Excimer Formation on a Polypeptide Carrying Two Pyrenyl Groups in the Middle of an α-Helical Main Chain

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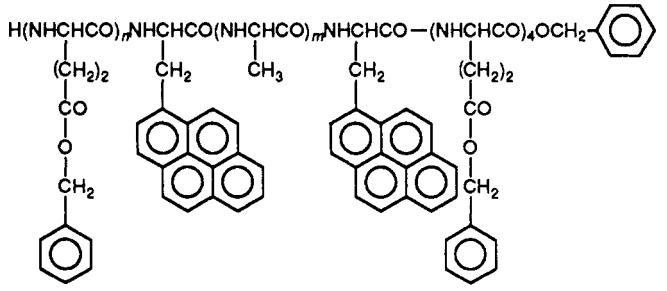
α-Helical polypeptides carrying two pyrenyl groups covalently linked to the main chain (I-0, I-2) were synthesized. Fluorescence spectra showed only small excimer emission, indicating the rigidity of the helical conformation. The temperature dependence of the peak position of excimer and that of the ratio of excimer/monomer quantum yield indicated that only a single excimer configuration is allowed for the I-0 polypeptide, but several configurations are present in the I-2 polypeptide. The conclusion is supported from the temperature dependence of the profile of circularly polarized fluorescence spectra. Possible polypeptide conformations in the ground state and in the excimer-forming state were proposed from conformational energy calculations. Fluorescence rise curves of excimer emission were analyzed to obtain the rate of excimer formation. The rise time of the excimer emission (30–70 ns) was much longer than those of local fluctuations of side-chain orientations but much shorter than those of unfolding processes of helix.

Introduction

Conformational fluctuation has been considered to play an important role in the biological activity of proteins.[1,2] Fluorescence probe techniques have been used to study the statics and dynamics of molecular conformations of polypeptides.[3] In particular, the decay curves of fluorescence anisotropy of tryptophan-containing proteins[4] and synthetic polypeptides[5] have been analyzed to give information on the local rotation of tryptophan residues and the tumbling motion of whole molecule. From these studies, information on the frequency of conformational fluctuation has been obtained. However, most of these studies provide information only on the average over a variety of librational and rotational modes in the polypeptide. The formation of excimer has been used to probe the dynamics of a specific mode of molecular motion, provided that the probe chromophores are attached at specific positions in the molecule.[6]

We have been synthesizing polypeptides consisting of synthetic amino acids carrying aromatic chromophores.[7-10] With the stepwise liquid-phase technique, peptides with a specific sequence of amino acids and hence peptides with aromatic chromophores at specific positions along the chain may be obtained. In this study we have prepared polypeptides of the structure shown here in order to probe a deformation mode of helical structure:



I-m (m = 0, 2); n = 34 (I-0), 28 (I-2)

All amino acids in the polypeptides are the L isomer. The long poly[Glu(OBzl)] segment at the left-hand side may render the polypeptide to take the α-helical conformation. The four Glu(OBzl) units at the right-hand side corresponds to one turn of the α-helix and may stabilize the helical conformation around the pyrAla units. Therefore, the polypeptide will be a suitable model compound for studying the deformation inside the helical structure that is probed by the statics and dynamics of excimer fluorescence.

One of the advantages of the excimer probes in the helical polypeptides is that circularly polarized fluorescence (CPF) spectroscopy is useful in obtaining information on the structure of excimer.[11,12] In this study, the CPF spectroscopy was used to specify the excimer conformation (final state) along with the CD spectroscopy to specify the ground-state conformation (initial state). In this way, the route of molecular motion to form the excimer was specified. From the fluorescence rise time of the excimer, the rate of the specific conformational deformation can be determined.

CPF spectra of the two polypeptides in tetrahydrofuran (THF) have been reported previously.[13] The fluorescence behavior of a model dipeptide, Ac-pyrAla-pyrAla-OMe, has been reported by De Schryver and co-workers.[14,15]

Experimental Section

Materials. Polypeptides I-m contain L-1-pyrenylalanine. The synthesis and the optical resolution of the unnatural amino acid have been described previously.[16] The polypeptides were obtained by a polymerization of Glu(OBzl) N-carboxyanhydride (NCA) using H-pyrAla-Ala$_m$-pyrAla-Glu(OBzl)$_4$-OBzl as initiator. All intermediates were checked for purity by TLC and IR and ^{1}H NMR spectroscopies. In the following, reagents for peptide synthesis will be represented by the usual abbreviations.[9]

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Boc-pyrAla-pyrAla-Glu(OBzl)$_4$-OBzl. The Boc group of Boc-pyrAla-Glu(OBzl)$_4$-OBzl[9] was removed by treatment with 4 N HCl/DOX. The HCl salt of the pentapeptide (160 mg, 0.12 mmol) was coupled with Boc-pyrAla-OH (46 mg, 12 mmol) in DMF (10 mL) by using EDC (25 mg, 0.13 mmol), HOBt (18 mg, 0.13 mmol), and TEA (17 μL). The mixture was stirred for 4 h under cooling with ice and for 1 day at room temperature. The solvent was evaporated and the residue was redissolved in ethyl acetate. The solution was washed with 10% citric acid, saturated NaCl, 3% $NaHCO_3$, and saturated NaCl and then dried on $MgSO_4$. The residue obtained after evaporation of the ethyl acetate was purified with a Sephadex LH-20/DMF column; yield 150 mg (78%). This compound did not melt sharply and slowly decomposed above 140 °C. Anal. Calcd for $C_{98}H_{94}N_6O_{17}$: C, 72.31; H, 5.82; N, 5.16. Found: C, 72.03; H, 5.81; N, 5.31.

Boc-pyrAla-Ala$_2$-OBzl. Boc-Ala$_2$-OBzl was synthesized from Boc-Ala-OH and TosOH Ala-OBzl. The dipeptide was dissolved in 4 N HCl/DOX and after 1 h the solvent was evaporated. The residue was washed with ether and dried under vacuum. The N-deprotected dipeptide was coupled with Boc-pyrAla-OH[9] by using EDC/HOBt/TEA as described above. The tripeptide was purified with a silica gel column using ethyl acetate as eluent.

Boc-pyrAla-Ala$_2$-pyrAla-Glu(OBzl)4-OBzl. Boc-pyrAla-Ala$_2$-OBzl (180 mg, 0.29 mmol) was dissolved in ethanol (50 mL) containing 10% Pd–C (90 mg). The mixture was stirred under hydrogen atmosphere at room temperature for 5 h. The catalyst was filtered off, and the solvent was evaporated. The residue was recrystallized from ethyl acetate/hexane mixed solvent. The free acid obtained above (39 mg, 0.07 mmol) was dissolved in THF (3.2 mL) and cooled to −10 to −20 °C. A mixed anhydride was prepared by adding *N*-methylmorpholine (8.3 μL, 0.07 mmol) and isobutyl chloroformate (9.9 μL, 0.07 mmol) to the cold solution. After 6 min HCl·pyrAla-Glu(OBzl)$_4$-OBzl (96 mg, 0.07 mmol) dissolved in DMF containing *N*-methylmorpholine (9.1 μL, 0.07 mmol) was added. The mixture was stirred for 2 h at −10 to −20 °C and a further 24 h at 0 °C. The mixture was diluted with chloroform, and the solution was washed with cirtic acid and $NaHCO_3$ solution. After the solvent was evaporated, the peptide was purified with a Sephadex LH-20/DMF column; yield 60 mg (46%). This compound did not melt sharply and slowly decomposed above 200 °C. Anal. Calcd for $C_{104}H_{104}N_8O_{19}$: C, 70.57, H, 5.92; N, 6.33. Found: C, 70.31; H, 5.87; N, 6.03.

Glu(OBzl)$_n$-pyrAla-Ala$_m$-pyrAla-Glu(OBzl)$_4$-OBzl (m = 0, 2). The Boc group of Boc-pyrAla-Ala$_m$-pyrAla-Glu(OBzl)$_4$-OBzl was removed with 4 N HCl/DOX. The peptide was then neutralized by washing the chloroform solution with 3% $NaHCO_3$. The peptide with free amino group was used to initiate the polymerization of Glu(OBzl) NCA in DMF, the NCA/amino group ratio being 30. The polypeptides obtained were fractionated with a Sephadex LH-60/DMF column to remove low-molecular-weight fractions.

Measurements. Spectroscopic measurements were made in THF or in dimethylformamide (DMF) solution. Fluorescence and CPF spectra and the decay measurements were made after bubbling the solution with argon gas for 20 min. The following spectrometers were used: absorption, Jasco Ubest-50; CD, Jasco J-600; fluorescence, Hitachi MPF-4; CPF, Jasco FCD-1.[11] Fluorescence decay curves were measured on a home-built single-photon-counting apparatus with an air-discharge lamp.[9] All the spectroscopic data were interfaced to an NEC PC9801 personal computer.

Results and Discussion

Circular Dichroism and Absorption Spectra. CD spectra of the I-0 and I-2 polypeptides were measured at room temperature. The CD patterns in the amide absorption region are typical for right-handed α-helix. The $\Delta\epsilon_{222}$ values are −11.2 and −10.6 for I-0 and I-2, respectively. The $\Delta\epsilon_{222}$ values indicate fully helical conformation for the two polypeptides.[17] Pyrenyl chromophores also show CD signals at their absorption wavelengths (Figure 1).

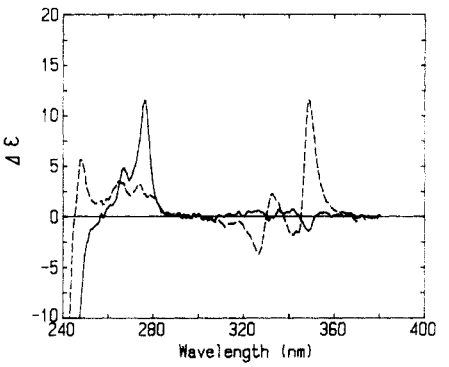

Figure 1. Circular dichroism of the I-0 and I-2 polypeptides in THF solution. The ordinate scale is the $\Delta\epsilon$ with respect to the molar concentration of pyrenyl groups ([Pyr] = 1 × 10^{-5} M).

Both polypeptides show positive CD peaks at the ^1B_b absorption band around 270 nm. A large positive signal is observed at the 0-0 peak of the ^1L_a absorption band of the I-2 polypeptide (349 nm), whereas a small negative peak is seen for the I-0 polypeptide. The opposite CD signals suggest opposite chirality for the relative orientations of the two pyrenyl groups in the two polypeptides in the ground state. The CD signals of pyrenyl chromophores of the I-0 and I-2 polypeptides in DMF were the same as those in THF.

Absorption spectra of the I-0 and I-2 polypeptides were measured in THF and in DMF solution. The intensity and the peak position were virtually the same as those of a model polypeptide containing only one pyrAla unit, Glu(OBzl)$_n$-pyrAla-Glu-(OBzl)$_4$-OBzl (II). The coincidence of the absorption spectra indicates the absence of ground-state interaction between two pyrenyl groups in the I-0 and I-2 polypeptides. This conclusion is supported by the coincidence of fluorescence excitation spectra of the I-0 and I-2 polypeptides monitored by the excimer fluorescence, with that of the model polypeptide II monitored by the monomer fluorescence.

The results of CD spectroscopy indicate that the polypeptides take α-helical conformation in solution. The pyrenyl chromophores are involved in the rigid helical chain to show moderately strong induced CD. No strong ground-state interaction is present between two pyrenyl groups in the polypeptides.

Conformational Analysis in the Ground State. Most stable ground-state conformations of the two polypeptides were predicted from conformational energy calculations using ECEPP potential functions.[18,19] The calculation was made on Ac-Ala$_4$-pyrAla-(1)-Ala$_m$-pyrAla(2)-Ala$_4$-OCH$_3$, in which the Ala units are replaced for Glu(OBzl) units, for simplicity. The main-chain conformation was fixed to the right-handed α-helix (ϕ = −57°, ψ = −47°, ω = 180°) and the four rotational angles of the two pyrAla units were varied. The most stable side-chain conformations obtained after the energy minimization were $\chi_1(1)$, $\chi_2(1)$, $\chi_1(2)$, $\chi_2(2)$ = 187°, 77°, 183°, 77° for the I-0 polypeptide and 187°, 76°, 186°, 77° for the I-2 polypeptide. No other conformational region was allowed for the side-chain orientations. The most probable conformations for the I-0 and I-2 polypeptides are illustrated in Figures 2a and 3a. The interchromophore center-to-center distance and the nearest edge-to-edge distance were 11.1 and 5.5 Å for the I-0 polypeptide and 8.2 and 5.2 Å for the I-2 polypeptide. These interchromophore distances are too long to form excimers unless large thermal fluctuations occur either in the helical main chain or in the side chains.

Fluorescence Spectra. Fluorescence spectra of the I-0 and I-2 polypeptides in DMF are shown in Figures 4 and 5, respectively.

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(19) A set of programs for carrying conformational energy calculations of peptides of arbitrary sequence of amino acids including unnatural aromatic amino acids, was developed by one of us (M.S.). The energy parameters for the unnatural amino acids were determined from a CNDO/ON MO calculation on *N*-acetylamino acid methylamide. We thank Professor H. A. Scheraga for sending us the CNDO/ON program.

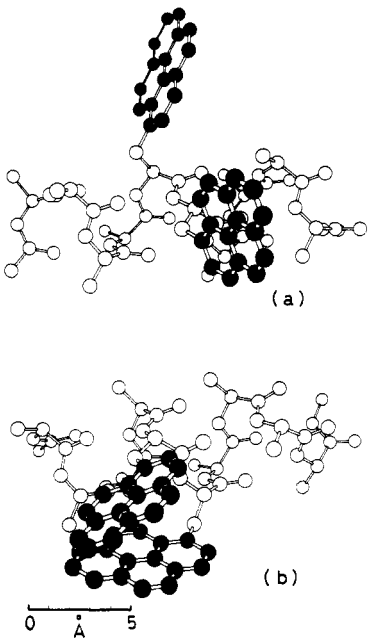

Figure 2. Most stable ground-state conformation (a) and a probable conformation for excimer formation (b) of Ac-Ala$_4$-pyrAla-pyrAla-Ala$_4$-NMA. NAMOD molecular display program[26] was used.

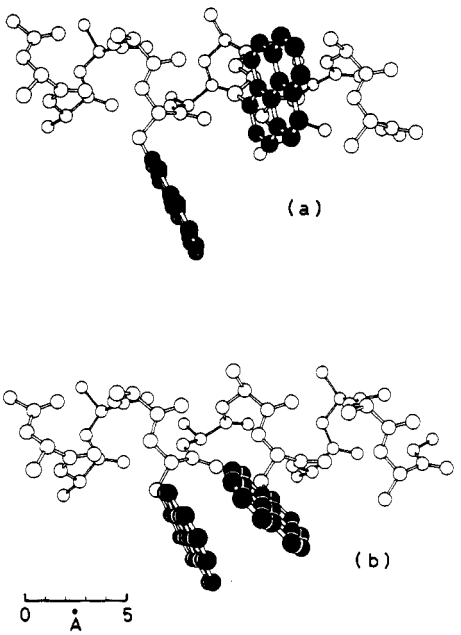

Figure 3. Most stable ground-state conformation (a) and one of the possible excimer-forming conformations (b) of Ac-Ala$_4$-pyrAla-Ala-Ala-pyrAla$_4$-NMA.

The fluorescence spectra of the two polypeptides consist mostly of monomer fluorescence, indicating that the pyrenyl groups are rigidly fixed to the α-helical polypeptide chain. The excimer formation, which requires considerable molecular deformation during the lifetime of excited pyrenyl group (>200 ns), is a minor process. Indeed, the excimer fluorescence decreases further at lower temperatures. The conclusion that the excimer formation is not important in the present polypeptides, is remarkable, since two pyrenyl groups are positioned fairly close to each other in the ground state (Figures 2a and 3a) and the chromophores are located near the end of a helix. Furthermore, since the excimer will be accumulated during the lifetime of the pyrenyl group, the occurrence of even very unusual conformations can play an important role in forming the excimer. Therefore, the small excimer emission experimentally observed demonstrates that the α-helical structure is a rigid molecular framework that can immobilize chromophores with a specific spatial order and orientation. The fluorescence

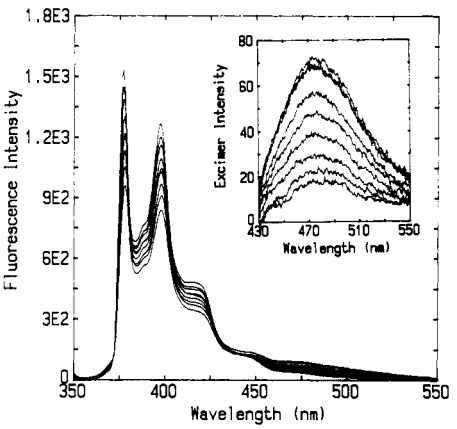

Figure 4. Fluorescence spectra of the I-0 polypeptide in DMF. [pyr] = 1×10^{-6} M, λ_{ex} = 345 nm. The insert shows the difference spectra of the I-0 polypeptide and the model polypeptide II, both being normalized at 377 nm. The temperature was +20, +10, 0, −10, −20, −30, −40, −50, and −60 °C, with increasing order of monomer fluorescence and decreasing order of excimer fluorescence.

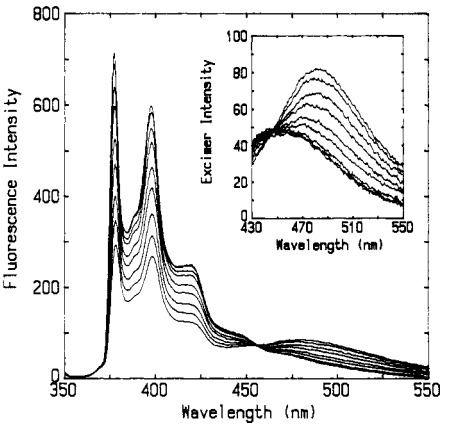

Figure 5. Fluorescence spectra of the I-2 polypeptide in DMF. [pyr] = 1×10^{-6} M, λ_{ex} = 345 nm. The insert shows the difference spectra of the I-2 polypeptide and the model polypeptide II, both being normalized at 377 nm. The temperatures was +20, +10, 0, −10, −20, −30, −40, −50, and −60 °C, with increasing order of monomer fluorescence and decreasing order of excimer fluorescence.

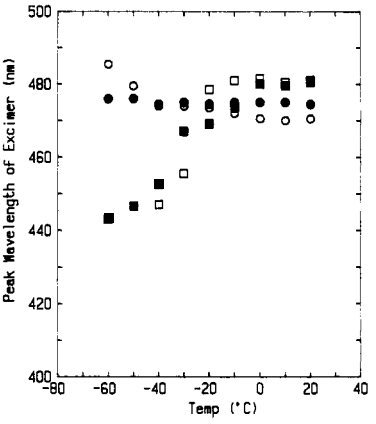

Figure 6. Temperature dependence of peak wavelength of the excimer of the I-0 polypeptide in DMF (●) and in THF (○) and of the I-2 polypeptide in DMF (■) and in THF (□).

behavior of the two polypeptides were similar in THF solution.

To study the nature of excimer fluorescence in more detail, differential fluorescence spectra between the polypeptide I-0 or I-2 and the model polypeptide II were obtained at each temperature. The subtraction was made after normalizing the fluorescence intensity of the model polypeptide II to that of the polypeptide I-0 or I-2 at the first peak of the monomer fluorescence (377–378 nm). The results are shown in the insert of Figures 4

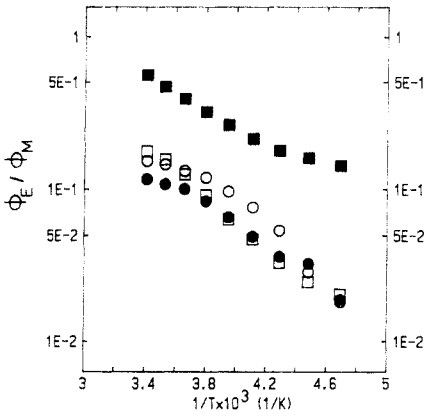

Figure 7. Arrhenius plot of the ratio of quantum yields of excimer and monomer fluorescence of the I-0 polypeptide in DMF (●) and in THF (○) and of the I-2 polypeptide in DMF (■) and in THF (□).

and 5. It is clear that the excimer peak position is insensitive to temperature for the I-0 polypeptide but is blue-shifted with lowering temperature for the I-2 polypeptide. The peak positions of the excimer are plotted against temperature in Figure 6. The shift of peak position in the I-2 polypeptide occurs sharply around −30 °C in DMF and in THF. Since no shift is observed in the I-0 polypeptide, the shift cannot be attributed to any conformational change in the helical main chain but can be interpreted in terms of the change of the local configuration of the excimer in the I-2 polypeptide.

From the analysis of fluorescence spectra, the ratios of the quantum yield of monomer emission and that of excimer emission were calculated and are plotted in the form of the Arrhenius plot (Figure 7). The profile of the plot for the I-0 polypeptide is typical for usual excimer systems,[20] and the downward curvature at high temperatures can be interpreted in terms of the increased contribution of the dissociation process. The excimer may not be dissociated below −20 °C in DMF and below −30 °C in THF. The situation is different in the case of the I-2 polypeptide. The Arrhenius plot showed an upward deviation below −40 °C. The deviation is undoubtedly related to the shift of the peak position of the excimer. Indeed, when the quantum yield of monomer emission and that of excimer emission were plotted separately, the former fell on a straight line but the latter deviated from it below −40 °C. The behavior in the Φ_e/Φ_m ratio and the peak position strongly indicates that the configuration of excimer in the I-2 polypeptide changes significantly at lower temperatures than −30 to −40 °C.

The activation energies calculated from the slope of the Arrhenius plot at the low-temperature region are 3.1 ± 0.1 (in DMF, 0 to −60 °C), 4.7 ± 0.2 (in THF, −20 to −60 °C) for the I-0 polypeptide and 2.6 ± 0.1 (in DMF, 20 to −40 °C), 4.0 ± 0.1 (in THF, 10 to −40 °C) for the I-2 polypeptide (kcal/mol). These activation energies correspond to those of the excimer formation in this temperature region, where the dissociation of excimers may not be important.[20] The activation energies are close to or lower than the values reported for Ac-pyrAla-pyrAla-OMe (4.7 kcal/mol)[14] and for dipyrenylpropane (2.9 to 5.3 kcal/mol),[21] which show much stronger excimer emissions than the present polypeptides. The relatively low activation energy and the low quantum yield indicate that the excimer formation in the polypeptides are restricted by entropy terms. That is, the excimer is formed when the polypeptide takes very unusual and specific conformations with relatively low energy.

Circularly Polarized Fluorescence (CPF) Spectra. The configuration of chiral excimers may be more clearly discussed on the basis of CPF spectroscopy.[10,11] Figures 8 and 9 show CPF spectra of the I-0 and I-2 polypeptides in the two solvents. The

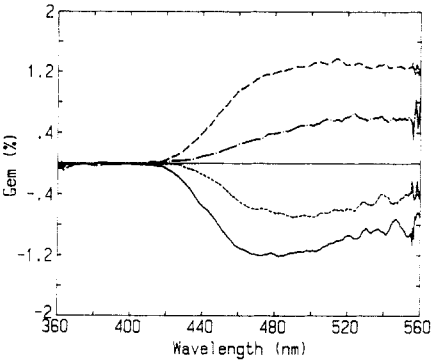

Figure 8. CPF spectra of the I-0 polypeptide at 20 °C (—) and at −40 °C (---) and of the I-2 polypeptide at 20 °C (——) and at −40 °C (-·-) in DMF.

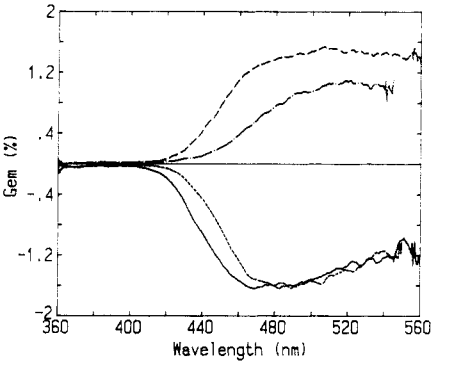

Figure 9. CPF spectra of the I-0 polypeptide at 20 °C (—) and at −40 °C (---) and of the I-2 polypeptide at 20 °C (——) and at −40 °C (-·-) in THF.

CPF spectra are presented with the Kuhn's dissymmetry factor $g_{em} = 2(I_L - I_R)/(I_L + I_R)$, as the ordinate, where I_L and I_R are the intensities of left- and right-circularly polarized fluorescence, respectively. For a single fluorescence transition, the g_{em} value should be flat over the whole transition band.

In all the CPF spectra recorded in this study, monomer fluorescence showed no circular polarization. The electronically isolated nature of the monomer excited state has been observed in other chiral systems carrying napthyl,[11] pyrenyl,[7] and anthryl[22] chromophores.

In contrast, excimer fluorescence is very strongly circularly polarized,[12] indicating that the excimer has a chiral configuration with a specific screw sense in the relative orientation of two pyrenyl groups. The strong CPF signal is in agreement with the conclusion from the temperature dependence of fluorescence spectra, i.e., a very specific excimer configuration is present in the polypeptide. On the other hand, excimer emissions of oligopeptides Boc-pyrAla$_2$-Glu(OBzl)$_4$-OBzl (III-0) and Boc-pyrAla-Ala$_2$-pyrAla-Glu(OBzl)$_4$-OBzl (III-2) showed only very small CPF signals ($g_{em} \sim 0$ for III-0 in THF and in DMF and III-2 in THF, $g_{em} \sim 1.4 \times 10^{-3}$ for III-2 in DMF), indicating that the excimers in the oligopeptides take no regular and specific configuration and consist of several kinds of configurations.

The sign of the CPF signal of the I-0 polypeptide is opposite from that of the I-2 polypeptide, indicating that the screw sense of the excimer configuration in the two polypeptide is opposite from each other, even though the main chain of the two polypeptides commonly takes a right-handed α-helical conformation. The g_{em} value is reasonably flat over the excimer fluorescence region in the two polypeptides. The constant g_{em} value suggests that only a single configuration of excimer is allowed in each polypeptide at each temperature.

The temperature dependence of CPF spectrum is noteworthy. In DMF solution, g_{em} values of the two polypeptides decreased with lowering temperature, and the decrease was more marked

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in the I-2 polypeptide (Figure 8). In THF solution, no temperature dependence was observed in the I-0 polypeptide, except for the effect of increasing contribution of monomer fluorescence without CPF signal. On the other hand, the I-2 polypeptide shows a significant decrease in the CPF intensity at the excimer band (Figure 9). The change of CPF spectrum indicates the change of excimer configuration in the I-2 polypeptide from a highly chiral one at high temperatures to a less chiral one at low temperatures. The temperature dependence of CPF spectra is in accord with the temperature dependence of excimer peak positions and that of excimer/monomer intensity ratios. All the spectroscopic data indicate that the configuration of excimer of the I-2 polypeptide changes around −30 to −40 °C. The configuration of excimer of the I-0 polypeptide is insensitive to temperature, especially in THF solution. The constant CPF intensity over the excimer-band region suggests only a single configuration is allowed in each polypeptide at each temperature. The excimer should have a chiral configuration with opposite screw senses for the I-0 and I-2 polypeptides.

Proposal of Possible Excimer Configurations. To estimate possible configurations of the excimer in the I-0 and I-2 polypeptides, conformational energy calculations were attempted. Two difficulties arise in the calculations. First, no reliable energy function is available for the excited-state interaction between two pyrenyl groups. Furthermore, within the framework of ECEPP system, the bond lengths and bond angles are not allowed to vary.[18] The change of the latter parameters will be important to predict the excimer configurations that may have higher conformational energies than those in the normal range of thermal fluctuations. However, since the observed activation energies were relatively low and the excimer formation is restricted by low conformational entropy, the conformational energy calculation was attempted within the framework of the ECEPP system. In the calculation, the π–π interaction between pyrenyl rings was not included. Other interatomic potentials including those between pyrenyl groups and the polypeptide main chain, were taken into account. First, only side-chain rotational angles, $\chi_1(1)$, $\chi_2(1)$, $\chi_1(2)$, and $\chi_2(2)$, were varied under the condition that the main chain is fixed to a right-handed α-helix. The range of the side-chain libration was limited within 3 kcal/mol in the energy contour map for $\chi_1(1)$ and $\chi_2(1)$ or for $\chi_1(2)$ and $\chi_2(2)$ around the most stable positions shown in Figures 2a and 3a. However, due to the bulkiness of pyrenyl group, the allowed range of the fluctuation was very small and the nearest center-to-center distances obtained were 6.9 Å for the I-0 polypeptide and 6.1 Å for the I-2 polypeptide. The result shows that the excimer cannot be formed only by the fluctuations of the side-chain rotational angles. Similarly, excimer conformation was not found in the energy calculation taking both fluctuations of the main-chain and the side-chain angles, when the fluctuation is limited within 3 kcal/mol from the most stable positions. This result is consistent with the finding that the excimer formation is a minor process in the polypeptides.

Since excimer conformations may be stabilized by the electronic stabilization energy between two pyrenyl groups and the excimer may be accumulated during the lifetime of the excited state of the pyrenyl group (>200 ns), the energy calculation should be extended to the regions of very high conformational energies. Therefore, in the second calculation, the main-chain angles as well as the side-chain angles were varied independently over wider ranges than in the first calculation. The range of main-chain libration was ±20° for ϕ and ψ around the standard α-helical conformation ($\phi = -57°$, $\psi = -47°$). This range of libration has been employed by Meirovitch et al.[23] in their Monte Carlo calculation of conformational entropy of α-helical polypeptide. The amide bond was fixed to a planar trans configuration. The side-chain angles were rotated from 0° to 360° at each main-chain conformation. In search for an excimer conformation of the I-0 polypeptide, conformational energy of Ac-pyrAla(1)-pyrAla(2)-$NHCH_3$ was calculated by varying $\psi(1)$, $\phi(2)$, $\chi_1(1)$, $\chi_2(1)$, $\chi_1(2)$, and $\chi_2(2)$ in the rotational range described above. Only one excimer conformation with $\psi(1) = 67°$, $\phi(2) = -77°$, $\chi_1(1) = 180°$, $\chi_2(1) = 180°$, $\chi_1(2) = 280°$, and $\chi_2(2) = 100°$ was found in this case. The conformation is shown in Figure 2b. In the excimer conformation, the center-to-center distance is 3.9 Å, and the nearest edge-to-edge distance is 2.5 Å. The two pyrenyl groups are arranged with a right-handed screw sense. The chirality factor defined by $\zeta = \mathbf{r}_{12} \cdot (\mathbf{m}_1 \times \mathbf{m}_2)$[24] ($\mathbf{r}_{12}$ = unit vector connecting the centers of pyrenyl groups, $\mathbf{m}_1$, $\mathbf{m}_2$ = unit vector along the long axis of each pyrenyl group) was +0.59. In the excimer conformation, only one hydrogen bond between the carbonyl group of pyrAla(1) and the NH group of Ala(5) was broken (O···H–N distance = 2.3 Å). Other hydrogen bonds that may be affected by the change of the main-chain rotational angles are bent to some extent, but the O···H–N distances change only slightly (elongation within 0.1 Å and shrinkage within 0.3 Å) from the normal distance (1.845 Å).

A similar calculation was made on the I-2 polypeptide. In the oligopeptide Ac-pyrAla(1)-Ala(2)-Ala(3)-pyrAla(4)-$NHCH_3$, several conformations suitable for excimer formation were found. The presence of multiple conformations is consistent with the finding of the temperature change of excimer configurations in the I-2 polypeptide. Among the possible conformations, those having a positive chirality factor may be excluded, since the CPF spectrum clearly indicates opposite screw senses for the excimers in the I-0 and I-2 polypeptides and the chirality factor is positive in the I-0 polypeptide both in the ground state ($\zeta = +0.32$) and in the excimer conformation ($\zeta = +0.59$). An excimer conformation of the I-2 polypeptide with the largest negative chirality factor is shown in Figure 3b. The rotational angles are $\psi(1) = -27°$, $\phi(2) = -57°$, $\psi(2) = -27°$, $\phi(3) = -67°$, $\psi(3) = -27°$, $\phi(4) = -57°$, $\chi_1(1) = 210°$, $\chi_2(1) = 60°$, $\chi_1(4) = 210°$, and $\chi_2(4) = 90°$. The chirality factor is −0.28. Since a relatively large number of bonds are involved in determining the relative orientation or pyrenyl groups in the I-2 polypeptide, the fluctuation of the bond lengths and bond angles may also be important in the excimer formation in the I-2 polypeptide.

Fluorescence Rise and Decay Analysis and the Rate of Excimer Formation. The experimental results and the theoretical considerations on the conformation of the polypeptides in the ground state and that in the excited state indicate that when the I-0 polypeptide is photoexcited the conformation changes from that in Figure 2a to that in Figure 2b. It is quite unusual that the starting conformation and the final conformation of the excimer formation are specified. Therefore the analysis of the rise and decay curve of the excimer emission will lead to the rate constant for a molecular motion along the specific route. The rise and decay curves were measured for the I-0 and I-2 polypeptides in the two solvents over the temperature range of +20 to −60 °C. The curves were commonly fitted to three-component exponential functions with a one-rise component, a fast decay component and a small contribution of a slow component. In the low-temperature region where the Arrhenius plot falls on a straight line, the inverse of the rise time of the excimer emission ($1/\tau$) corresponds to the sum of the rate constant of excimer formation (k_1) and that of the intrinsic decay of the monomer excited state of pyrenyl group (k_m):[20]

$$1/\tau = k_1 + k_m \quad (1)$$

The last rate constant was determined from the decay times of the model polypeptide II.[9] The rate constant of the excimer formation k_1 was calculated from k_m and τ and are plotted against temperature in Figures 10 and 11. The rate constants are on the order of $(1 - 3) \times 10^7$ s^{-1} over the temperature range −60 to +20 °C. The activation energy for the I-0 polypeptide is 1.2 kcal/mol in DMF and 0.6 kcal/mol in THF. The activation energies are smaller than those evaluated from the Arrhenius plot of the quantum yield ratios. At present, no reasonable explanation can be given for this disagreement. However, it should be pointed

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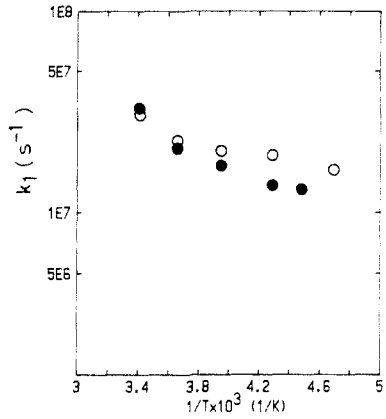

Figure 10. Arrhenius plot of the rate constant of the excimer formation of the I-0 polypeptide in DMF (●) and in THF (○).

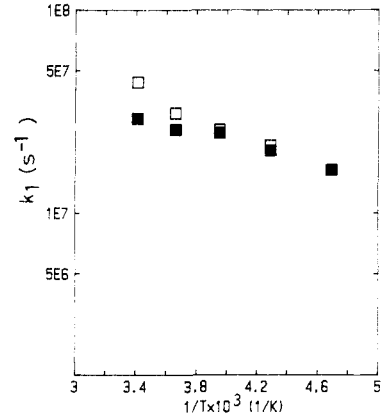

Figure 11. Arrhenius plot of the rate constant of the excimer formation of the I-2 polypeptide in DMF (■) and in THF (□).

out again that the activation energy is very small. The importance of the entropy term should be stressed for the excimer formation in this polypeptide system.

The rise time of the excimer formation was also measured for the I-2 polypeptide, and the rate constant of the excimer formation was calculated. The Arrhenius plot is shown in Figure 11. In this polypeptide, the final (excimer) conformation cannot be specified to a single conformation. However, the decay curves were also reasonably fitted to three-component exponentials with a single rise time. The rate of excimer formation is somewhat slower than that of the I-0 polypeptide at each temperature. The activation energy is again very small (0.9 kcal/mol in DMF, 1.2 kcal/mol in THF).

The rates of the excimer formation in the I-0 and I-2 polypeptides are much slower than the time scale of a local side-chain fluctuation measured by ^{13}C NMR relaxation method on the C^{β} of poly(γ-benzyl L-glutamate)[25] or by the anisotropy decay method on the tryptophan residue,[3,4] by about 2 orders of magnitude. This is reasonable, since the range of the librational motion must be much wider for the excimer formation. The present rate is even slower than the fluctuation of the whole helix of 21 amino acid polypeptide in solution (τ = 0.6 ns) and in lipid bilayer (9 ns).[4] On the other hand, the relaxation time for the main-chain motion accompanied with helix-coil transition is much longer than the present ones.[25a] Therefore, the range of the relaxation time found in the present experiment is consistent with the molecular motion proposed in Figures 2 and 3.

Conclusions

Two major conclusions are drawn from the present study. First, the helical polypeptide chain is rigid enough to fix side-chain groups, including such a large chromophore as pyrenyl group. Second, the relaxation time for the local unfolding of the helix conformation was found to be on the order of 30–40 ns.

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Registry No. I-0, 129314-68-7; I-2, 129215-26-5; Glu(OBzl) NCA, 3190-71-4; BOC-pyrAla-OH, 100442-89-5; BOC-pyrAla-Glu(OBzl)-OBzl, 121445-55-4; BOC-pyrAla-Ala$_2$-OBzl, 129149-42-4; BOC-Ala-OH, 15761-38-3; Ala-OBzl·TosOH, 42854-62-6; BOC-pyrAla-Ala$_2$-pyrAla-Glu(OBzl)$_4$-OBzl, 129173-90-6; *N*-methylmorpholine, 109-02-4.

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Temperature and Solvent Effects on Viscosity *B* Coefficients. Monovalent Ions in Acetonitrile at 15, 25, and 35 °C

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The viscosity *B* coefficients were measured for LiI, NaI, KI, $NaBPh_4$, and Bu_4NBPh_4 in acetonitrile at 15, 25, and 35 °C, and they were divided into the ionic components by using the reference electrolyte Bu_4NBPh_4. The ionic *B* coefficients were positive for all the monovalent ions studied at each temperature in this dipolar aprotic solvent. The temperature coefficients of the *B* values were quite small as in other nonaqueous solvents. The experimental results were compared with the values predicted by the dielectric friction theory to clarify the effect of ionic charge. The charge effect is examined also by correlating the ionic *B* coefficient with the dielectric constant of solvent.

Introduction

Although for a long time attention has been paid to the viscosity *B* coefficients for ions in aqueous solutions to investigate the effect of ions on the water structure, measurements of *B* coefficients in nonaqueous solutions are still scarce, in particular for its temperature dependence in aprotic solvents,[1] and the theoretical interpretation of *B* coefficients is not much developed after the achievement of Gurney.[2] To improve this situation, we carry out here a systematic study on the viscosity *B* coefficients for a variety

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