# Effect of the Electric Field Generated by the Helix Dipole on Photoinduced Intramolecular Electron Transfer in Dichromophoric α-Helical Peptides

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In natural photosynthesis, long-range electron transfer between donor−acceptor pairs embedded within a protein matrix occurs rapidly over long (>10 Å) distances.[1] It has been postulated that the electric field associated with the permanent dipole of α-helical sections of the proteins surrounding the photosynthetic reaction center influences the rate of the primary electron transfer event.[2a] The dipole of an α-helix (about 3.5 D per amino acid residue) generates an electrostatic field along the helix axis of $10^9$ V/m,[2] producing an effective positive charge at the amino end and an effective negative charge at the carboxyl end, each of magnitude $0.8 \times 10^{-19}$ C.[2b,c] This strong electric field plays an important role in the structure and function of proteins.[2a,3] In this work, we have investigated the effect of the position of probe chromophores relative to the direction of the electric field generated by the helix on the rate of intramolecular electron transfer reactions.

Helical oligopeptides **1** and **2** with pendant electron donor (D) and acceptor (A) chromophores (Figure 1) differ only by the positional reversal of the donor−acceptor (DA) pair along the dipolar helix. Photoinduced electron transfer between D and A generates a charge-separated pair ($D^{\bullet+}A^{\bullet-}$) which is oriented with the internal electric field in **2** and against the field in **1**. The potential energy of a charge-separated donor−acceptor pair in the presence of an electric field is higher or lower depending on this field orientation. Thus, when $D^{\bullet+}A^{\bullet-}$ is generated by electron transfer between neutral precursors, $\Delta G$ and, therefore, the electron transfer rate depend on this positioning. Namely, the driving force will be larger and the rate faster (if $\Delta G$ lies in the normal region) when $D^{\bullet+}A^{\bullet-}$ is against the field.[4,5] Hence, we anticipate that the alignment of electric field in **1** against the direction of photoinduced electron transfer should induce a faster rate of electron transfer in **1** than in **2**.

Peptides **1** and **2** were synthesized by standard solution-phase peptide coupling reactions,[6] the oligopeptide backbone having been prepared from L-alanine (Ala) and aminoisobutyric acid (Aib).[7,8] The donor (*N*,*N*-dimethylaniline) and the acceptor (pyrene) were incorporated into the backbone as substituents on L-alanine residues, and six residues separate the chromophores. As unfolding often occurs at peptide termini, D and A were separated from the terminus by three residues in order to fix their orientation. Two reference peptides, containing only the donor (**3**) or the acceptor (**4**), were also prepared for control experiments.

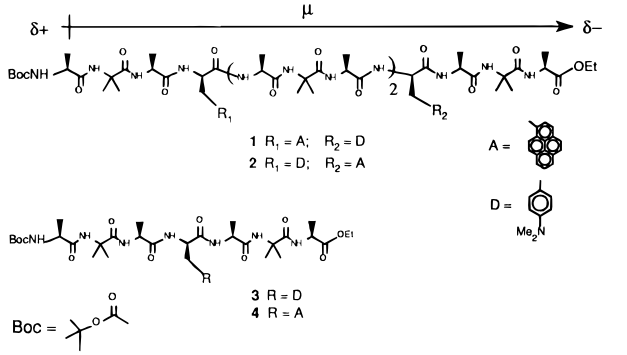


**Figure 1.** Dichromophoric oligopeptides **1** and **2** and reference peptides monosubstituted with donor (**3**) or acceptor (**4**). The molecular dipole $\mu$ is oriented from the amino end (positive) to the carboxyl end (negative).

To define the distance and the relative angular orientation between the appended D and A in **1** and **2**, it was necessary to establish the conformation of the backbones. The right-handed helical conformation of **1** and **2** was confirmed by analysis of their CD spectra in acetonitrile and in methanol, each of which showed a strong positive band at 190 nm and two negative bands near 210 and 220 nm.[9] In both solvents, the CD spectra of **1** and **2** were identical, suggesting that, on the time scale of the experiments, the two peptides have a very similar secondary structure. To distinguish between an α- or $3_{10}$-helical conformation, 2D $^1$H NMR spectra (COSY, NOESY, ROESY) of **1** and **2** were analyzed. Two kinds of NOE interactions were observed, $\beta$CH($i$)−αCH($i$ + 3) and NH($i$)−NH($i$ + 3), which are characteristic of an α-helix and not observable in $3_{10}$-helices.[10,11] We conclude that the preferential conformation of **1** and **2** is α-helical and that the two chromophores, roughly parallel to one another and to a plane perpendicular to the helix axis, are ∼10 Å apart.[12] In an α-helix, six residues correspond to two turns of coil. The methylenic units introduce one additional variable, the side-chain torsion. Conformational analysis of oligopeptides substituted with chromophores identical to the ones in **1** and **2** predicts that thermal fluctuation of side-

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(2) The permanent molecular dipole in an helical polypeptide is generated by the parallel alignment of the individual dipole moments of the amino acid residues to the helix axis. The reported value of $10^9$ V/m is calculated from vacuum electrostatics. For a review on the role of α-helix dipole on protein function and structure, see: (a) Hol, W. G. J. *Prog. Biophys. Mol. Biol.* **1985**, *45*, 149. (b) Hol, W. G. J.; van Duijnen, P. T.; Berendsen, H. J. C. *Nature* **1978**, *273*, 443. (c) Wada, A. *Adv. Biophys.* **1976**, *9*, 1.

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(6) (a) Bodanszky, M. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1994. (b) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: Berlin, 1993. (c) See supporting information for details about purification and characterization of **1**−**4**.

(7) $(Ala)_n$ peptides with $n > 4$ were nearly insoluble: Fox, M. A.; Meier, M. S.; Wall, C. G.; Miller, J. R. *J. Org. Chem.* **1991**, *56*, 5380. $(Ala\text{-}Aib)_n$ peptides were selected for this work because of their solubility in organic solvents and their proclivity to form α-helices when $n > 4$.

(8) Substituted Aib polypeptides are usually $3_{10}$-helical, but empirical rules suggest that Ala-Aib peptides with less than 35% Aib content are α-helical (**1** and **2** have a 28% Aib content): (a) Oyoda, K.; Kitakawa, Y.; Kimura, S.; Imanishi, Y. *Biopolymers* **1993**, *33*, 1337. (b) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocky, J.; Leplawy, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 487. (c) Butters, T.; Hütter, P.; Jung, G.; Pauls, N.; Schmitt, H.; Sheldrick, G. M.; Winter, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 889. (d) Karle, I. L.; Sukuman, M.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 9284.

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(10) 2D $^1$H NMR spectra were recorded in $CDCl_3$ using a General Electric GN-500 500 MHz spectrometer.

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(12) When donor and acceptor were separated by three residues in compounds analogous to **1** and **2** (one turn of coil, ∼5 Å $CH_2$ to $CH_2$), significant exciplex emission was observed, suggesting that the chromophores can access a parallel conformation. The $\lambda_{max}$ of the observed emission was compared with the $\lambda_{max}$ of exciplex emission of the system pyrene−dimethylaniline reported in the literature (450 nm), and it did not disappear upon dilution. Therefore, we conclude that the observed emission is due to an intramolecular exciplex.

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**Table 1.** Time-Resolved Fluorescence of **1** and **2**[a,b]

| | $\tau$ (weight)[c] | | $\kappa_{et1}$ | $\kappa_{et2}$[e] | $\kappa_{et1}/$ |
|---|---|---|---|---|---|
| | **1**[d] | **2** | ($\times 10^9$ s$^{-1}$) | ($10^9$ s$^{-1}$) | $\kappa_{et2}$ |
| solvent | | | | | |
| THF | 2.5 (52), 0.7 (42) | 33 (88), 81 (11) | 0.62 | 0.022 | 27 |
| $CH_2Cl_2$ | 3.9 (28), 1.5 (65) | 27 (84), 129 (15) | 0.48 | 0.020 | 24 |
| $CH_3CN$ | 2.7 (33), 0.9 (61) | 9 (97), 96 (2) | 0.71 | 0.090 | 8 |
| MeOH | 3.6 (60), 0.8 (33) | 10 (96), 70 (3) | 0.41 | 0.082 | 5 |
| $T$ (°C)[f] | | | | | |
| 22 | 2.5 (52), 0.7 (42) | 27 (84), 129 (15) | 0.62 | 0.023 | |
| −1 | 5 (37), 1.8 (56) | 69 | 0.35 | 0.011 | |
| −45 | 32 (27), 5.5 (70) | 209 | 0.07 | 0.001 | |

[a] All samples were degassed by bubbling nitrogen saturated with the solvent throughout the measurements. Concentrations were low (~30 $\mu$M) in order to avoid self-aggregation. The time-resolved fluorescence experiments were performed on a single-photon counting apparatus. The time resolution was 20 ps. [b] $\lambda_{exc} = 344$ nm, $\lambda_{obs} = 400$ nm. $T = 22$ °C. *c* Time decays are in nanoseconds. The number in parentheses indicates the relative weighting of the component. Time decay of the reference peptide **4** is $\tau_o = 300$ ns. [d] In compound **1** was observable a minor third component with a lifetime identical to that observable in **4**. The contribution of this component was small and constant (≤ 5%) in all solvents and temperatures examined. We assigned it to the presence of an impurity (racemized peptide or with pyrene only) in which intramolecular quenching does not occur. As a result, it is not included in the table. [e] Calculated from $K_i = 1/\langle\tau\rangle_i - 1/\tau_o$, where $\langle\tau\rangle_i = \tau'(A') + \tau''(A'')$, where $A$ is the amplitude. [f] In THF.

chain rotational angles for D and A are small ($\chi_1 = 15°$, $\chi_2 = 25°$ in D; $\chi_1 = 20°$, $\chi_2 = 8°$ in A).[13−15]

The absorption and fluorescence spectra of **1** and **2** in $CH_3CN$ are indistinguishable from one another and from the sum of the spectra of **3** and **4**. The fluorescence quantum yield of **2** was twice that of **1** ($\Phi_{f1} = 0.03$, $\Phi_{f2} = 0.06$, in $CH_3CN$, referenced to pyrene in ethanol[16] ), indicating a more efficient quenching in **1**.[17] In both compounds, the fluorescence lifetime was much shorter than that in the reference peptide **4** (~300 ns). The decay profile of **4** was monoexponential, whereas **1** and **2** had biexponential decay profiles (Table 1). The biexponential decays of **1** and **2** are likely due to the existence of two (or more) ground-state conformers, possibly differing by torsional angle (vide supra), on the time scale of the experiment.[18] In both **1** and **2**, the statistical weighting of the two components varied, although weakly, with solvent or temperature (Table 1). The variations probably reflect small torsional conformational changes. As anticipated, the ratio of $k_{et1}$ to $k_{et2}$ appears smaller in solvents with higher dielectric constants, where the difference in energies between the two charge-separated states $D^{\bullet+}A^{\bullet-}$ is reduced. However, the interpretation of the solvent effect is not straightforward, as the conformations of the oligopeptides, the magnitude of the electric field, and the electron transfer rates are all affected by solvation.

Peptides **1** and **2** are unsoluble in very low polarity solvents, such as hexane and cyclohexane, and methylene chloride is the lowest polarity solvent ($\epsilon = 9$) that could be employed. The dielectric constant ($\epsilon$) experienced at the interface between the solvated helical peptide and the bulk solvent may be substantially lower than that of the bulk solvent.[4b] As a consequence, the experimentally measured helix electric field can be up to 1 order of magnitude stronger than that expected on the basis of $\epsilon$ of the solvent. This may explain the differences observed in polar solvents, such as acetonitrile.[4b,19]

The electron transfer rates in **1** and **2** differ in all solvents and temperatures examined (Table 1). In all measurements, the decay times of **1** were shorter than those of **2**, and rate constants for photoinduced electron transfer in **1** were 5−27 times larger than in **2**, depending on the solvent. This observation is consistent with the electric field effect. In **1**, where the $D^{\bullet+}A^{\bullet-}$ state is in the low-energy orientation with respect to the helix-induced electric field, charge separation has a more negative $\Delta G$ and takes places with a higher rate. Higher rates should be expected for the process with the more negative $\Delta G$, since the pyrene−dimethylaniline couple ($\Delta G \cong -0.4$ eV) lies in the normal exothermic region,[13c,20] as confirmed by the variable temperature measurements in THF (the observed rates decrease with decreasing temperature, Table 1). With the data currently available, we cannot unambiguously exclude the possibility that part of the observed difference in the lifetimes of **1** and **2** could be a conformational effect. Although, in principle, **1** and **2** could experience slightly different local environments because of differential twisting upon switching the position of the chromophores, both CD and NMR data suggest that the two molecules sample very similar average conformations. Moreover, even if the biexponential decays reflect the presence of slightly different conformational preferences, both components of the decay of **1** are faster than **2** in all solvents examined.

The field-induced difference between the driving forces for intramolecular electron transfer in **1** and **2** was also observed by differential pulse voltammetry of **1** and **2** in acetonitrile. The $\Delta G_{el}$ of **1** is 100 mV more negative than that of **2**.[21] An estimate of $\Delta G°$ can be made as follows. In a field of $10^9$ V/m in a vacuum, two charge-separated species (10 Å apart) experience an electric field of 1 V. In acetonitrile ($\epsilon = 37$), the resulting field will be $1/37 = 0.027$ V. The energy gap ($\Delta G°$) between the two states (with and against the field) is then 0.054 eV. This value is remarkably close to the experimentally determined value of 0.100 V, especially considering that the effective dielectric constant may be lower (vide supra).

Our observations suggest that the orientation of a donor−acceptor pair with respect to the molecular electric field generated by the helix dipole of a peptide affects the electron transfer rates in the expected manner. Future work to provide further information about conformational effects and about the influence of the dipole on charge recombination is under way.

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**Supporting Information Available:** Experimental procedure for the synthesis and characterization and spectral data of compounds **1**−**4**, including COSY, NOESY, ROESY spectra of **1** and **2**; CD spectra and differential pulse voltammetry of **1** and **2**; and fluorescence decay profiles and Arrhenius plot of low-temperature fluorescence decays of **1** and **2** (39 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(18) Short (5−15 amino acids) peptides can adopt rapidly interconverting conformations in solution, and biexponential decays were observed in some of the studies of electron transfer in peptides (refs 13a,b, 14b).

(19) Although this effect has been observed in oligopeptides ($n = 21-40$), we have not tested the presence of a boundary effect in **1** and **2**, for instance with a solvatochromic dye molecule tethered to the peptides in the same positions as the donor or acceptor.

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(21) It was not possible to obtain precise values for the reduction potential of pyrene ($E^{red}_{pyr} = -2.10$ V, ref 19b) because of a small amount of adsorbed water in the samples. Since oxidation potentials are shifted by the same amount as the reduction potentials, we compared the difference between the oxidation potentials in **1** with the difference between the oxidation potentials in **2**: $E^{ox}_{pyr1} - E^{ox}_{dma1} = 0.55$ V; $E^{ox}_{pyr2} - E^{ox}_{dma2} = 0.43$ V.