)$_8$ (△), and poly(L-1-PyrAla)$_{20}$ (○).

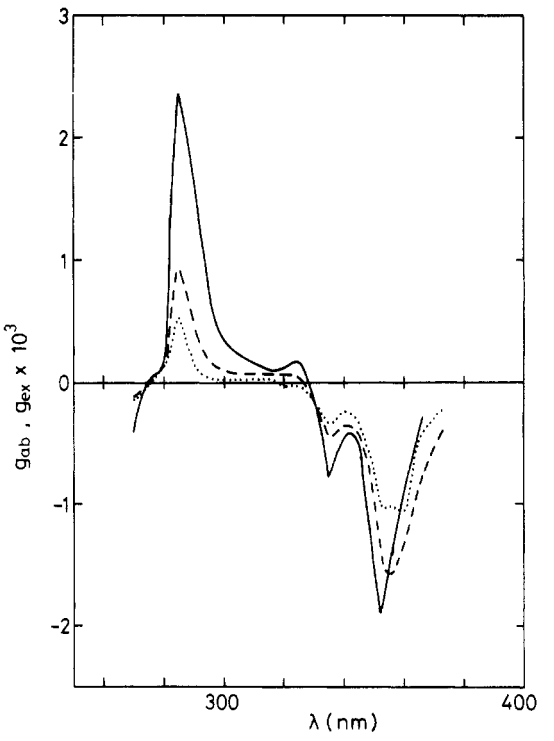


Figure 5. Fluorescence-detected circular dichroism of poly(L-1-PyrAla)$_{20}$ in dimethylformamide monitored at wavelengths longer than 520 nm (- - -) and at 420–460 nm (···). Absorption circular dichroism is also shown in the form of the dissymmetry factor (—); room temperature; [Pyr] = 2 × 10^{-5} M.

No significant temperature dependence was observed in the FDCD spectra over the range of 3–60 °C. The insensitivity to temperature is explained by the fact that the FDCD measures a concentration-normalized CD spectrum for each excimer-forming species. Thus, the relative populations of the two excimers do not affect the spectrum. Similar FDCD spectra were also observed for poly(L-1-PyrAla)$_8$ in DMF.

Fluorescence excitation spectra were also measured at different monitor wavelengths, but no appreciable de-

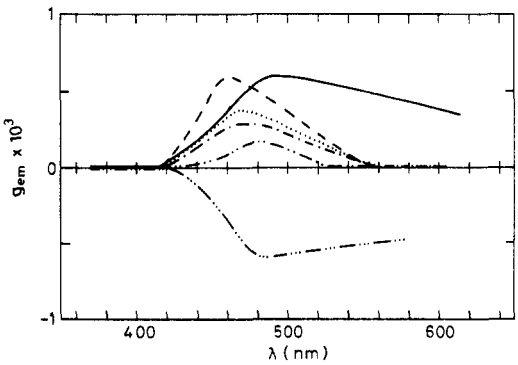


Figure 6. Circularly polarized fluorescence spectra of Ac-L-1-PryAla-OMe in dimethylformamide at 3 (- - -), 20 (···), 40 (- · -), and 60 (- ···) °C and in toluene (—) at room temperature. The CPF spectrum of Ac-D-1-PyrAla-OMe in toluene (- ···) is also shown; [Pyr] = 5×10^{-3} M, λ_{ex} = 280 nm.

pendence was detected in the ^1L_a absorption band (λ_{ex} = 310–360 nm). Since the FDCD and the excitation spectra exhibited no new peak in the ^1L_a and ^1B_b absorption band, the possibility of strong ground-state interaction between pyrenyl groups should be ruled out.

Circularly Polarized Fluorescence Spectra of *N*-Acetyl-L-1-pyrenylalanine Methyl Ester. CPF spectroscopy may be most powerful to elucidate the interactions of chiral fluorophores in the excited state. Figure 6 shows the CPF spectra of concentrated solutions of Ac-L- and -D-1-PyrAla-OMe in toluene and in DMF. Monomer fluorescence (λ_{em} = 360–400 nm) did not show fluorescence dissymmetry larger than the noise level of our CPF instrument ($g_{em} \sim 0.5 \times 10^{-4}$). The absence of dissymmetry for isolated fluorophores having a single chiral center has been established.[9] On the other hand, positive and negative dissymmetries of CPF are observed for the intermolecular excimers of Ac-L- and -D-PyrAla-OMe, respectively. In toluene the dissymmetry factor is almost constant over the entire excimer fluorescence region, indicating the presence of a single configuration for the excimer. The CPF spectrum of Ac-L-1-PyrAla-OMe in DMF indicates that the excimer fluorescence consists of at least two components: one fluoresces at a shorter wavelength with positive dissymmetry and the other at a longer wavelength with virtually no dissymmetry. The striking difference in the two solvents may be tentatively interpreted by the presence of two types of excimers, one being nonpolar (Pyr*–Pyr ↔ Pyr–Pyr*) which is predominant in nonpolar solvent and the other being polar (Pyr^+–Pyr^- ↔ Pyr^-–Pyr^+) which is favored in a polar environment. The nonpolar excimer may have a definite geometry, which is presumably a somewhat skewed sandwich-type one, and may show a significant CPF signal, whereas the polar excimer may consist of an assembly of a variety of configurations with somewhat longer interchromophore distance than that of the nonpolar one and, therefore, show no CPF dissymmetry. Evidently the above tentative interpretation should be confirmed by future studies. A similar discussion has been given for the excimers of [2,2](1,3)pyrenophane in polar and nonpolar solvents.[19]

Brittain et al. reported the CPF spectrum of the excimer of optically active 1-(1-hydroxyhexyl)pyrene in methanol.[20] They found a decrease of the magnitude of g_{em} below λ_{em} = 460 nm and interpreted the decrease as the presence of more than one emitting species. However, as is evident from our result (Figure 6), the decrease of the magnitude of g_{em} is due to a mixing of the monomer fluorescence which shows no CPF signal. Unfortunately, they reported neither the CPF spectrum at wavelengths longer than 520 nm nor those in nonpolar solvents.

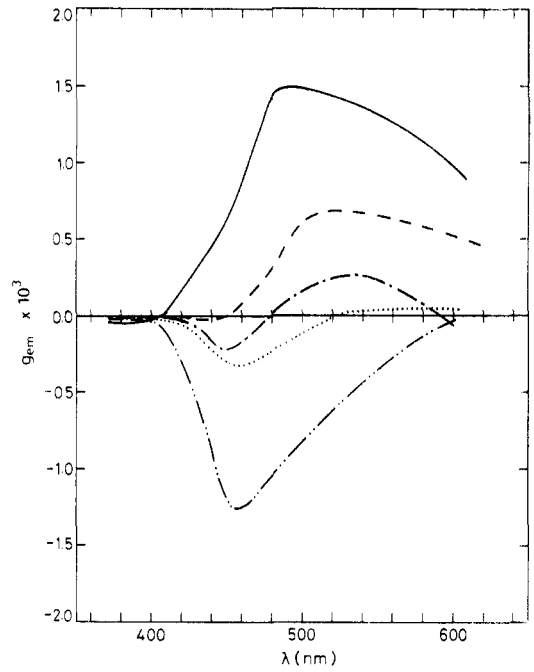


Figure 7. Circularly polarized fluorescence spectra of poly(L-1-PyrAla)$_8$ in dimethylformamide at 3 (—), 20 (- - -), 30 (- · -), 40 (···), and 60 (- ···) °C; [Pyr] = 6×10^{-5} M, λ_{ex} = 280 nm.

In Figure 6, the CPF intensity in DMF decreased with increasing temperature. The decrease suggests the increased thermal fluctuation of the excimer geometry at elevated temperatures.

Circularly Polarized Fluorescence Spectra of Poly(L-1-PyrAla) in DMF and Their Temperature Dependence. Figures 7 and 8 show CPF spectra of poly(L-1-PyrAla)$_8$ and poly(L-1-PyrAla)$_{20}$ at different temperatures, respectively. No fluorescence dissymmetry was observed at the monomer fluorescence region. Therefore, it is concluded that the lowest excited state of poly(L-1-PyrAla) is not delocalized over the neighboring pyrenyl groups but localized on a monomeric pyrenyl group. The localization may be related to the fact that no electronic dipole–dipole interaction between neighboring pyrenyl groups is possible, since the transition from the fluorescence state (^1L_b) to the ground state is forbidden.

At wavelengths longer than 420 nm, the presence of two kinds of excimers is evident in Figures 7 and 8. One component shows a negative CPF dissymmetry around 460 nm and is predominant at higher temperatures. The other exhibits a positive dissymmetry at wavelengths longer than 500 nm and becomes important at lower temperatures. The temperature dependence of the CPF spectra cannot be ascribed to a conformational transition of poly(L-1-PyrAla) in DMF, since CD spectra did not change significantly with temperature as described before.

The dramatic temperature dependence of CPF spectra may be interpreted not by the increase of the excimer with positive CPF at lower temperatures, but by the decrease of the negative CPF component, since the total excimer quantum yield decreased with decreasing temperature (Figure 4). Therefore, the excimer with negative CPF may be formed by a thermal motion of poly(L-1-PyrAla), whereas the excimer with positive dissymmetry requires no or little thermal activation. In view of the tentative assignment of the two excimers observed for Ac-L-1-Pyr-Ala-OMe, the excimer with negative CPF may be a sandwich-type nonpolar excimer formed by the thermal motion of the polymer chain and the excimer with

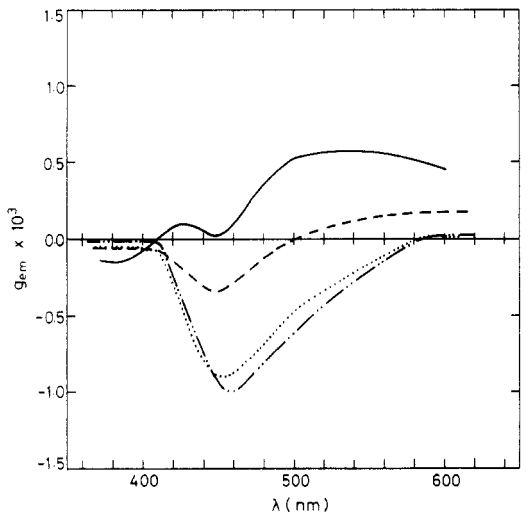


Figure 8. Circularly polarized fluorescence spectra of poly(L-1-PyrAla)$_{20}$ in dimethylformamide at 3 (—), 20 (- - -), 40 (···), and 60 (-·-·-) °C. [Pyr] = 6×10^{-5} M, λ_{ex} = 280 nm.

positive CPF may be a polar one but it has a definite geometry supported by the helical main chain and exhibits a significant CPF signal unlike the intermolecular case.

In the previous section it was shown that the FDCD monitored at wavelengths longer than 520 nm showed a larger g_{ex} value than that monitored around 460 nm. This indicates that the excimer with positive CPF receives more excitation energy from the helical part of poly(L-1-PyrAla) than from the random part, whereas the excimer with negative CPF receives more energy from the disordered part of the polymer, i.e., at the termini or at the point where D amino acid is present. Although some ambiguity due to the energy migration along the polymer still exists, it can be said that the excimer with positive CPF is formed at the helical part of the polymer chain, presumably with some side-chain fluctuations, whereas that with negative CPF is formed at the disordered part of the polymer where the main-chain conformation is randomized.

To summarize the CPF, FDCD, and other spectroscopic data, it is concluded that the excimer with negative fluorescence dissymmetry is formed in a disordered part by thermal motion of the polymer main chain and is considered to have a sandwich-type geometry which is electronically nonpolar. The excimer with positive dissymmetry is formed by small deformation of the side chains in the helical part of the polymer with little thermal activation and may have polar electronic character. The nature of the excimer formed at the disordered part may be similar to that reported for poly(vinylpyrene) which takes randomly coiled conformations in solution.[7,8] In the latter case, the excimer quantum yield was found to increase with the increase of temperature, due to a thermal activation of the molecular motion. The same tendency was also observed in poly(DL-1-PyrAla) as can be seen in Figure 4.

The configuration of the excimer with positive CPF signal deserves further consideration. It may be formed in the helical part of poly(L-1-PyrAla) with little thermal activation. A computer prediction of the stable conformation of helical poly(L-1-PyrAla) suggested that the pyrenyl chromophores are arranged so that the center-to-center interchromophoric distance between the nearest pyrenyl groups is 7.4 Å and the nearest C^{γ}–C^{γ} distance is 4.3 Å (for LH-ME-A conformation).[21] The interchromophoric distance is evidently too long to form excimers. The excimer can be formed only when the side-chain, or even the main-chain, conformation fluctuates significantly. The excimer with positive CPF has a polar character with a geometry different from the sandwich-type configuration and may be formed even for a highly skewed and relatively distant pair of pyrenyl chromophores. The latter configuration may be attained by a small fluctuation of side chains involved in the helical part of poly(L-1-PyrAla).

Table II
Results of Fluorescence Rise and Decay Curve Analysis of Poly(1-PyrAla's) in Dimethylformamide[a]

temp, °C	monitor, nm	G_1	τ_1, ns	G_2	τ_2, ns
		Poly(DL-1-PyrAla)$_{20}$			
60	460–480	−0.26	7.8	0.74	55
60	>560	−0.33	5.6	0.67	54
0	460–480	−0.26	9.3	0.74	76
0	>560	−0.43	2.3	0.57	80
		Poly(L-1-PyrAla)$_8$			
60	460–480	−0.50	3.0	0.50	63
60	>560	0.65	4.3	0.35	60
0	460–480			1.0	97
0	>560	0.73	6.1	0.27	82
		Poly(L-1-PyrAla)$_{20}$			
60	460–480	0.38	4.3	0.62	56
60	>560	0.74	7.8	0.26	56
0	460–480	0.64	3.5	0.36	85
0	>560	0.79	8.8	0.21	70

[a] The rise and decay curves were fitted to the general equation $I(t) = G_1 \exp(-t/\tau_1)) + G_2 \exp(-t/\tau_2)$.

One might argue that one of the excimers may be assigned to the "second excimer" which has been detected in such chromophoric polymers as poly(*N*-vinylcarbazole)[22,23] and poly(vinylnaphthalenes).[24] The second excimer has been characterized by partially overlapped chromophores and found at fluorescence wavelengths shorter than those of the normal excimers at low temperatures. In the case of poly(L-1-PyrAla), the excimer with shorter fluorescence wavelength increased its population at higher temperatures. Therefore, this excimer cannot be assigned to the second excimer.

Fluorescence Rise and Decay Curves. The fluorescence rise and decay curves of the excimers were measured at 0 and 60 °C in DMF. The curves were found to fit the following equation satisfactorily:

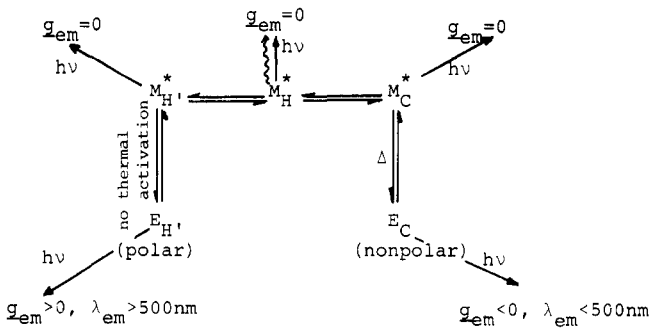
$$I(t) = G_1 \exp(-t/\tau_1) + G_2 \exp(-t/\tau_2) \qquad (4)$$

$$(\tau_1 < \tau_2, \quad |G_1| + |G_2| = 1)$$

Both G_1 and G_2 are positive when no delay in the fluorescence rise is observed. $G_1 = -0.5$ and $G_2 = 0.5$, when the excimer formation follows Birks kinetics.[25] The results of the curve-fitting analysis are collected in Table II. The results are in qualitative agreement with the above discussion. A delay in the fluorescence rise (negative G_1) was observed when the excimer fluorescence was monitored at λ_{em} = 460–480 nm for poly(L-1-PyrAla)$_8$ at 60 °C. The delay indicates that the excimer formation needs some relaxation process which may require thermal activation. No delay was observed when the excimer fluorescence was monitored at $\lambda_{em} > 560$ nm, indicating that the formation of the latter excimer requires no thermal activation. These results are consistent with the temperature dependence of CPF spectra. The only exception is the absence of delay in the excimer fluorescence of poly(L-1-PyrAla)$_{20}$ monitored at λ_{em} = 460–480 nm at 60 °C. A tentative explanation is that a type of molecular motion which leads to excimer formation can be rapid in the longer polymer at 60 °C.

As for poly(DL-1-PyrAla)$_{20}$, no appreciable difference was detected in the rise and decay curves monitored at 460–480

Scheme II



nm and at >560 nm and the delay of the fluorescence rise was observed in both cases at low and high temperatures. Therefore, it is suggested that in the DL polymer, a single excimer species exists which is formed after a substantial conformational relaxation.

A quantitative analysis of the rise and decay kinetics encounters a difficulty, because poly(L-1-PyrAla) shows two type of excimers which show largely overlapped fluorescences and a migration of excitation energy from one excimer to the other through the monomer excited state should be taken into account (see Scheme II). Only qualitative discussion as above is possible on the limiting cases where one excimer component dominates over the other.

Conclusions

In view of the above spectroscopic results, the excited state of poly(L-1-PyrAla) may be considred as in Scheme II. In the scheme M_H^*, $M_{H'}^*$, and M_C^* denote the excited monomers in the regular helix, in the helix with small side-chain fluctuation, and in the disordered part of the polymer, respectively. $E_{H'}$ and E_C are the excimers formed in the fluctuated part of the helix and in the disordered part, respectively. M_H^* and $M_{H'}^*$ should be distinguished because the excimer $E_{H'}$ forms with little thermal activation and a conformational calculation predicts the impossibility of excimer formation in the side chains of the regular helical part of poly(L-1-PyrAla). Moreover, if $E_{H'}$ is formed directly from M_H^*, the g_{ex} value monitored by the $E_{H'}$ fluorescence should *exceed* the g_{ab} value, but this was not true, as seen in Figure 5. It should be noted that M_H^* and M_C^* do not specify any particular pyrenyl groups, but represent an assembly of pyrenyl groups involved in the helical and disordered parts, among which an excitation energy migrates. Direct interconversion between $E_{H'}$ and E_C is unlikely, because they are located at different parts of the polymer.

It was demonstrated that chiroptical spectroscopy such as CD, CPF, and FDCD is powerful to elucidate the complex situation of the excited state of chromophoric polymers.

It is of interest that the M_H^* is deactivated very efficiently, especially when it is in the higher excited state (^1B_b), as shown by the small FDCD intensity (Figure 5). Since the higher excited state in the helical part forms an exciton state, as has been suggested by the splitting of the ^1B_a band (Figure 2), the efficient deactivation may be explained in terms of a frequent energy migration through the exciton state and an efficient trapping by an energy-deactivating site. Thus, one may expect an efficient energy flow into an energy-accepting site, if the latter is bound to a helix terminal. Similarly, an efficient and directed electron flow would be possible when an electron-accepting site is attached to the helix. To realize the molecular conducting system, chromophores whose lowest excited state can form the exciton state should be arranged along a helical backbone.

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