by ln A_0 and $1/T_0$. By comparing eq 13, with the Arrhenius type of behavior in eq 12, we find

$$\ln A = \ln A_0 + E_a/RT_0 \quad (14)$$

Thus a plot of ln A versus E_a/R will give a straight line with intercept of ln A_0 and slope of $1/T_0$. The plot of ln k_h versus $1/T$ for all the samples is shown in Figure 12. Ignoring the sample measured in dichloromethane solution, all the solid samples from ESR and TOF data intersect in the range of 150–300 K. Estimates of T_0 and A_0 are 190 K and 4×10^7 s^{-1}, respectively. It does seem that there is another factor operating, however, which may be a contribution due to changes in the dielectric constant, in addition to a compensation effect due to the absorption of dichloromethane, and to the presence of the cation radical and its counterion. A higher dielectric constant in dichloromethane solution compared to the films would also explain the high value of ln k_h for the solution ESR data.[10]

Figure 13 shows the plot of eq 14 for this data, including the TOF data. The fit to eq 14 is good, with a T_0 and A_0 of 225 K and 8×10^7 s^{-1}, in good agreement with the values from Figure 12. Again the dichloromethane data are anomalous, showing higher than expected A compared to the value of E_a, again as expected due to a change in dielectric constant. The conclusion is that the differences in k_h, A, and E_a observed within the ESR data, and in comparison with the TOF data, are due mainly to the compensation effect, with a less important effect due to changes in dielectric constant.

Conclusions

The mediation of hole-transport via the TPD$^+$ radical cation has been observed directly by using electron spin resonance in TPD films. In TPD films that are doped with tris(*p*-bromophenyl)-ammoniumyl hexachloroantimonate, or HNO_3, the Arrhenius activation parameters can be calculated for hole transport from the ESR data, giving $E_a = 10 \pm 2$ kJ/mol, $A = (1.7 \pm 1) \times 10^{10}$ s^{-1}, and $k_{h300} = (3.1 \pm 1) \times 10^8$. The value of E_a is 1/2 of that from time-of-flight measurements at zero field, while k_{h300} is a factor of 10 smaller, and A is a factor of 100 smaller. The presence of residual solvent and the ion-pairing of an TPD$^+$ ion with a dopant counterion appear be responsible for these differences. The differing results can be understood qualitatively and quantitatively in terms of the compensation effect, which has been previously applied to dark conductivity in organic semiconductors. In TPD/polycarbonate films the rate of hole transport is too low at zero field to produce significant changes in the ESR spectrum; thus the ESR results only show that the rate of hole transport must be less than 2×10^{-8} s^{-1} in these films. This is consistent with TOF data. In dichloromethane solution, an E_a of 9.7 kJ/mol was observed by using ESR. Extrapolating the solution data to solid TPD gave a rate of hole transport of 9.75×10^9 s^{-1}, a factor of 3 higher than that from TOF measurements. The lower E_a and higher rate in dichloromethane solution are consistent with the effect of the higher dielectric constant compared to an TPD film. This suggests that the rate-determining step for hole transport is the same in solution as it is in the solid state.

Distance and Orientation Dependence of Electron Transfer and Exciplex Formation of Naphthyl and *p*-Dimethylanilino Groups Fixed on a Helical Polypeptide Chain

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Polypeptides carrying a *p*-(dimethylamino)phenyl group (D) and a naphthyl group (N) at the middle of an α-helix chain were synthesized. The separation between D and N groups was varied by inserting different numbers (m) of alanyl units between them. The interchromophore center-to-center distance and the shortest edge-to-edge distance were estimated to be 8.3 and 5.4 (m = 0), 12.0 and 9.3 (m = 1), and 8.0 and 5.7 Å (m = 2). The D–N pair takes a head-to-tail orientation in the m = 0 polypeptide and a face-to-face orientation for the m = 2 polypeptide. Fluorescence from either D or N groups was markedly quenched and exciplex was formed in the m = 0 and 2 polypeptides, but neither quenching nor exciplex formation was observed for the m = 1 polypeptide. The absence of electron-transfer interactions in the m = 1 polypeptide indicates that the electron-transfer interactions in the polypeptides are occurring through space. The quenching efficiency was insensitive to the relative orientation of the D–N pair, but the exciplex formation was more effective in a face-to-face orientation than in a head-to-tail one. The exciplex emission of the m = 2 polypeptide in THF was circularly polarized ($g_{em} = 1.5 \times 10^{-3}$), indicating a specific chiral configuration of the exciplex.

Introduction

Photoexcitation of an electron donor–acceptor system leads to exciplex formation or electron transfer, depending on the nature of donor and acceptor, solvent, temperature, and other external factors.[1] Mataga and co-workers proposed that exciplex formation and electron transfer occur through different nonrelaxed charge-transfer (encounter) complexes.[2] However, it is still unknown what type of encounter complex leads to exciplex formation and what is favorable for the electron transfer, under conditions where other external factors are kept constant. In order to answer this question, a model system in which a donor–acceptor pair is fixed with a specific distance and orientation is necessary. However, such rigid systems usually show strong ground-state interactions without forming typical exciplexes. On the other hand, a flexible system, like 3-(*p*-(dimethylamino)phenyl)propyl-1-pyrene, does not seem to form an encounter complex with a single

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(1) For a review, see, Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401.
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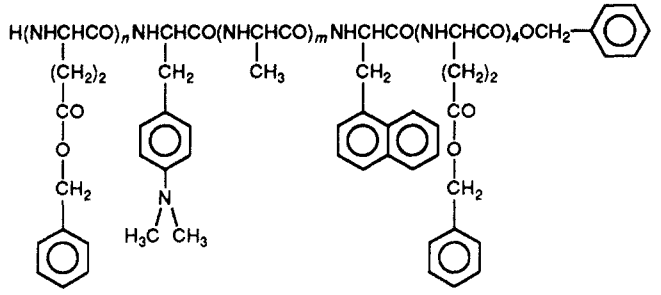
configuration. A semirigid intramolecular donor–acceptor pair may be the best system to study the exciplex formation/electron transfer competition.

The authors have been working on synthetic polypeptides along which different chromophores are arranged in a specific order.[3-5] Taking advantage of the semirigid helical conformation of polypeptides, one can arrange different chromophores in a specific order and orientation along the helix. In the polypeptide, the chromophores are not rigidly fixed, but allowed to fluctuate around a single stable orientation.

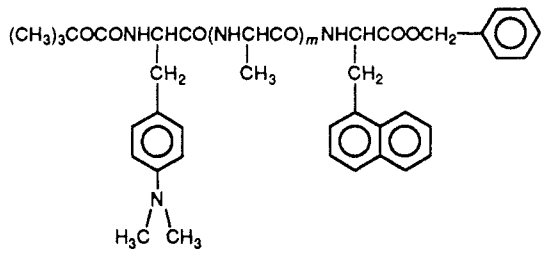
Chirality is another advantage of the polypeptide system. Circular dichroism (CD) gives detailed information on the chromophore orientation in the ground state, whereas circularly polarized fluorescence (CPF) provides information on the geometry of the complexes formed in the excited state.[6,7]

In this study, exciplex formation was studied on helical polypeptides carrying a single *p*-dimethylanilino (D) group and 1-naphthyl group (N) (I, m = 0, 1, 2). Two kinds of chromophores



Glu(OBzl)$_n$-dmaPhe-Ala$_m$-napAla-Glu(OBzl)$_4$-OBzl (I-m, m = 0, 1, 2)

were attached to the polypeptide in the form of β-arylalanines, i.e., L-*p*-(dimethylamino)phenylalanine (dmaPhe) and L-1-naphthylalanine (napAla). The distance and orientation of the D–N pair were varied by introducing m alanyl (Ala) units between the two artificial aromatic amino acid units. Four Glu(OBzl) units were attached to the C-terminal. The tetrapeptide corresponds to a single turn of α-helix, which may stabilize the α-helical conformation around the napAla unit. Oligopeptides carrying the same D–N pair were also prepared as flexible counterparts (II-m). Polypeptides carrying one *p*-(dimethylamino)phenyl (D)



Boc-dmaPhe-Ala$_m$-napAla-OBzl (II-m, m = 0, 1, 2)

group or one 1-naphthyl (N) group were also prepared in order to examine intrinsic properties of the D and N groups incorporated in the α-helix (III, IV). Peptides carrying D or N group were

Glu(OBzl)$_n$-napAla-Glu(OBzl)$_4$-OBzl (III)

Glu(OBzl)$_n$-dmaPhe-Glu(OBzl)$_4$-OBzl (IV)

also used as low-molecular-weight model compounds (V, VI).

Boc-Glu(OMe)-napAla-Glu(OMe)$_2$-OBzl (V)

Boc-Glu(OMe)-dmaPhe-Glu(OMe)$_3$-OMe (VI)

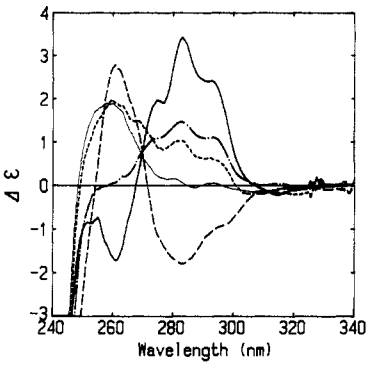


Figure 1. CD spectra of the polypeptide I-m and the model peptides in the absorption region of D and N groups: I-0 (—), I-1 (- - -), I-2 (– – –), III (–·–), and IV (—) in TMP at room temperature. The ordinate is the differential absorption coefficient with respect to the molar concentration of the D or N group. [D] = [N] = 4×10^{-5} M.

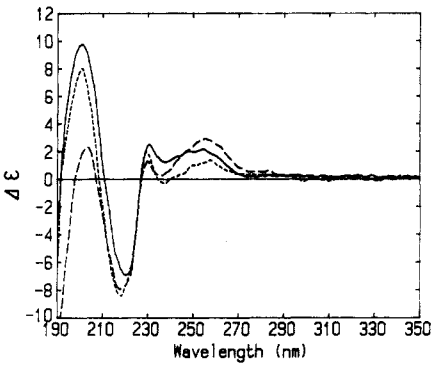


Figure 2. CD spectra of the peptides II-m in TMP at room temperature: II-0 (—), II-1 (- - -), II-2 (– – –). [D] = [N] = 2×10^{-5} M.

Results and Discussion

Circular Dichroism Spectra. A prerequisite condition of this study is that the D and N groups are linked to an α-helical polypeptide chain with a specific distance and orientation. This was confirmed by circular dichroism (CD) spectroscopy.

The main-chain conformation of a polypeptide in solution can be determined by CD spectroscopy in the region of amide absorption. CD spectra of the three kinds of polypeptides in trimethyl phosphate (TMP) showed a typical pattern of the right-handed α-helix. The differential molar absorption coefficient ($\Delta\epsilon$) at 222 nm with respect to the molar concentration of the amide unit was −13.3 (I-0), −12.7 (I-1), and −14.6 (I-2). These values indicate a fully helical conformation of the polypeptide main chain.[8]

CD spectra at the absorption region of the D and N groups are shown in Figure 1. It should be noted that homopolymer of γ-benzyl L-glutamate shows no CD signal in this region. The phenyl chromophores linked to the long spacer chain [–(CH_2)$_2$–CO–O–CH_2–] do not take specific orientations with respect to the helix axis. Contrary to the benzyl side groups, the D and N groups of the three polypeptides show moderately strong CD signals at their absorption wavelengths, indicating that their orientations are confined within a narrow conformational range. Profiles of the CD spectra of the three D–N polypeptides differ significantly from each other. The different CD profiles are interpreted in terms of different spatial arrangements of the D–N pairs and the dipole–dipole electronic interactions between the D and N groups.

The CD spectrum of I-1 is similar to the sum of the spectra of III and IV, indicating that the D and N groups in I-1 do not interact with each other. As will be shown from conformational analysis, the D and N groups in I-1 are located on the opposite side of the helix and separated by about 12 Å. Under these conditions, only the interaction between the chromophore and the

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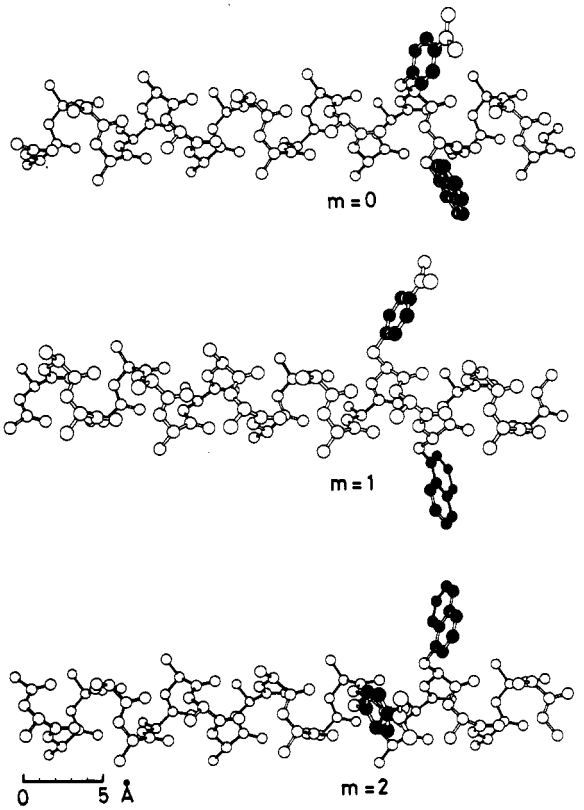

Figure 3. NAMOD (Version 3) molecular display of the most probable side-chain orientations of the polypeptides with different spacer chains intervening between D and N groups. Hydrogen atoms are omitted for simplicity, although they are included in the calculation.

polypeptide main-chain induces the CD. On the other hand, the D–N pairs in I-0 and I-2 are shown to be separated by about 8 Å, with different orientations. The interchromophore distances of I-0 and I-2 may be short enough to induce dipole–dipole interactions that cause the positive and negative splittings in the CD spectra.

CD spectra of the low-molecular-weight model compounds (II-*m*) carrying the same D–N pair were also measured (Figure 2). The three compounds show similar CD patterns, irrespective of the number of alanyl units intervening between D and N groups. Therefore, the CD spectra are regarded as the intrinsic CD of dmaPhe and napAla units, their orientations suffering no constraint from specific main-chain conformation.

Conformational Analysis. The results of CD spectroscopy of the polypeptides showed that the main-chain conformation is a right-handed α-helix and the orientations of the side-chain chromophores are constrained in a narrow conformational space which is determined by the steric interactions between the chromophore and the α- helical main-chain. The orientations of the D and N groups were determined by conformational energy calculation, assuming a regular right-handed α-helical main-chain ($\phi = -57°$, $\psi = -47°$, $\omega = 180°$). In the calculation, an alanyl unit was substituted for the γ-benzyl L-glutamyl unit for simplicity. The structure and energy parameters were taken from the ECEPP system.[9] The structural parameters for dmaPhe and napAla units were taken from the analogous compounds. The partial charges for the two artificial amino acids were calculated by the CNDO/ON MO method. The details of the parameters have been reported elsewhere.[10]

The most stable orientations of D and N groups on the helical polypeptide were estimated from a conformational energy minimization for Ac–$(Ala)_{24-m}$–dmaPhe–Ala_m–napAla–Ala_4–NMA (Ac = acetyl group; NMA = *N*-methylamide group; m = 0, 1, 2). The most stable orientations obtained were (χ_1^D, χ_2^D, χ_1^N, χ_2^N) = (185°, 265°, 183°, 257°) for m = 0, (188°, 266°, 187°, 257°) for m = 1, and (188°, 266°, 188°, 257°) for m = 2. The side-chain rotational angles are insensitive to m, indicating that the orientations of D and N groups are independent of each other in the polypeptide.

Figure 3 shows α-helical conformations of the three polypeptides with the most probable side-chain orientations.[11] The center-to-center (edge-to-edge) distances are 8.3 (5.4) Å for m = 0, 12.0 (9.3) Å for m = 1, and 8.0 (5.7) Å for m = 2. It is interesting to note that the D–N distances of the m = 0 and two polypeptides are about the same, but the orientations of the two chromophores differ markedly. A head-to-tail orientation is predicted for the m = 0 polypeptide, whereas a face-to-face orientation is predicted for the m = 2 polypeptide.

Thermal fluctuation of D and N groups on the α-helical polypeptide chain has been estimated. Fluctuation of the N group is very small near room temperature (thermal energy <1 kcal/mol). The allowed range of the bond rotations are 190 ± 15° for $\chi_1(C^\alpha–C^\beta)$ and 260 ± 5° for $\chi_2(C^\beta–C^\alpha)$. The D group fluctuates in much wider angles, e.g., χ_1 = 190 ± 15° and χ_2 = 270 ± 30°. Taking the thermal fluctuation into account, the shortest center-to-center distance of the D–N pair is calculated to be 7.8 Å (m = 0), 11.8 Å (m = 1), and 7.7 Å (m = 2).

Absorption Spectra. Absorption spectra of the three kinds of polypeptides were measured in TMP solution. The spectra were virtually indistinguishable from each other and from the model compounds carrying the D–N pair, in higher wavelength region than 275 nm where the contribution of the benzyl group can be ignored. The spectra were also virtually identical with the sum of the spectra for the model compounds carrying D or N groups. The coincidence indicates the absence of any ground-state interactions between D and N groups, except for the dipole–dipole exciton interaction as detected in the CD spectrum. The same conclusion was obtained from the absorption spectra in tetrahydrofuran (THF) solution.

Distribution of Photoenergy between the Two Chromophores. Since absorption spectra of D and N groups are largely overlapped, neither the D nor N group can be photoexcited selectively. For example, 83% of the incident photons are absorbed by the D group at 311.5 nm and 82% by the N group at 285 nm in TMP. The photoenergy initially absorbed by the D or N group will be redistributed between the two chromophores by nonradiative energy transfers. The Förster critical distances, r_0, for the energy transfer were calculated from the overlapping integrals of the absorption and fluorescence spectra in TMP of the model compounds containing N or D groups (V and VI)[12] to be 13.8 Å for r_0 ($N^* \rightarrow$ D) and 5.6 Å for r_0 ($D^* \rightarrow$ N). Those in THF were 13.9 and 5.9 Å, respectively. The rates of energy transfer are calculated according to the equation: $k_{et} = (1/\tau)(r_0/r)^6$. In the equation, r is the interchromophore distance estimated from the conformational energy calculation (8.3, 12.0, 8.0 Å for m = 0, 1, and 2, respectively), and τ is the intrinsic lifetime of the excited state of N and D groups as estimated from the fluorescence lifetimes of V (59 ns) and VI (3.8 ns), respectively. The rate constants for $N^* \rightarrow$ D transfer are 3.6×10^8 s^{-1} (m = 0), 3.9×10^7 s^{-1} (m = 1), and 4.5×10^8 s^{-1} (m = 2). Those for $D^* \rightarrow$ N transfer are 2.5×10^7 s^{-1} (m = 0), 2.7×10^6 s^{-1} (m = 1), and 3.1×10^7 s^{-1} (m = 2). It should be noted that in the above calculation the orientation factor κ^2 is taken to be 2/3, which is for random orientations of D and N groups. This assumption may be justified partly by the fact that no particular polarization is predominant in the absorbance and fluorescence transitions of N groups. Small changes in the orientation factor do not alter the following qualitative discussions.

The energy transfer competes with the formation of encounter complex and with the intrinsic deactivation through radiative and nonradiative paths. The latter rate constants are estimated from

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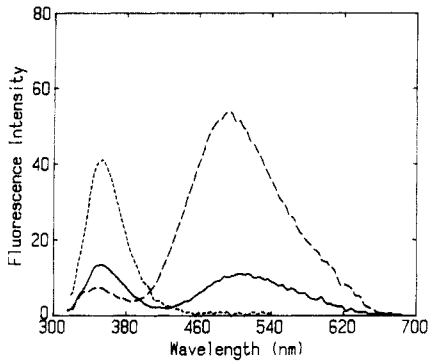

Figure 4. Fluorescence spectra of the polypeptides I-*m* in TMP at room temperature: I-0 (—), I-1 (---), I-2 (–––). λ_{ex} = 311.5 nm. [N] = [D] = 2×10^{-5} M.

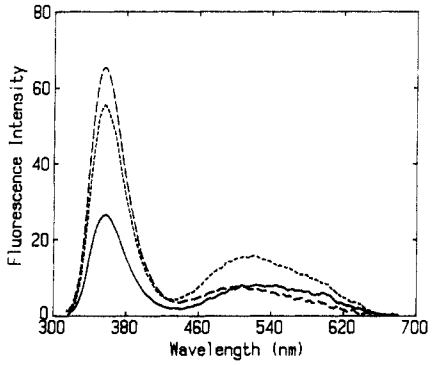

Figure 5. Fluorescence spectra of the peptides II-*m* in TMP at room temperature. λ_{ex} = 311.5 nm. II-0 (—), II-1 (---), II-2 (–––). [N] = [D] = 2×10^{-5} M.

the fluorescence decay times of the N group in III and the D group in IV to be 1.7×10^7 s^{-1} for N* and ca. 1×10^9 s^{-1} for D*. If one assumes the rate of the formation of encounter complex to be 2×10^8 s^{-1},[13] only 2% of photoenergy absorbed by D group will be transferred to N group before the formation of encounter complex in the case of the polypeptide I-0. The energy transfer efficiency will be 3% for the polypeptide I-2. On the other hand, the energy transfer from N* to D is competitive to the formation of encounter complex (62% for I-0, 67% for I-2). The rough estimation shows that the energy transfer from D* to N is negligible, whereas that from N* to D is predominant. In the following experiment, the polypeptide samples are photoexcited at 311.5 nm, where 83% of the photoenergy is initially absorbed by the D group. However, as a result of the energy redistribution, more than 90% of the photoenergy will be localized on the D group before the formation of encounter complexes in the case of the polypeptides I-0 and I-2.

Fluorescence Spectra. Figures 4 and 5 show fluorescence spectra of the polypeptides and the model compounds, respectively, in TMP at 20 °C. The exciting wavelength is 311.5 nm. The spectra show a striking dependence on the length of the spacer chain *m*. Only emission from a locally excited D group is observed in the polypeptide I-1. The monomer emission is largely quenched and the exciplex emission centered at 510 or 490 nm appears in the polypeptides I-0 and I-2, respectively. Similar behaviors were observed also in THF solution (Figure 7). Evidently, the different spatial arrangements of the D–N pairs cause the different

(13) The very rough estimation of the rate of the formation of encounter complex on the polypeptide was made from the preliminary measurement of the rise time of the exciplex emission. The rise time varied from less than 1 ns to about 17 ns, depending on the length of spacer chain *m*, solvent, and temperature. Furthermore, any single rise time could not explain the experimental rise curves of the polypeptides. The assignment of 5 ns to the rise time for the exciplex formation or the encounter complex formation is only tentative. The variation of the rise time within a reasonable range (1–17 ns) does not alter the qualitative discussion described in the text.

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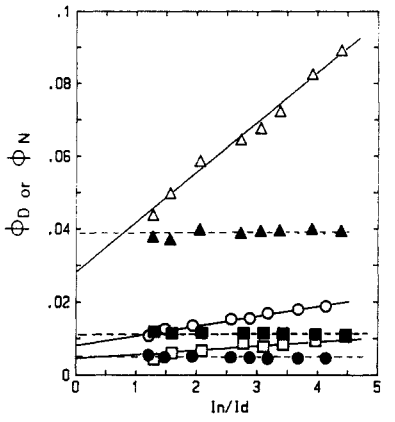

Figure 6. Quantum yield of D fluorescence of I-0 (○), I-1 (△), and I-2 (□) and that of N fluorescence of I-0 (●), I-1 (▲), and I-2 (■), plotted against the ratio of the absorption intensities of N and D groups measured at different wavelengths in the range of 285–300 nm.

fluorescence behaviors. The intrinsic ability of the dmaPhe-Ala-napAla sequence (m = 1) to form exciplex is higher than or comparable to those of the other two sequences, as can be seen in the fluorescence spectra of the model compounds (Figures 5 and 8). Therefore, the absence of exciplex in the polypeptide I-1 is explained in terms of unfavorable orientation of the D–N pair on the α-helix. The polypeptide I-2 shows much stronger exciplex emission than the polypeptide I-0 in TMP as well as in THF. Since the interchromophore distances are about the same for the two polypeptides, the stronger exciplex emission of the polypeptide I-2 may be interpreted in terms of favorable orientation of the D–N pair (face-to-face) and inadequate orientation (head-to-tail) in the polypeptide I-0.

Fluorescence peaks in the region of 310–420 nm should consist of the emissions from locally excited states of D and N groups. However, only D fluorescence was observed when the sample was excited at 311.5 nm, where 83% (in TMP) or 85% (in THF) of the photoenergy is intially absorbed by D groups (Figures 4, 5, 7 and 8). The absence of N fluorescence may be due to the energy transfer from N* to D. At other exciting wavelengths, a small contribution of N fluorescence was detected. The fluorescence intensities of the D and N groups were estimated separately by a spectrum resolution by means of the least-squares method. The fluorescence quantum yield of the locally excited D and N group ϕ_D and ϕ_N, i.e., the number of photons emitted from the D* and N* divided by those absorbed by the D and N group, respectively, were then calculated. The fluorescence quantum yield evaluated at various excitation wavelengths is plotted against the ratio of the photoenergies absorbed by the N group and the D group (Figure 6). The ϕ_N values are insensitive to the amount of the excited D groups, indicating the absence of energy transfer from D* to N group in the three polypeptides. This result is consistent with the above consideration on the rates of energy transfer. On the other hand, ϕ_D increases with increasing the amount of excited N groups. This clearly shows the contribution of energy transfer from N* to D group. From the intercept of the straight line in Figure 6, the ϕ_D values when only D groups are photoexcited were evaluated to be 0.008 (I-0), 0.028 (I-1), and 0.0044 (I-2). The ϕ_D of the polypeptide I-1 may be compared with that in the polypeptide IV (0.025). The similar quantum yield indicates the absence of quenching process by the presence of an N group nearby the D group in the polypeptide I-1. In our separate work, a similar polypeptide (m = 1) carrying a 1-pyrenyl group instead of 1-naphthyl group has been synthesized and the rate of electron transfer from the D group to the excited pyrenyl group has been measured.[5] The rate constant was on the order of 10^5 s^{-1}. Therefore, it is reasonable to conclude that no electron transfer quenching takes place in the present polypeptide with m = 1.

If one assumes ϕ_D of the polypeptide I-1 as the limiting value when no electron-transfer quenching occurs, the quantum yield of the electron transfer including exciplex formation, i.e., the quantum yield of the encounter-complex formation ϕ_{EC}, is cal-

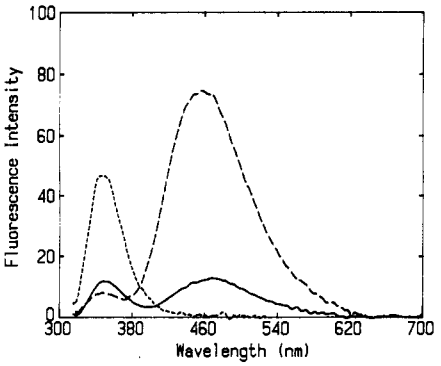

Figure 7. Fluorescence spectra of the polypeptides I-*m* in THF at room temperature: I-0 (—), I-1 (---), I-2 (–––). λ_{ex} = 311.5 nm. [N] = [D] = 2×10^{-5} M.

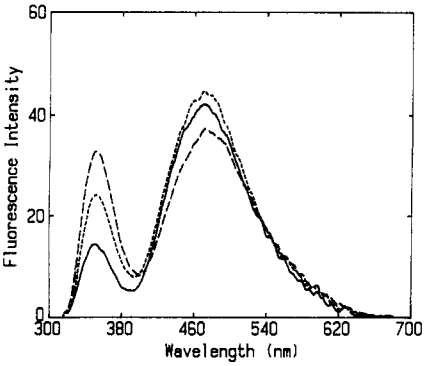

Figure 8. Fluorescence spectra of the peptides II-*m* in THF at room temperature: II-0 (—), II-1 (---), II-2 (–––). λ_{ex} = 311.5 nm. [N] = [D] = 2×10^{-5} M.

culated from $1 - \phi_D(\text{I-}m)/\phi_D(\text{I-1})$. The ϕ_{EC} values are 0.72, 0, and 0.84 for the polypeptides I-0, I-1, and I-2, respectively. Two important conclusions are drawn from these values. First, the electron transfer interaction occurs through space and through-bond interaction is negligibly small. The absence of through-bond interaction in this polypeptide system contrasts with the electron transfer between donors and acceptors rigidly fixed on saturated hydrocarbon spacers, where through-bond interactions play an important role in the electron transfer. Since the electronic potential barrier of the amide bond should be lower than that of the saturated hydrocarbon chain, the absence of through-bond interaction in polypeptides needs further investigation in future.

The second conclusion drawn from the ϕ_{EC} values is that the polypeptides I-0 and I-2 showed a similar efficiency in the encounter-complex formation. The center-to-center distances between D and N groups are estimated to be 8.3 and 8.0 Å for *m* = 0 and 2, respectively. On the other hand, the nearest edge-to-edge distances are 5.4 and 5.7 Å for m = 0 and 2, respectively. Probably, the present energy calculation was not accurate enough to allow interpretation of the small difference in the efficiency of encounter-complex formation in terms of different interchromophore distances estimated. It is concluded to a qualitative level that the efficiency of the encounter-complex formation with the face-to-face orientation is about the same as that with the head-to-tail orientation for nearly the same interchromophore distances.

Despite the similar efficiency of encounter-complex formation, the efficiency of exciplex formation of the polypeptide I-2 is much higher than that of the polypeptide I-0. This tendency was also observed in THF solution. A possible reason for the stronger exciplex of the polypeptide I-2 is that the D–N pair in the latter polypeptide thermally fluctuates more vigorously than that in the polypeptide I-0, due to increased rotational freedom of the spacer chain intervening between D and N groups. However, as is shown below, the energy barriers of the two polypeptides to form exciplex are about the same. This is expected if the α-helical main chain is rigid enough to allow only thermal fluctuations of the side chains for the exciplex formation. Therefore, the higher mobility of the D–N pair in the polypeptide I-2 may not be the major reason for the higher quantum yield of the exciplex formation. A qualitative conclusion is that the face-to-face configuration is more favorable for the exciplex formation than the head-to-tail configuration.

Actually, the face-to-face configuration in the polypeptide I-2 is very favorable for the exciplex formations. The intensity of the exciplex emission of the polypeptide I-2 is higher than those of the model peptides (II-0, II-1, II-2) in which the D–N pairs are connected by short and flexible chains (Figures 4 and 5 and 7 and 8). It should be noted that the favorable configuration of the encounter complex for exciplex may depend on the nature of the electron donating and accepting molecular orbitals. The presence conclusion might be changed when different donors and acceptors were used.

Results in Tetrahydrofuran Solution. The above conclusions might be altered when solvent of different polarity is used. In polar solvents the encounter complex takes loose configurations

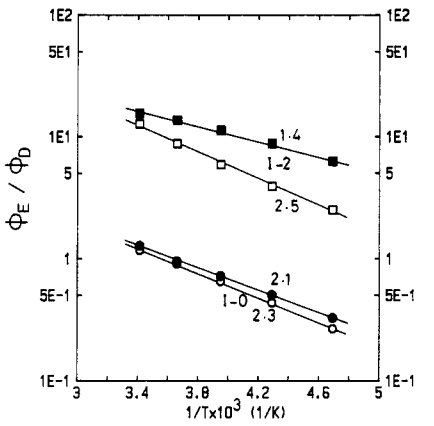

Figure 9. Arrhenius-type plot of the ratio of quantum yields of exciplex emission to the monomeric fluorescence of D group. λ_{ex} = 311.5 nm. I-0 (○), I-2 (□) in TMP and I-0 (●), I-2 (■) in THF. room temperature. The activation energies in kcal/mol are indicated in the figure.

and their formation may not depend sharply on the geometry of the D–N pair. This might be the case in the above experiment using TMP as a polar solvent (dielectric constant = 17.5). Fluorescence spectra were also measured in THF solution, a less polar solvent (dielectric constant = 7.6). Figure 7 shows the spectra of the three kinds of polypeptides and Figure 8 those of the model peptides in THF. The spectra of the polypeptides in THF are not much different from those in TMP solution, except for the blue shift of the exciplex emissions by 30–40 nm (λ_{em} = 470 nm for I-0 and 457 nm for I-2). The relative quantum yields of the exciplex and the D fluorescence are not much different from those in TMP solution. In contrast, the spectra of the model peptides in THF show much higher quantum yield of the exciplex emission. Presumably the effect of solvent may be more significant in the model peptides in which the D–N pair is in direct contact with solvent molecules. The model peptides in less polar solvent (THF) may favor exciplex formation rather than electron transfer quenching and also alter the ground-state conformations to increase compact conformations. In the polypeptide, the α-helical conformation is the most stable conformation in THF as well as in TMP and the configuration of the encounter complex or the exciplex will not be affected by the solvent. However, as will be described below, the effect of solvent is more pronounced in the temperature dependence of the exciplex formation and in the CPF spectrum, which may be more sensitive to the nature of environment.

The advantage of the face-to-face configuration over the head-to-tail one for the exciplex formation as well as the absence of electron transfer or exciplex formation in the polypeptide I-1 is also concluded in the less polar solvent.

Temperature Dependence. The exciplex formation was suppressed at low temperatures, indicating that the exciplex is formed by thermal fluctuations of the configuration of D–N pair. Figure 9 shows the Arrhenius plots for the ratios of the fluorescence

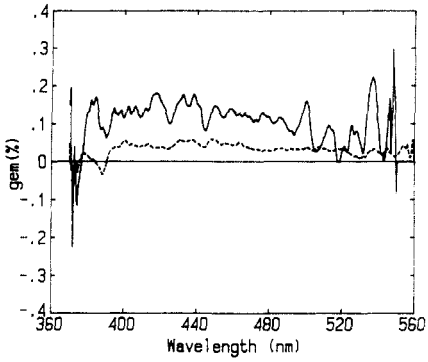

Figure 10. CPF spectra of the polypeptide I-2 in THF (—) and in TMP (---) at room temperature. λ_{ex} = 306 nm. [N] = [D] = 1×10^{-4} M.

quantum yields of the exciplex and the locally excited D group of the polypeptide I-0 and I-2 in TMP and in THF. The apparent activation energies were evaluated from the slope and are indicated in the figure. In TMP, the activation energies for the two polypeptides are the same. In THF, the activation energy of the polypeptide I-0 is higher than that of the polypeptide I-2, indicating that the conformational change required for the exciplex formation is more extensive in the former case. The different behaviors in the two solvents are interpreted as follows: in the polar solvent, an encounter complex with a loose structure is formed by the electron transfer before the exciplex formation, while in less polar solvent, the encounter complex that leads to exciplex may have a compact structure and its formation is governed by thermally assisted conformational change. Therefore, the activation energy varies sensitively by the ground-state conformation of the D–N pair.

Circularly Polarized Fluorescence Spectra. The structure of the exciplex is most conveniently studied by circularly polarized fluorescence (CPF) spectroscopy.[6,7] Figure 10 shows CPF spectra of the polypeptide I-2 in TMP and in THF. A positive CPF signal ($g_{em} = 1.5 \times 10^{-3}$) is observed in the exciplex emission of the polypeptide I-2 in THF. Only a small CPF signal ($g_{em} = 5 \times 10^{-4}$) was observed in TMP. No CPF signal was observed for the polypeptide I-0 in TMP and THF solutions. The positive CPF signal of the polypeptide I-2 in THF indicates a chiral configuration of the exciplex. The exciplex of the polypeptide I-2 may take a configuration which is similar to the ground-state configuration as shown in Figure 3. The smaller CPF signal of the polypeptide I-2 in TMP solution indicates a loose structure of the exciplex in the polar solvent. No CPF signal was observed for the oligopeptide II-2 either in THF or in TMP solution. The absence of the CPF signal indicates less defined and probably more than one exciplex configuration of the flexible oligopeptide.

Since a large conformational change may be required for the exciplex formation in the polypeptide I-0, the exciplex may also take several configurations. This may be a reason for the absence of CPF signal in the polypeptide I-0.

Conclusions

The electron-transfer-type interactions in the polypeptides were found to occur through space. The D–N pair with a face-to-face configuration was very favorable for exciplex formation, whereas that with a head-to-tail configuration was not adequate for the exciplex formation. The D–N pair separated by 12 Å did not show any evidence for electron transfer or exciplex formation.

Experimental Section

Materials. The synthesis of the oligopeptides was carried out by the conventional liquid-phase method. Each intermediate was identified by ^{1}H NMR and IR spectroscopy. The synthesis and optical resolution of L-1-naphthylalanine[13] and L-*p*-(dimethylamino)phenylalanine[5] have been reported. The purity of each intermediate was checked by IR, ^{1}H NMR, and thin-layer chromatography analysis.

The following abbreviations will be used: Glu for L-glutamic acid, Ala for L-alanine, dmaPhe for L-*p*-(dimethylamino)-phenylalanine, napAla for L-1-naphthylalanine, Boc for *tert*-butoxycarbonyl, OBz for benzyl ester, OMe for methyl ester, OSu for *N*-hydroxysuccinimide ester, $(Boc)_2O$ for di-*tert*-butyldicarbonate, TsOH for *p*-toluenesulfonic acid, DCHA for dicyclohexylamine, DCCI for dicyclohexylcarbodiimide, DCU for dicyclohexylurea, EDC for 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (water-soluble carbodiimide), HOBt for 1-hydroxybenzotriazole, TEA for triethylamine, MM for *N*-methylmorpholine, Pd–C for 10% palladium on activated carbon, IBCF for isobutyl chloroformate, DMF for dimethylformamide, THF for tetrahydrofuran, DOX for *p*-dioxane, NCA for *N*-carboxyanhydride.

Boc-dmaPhe-napAla-Glu(OBzl)$_4$-OBzl. The oligopeptide was synthesized by the fragment condensation of Boc-dmaPhe-napAla-OH[10] with Glu(OBzl)$_4$-OBzl by the mixed anhydride technique. Boc-dmaPhe-napAla-OH (62.6 mg, 0.124 mmol) was dissolved in THF (1.5 mL) and cooled to −20 °C and MM (13.9 μL) and then IBCF (16.6 μL) were added under stirring. After 6 min, HCl·Glu(OBzl)$_4$-OBzl (126 mg, 0.123 mmol) dissolved in THF (3.5 mL) was added and then MM (14 μL) was added. The mixture was stirred for further 1.5 h at −20 to −10 °C and then kept in a refrigerator for 12 h. The reaction mixture was poured into ethyl acetate and the solution was washed with 3% $NaHCO_3$, 10% NaCl, 10% citric acid, and 10% NaCl solutions. After drying on $MgSO_4$, the mixture was concentrated with evaporator. The product was purified by eluting through a silica-gel column in ethyl acetate. Yield 97 mg (53%); mp 197–202 °C. Anal. Calcd for $C_{84}H_{93}N_7O_{17}$: C, 68.51; H, 6.37; N, 6.66. Found: C, 68.42; H, 6.28; N, 6.61.

Glu(OBzl)$_n$-dmaPhe-napAla-Glu(OBzl)$_4$-OBzl (I-0). The above hexapeptide (20 mg, 0.014 mmol) was dissolved in formic acid (2 mL) and allowed to stand at room temperature for 7 h. The formic acid was removed by evaporation and 3% $NaHCO_3$ was added to the residue. The N-deprotected hexapeptide was extracted with ethyl acetate and the extract was washed with NaCl solution and dried on $MgSO_4$. Evaporation of the solvent gave 11.3 mg (0.0081 mmol) of the deprotected hexapeptide (58%). The deprotected peptide was dissolved in DMF (0.37 mL) and a DMF solution of Glu(OBzl) NCA (63.2 mg, 0.24 mmol/0.1 mL, [NCA]/[hexapeptide] = 30) was added. After 2 days, the characteristic IR peaks of the NCA disappeared. After another day, the polymer solution was poured into ether and the precipitate was collected. Yield 47 mg (74%). Fractions of higher molecular weight than the elution limit of Sephadex LH-60 gel (in DMF) were collected. The number average degree of polymerization ($\bar{n}$) of poly(γ-benzyl L-glutamate) unit was determined from the absorbance of the benzyl group (257.5 nm) to be 27.

Boc-Ala-napAla-OBzl. Boc-Ala-OH (0.49 g, 2.6 mmol) and TsOH·napAla-OBzl[10] (1.24 g, 2.6 mmol) were dissolved in DMF (10 mL). TEA (0.36 mL, 2.6 mmol), EDC (0.52 g, 2.7 mmol), and HOBt (0.37 g, 2.7 mmol) were added to the DMF solution at 0 °C. The mixture was stirred for 3 h under cooling with ice and for another day at room temperature. The solvent was evaporated and the residue was dissolved in ethyl acetate. The solution was washed with 10% citric acid, 10% NaCl, 3% $NaHCO_3$, and 10% NaCl solutions and then dried on $MgSO_4$. The solvent was evaporated and hexane was poured onto the residual oil. The solid that appeared was recrystallized from ethyl acetate/hexane mixture. Yield 0.87 g (71%); mp 79–81 °C. Anal. Calcd for $C_{28}H_{32}N_2O_5$: C, 70.57; H, 6.77; N, 5.88. Found: C, 70.47; H, 6.86; N, 5.77.

Boc-dmaPhe-Ala-napAla-OBzl (II-1). Boc-Ala-napAla-OBzl (0.4 g, 0.84 mmol) was dissolved in 3 N HCl/DOX and stored at room temperature for 1.5 h. The solvent was evaporated and the precipitate that appeared on adding excess ether was collected and washed with ether and dried under vacuum. The N-deprotected dipeptide and Boc-dmaPhe-OH (0.30 g, 0.97 mmol) were dissolved in DMF, and EDC (0.17 g, 0.88 mmol), HOBt (0.12 g, 0.88 mmol), and TEA (0.11 mL, 0.85 mmol) were added under cooling with ice. The mixture was stirred for 3 h under cooling and for 1 day at room temperature. The solvent was evaporated and the residue was redissolved in ethyl acetate. The ethyl acetate

solution was washed as above and dried on $MgSO_4$. The solid that appeared after evaporation of the solvent was collected and recrystallized from ethyl acetate/hexane mixture. Yield 0.44 g (79%); mp 167–170 °C. Anal. Calcd for $C_{39}H_{46}N_6O_4$: C, 70.25; H, 6.95; N, 8.40. Found: C, 69.95; H, 6.89; N, 8.18.

Boc-dmaPhe-Ala-napAla-OH. The benzyl ester of the above tripeptide (0.15 g, 0.23 mmol) was removed by catalytic hydrogenation as described for Boc-dmaPhe-napAla-OH. Yield 0.082 g (63%); mp 150–160 °C.

Boc-dmaPhe-Ala-napAla-Glu(OBzl)$_4$-OBzl. The above tripeptide with free carboxyl group was coupled with Glu-(OBzl)$_4$-OBzl by a similar procedure as described for the preparation of Boc-dmaPhe-napAla-Glu(OBzl)$_4$-OBzl. Yield, 56%; mp 235–238.5 °C. Anal. Calcd for $C_{87}H_{98}N_8O_{18}$: C, 67.69; H, 6.40; N, 7.26. Found: C, 67.60; H, 6.39; N, 7.16.

Glu(OBzl)$_n$-dmaPhe-Ala-napAla-Glu(OBzl)$_4$-OBzl (I-1). The polypeptide was prepared by the polymerization of Glu(OBzl) NCA using the N-deprotected heptapeptide as an initiator, $\bar{n}$ = 30. The procedure is the same as described for the preparation of I-0.

Boc-Ala$_2$-napAla-OBzl. Boc-Ala-napAla-OBzl (0.60 g, 1.2 mmol) was treated with 5 N HCl/DOX to remove the N-protecting group, as described for the deblocking of Boc-dmaPhe-Ala-napAla-OBzl. The dipeptide with free amino group was coupled with Boc-Ala-OH using EDC-HOBt as the coupling reagent in DMF. Yield 0.60 g (86%); mp 107–109 °C.

Boc-dmaPhe-Ala$_2$-napAla-OBzl (II-2). The above tripeptide (0.30 g, 0.55 mmol) was treated with 5 N HCl/DOX to remove the Boc group. The tripeptide was then coupled with Boc-dmaPhe-OH with EDC-HOBt in DMF. Yield 0.22g (53%); mp 132–134 °C. Anal. Calcd for $C_{42}H_{51}N_5O_7$: C, 68.37; H, 6.97; N, 9.49. Found: C, 68.16; H, 6.94; N, 9.44.

Boc-napAla-Glu(OBzl)$_4$-OBzl. Boc-napAla-OH (0.22 g, 0.70 mmol) was coupled with Glu(OBzl)$_4$-OBzl (0.76 g, 0.70 mmol) using DCCI-HOBt in dichloromethane. The peptide was purified with a silica gel/ethyl acetate column. Yield 0.63 g (71%); mp 135–142 °C. Anal. Calcd for $C_{73}H_{79}N_5O_{16}$: C, 68.37; H, 6.21; N, 5.46. Found: C, 68.19; H, 6.45; N, 5.46.

Boc-dmaPhe-Glu(OBzl)$_4$-OBzl. The procedure is the same as described for the preparation of Boc-napAla-Glu(OBzl)$_4$-OBzl. Yield 69%; mp 128–160 °C. Anal. Calcd for $C_{71}H_{82}N_6O_{16}$: C, 66.86; H, 6.48; N, 6.59. Found: C, 66.60; H, 6.46; N, 6.69.

Boc-Ala-napAla-Glu(OBzl)$_4$-OBzl. Boc-Ala-OH was coupled with napAla-GlunOBzl)$_4$-OBzl using EDC-HOBt in DMF. The detail of the procedure is the same as that in the preparation of Boc-napAla-Glu(OBzl)$_4$-OBzl. Yield 71%; mp 190–200 °C.

Boc-Ala$_2$-napAla-Glu(OBzl)$_4$-OBzl. The procedure is the same as that for the preparation of Boc-Ala-napAla-Glu(OBzl)$_4$-OBzl. Yield 74%; mp 234–240 °C.

Boc-dmaPhe-Ala$_2$-napAla-Glu(OBzl)$_4$-OBzl. The above pentapeptide was extended by repeating of N-deprotection with 5 N HCl/DOX and coupling with the corresponding Boc-amino acid using EDC-HOBt in DMF. The peptides were purified with a Sephadex LH-20/DMF column. The final yield was 34 mg (48%); mp 220–250 °C. Anal. Calcd for $C_{90}H_{103}N_9O_{19}$: C, 66.94; H, 6.43; N, 7.81. Found: C, 66.68; H, 6.27, N, 7.60.

Glu(OBzl)$_n$-dmaPhe-Ala$_2$-napAla-Glu(OBzl)$_4$-OBzl (I-2). The procedure is the same as described for the preparation of I-0, except for the solvent (chloroform) used for the extraction of the N-deprotected octapeptide. $\bar{n}$ = 31.

Glu(OBzl)$_n$-napAla-Glu(OBzl)$_4$-OBzl (III). The polypeptide was prepared by the polymerization of Glu(OBzl) NCA using napAla-Glu(OBzl)$_4$-OBzl as the initiator. $\bar{n}$ = 34.

Glu(OBzl)$_n$-dmaPhe-Glu(OBzl)$_4$-OBzl (IV). The polypeptide was similarly prepared by using dmaPhe-Glu(OBzl)$_4$-OBzl as the initiator. $\bar{n}$ = 23.

Boc-Glu(OMe)-napAla-Glu(OMe)$_2$-OBzl (V). Boc-napAla-Glu(OMe)$_2$-OBzl was synthesized by coupling Boc-napAla-OH with HCl·Glu(OMe)$_2$-OBzl. The Boc group of the tripeptide was removed with 4 N HCl/DOX and the N-deblocked peptide was coupled with Boc-Glu(OMe)-OH using EDC-HOBt in DMF. The tetrapeptide was purified with silica gel/ethyl acetate column. Mp 121–123 °C. Anal. Calcd for $C_{43}H_{54}N_4O_{13}$: C, 61.86; H, 6.52; N, 6.71. Found: C, 61.80; H, 6.53; N, 6.72.

Boc-Glu(OMe)-dmaPhe-Glu(OMe)$_3$-OMe (VI). HCl·Glu-(OMe)$_3$-OMe (1.8 g, 3.8 mmol) and Boc-dmaPhe-OH (1.2 g, 3.9 mmol) were dissolved in DMF (12 mL) and EDC (0.8 g, 4.2 mmol), and HOBt (0.56 g, 4.1 mmol), and TEA (0.49 mL, 3.8 mmol) were added under cooling with ice. The mixture was stirred for 3 h and for 1 day at room temperature. The solvent was evaporated and the residue was redissolved in ethyl acetate. The latter solution was washed as usual and dried on $MgSO_4$. The solid appeared after evaporation was collected and recrystallized from ethyl acetate/hexane to yield Boc-dmaPhe-Glu(OMe)$_3$-OMe. Yield 1.9 g (69%); mp 118–125 °C. Anal. Calcd for $C_{35}H_{53}N_5O_{13}$: C, 55.91; H, 7.11; N, 9.32. Found: C, 55.77, H, 7.06; N, 9.19.

Boc-dmaPhe-Glu(OMe)$_3$-OMe (0.1.5 g, 0.19 mmol) was dissolved in formic acid (10 mL) and stored at room temperature for 6 h. The formic acid was evaporated and the residue was washed with ether. The N-deprotected tetrapeptide was coupled with Boc-Glu(OMe)-OH by the mixed anhydride method in THF. The product was purified by eluting through a silica gel/ethyl acetate column. Yield 42 mg (26%); mp 164.5–170 °C. Anal. Calcd for $C_{41}H_{62}N_6O_{16}$: C, 55.02; H, 6.98; N, 9.39. Found: C, 54.76; H, 6.98; N, 9.37.

Measurements. Trimethyl phosphate (TMP) was used as a solvent throughout this study, because of its ability of solubilizing polypeptides and its transparency down to 190 nm. TMP was purified by fractional distillation. Fluorescence spectra and the decay curves were measured in a quartz cell equipped with a Teflon stopcock. Before each measurement the solution was bubbled with argon gas for 20 min. The following instruments were used: JASCO Ubest-50 for absorption spectroscopy, Hitachi MPF-4 for fluorescence spectroscopy, JASCO J-20 and J-600 for CD spectroscopy, and a home-built single-photon counting apparatus (electronics: Ortec) for fluorescence decay measurement. The decay curves were fitted to exponential functions by an iterative reconvolution method. All the output of the spectrometers were interfaced to a microcomputer (NEC PC9801) and the spectroscopic data were processed on it.

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