Photoinduced Electron Transfer on a Single α-Helical Polypeptide Chain

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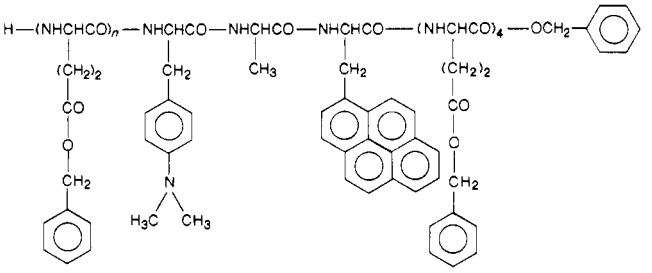
Abstract: Electron transfer on an α-helical polypeptide carrying the sequence L-*p*-(dimethylamino)phenylalanine (dmaPhe)-L-alanine-L-1-pyrenylalanine (pyrAla) at the midpoint of an α-helical poly(γ-benzyl L-glutamate) chain was studied. Conformational energy calculation for the side-chain orientations predicted that only one type of orientation is allowed for both the dmaPhe and the pyrAla units. The center-to-center (edge-to-edge) distance between the two chromophores was estimated to be 13.2 (9.4) Å. The fluorescence spectrum showed no exciplex emission in the polypeptide, in contrast to the strong exciplex observed for a model tripeptide having the same dmaPhe-Ala-pyrAla sequence. The rate of electron transfer was calculated from the decay times of pyrenyl fluorescence of the polypeptide in trimethyl phosphate and in tetrahydrofuran solutions. The k_{et} was on the order of 10^5 (s^{-1}). The activation enthalpy was 1.4 kcal mol^{-1} in trimethyl phosphate and smaller than 1 kcal mol^{-1} in less polar solvents near room temperature. It was even smaller at lower temperatures. The activation entropy was less than −25 eu, suggesting a nonadiabatic electron transfer. In contrast to the slow electron transfer in the polypeptide, the rate constant for the model tripeptide was on the order of 10^7–10^8 (s^{-1}) around room temperature, and the activation enthalpy was higher than that in the polypeptide case.

Electron transfer (ET) in biological systems has been studied on modified proteins carrying metal complexes[1-5] and on synthetic polypeptides carrying an electron donor–acceptor pair at both ends of a chain.[6,7] The protein system is complicated because of the presence of side-chain aromatic groups that work as electron mediators and the ill-defined structures of the polypeptide main chain. The structure of the synthetic polypeptide system is much simpler and well-defined. Therefore, synthetic polypeptides are suited for obtaining basic information on the ET. However, as recently pointed out by Schanze and Sauer,[7] the terminal portions of an α-helical polypeptide chain are often unfolded and the end-to-end distance or the terminal donor–acceptor distance becomes statistical.

In this article, we will report the first attempt to attach an organic electron donor, *p*-dimethylanilino (D) group, and an organic photosensitizer, pyrenyl (P) group, at the midpoint of an α-helical poly(γ-benzyl L-glutamate) chain (I). The chromophores are linked to the main chain by the shortest, spacer (methylene group) to minimize their orientational freedom.

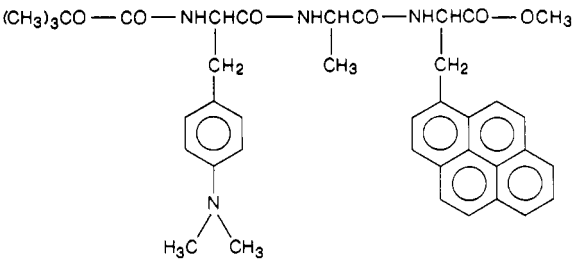


n = 45

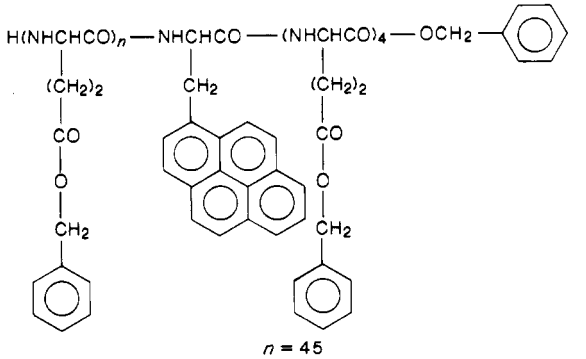
I, Glu(OBzl)$_{45}$-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$-OBzl (GnDAPG4)

Poly(γ-benzyl L-glutamate) is a typical polypeptide that takes an α-helical conformation in various solvents. The tetrapeptide unit of γ-benzyl L-glutamate on the right-hand side of I corresponds to one turn of the α-helix and is expected to stabilize the α-helical conformation at the pyrAla unit. As a flexible model compound, an oligopeptide carrying the same D–P pair (II) was also synthesized.

To examine the intrinsic property of the pyrenyl and (dimethylamino)phenyl groups fixed on the polypeptide chain, polypeptides carrying only one pyrenyl group (III) and one (dimethylamino)phenyl group (IV), respectively, were also prepared.

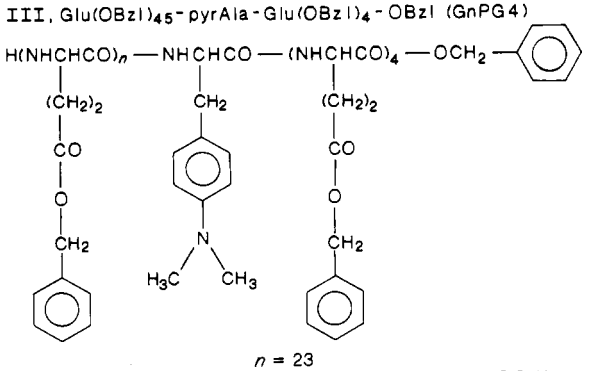


II, Boc-dmaPhe-Ala-pyrAla-OMe (BDPM)



n = 45

III, Glu(OBzl)$_{45}$-pyrAla-Glu(OBzl)$_4$-OBzl (GnPG4)



n = 23

IV, Glu(OBzl)$_{23}$-dmaPhe-Glu(OBzl)$_4$-OBzl (GnDG4)

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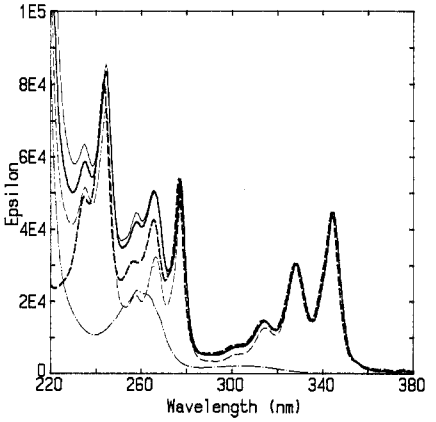
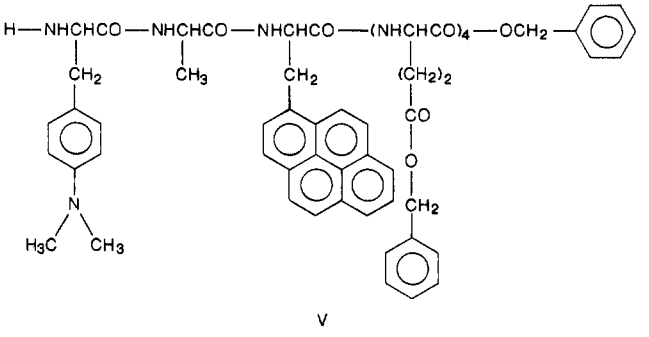


Figure 1. Absorption spectra of polypeptide I (—), tripeptide II (▪ ▪), polypeptide III (—), polypeptide IV (–·–), and the sum of the spectra of III and IV (---) in TMP at room temperature. The polypeptide concentrations were determined by the absorbance at 345 nm using the molar absorption coefficient of the tripeptide (ϵ_{344} = 4.47 × 10^4).

Results and Discussion

Synthesis and Characterization of the Samples. The polypeptide I was prepared by the polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride [Glu(OBzl)NCA] by using an oligopeptide (V) as the initiator. The oligopeptide with a (*tert*-butyloxy)-



V

carbonyl (Boc) protecting group at the terminal nitrogen was synthesized by a conventional liquid-phase method and characterized by IR, ^{1}H NMR, and UV spectra and by elemental analysis. After the Boc group was removed, the oligopeptide was mixed with the NCA in dimethylformamide. The polymerization was completed after 4 days. The number-average degree of polymerization of the poly(γ-benzyl L-glutamate) unit, $\bar{n}$, in the final polypeptides was determined from the absorption intensity of the benzyl group in the UV spectruym ($\epsilon_{257.5}$ = 218) of the polypeptide (difference between the bold-faced solid line and the bold-faced broken line in Figure 1). The model oligopeptide II was synthesized similarly by a liquid-phase method. The polypeptides III and IV were prepared similarly by the polymerization of Glu(OBzl)NCA using the corresponding oligopeptide as the initiator.

Absorption and Circular Dichroic Spectra. The UV absorption spectra of the polypeptides and the oligopeptide were measured in trimetyl phosphate (TMP) (Figure 1). The spectrum of I is essentially the same as the sum of the spectra of III and IV. This indicates that the D and P chromophores are properly introduced in the polypeptide and that any strong ground-state electronic interaction is absent between the D and P groups in polypeptide I. The ground-state interaction was also not seen in the model oligopeptide II. Therefore, the absence of the ground-state interaction is not a result of the geometry of the D–P pair fixed on the polypeptide chain but of the electronic property of the D–P pair in TMP.

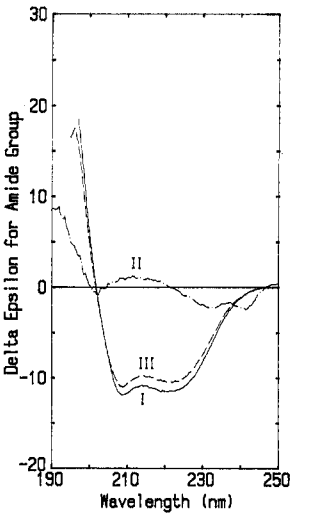


Figure 2. CD spectra of polypeptide I (—), polypeptide III (---), and tripeptide II (–·–) in TMP at room temperature. The ordinate is the value with respect to the molar concentration of amide group.

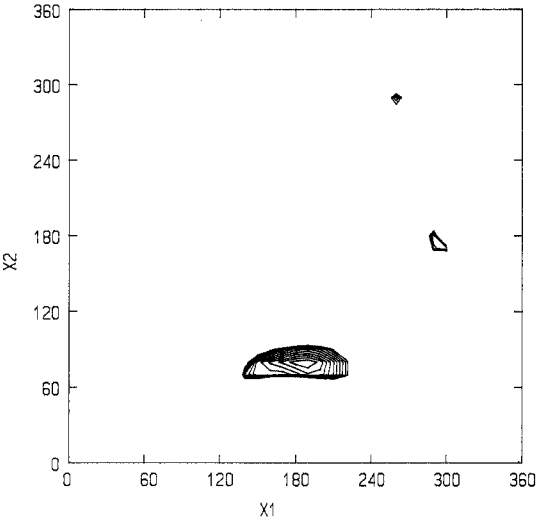


Figure 3. Energy contour map for the side-chain orientation of the pyrenyl group in Ac-$(Ala)_4$-pyrAla-$(Ala)_4$-Nh(CH_3) in an α-helical main-chain conformation. The interval of the contour lines is 0.5 kcal mol^{-1}.

Circular dichroic (CD) spectra of the polypeptides and the model peptide in the far-UV region are shown in Figure 2. The CD patterns of the polypeptides are those of a right-handed α-helix. The Δε values at 222 nm (−11.6 for I and −10.3 for III) are very close to the value reported for 100% α-helical polypeptides (Δε = −10.8 to −12).[8]

Conformational Analysis. The CD spectra indicated a right-handed α-helical main chain of the polypeptides I and III. To estimate the most probable orientations and the extent of thermal fluctuations of the side-chain chromophores, conformational energy calculation was carried out assuming the α-helical main chain (ϕ = −57°, ψ = −47°, ω = 180°).[17] For simplicity, the Glu(OBzl)

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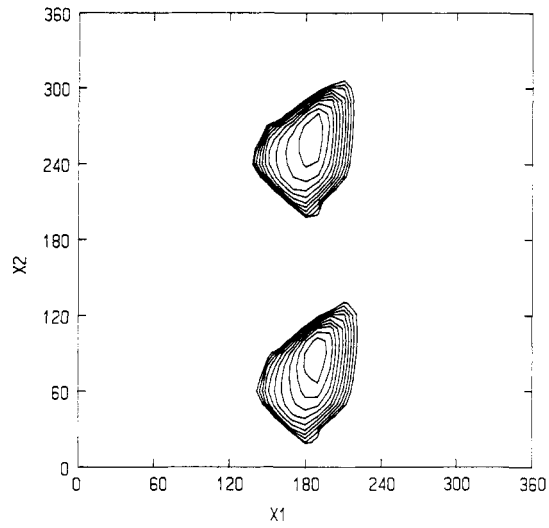

Figure 4. Energy contour map for the side-chain orientation of the (dimethylamino)phenyl group in Ac-$(Ala)_4$-dmaPhe-$(Ala)_4$-$NH(CH_3)$ in an α-helical main-chain conformation. The interval of the contour lines is 0.5 kcal mol^{-1}.

units were replaced with alanine units in the calculation.

First, energy contour maps for the rotations of side-chain angles $\chi_1(C^\alpha-C^\beta)$ and $\chi_2(C^\beta-C^\gamma)$ of the pyrAla unit or the dmaPhe unit incorporated in an α-helical Ac-$(Ala)_4$-X-$(Ala)_4$-$NH(CH_3)$ (X = dmaPhe or pyrAla) were calculated. The contour map for the isolated pyrAla unit is shown in Figure 3. The orientation of the P group is highly constrained in a narrow range around $\chi_1 = 190 \pm 20°$ and $\chi_2 = 80° \pm 8°$ near room temperature (thermal energy < 1 kcal mol^{-1}). The side-chain energy contour map for the isolated D group in the α-helix is shown in Figure 4. Since the rotation around χ_2 has C_2 symmetry, the top half of Figure 4 is the same as the bottom half. The D group can fluctuate over a wide area in the conformational space ($\chi_1 = 190 \pm 15°$, $\chi_2 = 90 \pm 25°$), but again, only one energy minimum is accessible. The energy contour map of the D group in Ac-$(Ala)_4$-dmaPhe-Ala-pyrAla-$(Ala)_4$-$NHCH_3$ was also calculated. The contour map was, however, virtually the same as that in Figure 4, indicating the P group does not affect the orientation of the D group in the helical polypeptide. Indeed, the energy minimization for Ac-$(Ala)_8$-dmaPhe-Ala-pyrAla-$(Ala)_4$-$NH(CH_3)$, varying the four side-chain angles of the D and P groups, converged near the stable orientations found in the isolated D and P groups: (χ_1, χ_2) = (187°, 77°) for P group and (190°, 93°) for D group. The minimum-energy conformation is shown in Figure 5. In the figure, the center-to-center and the edge-to-edge distances between the D and P groups are 13.2 and 9.4 Å, respectively.

The distribution of the interchromophore distance between D and P groups due to the fluctuation of the D group over the allowed range (thermal energy < 1 kcal mol^{-1}) in the contour map (Figure 4) was calculated. The shortest possible edge-to-edge distance was 9.26 Å, indicating that the thermal fluctuation does not alter the interchromophore distance significantly.

As for the thermal fluctuation of the helical main chain, energy contour maps were calculated for three pairs of rotational angles in Ac-$(Ala)_8$-dmaPhe-Ala*-pyrAla-$(Ala)_4$-$NH(CH_3)$: ψ-(dmaPhe)-ϕ(Ala*), ϕ(Ala*)-ψ(Ala*), and ψ(Ala*)-ϕ(pyrAla). The α-helical conformation was assumed for the rest of the main chain, and the side chains were set to be in the minimum-energy orientations. The three maps showed only one allowed region at the rotational angles corresponding to the right-handed α-helical conformation. The range of thermal fluctuation is very small (<±5°) near room temperature. The result suggests that the fluctuation in the main chain is much less significant than that in the side chains, especially in the D group.

Conformational analysis of the model tripeptide was also carried out. After 10 rotational angles were varied (ϕ and ψ for dmaPhe, Ala, and pyrAla units, and χ_1 and χ_2 for the side chains of dmaPhe and pyrAla units), several low-energy conformations were found. The minimum-energy conformation is illustrated in Figure 6. The conformation has a close D–P pair (center-to-center distance = 6.2 Å and the distance between the nitrogen to the nearest edge of the P group = 3.6 Å). There are several other conformations for the tripeptide having comparable energies but with longer D–P distances.

It is concluded from the CD spectra and the conformational calculation that the main chain of I is in an α-helix and the side-chain chromophores are fluctuating around one energy minimum. The range of the fluctuation is very small for the P group, whereas that for the D group is fairly large. However, the fluctuation of the D group does not alter the interchromophore distance significantly. The tripeptide does not seem to take a single conformation, and in some of the possible conformations, the D–P distance can be very close.

Fluorescence Spectra. Fluorescence spectra of polypeptides I and III and the model tripeptide in THF (tetrahydrofuran) are shown in Figure 7. The tripeptide shows a strong exciplex emission, but no exciplex was detected for polypeptide I. The difference is unambiguously attributed to the different conformations of the polypeptide (Figure 5) and of the tripeptide (Figure 6). In the polypeptide, the D–P pair is widely separated in the ground-state minimum-energy conformation, and the fluctuation in the interchromophore distance is not so large as to enable the exciplex formation. The tripeptide may have D and P very close to each other in some of the ground-state conformations, and even small thermal fluctuations around those conformations may form the exciplex in the excited state.

The fluorescence quantum yield of the monomer excited state of polypeptide I is smaller by 17% at 20 °C and by 10% at −40 °C in TMP than that of polypeptide III. The reduced quantum yield of I is most reasonaly explained in terms of a long-range ET quenching by the D group. The total quantum yield of the tripeptide was much smaller than that of III, indicating that ET quenching is the major deactivation process for the excited state of the tripeptide.

The fluorescence spectra in TMP solution were also measured. The tripeptide showed a very weak exciplex emission around 590 nm, but absolutely no exciplex was detected in polypeptide I. Again, the quantum yield of polypeptide I was a little smaller than that of III and that of the tripeptide was much smaller than that of III.

Fluorescence Decay Analysis. The fluorescence decay curves of polypeptides I and III were measured on a single-photon-counting apparatus. Typical decay profiles are shown in Figure 8. Both of the decay curves of the fluorescence from the P group in polypeptides I and III obeyed first-order kinetics over more than 99% decay in TMP, in THF, and in a 1:1 mixture of MTHF (2-methyltetrahydrofuran) and THF. The fit to the single-exponential convolution curve was satisfactory in most cases (χ^2 < 1.1). However, a small deviation was observed at low temperatures. For example, a two-component decay was found for I in the MTHF/THF mixed solvent at −196 °C [τ_1 = 70.6 ns (0.15), τ_2 = 312 ns (1.29), χ^2 = 1.05; the numbers in parentheses indicate

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(17) Throughout this paper, the IUPAC-IUB recommendation was followed for the definition of the rotational angles in polypeptides (reference: *Biochemistry* **1970**, *9*, 3471).

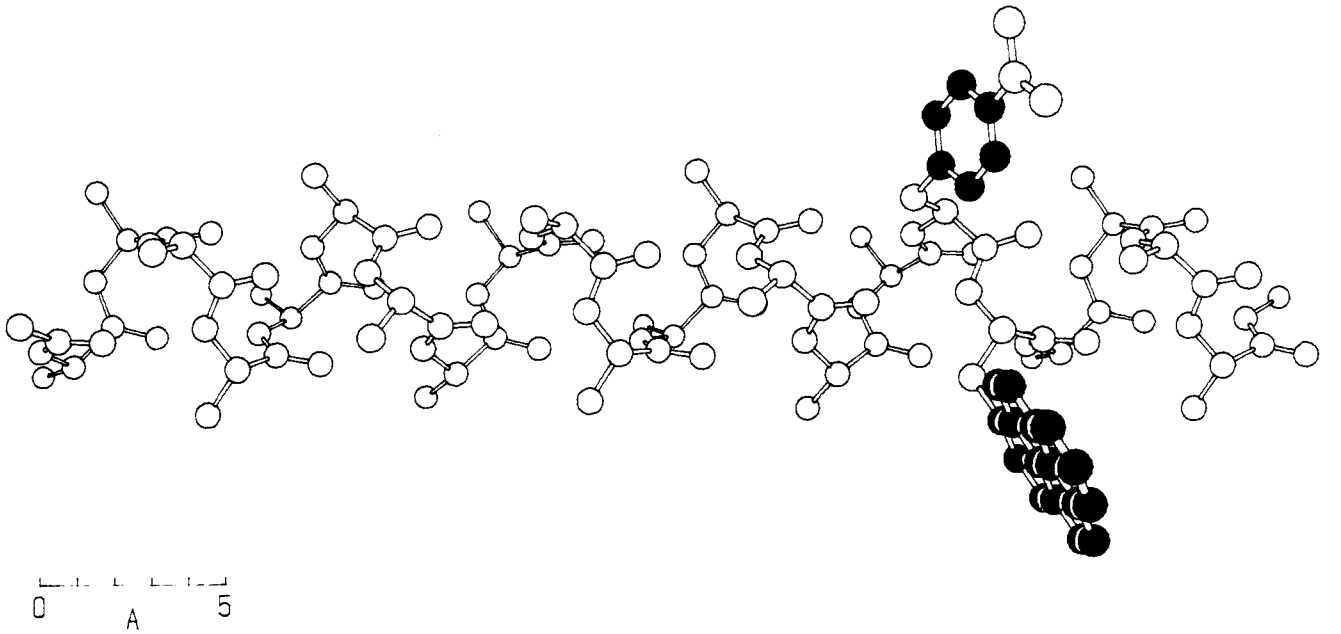

Figure 5. Most probable conformation of Ala_n-dmaPhe-Ala-pyrAla-Ala_4 predicted from the side-chain energy minimization. All hydrogen atoms are omitted but are included in the energy calculation. (χ_1, χ_2) = (190°, 93°) for D and 187°, 77°) for P. The main chain was in α-helix (ϕ = −57°, ψ = −47°, ω = 180°). The NAMOD (version 3) molecular display program was used (ref 16).

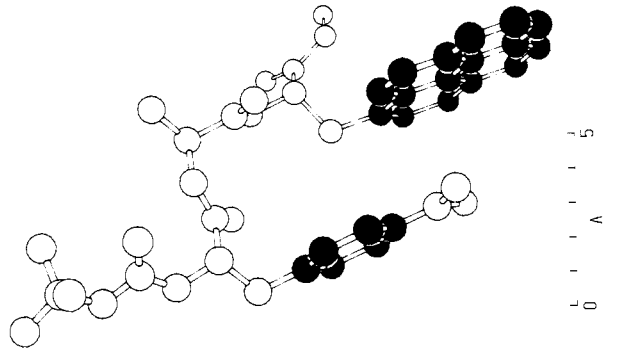

Figure 6. Most stable conformation of tripeptide II. See also the footnote of Figure 5.

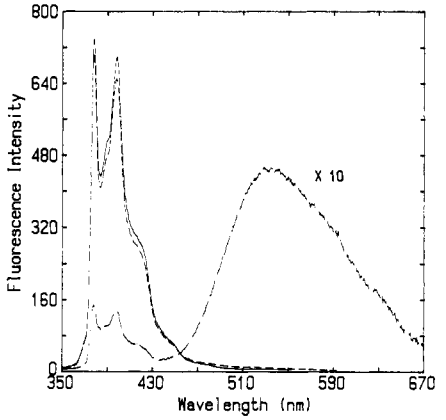

Figure 7. Fluorescence spectra of polypeptide I (—), polypeptide III (- - -), and tripeptide II (-·-) in THF at room temperature. λ_{ex} = 345 nm.

the preexponential factor]. Since the contribution of the fast-decaying component is small, it was not taken into consideration in the following discussion.

The decay times of I were smaller than those of III in all temperatures and solvents examined. Typically, decay times of I in TMP were 192 ns (20 °C) and 268 ns (−60 °C), and those of III were 243 ns (20 °C) and 290 ns (−60 °C). In MTHF/THF mixed solvent, they were 229 ns (20 °C) and 312 ns (−196 °C)

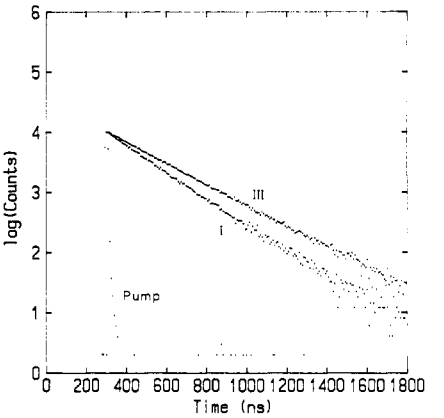

Figure 8. Fluorescence decay curves of polypeptide III (upper) and polypeptide I (lower) in TMP at 20 °C. λ_{ex} = 345 ± 5 nm, λ_{em} = 385 ± 5 nm. Both of the decay curves were fitted to single-exponential functions with the decay times of 242 ns for III and 191 ns for I.

for I and 255 ns (20 °C) and 334 ns (−196 °C). The faster decay of I may be interpreted in terms of a long-range ET quenching by the D group, as has been suggested from the static spectroscopy.

Electron-Transfer Rate Constant. Both the static spectra and the decay curve analyses suggested a long-range ET in polypeptide I. The ET rate constant, k_{et}, was obtained from the fluorescence quantum yields of I (η) and of III (η_0) as

$$k_{et} = (\eta_0/\eta - 1)/\tau_0 \quad (1)$$

where τ_0 is the lifetime of the P group in polypeptide III. The ET rate constant may be calculated also from the lifetimes of I (τ) and III (τ_0) as

$$k_{et} = 1/\tau - 1/\tau_0 \quad (2)$$

The ET rate constants were calculated and are plotted as functions of temperature in Figure 9. The two kinds of ET rate constants in TMP solution agree with each other quite well. Since the ET rate is much slower in THF or in MTHF/THF mixed solvent than in TMP, no physically significant rate constants could be obtained from the static spectroscopy. Therefore, only those obtained from the decay data are shown in the figure.

The ET rate constants show two characteristic features when compared with those reported for other rigid D–A systems; i.e.,

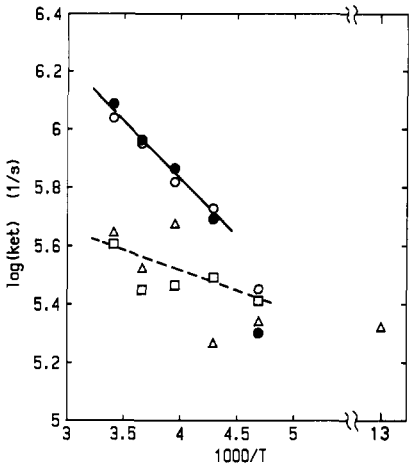

Figure 9. Arrhenius plot of the electron-transfer rate constants in polypeptide I. Solvent: TMP (○), THF (□), and MTHF/THF (1/1) mixture (△). The rate constants obtained from the fluorescence quantum yield in TMP (●) are also plotted.

(1) the rate constant is much smaller than those reported for rigid hydrocarbon systems, and (2) the activation energy is very small or virtually zero. Rate constants on the order of 10^{10} s^{-1} have been reported for a dimethoxynaphthalene (donor) and dicyanoethylene (acceptor) pair fixed on a saturated hydrocarbon framework, with a D–A distance of 11.4 Å (center-to-center) or 9.4 Å (edge-to-edge).[9] This ET rate constant is larger than the present data by 5 orders of magnitude, even though the D–A separation of the two systems is about the same! This remarkable difference cannot be interpreted in terms of the difference in the driving forces of the two ET processes ($\Delta G(\infty) = -0.4$ eV for the present system and −0.98 eV for the dimethoxynaphthalene–dicyanoethylene pair). The two ΔG's lie near the optimally exothermic region.

On the other hand, the rate constant on the order of 10^5 s^{-1} has been reported for synthetic polypeptide systems,[6b,7] where the donor–acceptor separation is around 9 Å. The present data also fall into this category. Although a detailed comparison is difficult due to the different nature of the D–A pairs in the hydrocarbon and the polypeptide systems, it may be concluded that there should be some fundamental difference between the ET processes in the two types of molecular frameworks. The contribution of through-bond interaction in the hydrocarbon systems may be one of the possible reasons.[9,10]

The activation enthalpy calculated from the rate constants is 1.4 kcal mol^{-1} in TMP near room temperature. In MTHF/THF mixed solvent, it is less than 0.4 kcal mol^{-1} near room temperature and nearly 0 at lower temperatures than −60 °C. The small or zero activation enthalpy contrasts those reported for oligoprolines carrying a redox pair of metal complexes at the two ends of a chain (10–20 kcal mol^{-1}).[6a,b] However, since a large part of the activation energy of the oligoproline systems is due to the inner-sphere reorganization energy of the metal complex, the high activation energy does not indicate large conformational changes required for the ET.

The activation entropy of the present system was −26 eu in TMP and around −32 eu in THF or in THF/MTHF mixture. The large negative values indicate a nonadiabatic mechanism of the ET in the present system.[5,11]

Electron Transfer in the Model Tripeptide. The model tripeptide II showed a very small quantum yield compared with that of the isolated P group, indicating that the ET quenching is the major deactivation process of the excited state, together with the exciplex formation in less polar solvents. The ET rate constant of tripeptide III may be determined from the lifetime of monomer fluorescence using eq 2. however, the decay curve did not fit a single-exponential function. More than three exponential components were needed to fit the decay data, either in THF or in TMP solution. A typical decay curve is shown in Figure 10. The multicomponent decay is most reasonably attributed to the presence of several ground-state conformations as predicted from the conformational

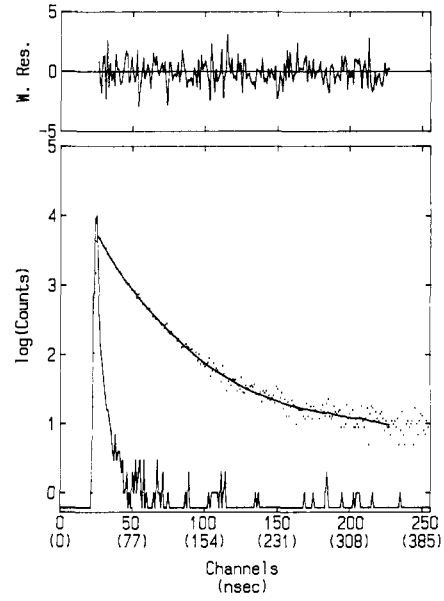

Figure 10. Fluorescence decay curve of tripeptide II in TMP at 0 °C. $\lambda_{ex} = 345 \pm 5$ nm, $\lambda_{em} = 385 \pm 5$ nm. The decay curve is fitted to a three-component exponential function.

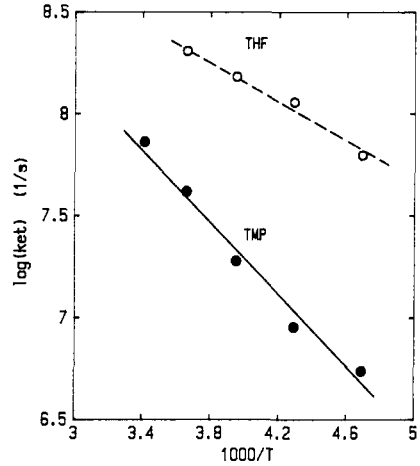

Figure 11. Arrhenius plot of the electron-transfer rate constants of tripepide II in TMP (●) and in THF (○). The rate constants were determined from the fluorescence quantum yields and the decay times of polypeptide III.

calculation. Another possible reason for the multicomponent decay is that a small amount of impurity carrying a pyrenyl group only is contained in the tripeptide sample. The presence of an isolated pyrenyl group may explain the longest component in the decay curve (component with the 178-ns lifetime in Figure 10). However, taking the presence of the possible isolated pyrenyl group into account, the decay curves did not fit single-exponential functions. Therefore, the presence of multiple conformations is needed to interpret the decay curve, and it is difficult to calculate the ET rate constant from the decay curve.

An "average" ET rate constant was calculated from the quantum yield of tripeptide II using eq 1. The quantum yield and the lifetime for an isolated P group were taken from the data on polypeptide III. The results are plotted as a function of temperature in Figure 11. The rate constants are on the order of 10^7–10^8 s^{-1}. They are much larger than those of the polypeptide systems. The fast ET in the tripeptide indicates that the low-energy conformations that are dominant in the ground state have shorter D–P distances than the D–P distance in polypeptide I. This finding agrees with the prediction from the conformational calculation described above. The difference between polypeptide I and tripeptide II clearly indicates the importance of fixing the donor–acceptor pair in the study of the ET process.

It may be worth noting that the ET in tripeptide II is faster in THF than in TMP, but the inverse is true in polypeptide I. This may be interpreted in terms of different distributions of the ground-state conformations in the tripeptide according to the nature of solvent. It has been reported that extended conformations are favored for oligopeptides in polar aprotic solvents.[12] Therefore, a likely explanation is that the average D–P distance is longer in TMP than in THF for the tripeptide.

The activation parameters for the tripeptide are as follows: $\Delta H^{\ddagger}$ = 3.8 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −10 eu in TMP and $\Delta H^{\ddagger}$ = 1.7 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −14 eu in THF. Since these parameters are calculated from the average ET rate constant for several conformations, they cannot be compared directly with the parameters for polypeptide I. However, the higher activation enthalpies of tripeptide II suggest that some conformational change is needed to induce the ET in the tripeptide.

Conclusions

A slow (10^5-s^{-1}) and nonadiabatic ET was observed between D and P fixed on a helical polypeptide chain with an interchromophore edge-to-edge distance of 9.4 Å. The activation enthalpy was very small (<1.4 kcal mol^{-1}). The ET was on the order of 10^7–10^8 s^{-1} when the same D–P pair is involved in the tripeptide, which does not take any regular conformation. Experiments using similar polypeptides carrying the same D–P pair with different spatial arrangements are in progress.

Experimental Section

Materials. The polypeptide contains two artificial aromatic amino acids, L-*p*-(dimethylamino)phenylalanine (dmaPhe) and L-1-pyrenylalanine (pyrAla). The former was prepared in the form of the *N*-Boc derivative by a reductive methylation of ((*tert*-butyloxy)carbonyl)-L-*p*-nitrophenylalanine according to the procedure reported by Bergel and Stock.[13] The synthesis and optical resolution of pyrAla has been reported.[14] Oligopeptides II, IV, and V were synthesized by conventional liquid-phase techniques. Each intermediate was checked for purity by IR, ^{1}H NMR (90 MHz), elemental analysis, and TLC.

The following abbreviations will be used: Boc, (*tert*-butyloxy)carbonyl; OMe, methyl ester; OBzl, benzyl ester; OSu, *N*-hydroxysuccinimide ester; nitroPhe, L-*p*-nitrophenylalanine; dmaPhe, L-*p*-(dimethylamino)phenylalanine; pyrAla, L-1-pyrenylalanine; Ala, L-alanine; Glu(OBzl), γ-benzyl L-glutamate; $(Boc)_2O$, di-*tert*-butyldicarbonate; DCHA, dicyclohexylamine; TsOH, *p*-toluenesulfonic acid; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (water-soluble carbodiimide); HOBt, 1-hydroxybenzotriazole hydrate; DMF, dimethylformamide; TEA, triethylamine; NCA, *N*-carboxyanhydride; DOX, *p*-dioxane.

Boc-nitroPhe. NitroPhe (8.24 g, 39.2 mmol) was suspended in a DOX/H_2O (2/1) mixture (40 mL) and cooled with ice. $NaHCO_3$ (4.62 g, 55 mmol) and $(Boc)_2O$ (9.87 g, 45 mmol) were added and stirred for 30 min at the ice temperature. Stirring was continued for 6 h further at room temperature. The solution was acidified by 5% $KHSO_4$ and extracted with ethyl acetate. The extract was washed with water and dried on $MgSO_4$. The solvent was evaporated, and the residual oil was solidified by adding hexane. Yield 9.16 g (75%), mp 122–124 °C. Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.04; H, 5.87; N, 9.07.

Boc-dmaPhe. Boc-nitroPhe (3.04 g, 9.80 mmol) was dissolved in ethanol (40 mL), and sodium acetate (2.7 g), 35% formaldehyde solution (5.4 mL), and 10% palladium carbon (1.7 g) were added. The mixture was hydrogenated under hydrogen atmosphere for 36 h at room temperature. The catalyst was filtered off, and the solvent was evaporated. The residue was redissolved in ethyl acetate and the solution was dried on $MgSO_4$. The ethyl acetate was evaporated, and the residue was solidified by adding hexane. Yield 2.01 g (67%). The ^{1}H NMR indicated the presence of a small amount of sodium acetate. But the compound was used for the next step without further purification.

Boc-Glu(OBzl)$_n$-OBzl (n = 2–4). Boc-Glu(OBzl)·DCHA salt and an equimolar amount of TosOH and Glu (OBzl)-OBzl were mixed in DMF, and equimolar amounts of EDC and HOBt were added under cooling with ice. The mixture was stirred overnight and treated as usual to obtain crystalline Boc-Glu(OBzl)$_2$-OBzl. The Boc group was removed with 3 N HCl/DOX, and the chain extension was repeated using Boc-Glu(OBzl)·DCHA. The yield for each step was about 80%. The melting points and the results of elemental analysis were as follows: mp 72–73 (n = 2), 83–85 (n = 3), 78–82 °C (n = 4). Anal. Calcd for $C_{48}H_{55}N_3O_{12}$ (n = 3): C, 66.58; H, 6.40; N, 4.85. Found: C, 66.51; H, 6.34; N, 4.93. Anal. Calcd for $C_{60}H_{68}N_4O_{15}$ (n = 4): C, 66.41; H, 6.32; N, 5.16. Found: C, 66.12; H, 6.18; N, 5.12.

Boc-pyrAla. PyrAla (0.338 g, 1.17 mmol) and $NaHCO_3$ (0.209 g, 2.49 mmol) were suspended in a DOX/H_2O (15 mL/5 mL) mixture and cooled in an ice bath. $(Boc)_2O$ (0.281 g, 1.29 mmol) was added dropwise under vigorous stirring. The mixture was stirred for 1 h under cooling with ice and for 12 h at room temperature. It was concentrated under reduced pressure and acidified with 5% $KHSO_4$ solution. The oil was extracted with ethyl acetate, and the extract was washed with saturated NaCl solution and dried over $MgSO_4$. The solvent was evaporated, and the residue was solidified by adding excess hexane. The crude crystal was recrystallized from chloroform/hexane. Yield 0.253 g (56%); mp 178–179 °C. Anal. Calcd for $C_{24}H_{24}NO_4$: C, 73.83; H, 6.20; N, 3.59. Found C, 74.00; H, 5.91; N, 3.55.

Boc-pyrAla-Glu(OBzl)$_4$-OBzl. Boc-Glu(OBzl)$_4$-OBzl (97 mg, 0.090 mmol) was dissolved in 3 N HCl/DOX and kept at room temperature. After 1.5 h, the solvent was evaporated and the residue was solidified by adding ether. The solid was washed repeatedly with ether and dried under vacuum. The obtained N-deprotected tetrapeptide hydrochloride and Boc-pyrAla (35 mg, 0.090 mmol) were coupled in DMF by using equimolar amounts of EDC and HOBt as usual. After the mixture was allowed to stand at room temperature for 24 h, the solvent was evaporated and the residue was redissolved in ethyl acetate. The ethyl acetate solution was washed with 10% citric acid solution, water, 5% $NaHCO_3$ solution, and water and dried over $MgSO_4$. The solvent was then evaporated, and the residue was solidified with hexane. Yield 0.105 g (85%); mp 156–170 °C (the wide range of the melting temperature may be due to the conformational distribution in the oligopeptide. No impurity was found in the TLC and in the ^{1}H NMR spectrum). Anal. Calcd for $C_{79}H_{81}N_5O_{16}$: C, 69.95, H, 6.02; N, 5.16. Found: C, 69.70; H, 6.05; N, 5.29.

Boc-Ala-pyrAla-Glu(OBzl)$_4$-OBzl. Boc-pyrAla-Glu(OBzl)$_4$-OBzl (0.497 g, 0.37 mmol) was treated with 3 N HCl/DOX as above to remove the Boc group. The pentapeptide hydrochloride obtained was coupled with Boc-Ala (76 mg, 0.41 mmol) as described above. The hexapeptide obtained was purified by column chromatography (silica gel/ethyl acetate). Yield 0.279 g (53%); mp 158–178 °C.

Boc-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$-OBzl. The Boc group of Boc-Ala-pyrAla-Glu(OBzl)$_4$-OBzl (67 mg, 0.047 mmol) was removed as described above, and the resulting hexapeptide hydrochloride was coupled with Boc-dmaPhe as described above but using dichloromethane as the solvent. The heptapeptide was purified by a Sephadex column (LH-20/DMF) and recrystallized from a chloroform/hexane mixture. Yield 41 mg (54%); mp 150–210 °C. Anal. Calcd for $C_{93}H_{100}N_8O_{18}$: C, 69.04; H, 6.23; N, 6.93. Found: C, 68.79; H, 6.01; N, 6.73.

Glu(Obzl)$_n$-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$-OBzl (I). Boc-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$-OBzl was dissolved in formic acid and kept at room temperature for 5 h. The formic acid was evaporated, and the residue was redissolved in chloroform. The solution was washed with 5% $NaHCO_3$ solution and dried over $MgSO_4$. The solvent was evaporated, and the residue was dried under vacuum. The heptapeptide with free terminal nitrogen was dissolved in DMF, and 30-fold amount of Glu(OBzl)·NCA was added. The polymerization was carried out for 4 days at room temperature, and the polymer was precipitated with ether. The polymer was fractionated by a Sephadex LH-60 column, and the low-molecular-weight component was removed. The number-average degree of polymerization, $\bar{n}$, of the polymerized Glu(OBzl) unit was determined to be 45, from the difference of the absorption intensity at 257.5 nm between the polypeptide and Boc-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$-OBzl (ϵ(OBzl) = 218).

Glu(OBzl)$_n$-pyrAla-Glu(Obzl)$_4$-OBzl (III). The polymerization of Glu(OBzl)·NCA was also carried out using pyrAla-Glu(OBzl)$_4$-OBzl prepared by the acid treatment of Boc-pyrAla-Glu(OBzl)$_4$-OBzl. The procedure is the same as described above. The number-average degree of polymerization, $\bar{n}$, was 45.

Glu(OBzl)$_n$-dmaPhe-Glu(OBzl)$_4$-OBzl (IV). This polypeptide was prepared by using dmaPhe-Glu(OBzl)$_4$-OBzl as the initiator for the polymerization of Glu(OBzl)·NCA. The synthesis of the initiator will be reported elsewhere. The number-average degree of polymerization, $\bar{n}$, was 23.

Boc-Ala-pyrAla-OMe. PyrAla-OMe·HCl (75 mg, 0.22 mmol) and Boc-Ala (42 mg, 0.24 mmol) were coupled by the usual EDC/HOBt method in DMF. The product was recrystallized from an ethyl acetate/hexane mixture. Yield 90 mg (85%); mp 163–165 °C. Anal. Calcd for $C_{28}H_{30}N_2O_5$: C, 70.87; H, 6.37; N, 5.90. Found: C, 70.59; H, 6.37; N, 5.82.

Boc-dmaPhe-Ala-pyrAla-OMe (II). Boc-Ala-pyrAla-OMe (44 mg, 0.093 mmol) was dissolved in 3 N HCl/DOX and kept at room temperature for 1 h. The solvent was evaporated, and the residue was washed with ether. The dipeptide hydrochloride was dissolved in dichloromethane (3 mL) containing triethylamine (0.017 mL, 0.12 mmol) and

Boc-dmaPhe (40 mg, 0.11 mmol). EDC (21 mg, 0.11 mmol) and HOBt (15 mg, 0.11 mmol) were then added under cooling with ice. The stirring was continued overnight. The solvent was evaporated, and the residue was redissolved in chloroform. The chloroform solution was washed with 10% citric acid solution, water, 3% $NaHCO_3$ solution, and water and dried over $MgSO_4$. The solvent was evaporated. The residue was purified by column chromatography (silica gel/ethyl acetate) and recrystallized from ethyl acetate/hexane. Yield 41 mg (67%); mp 182–185 °C. Anal. Calcd for $C_{39}H_{44}N_4O_6$: C, 70.46; H, 6.67; N, 8.43. Found: 70.48; H, 6.88; N, 8.13.

Measurements. Spectroscopic measurements were carried out in TMP, THF, and a 1:1 mixture of THF and MTHF. The solvents were distilled before use. The sample solution was put into a quartz cuvette equipped with a Teflon stopcock. Argon gas was bubbled for 15 min before each measurement. The following spectrometers were used. Absorption, Jasco Ubest-50; fluorescence, Hitachi MPF-4; CD, Jasco J-600. The output of the spectrometers were interfaced to an NEC PC9801 personal computer. Fluorescence rise and decay curves were measured on a home-build single-photon-counting apparatus equipped with Ortec electronics. An air discharge lamp (fwhm = 3 ns) was used as the light source. The excitation and the emission wavelengths were selected by a combination of appropriate interference filters and glass filters. The excitation wavelength was 340–350 nm, and the emission wavelength was 380–390 nm for the monomer emissions and >550 nm for the exciplex emissions. The decay curves were analyzed by an iterative reconvolution method.

Conformational Analysis. The conformational calculation was carried out by using a set of programs for the conformational energy analysis of peptides of any amino acid sequence including the artificial ones. The software was developed by one of the authors (M.S.). The ECEPP parameters[15] were employed in the calculation, except for the two artificial amino acids, for which the structural parameters were taken from model compounds, and the energy parameters were calculated by a CNDO/ON MO calculations. The details of the parameters for the artificial amino acids will be reported elsewhere. The molecular model was drawn by using the NAMOD version 3 program.[16] The software was run on a NEC PC9801 personal computer.

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Registry No. I, 121445-62-3; II, 121445-60-1; III, 121445-63-4; IV, 121445-64-5; V, 121472-33-1; nitroPhe, 949-99-5; BOC-nitroPhe, 33305-77-0; BOC-dmaPhe, 105115-92-2; BOC-Glu(OBzl)·DCHA, 13574-84-0; Glu(OBzl)-OBzl·TosOH, 2791-84-6; BOC-Glu(OBzl)$_2$-OBzl, 89092-61-5; BOC-Glu(OBzl)$_3$-OBzl, 121445-54-3; BOC-Glu-(OBzl)$_4$-OBzl, 89107-69-7; pyrAla, 87147-90-8; BOC-pyrAla, 100442-89-5; BOC-pyrAla-Glu(OBzl)$_4$-OBzl, 121445-55-4; BOC-Ala, 15761-38-3; BOC-Ala-pyrAla-Glu(OBzl)$_4$-OBzl, 121445-56-5; BOC-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$-OBzl, 121445-57-6; Glu(OBzl) NCA, 3190-71-4; dmaPhe-Glu(OBzl)$_4$-OBzl, 121445-58-7; pyrAla-OMe·HCl, 107987-11-1; BOC-Ala-pyrAla-OMe, 121445-59-8; pyrAla-Glu(OBzl)$_4$-OBzl, 121472-34-2; Ala-pyrAla-OMe·HCl, 121445-61-2.

Long-Range Triplet Hydrogen Abstraction. Photochemical Formation of 2-Tetralols from β-Arylpropiophenones

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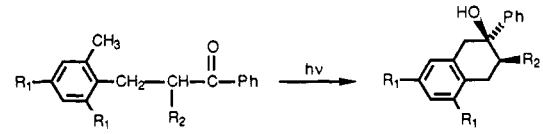
Abstract: The four ketones β-(*o*-tolyl)- and β-mesitylpropiophenone and -isobutyrophenone all undergo photocyclization to 2-tetralols via triplet-state ε-hydrogen abstraction. The intermediate 1,6-biradicals cyclize in 100% efficiency. The triplets react in very low quantum efficiency; rate constants for ε-hydrogen abstraction must compete with rapid (10^9 s^{-1}) CT quenching by the β-aryl group and range from $<3 \times 10^4$ for β-(*o*-tolyl)propiophenone to 3×10^6 s^{-1} for β-mesitylisobutyrophenone. The mesityl ketones are 10 times more reactive than the tolyl ketones; and the isobutyrophenones are 10 times more reactive than the propiophenones. The latter reactivity differences are ascribed to conformational constraints imposed by α-alkylation, contraints that actually increase the population of conformations close to that required for reaction.

The fortuitous preparation of β-(*o*-tolyl)propiophenone by the irradiation of the α isomer in the solid state[1] prompted us to investigate such structures for possible photoreactivity. We report that several β-(*o*-tolyl)propiophenones undergo triplet-state ε-hydrogen abstraction in competition with the well-known *rapid* internal CT quenching[2,3] to yield 1,2,3,4-tetrahydro-2-naphthols in high chemical but low quantum yields. These results add new information to two topics of considerable current interest: how rate constants for such remote hydrogen abstractions depend on molecular conformation[4,5] and how the behavior of triplet-generated biradicals varies with the distance between radical centers.[6]

Results

The four compounds in Scheme I have been studied. They were prepared by different procedures as described in the Experimental Section. Cyclohexane solutions approximately 0.003 M in ketone were irradiated for 2 weeks with only Pyrex filters. Compound **2** underwent only 25% conversion while compounds **3** and **4** were completely reacted. Collection by preparative gas chromatography

Scheme I



1 $R_1 = R_2 = H$
2 $R_1 = CH_3$ $R_2 = H$
3 $R_1 = H$ $R_2 = CH_3$
4 $R_1 = R_2 = CH_3$

Table I. Photokinetics of β-(*o*-Tolyl)propiophenones[a]

ketone	$\Phi(C_6H_6)$	Φ(MeOH)	τ_T,[b] ns	$k_{\epsilon\text{-H}}$,[c] $\times 10^7$ s^{-1}
1	<0.000 05		1.4[d]	<0.003
2	0.000 17	0.0003	1.6	0.010
3	0.000 26	0.0012	1.0 (0.9)[d]	0.026
4	0.002 0	0.012	1.1	0.18

[a] All measurements represent the average of duplicate runs, with precision typically of ±8%. [b] Measured by quenching with 2,5-dimethyl-2,4-hexadiene in benzene. [c] $k = \Phi/\tau$. [d] From ref 3.

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