Photoinduced Electron Transfer on a Single α-Helical Polypeptide Chain. Evidence of a Through-Space Mechanism

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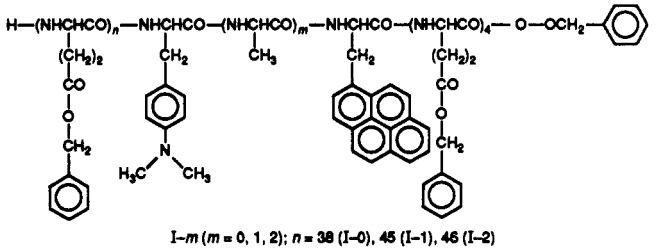
α-Helical polypeptides containing a single pair of L-*p*-(dimethylamino)phenylalanine and L-1-pyrenylalanine were synthesized. The distance and orientation of the (dimethylamino)phenyl (D) and pyrenyl (P) group were varied by inserting different numbers (*m*) of L-alanine units between the two artificial aromatic amino acids. The edge-to-edge distances between D and P groups, predicted from conformational energy calculations, were 5.4 Å for $m = 0$, 9.4 Å for $m = 1$, and 5.5 Å for $m = 2$. This indicates that the D–P pair in the $m = 2$ polypeptide is separated by a larger number of bonds but located closer than the D–P pair in the $m = 1$ polypeptide. The D–P pair in the $m = 0$ polypeptide was arranged in a head-to-tail manner, but that in the $m = 2$ polypeptide had a face-to-face arrangement. The rates of photoinduced electron transfer (ET) from the D to P* group at −20 °C in trimethyl phosphate solution were evaluated from the decay time of P* fluorescence to be 1.9×10^7 ($m = 0$), 6.6×10^5 ($m = 1$), and 2.1×10^7 s^{-1} ($m = 2$). The results show that the ET is occurring through space, and the possibility for a helical polypeptide chain as an electron mediator was excluded, at least for this relatively short distance ET. The ET rate was not very sensitive to the orientation of the donor and acceptor groups.

Introduction

Electron transfer (ET) on a single α-helical polypeptide chain can be a simple model for studying biological electron transfers.[1-5] An α-helical polypeptide chain has been shown to be an excellent molecular framework that supports a variety of chromophores in a spatially fixed arrangement along the helical rod. For instance, no or only very little excimers are observed in helical polypeptides carrying naphthyl[6,7] or pyrenyl groups,[8-10] indicating that the chromophores are unable to fluctuate thermally to form excimers during the lifetime of naphthyl (50 ns) or pyrenyl (200 ns) groups. In a previous work,[11] we have studied the rate and the activation energy of ET on a synthetic polypeptide Glu(OBzl)$_n$-dmaPhe-Ala-pyrAla-Glu(OBzl)$_4$ consisting of a pair of L-*p*-(dimethylamino)phenylalanine (dmaPhe) and L-1-pyrenylalanine (pyrAla) placed inside a helical poly(γ-benzyl L-glutamate) chain. In this polypeptide, the nearest edge-to-edge distance between the dimethylanilino (D) group and the pyrenyl (P) group was fixed to be 9.4 Å. The ET rate constant was on the order of 10^5 (s^{-1}), and the activation enthalpy was 1.4 kcal/mol or less. The study was extended to a series of polypeptides carrying the sequence of -dmaPhe-Ala$_m$-pyrAla- in the middle of a poly[Glu(OBzl)] chain (I-*m*). The results are reported in this paper. The three



I-*m* (*m* = 0, 1, 2); *n* = 38 (I-0), 45 (I-1), 46 (I-2)
Glu(OBzl)$_n$-dmaPhe-Ala$_m$-pyrAla-Glu(OBzl)$_4$

kinds of polypeptides will have different distances and orientations for the D–P pair along a helical main chain. These polypeptides will be adequate model systems to investigate the distance and orientation dependence of the ET process. It should be noted that the attachment of four Glu(OBzl) units at the right terminal of the polypeptide is of crucial importance to stabilize the helical conformation at the D–P pair.

Previously, we have also reported the synthesis and fluorescence spectra of a series of similar polypeptides carrying a 1-naphthyl (N) group, instead of the 1-pyrenyl group.[12] Since the absorption bands of D and N groups largely overlap, neither the D group nor the N group was photoexcited selectively. Therefore, the rate of elemental ET process could not be measured for the D–N pair. In the present case, the rate of ET from the D to P* group can be measured directly by following the decay of fluorescence of the P group after a selective photoexcitation of the P group.

Results and Discussion

Absorption and Circular Dichroism Spectra. The absorption spectra of the three kinds of polypeptides in tetrahydrofuran (THF) and in trimethyl phosphate (TMP) solutions were virtually the same as the sum of the spectra of model peptides containing only D and P groups, respectively. The absence of ground-state interaction between D and P groups has been reported in the case of I-1.[11] CD spectra of the three polypeptides in TMP showed a typical CD profile for a full right-handed α-helical conformation [$\Delta\epsilon_{222} = -11.2$ (I-0), −11.6 (I-1), and −10.6 (I-2)].[13] The full helical conformation is also supported by the observation of single C$^\alpha$H proton peak at 3.95 ppm in the ^{1}H NMR spectrum in $CDCl_3$. If a coiled conformation coexists, the C$^\alpha$H proton should appear at 4.62 ppm[14-16]

Conformational Analysis. Since the CD spectra showed the occurrence of a right-handed α-helical conformation for the three kinds of polypeptides, conformational energy calculations were carried out to predict the most stable orientations of the side-chain chromophoric groups, assuming a standard α-helical conformation for the main chain.[11,17] The calculation was performed on Ac-

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(1) Isied, S. S.; Vassilian, A. *J. Am. Chem. Soc.* **1984**, *106*, 1726.
(2) Isied, S. S.; Vassilian, A.; Magnuson, R. H.; Schwarts, H. A. *J. Am. Chem. Soc.* **1985**, *107*, 7432.
(3) Isied, S. S.; Vassalian, A.; Wishart, J. F. *J. Am. Chem. Soc.* **1988**, *110*, 635.
(4) Schanze, K. S.; Sauer, K. *J. Am. Chem. Soc.* **1988**, *110*, 1180.
(5) Faraggi, M.; De Felippis, M. R.; Klapper, M. H. *J. Am. Chem. Soc.* **1989**, *111*, 5141.
(6) Sisido, M.; Egusa, S.; Imanishi, Y. *J. Am. Chem. Soc.* **1983**, *105*, 1041, 4077.
(7) Sisido, M.; Imanishi, Y. *Macromolecules* **1986**, *19*, 2187.
(8) Egusa, S.; Sisido, M.; Imanishi, Y. *Macromolecules* **1985**, *18*, 882.
(9) Sisido, M. *Macromolecules* **1989**, *22*, 3280.
(10) Inai, Y.; Sisido, M.; Imanishi, Y. *J. Phys. Chem.* **1990**, *94*, 8365.
(11) Sisido, M.; Tanaka, R.; Inai, Y.; Imanishi, Y. *J. Am. Chem. Soc.* **1989**, *111*, 6790.
(12) Inai, Y.; Sisido, M.; Imanishi, Y. *J. Phys. Chem.* **1990**, *94*, 6239.
(13) Woody, R. W. *J. Polym. Sci., Macromol. Rev.* **1977**, *12*, 181.
(14) Suzuki, Y.; Inoue, Y.; Chujo, R. *Biopolymers* **1975**, *14*, 1223.
(15) Silvermann, D. N.; Scherage, H. A. *Biochemistry* **1971**, *10*, 1340.
(16) Bradbury, E. M.; Cary, P. D.; Crane-Robinson, C.; Hartmann, P. G. *Pure Appl. Chem.* **1973**, *36*, 53.
(17) Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361.

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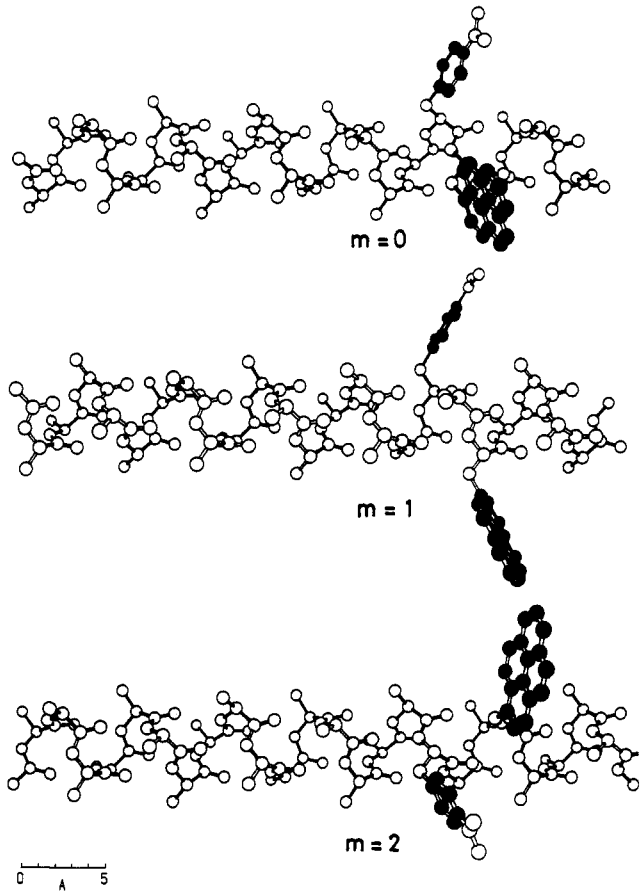

Figure 1. Most probable side-chain orientations of the three kinds of polypeptides predicted from the ECEPP conformational calculation. The NAMOD molecular display program was used (ref 18).

Ala_4-dmaPhe-Ala_m-pyrAla-Ala_4-$NHCH_3$. The main-chain rotational angles were fixed to be $\phi = -57°$, $\psi = -47°$, and $\omega = 180°$. Only the side-chain rotational angles of dmaPhe [χ_1(D), χ_2(D)] and pyrAla [χ_1(P), χ_2(P)] were varied. As in the case of I-1, only a single stable orientation was found for D and P groups in the polypeptides. The most stable orientations of the side chains were [χ_1(D), χ_2(D), χ_1(P), χ_2(P)] = (185.4°, 83.8°, 182.8°, 77.0°) for $m = 0$, (186.9°, 84.5°, 186.5°, 76.8°) for $m = 1$, and (186.9°, 82.2°, 187.2°, 77.0°) for $m = 2$. The ball-and-stick molecular models are illustrated in Figure 1.[18] It should be mentioned that the structure of the $m = 0$ polypeptide instead of the $m = 1$ one has been erroneously shown in Figure 5 of ref 11. The interchromophore center-to-center (edge-to-edge) distances predicted from the calculation are 9.1 (5.4) for $m = 0$, 13.2 (9.4) for $m = 1$, and 9.0 (5.5) for $m = 2$ (in angstroms).

Interestingly, the D–P pair in the $m = 2$ polypeptide is linked by a larger number of covalent bonds (13) than that in the $m = 1$ polypeptide (10), but the interchromophore distance in the $m = 2$ polypeptide (5.5 Å) is shorter than that in the $m = 1$ one (9.4 Å). This unusual geometrical situation is a result of the helical structure and provides an excellent example that can discriminate whether the ET is occurring through bond or through space (or through solvent).

Furthermore, the $m = 0$ and $m = 2$ polypeptides have about the same interchromophore distances, but the orientation of the D–P pair in the former polypeptide is close to a head-to-tail type, whereas that of the latter is almost a sandwich type. Therefore, a comparison of the ET rates in the I-0 and I-2 polypeptides may give information on the orientational dependence of the ET.

The thermal fluctuation of a P group in the polypeptide is predicted to be very small ($\chi_1 = \pm 20°$, $\chi_2 = \pm 8°$) near room temperature (potential energy <1 kcal/mol). The fixed orientation

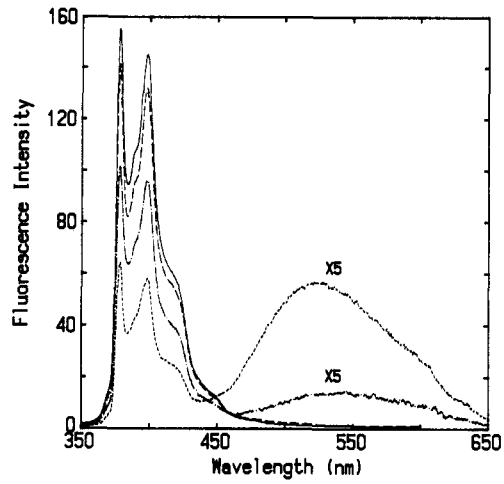

Figure 2. Fluorescence spectra of the I-0 (–·–), I-1 (---), I-2 (···), and II (—) polypeptide in THF at 20 °C. The scales for the I-0 and I-2 polypeptides are enlarged by a factor of 5. [Pyr] = 1×10^{-6} M.

of the pyrenyl group is supported by the observation of strong induced CD, especially in the case of I-0 ($\Delta\epsilon_{276} = 33$ in TMP). Single orientation was also predicted for the D group with a larger area of thermal fluctuation ($\chi_1 = \pm 15°$, $\chi_2 = \pm 25°$). The relaxation time of the rotational motion of a single tryptophyl group in a helical polypeptide has been measured by the fluorescence–anisotropy decay method as 0.1–0.2 ns in solution.[19] If the same relaxation time is applied to the present case, the D group may be fluctuating very frequently in the allowed range, before ET occurs within 10 ns.

As a result of thermal fluctuations in the χ_1 and χ_2 angles, the edge-to-edge distance between D and P groups will be varied. The nearest possible edge-to-edge distances when the D group fluctuates within the above-mentioned range (energy <1 kcal/mol) were evaluated as 5.0 ($m = 0$), 9.2 ($m = 1$), and 5.2 Å ($m = 2$). The edge-to-edge distances may not be altered much from the most stable conformations by the thermal fluctuation.

Fluorescence Spectra. Fluorescence spectra of the three kinds of polypeptides and that of a polypeptide containing a single pyrAla residue, $Glu(OBzl)_n$-pyrAla-$Glu(OBzl)_4$ (II), are shown in Figure 2. The spectra show a marked dependence on the arrangement of the D–P pair along the helix. The fluorescence spectrum of the I-1 polypeptide is not much different from that of the reference polypeptide II, except for an about 10% decrease of monomer fluorescence. The small but definite fluorescence quenching has been attributed to an ET from D group to P* group.[11] The monomer fluorescence of the I-0 and I-2 polypeptides is markedly quenched, and a moderate amount of exciplex is formed. The amount of exciplex is smaller than that expected from the close arrangement of the D–P pair, as shown in Figure 1. One of the reasons for the small exciplex emission may be the polar solvent employed in this study. In fact, in TMP solution, the exciplex emission further decreased for the I-2 polypeptide and virtually disappeared for the I-0 polypeptide. However, a major reason for the weak exciplex emission may be the rigid arrangement of the D and P groups on the helical polypeptide chain. Indeed, an oligopeptide carrying a D–P pair that is not located inside the helix, Boc-dmaPhe-Ala-pyrAla-$Glu(OBzl)_4$-OBzl, showed a much stronger exciplex emission than the corresponding polypeptide (I-1). The exciplex in the polypeptides decreased further with lowering temperature. The ratios of the quantum yields of exciplex to monomer (q_E/q_M) were 0.28 (I-0) and 1.7 (I-2) in THF at 20 °C, but they were 0.05 (I-0) and 0.53 (I-2) at −60 °C. The quantum yield ratios of the I-2 polypeptide in TMP were 0.26 at 20 °C and 0.09 at −60 °C. These data indicate that the exciplex formation is a minor process in the polypeptides, especially at low temperatures and in polar solvents. Instead, the ET quenching

(18) The NAMOD molecular display program was used. See: Beppu, Y. *Comput. Chem.* **1989**, *13*, 101.

(19) Vogel, H.; Nilsson, L.; Rigler, R.; Voges, K.-P.; Jung, G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5067.

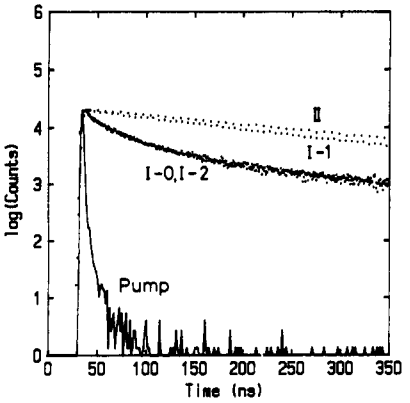

Figure 3. Decay curves of pyrenyl fluorescence of the I-0, I-1, I-2, and II polypeptides in TMP at −20 °C.

of P* is the major photophysical process in the three kinds of polypeptides. Only ET quenching is occurring in the I-1 polypeptide.

The increase of exciplex emission is not parallel to the decrease of monomer fluorescence. The exciplex quantum yield in THF at 20 °C was 0.03 for the I-0 polypeptide and 0.11 for the I-2 polypeptide, whereas the quenching efficiency of monomer fluorescence was 87% for the I-0 polypeptide and 92% for the I-2 polypeptide. Since the interchromophore distances in the I-0 and I-2 polypeptides are considered to be the same, the different quantum yields of exciplex formation could be attributed to the different orientations of the chromophoric groups; i.e., the sandwich-type orientation of the I-2 polypeptide is more favorable for exciplex formation than the head-to-tail-type one in the I-0 polypeptide. The preference of the sandwich-type orientation for exciplex formation has also been observed in similar polypeptides containing the 1-naphthyl group, instead of the 1-pyrenyl group.[12]

The fluorescence spectra indicated that the quenching efficiency depends markedly on the relative arrangement of D and P groups. The quenching efficiency should be the sum of the efficiencies of exciplex formation and of ET. However, since the exciplex formation is a minor process, the former will be neglected in the following discussion.

Fluorescence Decay Analysis and Rates of Electron Transfer. Fluorescence decay curves of the I-0, I-1, I-2, and II polypeptides are compared in Figure 3.[20] The decay curves of the I-0 and I-2 polypeptides deviated from a single-exponential decay. The deviation was observed at all temperatures examined, and the decay time of the slow component was similar to that of the reference polypeptide II at each temperature. Therefore, we have assigned the slow component as the contribution from a deficient polypeptide that has a pyrenyl group but lacks the D group on the same chain. A part of the D groups might have been oxidized or protonated during the synthesis of the polypeptide and cannot work as the ET quencher. This assignment is supported by the fact that the fraction of the slow component is almost constant (about 20%) over the temperature range examined. Therefore, the decay curves were fitted to two-component exponential functions, under the restriction that the decay time of the slow component being fixed to that of the reference polypeptide II at the same temperature. The results of the decay analysis are listed in Table I. The fitting is fairly good in most cases, and the fraction of the slow component is constant, irrespective of temperature.

The inverse of the fast decay time (τ_f) should be the sum of the rate constant of an intrinsic deactivation process of the P group in the polypeptide and that of ET from D to P*.

$$1/\tau_f = k_M + k_{et} \quad (1)$$

The intrinsic deactivation rate constant (k_M) may be evaluated from the inverse of the decay time of the reference polypeptide II. Typical values of the ET rate constants (k_{et}) are listed in Table II.

TABLE I: Results of Two-Component Analysis of the Fluorescence Decay Curves of I-0 and I-2 in TMP and THF[a]

polypeptide (solvent)	temp, °C	τ_f (weight)	τ_s (weight)	χ^2
I-0 (TMP)	20	25.6 (0.80)	242 (0.20)	1.55
	0	31.1 (0.80)	250 (0.20)	1.33
	−20	44.0 (0.81)	269 (0.19)	1.19
	−40	56.9 (0.80)	280 (0.20)	1.33
	−60	66.7 (0.77)	290 (0.23)	1.43
I-2 (TMP)	20	16.0 (0.79)	242 (0.21)	1.30
	0	23.7 (0.80)	250 (0.20)	1.34
	−20	41.2 (0.81)	269 (0.21)	1.24
	−40	63.0 (0.77)	280 (0.23)	1.47
	−60	72.8 (0.70)	290 (0.30)	1.46
I-0 (THF)	20	24.1 (0.82)	256 (0.18)	1.50
	0	30.6 (0.82)	262 (0.18)	1.32
	−20	40.5 (0.83)	272 (0.17)	1.39
	−40	54.0 (0.83)	284 (0.17)	1.21
	−60	65.6 (0.82)	296 (0.18)	1.11
I-2 (THF)	20	9.4 (0.82)	256 (0.18)	1.02
	0	14.6 (0.83)	262 (0.17)	1.25
	−20	27.9 (0.83)	272 (0.17)	1.04
	−40	55.5 (0.81)	284 (0.19)	1.33
	−600	98.0 (0.76)	296 (0.24)	1.03

[a] The decay times (in nanoseconds) of the slow-decaying component (τ_s) are fixed to the decay times of the model polypeptide carrying no D group (II). The decay data of the I-1 polypeptide are given in ref 11.

The ET rates depend not on the number of bonds between the D and P groups, but on the spatial distance between the two groups. The distance dependence clearly indicates a through-space (or through-solvent) mechanism for the ET in this series of polypeptides. Contribution of a through-bond mechanism, if it is present at all, should be much less important than the through-space mechanism. This conclusion contrasts with the through-bond mechanism postulated for the ET's occurring on rigid nonconjugated bridges.[21-24] A possible role of the electronic levels of a polypeptide chain to promote the long-range ET has been discussed.[25] However, the present results clearly eliminate this possibility, as far as the ET across a relatively short distance of around 10 Å is concerned.

The absence of ET promoted by the peptide chain is further confirmed by the relatively slow rate of ET. In the ET on a saturated hydrocarbon chain, the rate has been reported on the order of 10^9–10^{10} (s^{-1})[21,22,24] when the edge-to-edge distance of the chromophores is about 10 Å, which is near the optimal exothermic condition. The present ET rate constants are much lower than the above values, although the electronic driving force of the D–P pair (−0.4 eV) lies near the optimal region.[11]

An exponential dependence of the ET rate constant on the interchromophore edge-to-edge distance r has been proposed.[26]

$$k_{et} = A \exp[-\beta(r - r_0)] \quad (2)$$

The r_0 is the contact distance. Having the above ET rate constants fitted to eq 2, the β value should be approximately 0.93 in TMP and 1.3 in THF. These values seem to be reasonable as compared with those reported for ET in proteins.[26-28] However, because

(20) The decay data were obtained mostly on a single-photon counting system with an air-discharge lamp. A part of the decay curves was also measured on a single-photon counting system with a picosecond laser source. The two measurements gave virtually the same results.

(21) Oevering, H.; Paddon-Row, M. N.; Heppener, M.; Oliver, A. M.; Cotsaris, E.; Verhoeven, J. W.; Hush, N. S. *J. Am. Chem. Soc.* **1987**, *109*, 3258.
(22) Closs, G. L.; Piotrowiak, P.; MacInnis, J. M.; Fleming, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 2652.
(23) Ohta, K.; Closs, G. L.; Morokuma, K.; Green, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 1319.
(24) Closs, G. L.; Calcaterra, T. T.; Green, N. J.; Penfield, K. W.; Miller, J. R. *J. Phys. Chem.* **1986**, *90*, 3673.
(25) McLendon, G. *Acc. Chem. Res.* **1988**, *21*, 160.
(26) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.

TABLE II: Rate Constants of Electron Transfer and the Activation Parameters

	$10^6 k_{et}$, s^{-1}						
m	20 °C	0 °C	−20 °C	−40 °C	−60 °C	$H^{\ddagger}$,[b] kcal/mol	$S^{\ddagger}$,[b] eu
				In THF			
0	38	29	21	15	12	1.3 (0.1)	−19 (0.3)
1[a]	0.40	0.28	0.29	0.31	0.26	<1	−32 (0.7)
2	102	65	32	15	6.8	3.8 (0.1)	−8.8 (0.2)
				In TMP			
0	58	38	21	12	10	1.2 (0.1)	−20 (0.3)
1[a]	1.1	0.89	0.66	0.54	0.28	1.4 (0.1)	−26 (0.6)
2	35	27	19	14	12	2.3 (0.2)	−16 (0.7)

[a] Data taken from ref 11. [b] Numbers in parentheses are the corresponding error ranges.

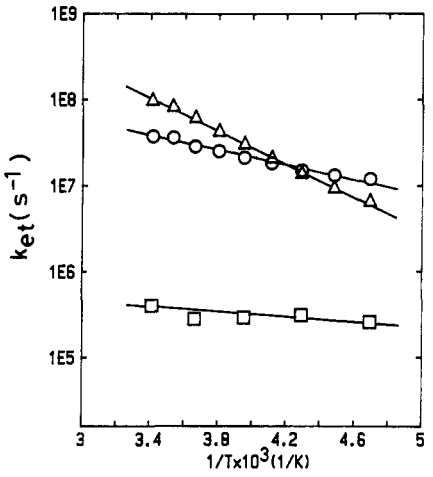

Figure 4. Arrhenius plot of the ET rate constants for the I-0 (○), I-1 (□), and I-2 (△) polypeptides in THF. Values for the I-1 polypeptide are taken from ref 11.

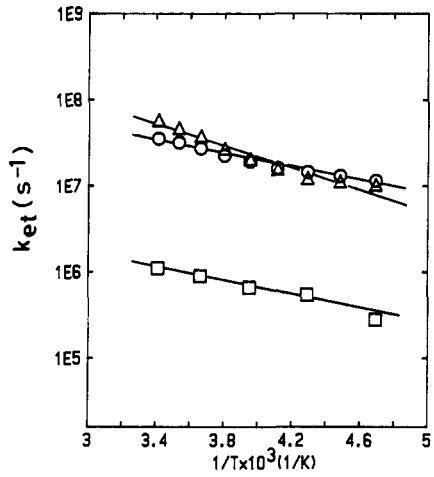

Figure 5. Arrhenius plot of the ET rate constants for the I-0 (○), I-1 (□), and I-2 (△) polypeptides in TMP. Values for the I-1 polypeptide are taken from ref 11.

of the accidental agreement of the r values of the I-0 and I-2 polypeptides, we cannot go further into detailed discussion on the distance dependence of ET.

The I-0 and I-2 polypeptides possess nearly the same interchromophore distances, but the orientation of the D–P pair in the I-0 polypeptide is a head-to-tail type and that in the I-2 polypeptide is near a sandwich type. Despite the different orientations, the ET rates are not much different from each other. The sandwich-type orientation appears to be slightly preferred for ET near room temperatures, but the inverse is true below −30 °C. The small or no dependence on the orientation of chromophores in the polypeptide system contrasts with the observations on the nonconjugated bridges, where a small but definite orientation dependence has been reported.[23,24]

Temperature Dependence. The ET rate constants were measured over the temperature range from 20 to −60 °C for the I-0 and I-2 polypeptides and are plotted as a function of temperature in Figures 4 and 5, along with the data for the I-1 polypeptide that have been reported previously.[11] The data were replotted in the form of the Eyring plot, and the activation parameters were calculated. They are listed in Table II. The activation enthalpies for the I-0 and I-1 polypeptides are similar to or less than those of the solvent viscosity. Therefore, no large conformational change is associated with the ET in the two kinds of polypeptides. For ET processes in some proteins, a mechanism involving "conformational gating" has been proposed and experimentally supported.[29,30] However, the gated ET mechanism cannot be applied to the present system, at least for the I-0 and I-1 polypeptides.

The activation enthalpy for the I-2 polypeptide is a little higher than those of other two kinds of polypeptide. The higher activation enthalpy may indicate that some conformational deformations are involved or a large solvent reorganization is needed in the ET of the I-2 polypeptide. Since the interchromophore distances are about the same for the I-0 and I-2 polypeptides, the former interpretation seems more likely for the present time. The activation parameters for photophysical processes including ET, energy transfer, exciplex formation, and excimer formation on helical polypeptide chain will be discussed after the above processes are studied on Glu(OBzl)$_n$-X-Ala$_m$-Y-Glu(OBzl)$_4$ systems.

Conclusions

Two major conclusions were drawn from the present experiment. First, the ET between chromophores on an α-helical polypeptide chain occurs by a through-space mechanism, and the possibility of electronic assistance by the polypeptide helix was excluded, as far as the ET across the distance shorter than 10 Å is concerned. Second, the ET process is insensitive to the relative orientation of the electron donor and acceptor groups. These conclusions are in contrast to the observations on nonconjugated hydrocarbon systems and to the prevailing proposals for the ET process in proteins. However, the present data do not exclude the possibility of a very long range ET (>10 Å) through a polypeptide chain that occurs over the time scale of milliseconds or longer.

Experimental Section

Materials. The polypeptides were prepared in a similar manner as described for the I-1 polypeptide.[11] Only those newly synthesized in this study are presented here. Abbreviations used previously are employed.

Boc-dmaPhe-pyrAla-Glu(OBzl)$_4$-OBzl. The Boc group of Boc-pyrAla-Glu(OBzl)$_4$-OBzl was removed with 4 N HCl/DOX to give HCl-pyrAla-Glu(OBzl)$_4$-OBzl. The latter (53 mg, 0.04 mmol) and Boc-dmaPhe (17 mg, 0.055 mmol) were dissolved in THF (2 mL) and cooled with ice. DCC (11 mg, 0.05 mmol), HOBt (6 mg, 0.04 mmol) in THF (2 mL), and TEA (5.8 μL, 0.04 mmol) were then added to the mixture, and the mixture was stirred

(27) Auxup, A. W.; Albin, M.; Mayo, S. L.; Crutchley, R. J.; Gray, H. B. *J. Am. Chem. Soc.* **1988**, *110*, 435.
(28) Gray, H. B. *Chem. Soc. Rev.* **1986**, *15*, 17.
(29) Hoffman, B. M.; Ratner, M. A. *J. Am. Chem. Soc.* **1987**, *109*, 6237.
(30) McLendon, G.; Pardue, K.; Bak, P. *J. Am. Chem. Soc.* **1987**, *109*, 7540.

for 3 h under cooling. The stirring was continued for 1 day at room temperature. The mixture was diluted with ethyl acetate, and the solution was washed successively with 10% NaCl, 10% citric acid, 10% NaCl, and 3% $NaHCO_3$ solutions. The solution was dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified with a silica gel column in ethyl acetate: yield 35 mg (55%); mp 156–180 °C. Anal. Calcd for $C_{90}H_{95}N_7O_{17}$: C, 69.89; H, 6.19; N, 6.34. Found: C, 69.84; H, 6.06; N, 6.18.

Glu(OBzl)$_n$-dmaPhe-pyrAla-Glu(OBzl)$_4$-OBzl (I-0). The above hexapeptide was dissolved in formic acid and kept at room temperature for 5 h. The formic acid was removed by evaporation, and 3% $NaHCO_3$ was added to the residue. The N-deprotected hexapeptide was extracted with chloroform, and the extract was washed with NaCl solution and dried on $MgSO_4$. The solvent was evaporated, and the residue was dried under vacuum. The hexapeptide with a free amino group was dissolved in DMF, and a 30-fold amount of Glu(OBzl) NCA was added. The polymerization was continued for 4 days at room temperature. The polymer was precipitated with ether and fractionated with a Sephadex LH-60 gel. Only the highest molecular weight fraction was used for the spectroscopic study. The number-average degree of polymerization $\bar{n}$ of the polymerized Glu(OBzl) unit was determined to be 38 from the difference of the absorption intensity at 257.5 nm.[11]

Boc-dmaPhe-Ala$_2$-OBzl. Boc-Ala$_2$-OBzl (660 mg, 1.9 mmol) was treated with 4 N HCl/DOX (4 mL) to obtain HCl·Ala$_2$-OBzl. The latter was coupled with Boc-dmaPhe (750 mg, 2.4 mmol) in dichloromethane with EDC and HOBt as a coupling reagent. The procedure is the same as described above. The product was purified with a silica gel column with ethyl acetate as an eluent: yield 575 mg (57%); mp 151–153 °C. Anal. Calcd for $C_{29}H_{40}N_4O_6$: C, 64.42; H, 7.46; N, 10.34. Found: C, 64.40; H, 7.48; N, 10.28.

Boc-dmaPhe-Ala$_2$-OH. Boc-dmaPhe-Ala$_2$-OBzl (0.22 g, 0.41 mmol) was dissolved in ethanol (40 mL), and 10% palladium–carbon (0.10 g) was added. The mixture was stirred for 2.5 h under hydrogen atmosphere at room temperature. The catalyst was filtered off, and the solvent was evaporated. The crude product was purified by gel chromatography (Sephadex LH-20/methanol): yield 101 mg (55%); mp 102–106 °C.

Boc-dmaPhe-Ala$_2$-pyrAla-Glu(OBzl)$_4$-OBzl. The free acid obtained above (41 mg, 0.09 mmol) was dissolved in anhydrous THF (1 mL) and cooled to −10 to −15 °C. *N*-Methylmorpholine (10 μL, 0.09 mmol) and isobutyl chloroformate (12 μL, 0.09 mmol) were added, and the mixture was stirred at −10 °C. After 6 min, pyrAla-Glu(OBzl)$_4$ HCl salt (118 mg, 0.09 mmol) in DMF (1 mL) containing *N*-methylmorpholine (10 μL, 0.09 mmol) were added, and the mixture was stirred for 1 h at −10 to −15 °C. The mixture was left standing for 12 h at 0 °C and diluted with dichloromethane. The solution was washed successively with 10% NaCl, 10% citric acid, 10% NaCl, 3% $NaHCO_3$, and 10% NaCl solutions and dried over $MgSO_4$. After the solvent was evaporated, the residue was purified by gel chromatography (Sephadex LH-20/DMF): yield 48 mg (32%); mp 250–256 °C. Anal. Calcd for $C_{96}H_{105}N_9O_{19}$: C, 68.27; H, 6.27; N, 7.46. Found: C, 68.27; H, 6.51; N, 7.36.

Glu(OBzl)$_n$-dmaPhe-Ala$_2$-pyrAla-Glu(OBzl)$_4$-OBzl (I-2). After removing the Boc group, the above octapeptide was used as the initiator of the polymerization of Glu(OBzl) NCA. The procedure was the same as described for the I-0 polypeptide, $\bar{n}$ being 46.

Measurements. The instruments employed and the procedure were the same as described previously.[11] The decay curves were measured on a home-built single-photon counting system using an air-discharge light source.[11] A part of the fluorescence decay curves was also measured on a single-photon counting system using a synchronous cavity-dumped dye laser pumped by a mode-locked CW Nd:YAG laser (Spectra Physics).[31]

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(31) Ikeda, T.; Lee, B.; Kurihara, S.; Tazuke, S.; Itoh, S.; Yamamoto, M. *J. Am. Chem. Soc.* **1988**, *110*, 8299.