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One-Dimensional Aromatic Crystals in Solution. 9. Synthesis, Conformation, and Chiroptical Spectroscopy of Poly[Lys(Z)$_2$-pyrAla] in Solution[1]

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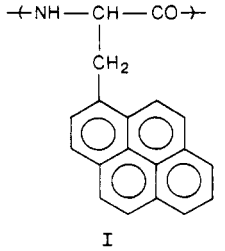
ABSTRACT: A sequential polypeptide having a repeating unit of [Lys(Z)$_2$-pyrAla] was synthesized [Lys(Z) = $^\epsilon N$-benzyloxycarbonyl-L-lysine, pyrAla = L-1-pyrenylalanine]. The polypeptide main chain was in a right-handed α-helical form in trimethyl phosphate solution. A one-dimensional array of pyrenyl groups along the helix was suggested from a side-chain conformational energy calculation and a theoretical circular dichroism calculation. The fluorescence spectra consisted of a monomer and a less intense excimer emission. The excimer emission was strongly left circularly polarized. The chiral configuration of the excimer indicated that the excimer is formed at an excimer-forming site located at the middle part of the helix.

A helical polypeptide chain, being rigid and regular, has been shown to be a good framework to support chromophores, keeping specific distance and orientation between the neighboring ones.[2,3] In the previous papers of this series, homopolypeptides of 1- and 2-naphthylalanines[4,5] and 1-pyrenylalanine[6,7] and also sequential polypeptides having 1-naphthylalanine as one of the constituents[8] have been reported. Circular dichroic (CD) spectra indicated that the chromophoric polypeptides are in helical conformations and the side chains are arranged regularly along the helix.

Previously,[6] we have shown that poly(L-1-pyrenylalanine) [p(pyrAla), (I)] exhibited two types of excimer fluorescence: one emits around 460 nm and is right circularly polarized, the other emits at longer wavelengths than 500 nm and is left circularly polarized. The former excimer is predominant at high temperatures (60 °C), whereas the latter is favored at low temperature (3 °C). A likely reason for the multiple excimers is that each pyrenyl group in p(pyrAla) may interact with more than one neighboring group as shown by the dotted lines in Figure 1. In this sense, homopolypeptides of aromatic amino acids cannot support a one-dimensional chromophoric



I

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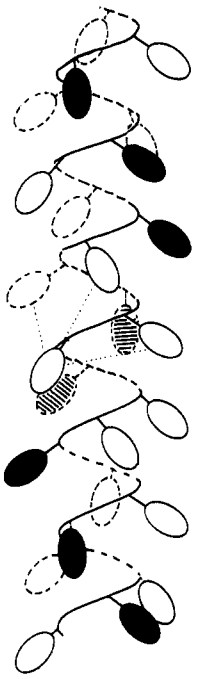
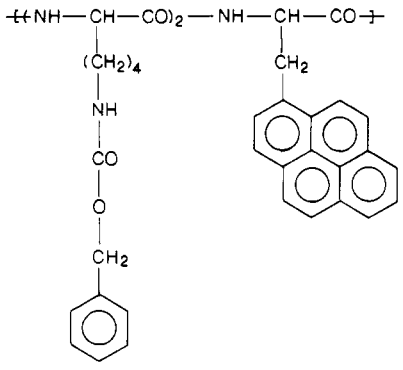


Figure 1. Illustration of the one-dimensional array of chromophores (black ovals) along a helical polypeptide chain. In a homopolypeptide of aromatic amino acids, multiple interchromophore interactions are possible as indicated by dotted lines.

array. If one properly replaces the pyrenylalanines by nonchromophoric amino acids, a sequential polypeptide carrying a one-dimensional array of pyrenyl chromophores will be obtained. This principle is illustrated in Figure 1, where the black ovals represent the one-dimensional array of chromophores. The polypeptide actually prepared in this study is poly[Lys(Z)$_2$-pyrAla] [p(L_2P), II, Lys(Z) =



II

'*N*-benzyloxycarbonyl-L-lysine]. To examine the effect of the Lys(Z) unit on the spectroscopic properties of the pyrenyl polypeptide, another sequential polypeptide with two γ-methyl L-glutamate [Glu(OMe)] units as the spacer was also prepared [p(G_2P)]. Since the Lys(Z) unit as well as the Glu(OMe) unit favors an α-helical conformation, the two sequential polypeptides may favor an α-helical conformation in the usual helix-supporting solvents.

The pyrenyl polypeptides were subjected to spectroscopic measurements including chiroptical spectroscopy, such as circular dichroism (CD) and circularly polarized fluorescence (CPF). A series of similar sequential polypeptides carrying L-1-naphthylalanine instead of L-1-pyrenylalanine have been prepared [p(L_m*N*), m = 1–4].[8] However, since the naphthyl polypeptides showed no excimer emission, the multiplicity of the side-chain interactions was not studied.

Oligopeptides containing pyrenylalanine units have been reported by other workers.[9-13]

Experimental Section

Materials. P(L_2P) and p(G_2P) were prepared by the polymerization of the corresponding tripeptide activated esters, Lys(Z)-pyrAla-Lys(Z)-OSu and Glu(OMe)$_2$-pyrAla-OSu (OSu = *N*-hydroxysuccinimide ester). The latter oligopeptides were synthesized by a stepwise coupling of the constituent amino acids, as described below. All intermediates were checked by ^{1}H NMR, IR, and TLC methods. *N*-Acetyl-L-1-pyrenylalanine methyl ester (Ac-pyrAla-OMe) prepared previously[6] was used as a reference compound.

Boc-Lys(Z)-pyrAla-OMe. *N*-*tert*-Butyloxycarbonyl, *N*-benzyloxycarbonyl-L-lysine [Boc-Lys(Z), commercially available, 1.1 g, 2.9 mmol], L-1-pyrenylalanine methyl ester hydrochloride[6] (pyrAla-OMe HCl, 1.0 g, 2.9 mmol), dicyclohexylcarbodiimide (DCC, 0.61 g, 2.9 mmol), and triethylamine (TEA, 0.41 mL, 2.9 mmol) were mixed in chloroform/dimethylformamide (DMF, 10 mL/20 mL) mixed solvent. The mixture was stirred for 3 h at 0 °C and for 16 h further at room temperature. The solvents were evaporated under vacuum, and ethyl acetate (30 mL) was added. The precipitate (dicyclohexylurea, DCU) was removed by filtration, and the solution was stored in a refrigerator to precipitate further DCU. The ethyl acetate solution was washed with 10% NaCl, 10% citric acid, 4% $NaHCO_3$, and 10% NaCl solutions and dried with Na_2SO_4. Colorless crystals appeared after concentrating the solution and adding ethyl ether; yield 1.1 g (57%), mp 127–129 °C. Anal. Calcd for $C_{39}H_{44}N_3O_7$: C, 70.25; H, 6.65; N, 6.30. Found: C, 70.42; H, 6.57; N, 6.40.

Boc-Lys(Z)-pyrAla-OH. Boc-Lys(Z)-pyrAla-OMe (0.20 g, 0.30 mmol) was dissolved in methanol (5 mL), and 0.55 mL of 1 N NaOH (0.55 mmol) was added. The ester hydrolysis was followed by TLC (ethanol), and after 7 h the ester almost disappeared. Methanol was evaporated under vacuum, and 0.5 N citric acid (10 mL) was added. The precipitates were collected, washed with water, and dried under vacuum. The removal of methyl ester was confirmed by the ^{1}H NMR spectrum; yield 0.18 g (92%).

Boc-Lys(Z)-pyrAla-Lys(Z)-OSu. Boc-Lys(Z)-OSu (commercially available, 0.5 g) was mixed with trifluoroacetic acid (TFA, 4 mL) under cooling by ice. After 30 min, ether (30 mL) was added. The oil separated was repeatedly washed with ether and dried under vacuum. The procedure gave Lys(Z)-OSu TFA salt, which was not purified further at this stage.

Boc-Lys(Z)-pyrAla-OH (0.18 g, 0.28 mmol) and *N*-methylmorpholine (MM 39 μL, 0.35 mmol) were dissolved in tetrahydrofuran (THF, 3 mL) and cooled to −4 °C. Isobutyl chloroformate (46 μL, 0.35 mmol) was added to the mixture, and the mixture was stirred for 6 min at −4 °C. The Lys(Z)-OSu TFA freshly prepared (0.17 g, 0.35 mmol) in THF (1 mL) and MM (39 μL) were then added and stirred for 30 min. The mixture was stored in refrigerator overnight. The TFA MM salt was removed by filtration, and the solvent was evaporated. The residue was dissolved in ethyl acetate and solidified by adding ether under cooling. The product was recrystallized from ethyl acetate/ether; yield 0.20 g (72% from Boc-Lys(Z)-pyrAla-OH), mp 155–160 °C. Anal. Calcd for $C_{56}H_{63}N_6O_{12}$: C, 66.45; H, 6.27; N, 8.30. Found: C, 65.90; H, 6.20; N, 8.13.

Poly[Lys(Z)$_2$-pyrAla] [p(L_2P)]. Boc-Lys(Z)-pyrAla-Lys(Z)-OSu (51 mg) was dissolved in TFA (0.5 mL) under ice cooling. After 15 min, ether was added, and the precipitate was washed with ether and dried under vacuum. ^{1}H NMR spectroscopy (in dimethyl sulfoxide-d_6) indicated a complete removal of the Boc group. The N-deblocked tripeptide active ester (43 mg, 0.041 mmol) was dissolved in dimethylformamide (0.1 mL), and TEA (8.7 μL, 0.062 mmol) was added. The mixture stood for 3 days at room temperature in the dark, and further TEA (5 μL) was added. After a further 1 week, an excess amount of methanol was added, and the precipitate was washed with water/methanol mixture and then with pure methanol, yield 19 mg (57%). Gel chromatographic analysis (Sephadex LH-60 in DMF) indicated that the molecular weight is near to or a little larger than 10^4, which corresponds to the number of Lys(Z)$_2$-pyrAla units of 12 or a little more. The polypeptides was soluble in polar aprotic solvents, such as DMSO, DMF, and TMP.

Poly[Glu(OMe)$_2$-pyrAla] [p(G_2P)]. The sequential polypeptide was prepared by a polymerization of Glu(OMe)$_2$-pyrAla-OSu in DMF. The latter peptide was synthesized by a coupling

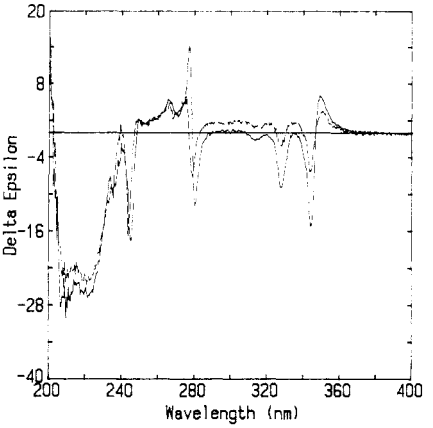


Figure 2. Circular dichroism of p(L_2P) and p(G_2p) in TMP: p(L_2P), [Pyr] = 3.9×10^{-5} M at 25 °C (—); p(G_2P), [Pyr] = 2.2×10^{-5} M at 25 °C (---).

of Boc-[Glu(OMe)$_2$-OH with pyrAla-OSu. The molecular weight of the polypeptide estimated from the gel chromatographic analysis was around a few thousand.

Measurements. The polypeptide sample was only soluble in polar aprotic solvents. Trimethyl phosphate (TMP) was chosen for spectroscopic measurements, because of its transparency down to 190 nm. TMP was twice distilled to remove fluorescent impurities contained in the commercial product. The polypeptide solution was bubbled with argon gas for 10 min before the measurement of fluorescence spectra. The following instruments were used: absorption, Hitachi 320; CD, Jasco J-500; fluorescence, Hitachi F4000; CPF, Jasco FCD-1.

Energy Calculations. Conformation energy calculations were carried out using the structure and energy parameters of the ECEPP system.[14] The parameters for a pyrAla unit were the same as reported before.[7] The program written on MS-Fortran was run on an NEC PC9801 personal computer.

Theoretical CD Calculation. Theoretical CD curves were computed on the basis of the exciton theory, taking amide $n\pi^*$, $\pi\pi^*$ transitions and pyrenyl ^{1}Ba, ^{1}Bb, ^{1}La, and ^{1}Lb transitions into consideration. The program was essentially the same as that used previously for poly[L_mN].[8] The parameters for the electronic properties of the pyrenyl group were the same as those used for poly(pyrAla).[7] The calculation was carried out for a decamer of [Lys(Z)$_2$-pyrAla] unit. It was found that the theoretical CD curves were virtually independent of the chain length above the octamer. The CD curve was drawn by assigning a Guassian width of 4 nm to each rotational strength obtained from the quantum mechanical calculation. The computation was carried out on a Hitachi M660 machine.

Results and Discussion

CD Spectra and Conformation. Figure 2 shows CD spectra of p(L_2P) and p(G_2P) in TMP. The ordinate is expressed by $\Delta\epsilon = \epsilon_L - \epsilon_R$ calculated with respect to the molar concentration of pyrenyl groups. Therefore, the $\Delta\epsilon$ value with respect to the amide group is one-third of the value presented in the figure. The CD pattern around 200–230 nm resembles that of right-handed α-helical polypeptides, although the $\Delta\epsilon$ value at 222 nm (−8 to −8.7 with respect to the amide chromophore) is smaller than the value reported for the 100% helix of long polypeptides (−10.6 to −12.1). The α-helical main-chain conformation of p(L_2P) is supported by a recent analysis of the CD spectrum in the vibrational region (VCD).[15] The pattern of the VCD spectrum at the amide I and II bands indicated a right-handed α-helical main-chain conformation of p(L_2P) in DMSO solution.

The CD spectrum of p(G_2P) is virtually the same as that of p(L_2p), with somewhat smaller intensity. The resemblance suggests that the main-chain conformation and the side-chain orientation of the two polypeptides are virtually the same and that the CD pattern is not affected by the nonchromophoric side chains.

The CD signals of the pyrenyl absorption band of the polypeptides are much stronger than those of a model compound Ac-L-pyrAla-OMe,[6] and the CD patterns are very different from the model, indicating interchromophore interactions between pyrenyl groups arranged along the polypeptides. The splittings of the 0–0 transitions of the ^{1}La (~350 nm) and the ^{1}Bb (~275 nm) band into negative and positive peaks indicate the exciton-type interactions over the neighboring pyrenyl chromophores whose spatial positions and orientations are constrained by the helix conformation. The signs of the splitting are opposite for the 0–0 peak of the ^{1}La band and that of the ^{1}Bb band, i.e., the positive peak is positioned at the longer wavelength side in the former case, whereas the inverse is observed in the latter case. The opposite signs of the exciton splitting suggest that the screw senses of the transition moments belonging to two succeeding pyrenyl groups are opposite for the ^{1}La and ^{1}Bb transitions. The ^{1}La transition moment is parallel to the long axis of pyrene, and the ^{1}Bb transition moment is perpendicular. But, irrespective of the polarization, the screw sense of the succeeding transition moments should be the same for the two transitions. Therefore, the opposite signs of the exciton splitting cannot be explained in terms of a simple chirality rule.[16]

CD intensities of the polypeptides are much smaller than that of the corresponding naphthyl polypeptide, p(L_2N), where a $\Delta\epsilon$ value up to 182 with respect to naphthyl group has been observed at the ^{1}Bb absorption band. Possible reasons for the small CD intensity are as follows. First, the order of the helical main chain and/or the side chain can be less regular than the p(L_2N) case, due to the bulk pyrenyl groups. As for the main-chain conformation, the $\Delta\epsilon_{222}$ value calculated with respect to the amide group is a good measure for the helix content. The $\Delta\epsilon_{222}$ value of p(L_2P) (−8.7) is smaller in magnitude than the value expected for a long polypeptide chain in a 100% helix conformation (−10.6 to −12.1). However, since the degree of polymerization of p(L_2P) is around 12 or only a little more, the small $\Delta\epsilon_{222}$ value may be attributed mainly to the short helix length and not solely to the randomness of the main-chain conformation. Although the $\Delta A/A$ value of the VCD spectrum at the amide I band of p(L_2P) (1.5×10^{-4} in DMSO) was smaller than that of p(L_2N) (1.8×10^{-4}),[15] the difference is not as large as observed in the electronic CD. These data indicate that the small electronic CD may be attributed either to the randomness of side-chain orientation of p(L_2P) or p(G_2P) or to a specific spatial arrangement of the pyrenyl groups that induces small CD.

The orientational mobility of the pyrenyl side chains was evaluated from the conformational energy calculation as will be described next. The side-chain orientation was found to be highly constrained in a small range of angles, provided that the main-chain conformation is fixed to the α-helix. The constraints of the side-chain orientations were supported by the temperature independence of the CD spectrum over the range 5–50 °C, although some sharpening was observed at low temperatures. This suggests that the major reason for the small CD intensity is neither the incomplete helical structure of the main chain nor the random orientation of the side chains. It will be shown below that the small CD is explained in terms of a specific arrangement of the pyrenyl chromophores that accidentally induces small CD.

Conformational Energy Calculation on the Side-Chain Orientation. A conformational energy calculation

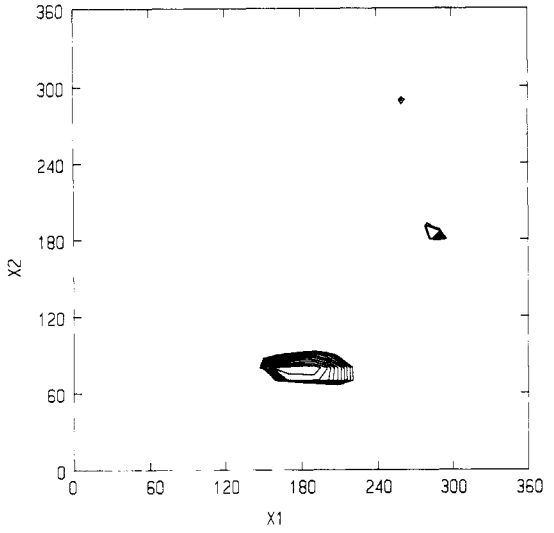


Figure 3. Energy contour map for the side-chain orientation of right-handed α-helical [Ala-Ala-pyrAla] (n = 10). The interval of the contour lines is 0.5 kcal mol^{-1}.

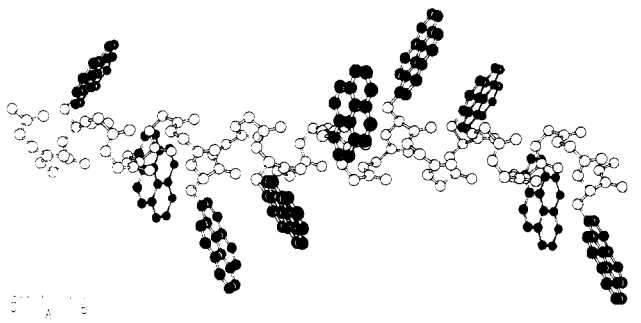


Figure 4. Most probable conformation of p(L_2P). The main chain is a right-handed α-helix. The side-chain rotational angles are χ_1 = 180°, χ_2 = 80°. The hydrogen atoms and the side chain of the nonchromophoric amino acid were omitted, although they are included in the energy calculation. The molecular model is drawn by using the NAMOD program.[26]

was carried out to predict the side-chain orientation of the pyrenyl groups, by using empirical potential functions. An energy contour map for the rotation of the pyrenylmethyl group in the sequential polypeptide (Ala-Ala-pyrAla)$_{10}$ [p(A_2P)] was calculated. The Lys(Z) or Glu(OBzl) unit was replaced by an Ala unit in the calculation. This simplification may be justified by the observation of virtually the same CD spectra for p(G_2P) and p(L_2P). The α-helical main chain was assumed (ϕ = −57°, ψ = −47°, ω = 180°) in the calculation. The side-chain rotational angles (χ_1, χ_2) were assumed to be the same for all pyrAla units. The contour map is shown in Figure 3. Virtually a single energy minimum was found to be allowed for the pyrenylmethyl group, and the range of thermal fluctuation is not large near room temperature. The result indicates that the orientation of the pyrenyl group is virtually fixed, provided that the main chain is in the α-helical conformation.

Figure 4 shows the α-helical conformation of p(A_2P) in the most probable side-chain orientation. It is seen that a one-dimensional array of pyrenyl groups is attained, that is, each pyrenyl group can interact with only the nearest pyrenyl groups along the polypeptide chain. The nearest interchromophore distance is 8.5 Å (center-to-center) or 5.4 Å (edge-to-edge).

Theoretical CD Calculation. A theoretical CD curve was calculated for the α-helical main chain. To examine the effect of side-chain orientations, the side-chain angles (χ_1, χ_2) were varied around the most stable position shown

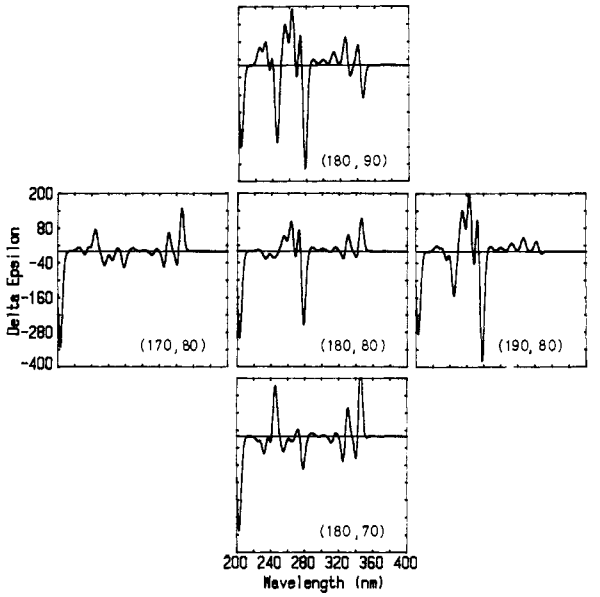


Figure 5. Theoretical CD spectra calculated for various side-chain orientations. The side-chain rotational angles (χ_1, χ_2) are shown in the figure. The main chain was fixed to a right-handed α-helix (ϕ = −57°, ψ = −47°, ω = 180°).

in Figure 4. The results are shown in Figure 5. It is seen that the most stable orientation (180°, 80°) coincides with the point where the exciton splittings change their signs. For instance, the change in χ_1 from 170° to 190° induces the change in the sign of the exciton splitting at the ^{1}Ba band. Similarly, the exciton splittings at the ^{1}Ba and ^{1}La bands were reversed by changing the χ_2 from 70° to 90°. Therefore, the small CD experimentally observed is explained in terms of the accidental coincidence of the chromophore arrangement to the critical geometry for the CD spectrum.

The profile of the theoretical CD curve at the most stable side-chain orientation agrees qualitatively with the experimental CD. The signs of the exciton splittings at the 0–0 peaks of the ^{1}Ba, ^{1}Bb, and ^{1}La band agree with those experimentally observed (Figure 2). No other side-chain orientations can reproduce correctly the experimental splittings. Therefore, the theoretical calculation strongly supports the side-chain orientation predicted from the energy calculation.

The theoretical CD intensity is, however, larger than the experimental one by more than an order of magnitude. This may be partly interpreted in terms of the thermal fluctuations of the side chains which mix positive and negative CD contributions. However, the larger CD intensity has been calculated for other chromophoric polypeptides, including poly(L_mN),[8] where less of an effect of side-chain fluctuation may be expected, since the stable orientation does not coincide with the critical orientation for the CD spectrum. I believe that one of the major reasons for the large theoretical CD is the neglect of the higher order interaction terms in the exciton theory employed. For example, the electronic interaction that leads to the hypochromicity in the absorption spectrum is not taken into consideration. The introduction of these terms in the theoretical CD calculation will be a matter of future study.

Absorption Spectra. Figure 6 compares absorption spectra of p(L_2P) and Ac-pyrAla-OMe in TMP. The peak positions of p(L_2P) are slightly red-shifted as compared with the model (1.5 nm at the ^{1}La band and 1.0 nm at the ^{1}Bb band). Furthermore, a considerable decrease in the absorbance is observed for p(L_2P). These phenomena are

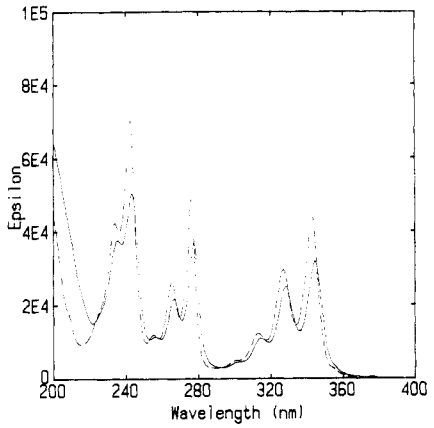


Figure 6. Absorption spectra of p(L_2P) (—) and Ac-L-pyrAla-OMe (---) in TMP at 25 °C.

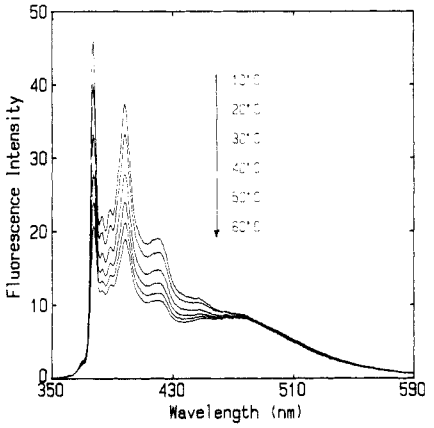


Figure 7. Fluorescence spectra of p(L_2P) in TMP at six different temperatures; λ_{ex} = 328 nm.

interpreted by the small interchromophore interactions as have been indicated in the CD spectrum. However, no strong ground-state interaction, such as the dimer formation as observed in some molecular aggregates containing pyrenyl chromophores,[17-19] was detected. The absence of the strong ground-state interaction is further confirmed by the coincidence of the absorption spectrum with the fluorescence excitation spectra monitored at the monomer and the excimer wavelengths (Figure 8). These results are consistent with the molecular structure of Figure 4, in which the interchromophore separation is about 8.5 Å.

Fluorescence Spectra. Fluorescence spectra of p(L_2P) over the temperature range 10–60 °C are shown in Figure 7. The spectra are dominated by the monomer emission of pyrenyl group, and the excimer emission is minor near room temperatures. The total (monomer + excimer) fluorescence quantum yield was 0.54 at 10 °C and 0.36 at 60 °C. The amount of excimer is much less than those in other polymers carrying a pendant pyrenyl group with a similar chromophore density.[20] The excimer emission of p(L_2P) is also smaller than that in p(DL-pyrAla) or p(L-pyrAla).[6] The suppression of the excimer formation is evidently the result of the spatial fixation of the pyrenyl groups along the regular and rigid polypeptide helix. With an increase in the temperature, the monomer emission decreased considerably, but the amount of the excimer emission remained constant or increased only slightly. The monotonic temperature dependence suggests that the population of the excimer is kinetically determined in this temperature range. The deactivation rate of the monomer excited state may be more sensitive to temperature than the formation and the deactivation rates of the excimer.

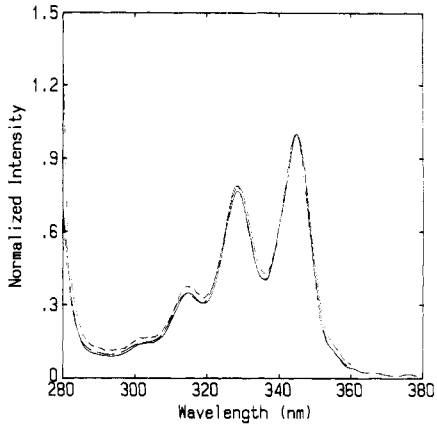


Figure 8. Fluorescence excitation spectra of p(L_2P) in TMP monitored at 378 nm (—) and at 550 nm (---). An absorption spectrum (-·-) is also shown. The three spectra are normalized at the peak wavelength of the ^{1}La band (344.5 nm).

Furthermore, the deactivation of the monomer and the formation of the excimer seem to be independent processes, that is, the excimer is not formed by the expense of the monomer excited state. The independence of the two processes has been suggested also from the preliminary results on the analysis of decay curves of the monomer and the excimer emissions. The details of the excimer kinetics is being studied by using a time-resolved fluorescence spectroscopy.

Although its formation is suppressed when compared with other pyrenyl polymers, the excimer is actually observed. Therefore, the location of the excimer-forming site should be clarified. There are two possible excimer sites in the polypeptide. First, an excimer may be formed at an unfolded part of the helix, particularly at the two ends. In this case the excimer may not take any special configuration. Second, an excimer may be formed at the middle part of the helix by a thermal fluctuation of the main-chain and the side-chain conformations. In thise case, the excimer may take a specific configuration, due to the constraints by the helical conformation. In either case, an efficient energy transfer along the one-dimensional array may carry the photoenergy to the excimer site. Since the interchromophore distance (8.5 Å) is a little shorter than the Forster critical distance for the energy transfer (10.0 Å),[21] the energy migration may be very efficient. The orientation factor of the energy transfer (κ^2) should be close to $^2/_3$, since no definite polarization dominates in the absorption and fluorescence transitions of the pyrenyl group.

In the following section, fluorescence excitation spectroscopy and circularly polarized fluorescence (CPF) spectroscopy were used to clarify the nature of the excimer-forming site.

Fluorescence Excitation Spectra. Figure 8 compares the excitation spectra monitored at the monomer emission (378 nm) and at the excimer emission (550 nm) with the absorption spectrum. The three spectra are normalized at their peak wavelengths. The peak wavelengths were, however, coincident with each other (344.5 nm). The coincidence of the three spectra indicates that the excimer emission is not originated from any aggregated species in the ground state.

Circularly Polarized Fluorescence Spectra. CPF spectroscopy measures the difference of the fluorescence intensities that are left (I_L) and right (I_R) circularly polarized.[22] The CPF spectrum is expressed by the Kuhn dissymmetry factor, $g_e = 2(I_L - I_R)/(I_L + I_R)$. Figure 9 shows CPF spectra of p(L_2P) in TMP. No CPF signal is observed at the monomer emission region, indicating that

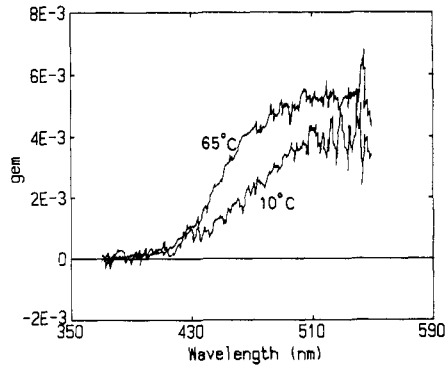


Figure 9. Circularly polarized fluorescence spectra of p(L_2P) in TMP at 10 and 65 °C; λ_{ex} = 320 nm.

the monomeric excited state is localized electronically and no chiral perturbation exists in the chromophoric array of p(L_2P). This result is understandable, since the lowest excited state of the pyrenyl group (^{1}Lb) has a forbidden transition to the ground state and little dipole–dipole coupling is expected between neighboring pyrenyl groups. The absence of the CPF signal in the monomer emission region has been observed also in other aromatic polypeptides[23] and in other chromophoric assemblies, including cholesteric liquid crystals[24] and bilayer membranes.[25]

A strong CPF signal ($g_e = 5 \times 10^{-3}$) is observed in the excimer region. The strong CPF signal indicates a chiral structure of the excimer. The high chirality then suggests that the excimer is formed at the middle of the helix by some conformational fluctuation and not at the ends of the polypeptide where the chain conformation is randomized. The CPF signal appears to be flat above 500 nm, indicating that only a single excimer component is present above 500 nm. Furthermore, the CPF spectrum is not sensitive to the temperature, except for a small change that may be caused by the increased relative population of the excimer at elevated temperatures. The CPF pattern of p(L_2P) contrasts with that of p(pyrAla), which showed negative (around 460 nm) and positive (>500 nm) signals.[6] The population of the negative component increased at high temperatures, while the positive component is dominant at low temperatures. The CPF spectra of p(pyrAla) clearly indicated that at least two types of excimers with different chiral configurations are present, and their relative population changes with temperature.

The difference in the CPF spectra of p(pyrAla) and p(L_2P) is most reasonably interpreted by the different arrangements of the pyrenyl groups along the two helical polypeptides. As is clear from Figures 1 and 4, only a single type of excimer is possible in p(L_2P), if the helix conformation is not completely destroyed. Presumably, the left circularly polarized excimer of p(pyrAla), which was predominant at low temperatures, corresponds to the left circularly polarized one of p(L_2P) and, therefore, may be formed by a 1–4 type interaction. The right circularly polarized component of p(pyrAla) may be attributable to 1–2 or 1–5 type interaction. This assignment may be important, since, to our knowledge, no qualitative data have been reported on the relation between the sign of CPF signal and the absolute configuration of an excimer.[22]

Conclusions

A sequential polypeptide carrying a one-dimensional array of pyrenyl chromophores was synthesized. The main-chain conformation was a right-handed α-helix and the side-chain conformation was predicted from the conformational energy calculation. The most probable conformation was supported by the theoretical CD calculation. The excimer emission was smaller than those in other pyrenyl polymers, but some excimer was still observed. The excimer emission was found to be strongly left circularly polarized, indicating that the excimer is formed at the middle of the helix by a deformation of the helix conformation.

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Registry No. p(L_2P) (homopolymer), 109720-17-4; p(L_2P) (SRU), 107874-52-2; p(G_2P) (homopolymer), 120332-15-2; p(G_2P) (SRU), 120332-18-5; p(A_2P) (homopolymer), 120332-17-4; p(A_2P) (SRU), 120332-19-6; BOC-Lys(Z)-OH, 2389-45-9; H-pyrAla-OMe·HCl, 107987-11-1; BOC-Lys(Z)-pyrAla-OMe, 120332-05-0; BOC-Lys(Z)-pyrAla-OH, 120332-06-1; BOC-Lys(Z)-OSu, 34404-36-9; H-Lys(Z)-OSu·TFA, 120332-08-3; BOC-Lys(Z)-pyrAla-Lys(Z)-OSu, 120332-09-4; H-Lys(Z)-pyrAla-Lys(Z)-OSU·TFA, 120332-10-7; H-Glu(OMe)-Glu(OMe)-pyrAla-OSu, 120332-11-8; BOC-Glu(OMe)-Glu(OMe)-OH, 120332-12-9; H-pyrAla-OSu, 120332-13-0.

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