Intramolecular Excited State Electronic Coupling Along an α-Helical Peptide

Traci L. Batchelder, Robert J. Fox, III, Mark S. Meier, and Marye Anne Fox*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712

Received January 23, 1996[⊗]

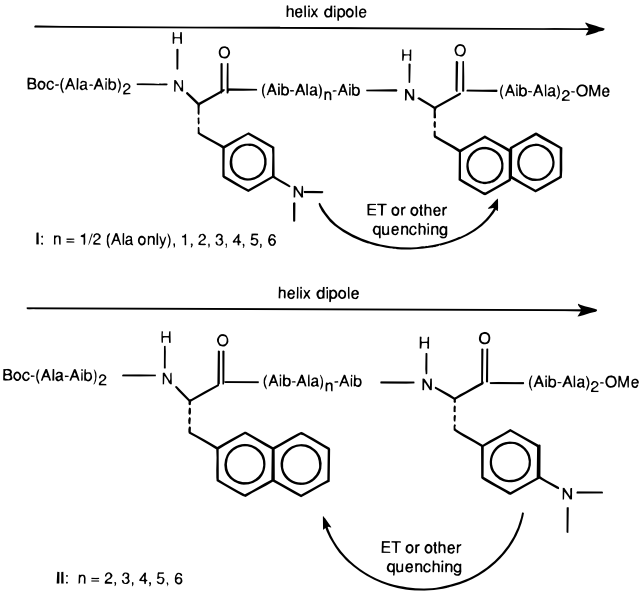
Several families of peptides composed of alternating L-alanine (Ala) and α-aminoisobutyric acid (Aib) residues with an appended *N*,*N*-dimethylanilino and/or 2-naphthalenyl group exist in MeOH and $CDCl_3$ as α-helices. Steady state and time-resolved fluorescence measurements show that the distance and dihedral angle between the appended donor and acceptor and the alignment of the vectors for intramolecular charge transfer interaction (from donor to acceptor) with or against that of the helical dipole moment significantly influence the efficiency of photoinduced electronic coupling and, hence, of intramolecular fluorescence quenching.

Introduction

There has been considerable interest over several decades in devising means to achieve efficient photoinduced charge-separation over distances that are large on a molecular scale because of the potential utility of these instantaneously generated long-lived ion pairs in lithography, photoimaging, and molecular electronic devices. In addition, polymers and supramolecular arrays incorporating photoresponsive electron donors and/or acceptors can provide a simple model for those features that control the key electron transfer events in photosynthesis.

Several different modes can operate to provide the electronic coupling necessary for efficient electron transfer or excited state quenching. These include through-bond (both covalent and hydrogen bonds) and through-space/through-solvent pathways.[1−9] Many small peptides functionalized by a variety of organic and inorganic donors and acceptors participate in electron transfer by pathways involving strong electron coupling through covalent bonds.[1−12] In such compounds, the observed rates of electron transfer decrease exponentially with increased separation between the donor/acceptor pair. From studies of electron transfer in natural peptides, order-of-magnitude electronic couplings have been assigned for interactions taking place through covalent or hydrogen bonds and through space (i.e., by noncovalent, solvent-mediated interactions).[9,12] Such estimates suggest that the integrated importance of multiple hydrogen bond pathways or through-solvent interactions can be comparable to that derived from covalent bond pathways.

In addition, the dipole moment of a peptide is known to have a magnitude of about 3.5 Debye per helical turn (1.5 Å) and can be approximated as an electric field of about 10^9 V/m near the termini.[13] Accordingly, we have recently shown that the alignment of a donor−acceptor pair with or against the macroscopic dipole moment induced by an α-helical backbone might significantly influence observable electron transfer rates in such peptides.[14]



To further investigate the dynamics of intramolecular electronic coupling that can influence both the efficiency of electron transfer and other excited state quenching processes, two families **I** and **II** of alternating Ala-Aib peptides bearing an appended donor and acceptor have been synthesized. Three questions have been addressed: (1) the effect of the appended photoactive units on the secondary structure of the peptide; (2) the effect of the distance and relative orientation (as measured by

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1996.

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S0022-3263(96)00149-1 CCC: $12.00

dihedral angle) between the donor and acceptor on observed emission; and (3) whether the peptide's net dipole moment can influence the efficiency of photoinduced intramolecular electronic coupling.

To address such questions, a rigid, conformationally inflexible backbone is required. A helical peptide is a particularly apt backbone because its rigid structure, attained through a network of intramolecular hydrogen bonds, allows for controlled positioning of a desired donor or acceptor at one or more fixed sites along the chain. Helix formation in short peptides is enhanced by organic solvents, e.g., MeOH,[15,16] and by using L-alanine (Ala) and α-aminoisobutyric acid (Aib) as component amino acids.[17−25] Furthermore, the inclusion of Aib units greatly enhances the solubility of the functionalized peptide from that attained with the analogous polyalanine.

N,*N*-Dimethylanilino (DMA) and 2-naphthalenyl (Np) groups were chosen, respectively, as potential pendent electron donor and acceptor because of their relative redox potentials.[26] From the measured redox potentials of Np-Ala and DMA Ala and the singlet energy of Np-Ala, we estimate electron transfer from DMA to the fluorescent singlet state of Np to be exothermic by about 0.7 V. Series **I** peptides have the DMA/Np pair oriented so that the direction of the photoinduced electron transfer vector (from DMA to Np) corresponds with that of the dipole moment (from the N-terminus to the C-terminus in an α-helix of L-amino acids). Individual members of the series differ only in the number of amino acid residues that separate the donor from the acceptor. Each donor−acceptor peptide sequence is capped by a four amino acid residue to ensure that the peptide resists unwinding and remains helical within the region of the observed photoinduced electron transfer.[26] In Series **II** peptides, the sequence of the donor and acceptor along the peptide is reversed, while maintaining the same separation between the groups and the same amino acid sequence. A third control group of peptides, Series **III**, consists of a peptide bearing neither an appended donor nor acceptor, **IIIa**, an analogous peptide with only the appended electron donor, **IIIb**, and one with only the appended electron acceptor, **IIIc**.

Boc-(Ala-Aib)$_2$(Ala)(Aib)(AlaAib)$_4$AlaOMe

IIIa

Boc-(AlaAib)$_2$(DMA-Ala)(Aib)(AlaAib)$_4$-AlaOMe

IIIb

Boc(Aib)(AlaAib)$_5$(NpAla)(AibAla)$_2$OMe

IIIc

Results and Discussion

A. Conformational Analysis. Peptide secondary structures typically fall into one or a combination of three general categories: helices, β sheets, or random coils. Circular dichroism (CD) is frequently used to distinguish these structures, with a helix typically exhibiting a maximum at 190 nm and two minima at 206 and 220 nm.[15,27−29] When measured as 2 mM solutions in MeOH or $CDCl_3$, all peptides described here show such CD characteristics, Figure 1. The CD spectra of families **I** and **II** also exhibit fine structure assigned to the DMA and Np moieties, as shown for the series **I** peptides shown in Figure 2.

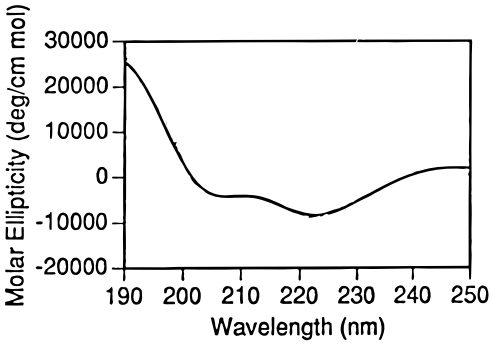

Figure 1. Circular dichroism spectrum of **IIIa** (2×10^{-5} M in MeOH).

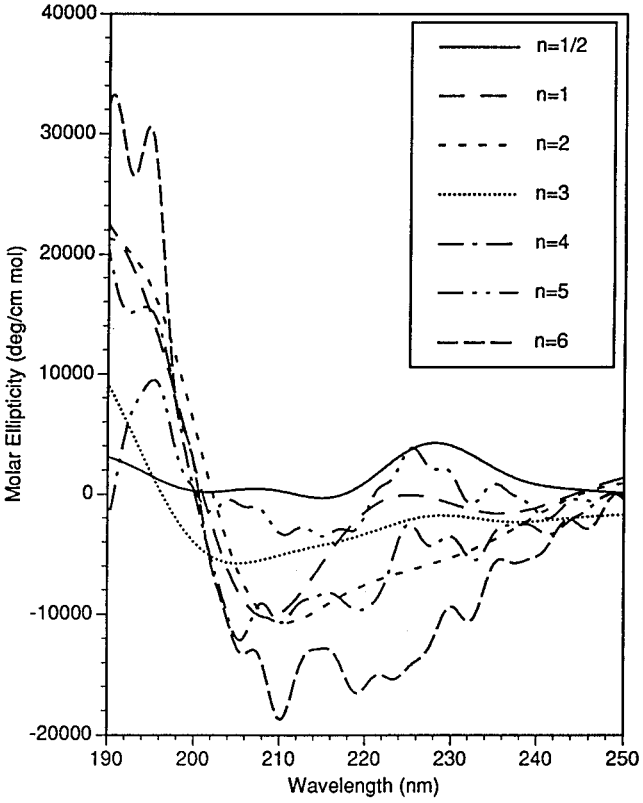

Figure 2. Circular dichroism spectra of **I** (2×10^{-5} M in MeOH).

CD alone cannot unambiguously distinguish between α and 3_{10} helices. However, because an α helix has the

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Table 1. DMSO Perturbation of the ^{1}H NMR Spectrum of IIIa

N−H resonances[a]	δ ($CDCl_3$)[b]	δ (80 $CDCl_3$: 20 DMSO)[b,c]	$\Delta\delta$ (ppm)
d_1	7.36	7.08	0.28
s_1	7.40	7.10	0.30
s_2	7.45	7.15	0.30
s_3	7.49	7.13	0.36
s_4	7.41	7.11	0.30
d_2	7.54	7.19	0.35
s_5	7.51	7.27	0.24
d_3	7.63	7.27	0.36
d_4	7.60	7.29	0.31
d_5	7.64	7.34	0.30
d_6	7.66	7.35	0.31
s_6	7.66	7.41	0.27
d_7	7.70	7.45	0.25
d_8	7.81	7.50	0.31
s_7	7.74	7.51	0.23

[a] Unassigned N−H resonances listed in order of increasing downfield chemical shift; d = doublet; s = singlet. [b] In δ units from tetramethylsilane internal standard. [c] Composition by volume.

Table 2. DMSO Perturbation of the ^{1}H NMR Spectrum of Ia

N−H resonances[a]	95 $CDCl_3$: 5 DMSO[b−d]	83 $CDCl_3$: 17 DMSO[b,c]	$\Delta\delta$ (ppm)
s_1	5.99	5.80	0.19
d_1	6.36	6.44	−0.08
d_2	7.03	6.90	0.13
d_3	7.44	7.19	0.25
d_4	7.50	7.30	0.20
d_5	7.63	7.33	0.30
s_2	7.58	7.36	0.22
s_3	7.54	7.46	0.08
s_4	7.66	7.46	0.20
s_5	7.73	7.47	0.26
d_6	7.65	7.52	0.13
s_6	7.70	7.60	0.10
d_7	7.79	7.67	0.12
d_8	7.83	7.68	0.15
s_7	7.96	7.81	0.15

[a] Unassigned N−H resonances listed in order of increasing downfield chemical shift; d = doublet; s = singlet. [b] In δ units from tetramethylsilane internal standard. [c] Composition by volume.[d] In neat $CDCl_3$, the resonances were too broad for easy resolution.

carbonyl oxygen of the first amino acid residue hydrogen bonded to the amide proton of the fifth amino acid residue, whereas a 3_{10} helix has the carbonyl oxygen of the first amino acid residue hydrogen bonded to the amide proton of the fourth amino acid residue, these structures can be distinguished by NMR spectroscopy. The number of amide protons not involved in intramolecular hydrogen bonding also differs in the two types of helices: in a 3_{10} helix, the number of "free" amide protons is two, whereas in the α helix there are three. Because the amide protons not involved in intramolecular hydrogen bonds are more solvent exposed, quantitative measurement of the perturbation of the ^{1}H NMR spectrum induced by adding aliquots of DMSO-d_6 to a chloroform solution of these peptides can distinguish these helical types.[21] The changes in chemical shifts of the resonances of solvent-exposed protons are greater than those participating in intramolecular hydrogen bonds. Solvent perturbation of the NMR spectra of these peptides reveals that, in each case studied, three resonances are shifted significantly (≥0.25 ppm) which indicates α helicity, Tables 1 and 2. Two-dimensional NMR spectroscopy also shows three NH resonances to be free from hydrogen bonding in each of these peptides.

Table 3. Fluorescence Lifetimes and Geometries for Series I Peptides[a]

peptide I, *n*	τ (ns ± 3 ns)	r_{DA} (Å)[b]	D/A dihedral angle,[b] deg
1/2	24	6	40
1	53	8	140
2	57	11	20
3	60	14	180
4	63	17	20
5	68	20	140
6	80	23	60

[a] As 10^{-5} M solutions in methanol. [b] Calculated by molecular mechanics as the separation and projected dihedral angle between appended CH_2Ar groups fixed along an α helix with alternating Ala and Aib units.

Table 4. Fluorescence Lifetimes and Geometries for Series II Peptides[a]

peptide II, *n*	τ (ns ± 3 ns)	r_{DA} (Å)	D/A dihedral angle,[b] deg
2	28	11	20
3	44	14	180
4	44	17	20
5	59	20	140
6	65	23	60

[a] As 10^{-5} M solutions in methanol. [b] Calculated by molecular mechanics as the separation and projected dihedral angle between appended CH_2Ar groups fixed along an α helix with alternating Ala and Aib units.

This result initially appears to contradict previous reports in which closely related peptides have been found to be 3_{10} helical.[23,25,30] In short peptides, the Aib residue typically induces 3_{10} helicity because of the more favorable backbone torsional angles in the 3_{10} arrangement, but as the chain length of the peptide increases, the molar fraction of Aib in the peptide must be greater than about 60% to continue this effect.[19,31] Since the Aib content in these peptides is about 50%, α helicity is observed in Series **I**−**III**, unlike previously described peptides that were richer in Aib. In an α helix, the edge-to-edge distance between the donor and the acceptor in each peptide and the relative orientation of the donor and acceptor (dihedral angle along the helical axis) can be calculated, Tables 3 and 4.

As has been observed in the solid state,[32] these results show that incorporation of photoactive side chains onto component amino acids does not appreciably alter the propensity for helix formation from that observed in unsubstituted natural amino acids. Furthermore, hydrophobic side chains, in general, are known to promote α helicity,[33] as has also been shown specifically for synthetic peptides bearing pyrenyl groups.[27,28] Thus, the naphthalenyl and dimethylanilino groups employed here follow the same general trends toward enhancing α-helicity that have been reported for other aryl substituents.

B. Efficiency of Photoinduced Electronic Coupling. The efficiency of the photoinduced electron transfer or intramolecular electronic coupling between a donor and an excited acceptor placed strategically along these helical peptides can be probed by comparing the relative fluorescence quantum yields of the peptides substituted

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with both the DMA donor and the fluorescent Np acceptor with that of the corresponding peptide substituted with Np alone. In Series **I** and **II**, the dimethylanilino group and the 2-naphthalenyl group have partially overlapping absorbance and emission spectra (see Supporting Information). This spectral overlap makes strictly quantitative interpretation of fluoresence yields difficult.[34] Even so, the presence of the DMA donor in **I** and **II** caused a significant reduction of the observed quantum yield of fluorescence from that observed in **IIIc** (about 0.26 ± 0.02), with fluorescence yields in Series **I** being about 50% higher than those observed in the analogous members of Series **II**.

Although these systems were designed to permit exothermic electron transfer on the excited state surface (*vide supra*), the spectral overlap problem also makes it impossible to unambiguously assign the observed fluorescence quenching to a discrete electron transfer event. Whether this quenching is indeed electron transfer or is instead a manifestation of another intramolecular quenching event deriving from the altered electronic coupling afforded by these structural changes must therefore be considered an open question. In either case, the influence of the geometric changes on the magnitude of excited state intramolecular coupling is certain.

For greater quantitative precision, the rates of intramolecular quenching of the singlet excited state of the appended Np group by the DMA group were examined by single photon counting. By employing this method, the contribution of the appended DMA to the observed fluoresence can be resolved qualitatively and analyzed separately from that of the overlapping naphthalenyl group because the fluorescence lifetime of the appended DMA is much shorter than that of an appended Np group.

If only through-bond coupling were operative, increasing separations between donor and acceptor would be expected to result in lower rates of electron transfer in this series. From the measured fluorescence lifetimes (qualitatively corrected for overlapping absorption), the rates of electron transfer are then estimated from:

$$k_{et} = 1/\tau_1 - 1/\tau_0$$

where τ_1 is the lifetime of the donor- and acceptor-substituted peptide (**I** or **II**), and τ_0 is the lifetime of **IIIc**, the model peptide. In Tables 3 and 4 are listed the corrected fluorescence lifetimes thus obtained. These results show only a weak distance dependence for intramolecular quenching, as is consistent with previous reports of weak electron coupling in nonhelical polyprolines bearing inorganic donors and acceptors.[35−37] Such weak distance dependence is fully consistent with strong electronic coupling through the peptide, or even with a characterization of the backbone as a "peptide wire," a term introduced by Klapper and co-workers to describe the strong electron coupling observed in peptides containing tyrosine and tryptophan.[38]

Any contributions of through-space coupling would be maximal when the donor−acceptor pair are aligned along the helix, i.e., when the dihedral angle between the attached groups along the helical axis is minimized. Thus, our observation of faster rates than predicted from a through-bond coupling mechanism when $\Theta < 60°$ and of somewhat slower-than-predicted rates in peptides when $\Theta > 120°$ implicate possible through-space coupling in addition to efficient through-bond coupling. In addition, the effect is maximized when the redox sites are closer together ($n < 4$), which further supports the argument that through-space coupling in fact influences the observed excited state intramolecular electronic coupling.

The effect of the helix dipole on intramolecular fluorescence quenching efficiency can also be seen from this data. Thus, modestly enhanced fluorescence quenching in the Series **II** peptides (in which the direction of electron transfer (or intramolecular charge-transfer contributions) is antiparallel to the helix dipole and, hence, electrostatically favored) is observed in comparison with that seen in Series **I** (in which these same vectors are parallel and, hence, electrostatically disfavored). A clearer illustration of the effect of the α-helical dipole moment on the observed intramolecular electron transfer rate has also been observed in similar pyrenyl-substituted peptides in which the problematic spectral overlap between the appended donor and acceptor are minimized.[14]

In conclusion, alternating Ala/Aib peptides bearing pendent aryl groups capable of acting as excited state electron donors and acceptors exist in organic solvents primarily as α-helices. Photoinduced intramolecular electronic coupling is not solely governed by the number of intervening covalent bonds that separate donor and acceptor. Rather, the relative orientation of the interacting aryl groups and the alignment of the helical dipole moment with or against a redox gradient also affect the observed efficiency of the observed fluorescence quenching.

Acknowledgment. This work was supported by the Office of Basic Energy Sciences, U.S. Department of Energy. We gratefully acknowledge the assistance of Dr. Donald O'Connor of the Center for Fast Kinetics Research in the execution of the single photon counting experiments.

Supporting Information Available: Syntheses and full spectroscopic characterization of all peptides **I**−**III** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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