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Long-Range Electronic Interactions in Peptides: The Remote Heavy Atom Effect

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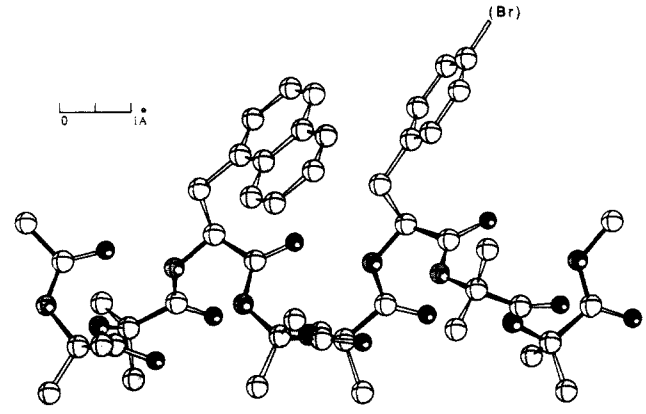
Introduction. A proper understanding of electronic interactions between nonconjugated molecular components depends heavily upon the ability to design and build molecular structures that are rigid and whose intercomponent geometry is well defined. The intramolecular heavy atom effect,[1] a variant of the extensively studied intermolecular heavy atom effect ($S_1 \rightarrow T_1$),[2] enables a direct study of the dependence of remote electronic interactions upon molecular structure, but has only been studied at a separation of three σ bonds or less. Polypeptides, a basic architectural unit in nature, have been exploited in the past to study singlet excitation transfer,[3] electron transfer,[4] and excitonic interactions.[5] We chose to focus on rigid helical oligopeptides which fold to bring two electronic partners positioned one turn apart into close proximity even though the through-bond separation may remain large. This offers the opportunity to explore mechanisms of electronic interactions such as the question[6] of covalent (through-bond) vs noncovalent (through-space) mechanisms. A further motivation is that the long-range spin-exchange interactions responsible for the remote heavy atom effect may be intimately connected to and shed light upon mechanisms of long-range electronic tunneling.[7] We report here bromine-induced enhanced intersystem crossing in the naphthalene chromophore at 13 σ bond separation in α-aminoisobutyric acid (Aib) rich oligopeptides containing β-(1′-naphthyl)-L-alanine (Nap) and *p*-bromo-L-phenylalanine (Bph).

The Ground State. In the present study, two octapeptides and two dipeptides were synthesized.[8] The dipeptides, H-Nap-Bph-OMe (bromo dimer, Br-Dim) and H-Nap-Phe-OMe (control dimer, C-Dim), contained no Aib residues and thus had no conformational bias. The control and the bromo octamers (C-Oct and Br-Oct, respectively) contained six host Aib residues to induce a strong helical bias[9] and two guest aromatic residues (see Figure 1). The guest aromatic residues in both octamers were installed



Ac-Aib-Aib-Nap-Aib-Aib-Phe-Aib-Aib-NHMe

Figure 1. The Aib-rich control octamer (C-Oct) displayed in a 3_{10} helical conformation ($i \leftarrow i + 3$ hydrogen-bonding pattern). This structure is consistent with ^{1}H NMR data and exploratory energy minimization results[17] and is similar to that determined by Toniolo and co-workers for the *p*-BrBz-$(Aib)_8$-O-*t*-Bu octamer.[12] The pitch of the 3_{10} helix is close to 6 Å (5.85 Å in the X-ray structure of Toniolo's octamer). The Br represents the location of the bromine in Br-Oct, in which Bph replaces Phe. The dark atoms are carbonyl oxygens.

Table I. Steady-State and Time-Resolved Fluorescence Data[a,b]

	relative fluorescence quantum yields[c]	
solvent	Br-Oct/C-Oct	Br-Dim/C-Dim[d]
CH_3OH	0.36	0.79
CH_3CN	0.32	0.82
CH_3CN/THF (1:1)	0.34	
THF	0.28	
THF/isooctane (1:1)	0.28	

	lifetime: τ_F, ns	
solvent	Br-Oct[e,f]	C-Oct
CH_3OH	28.4 ± 0.6	60.6 ± 0.6
CH_3CN	26.6 ± 0.6	54.4 ± 2.7

[a] All samples were degassed by freeze–pump–thaw. λ_{ex} = 290 nm. [b] The concentrations (~10 μM) were far below that required to initiate self-aggregation (0.6 mM) in similar octapeptides.[10a] The fluorescence quenching in the octamer was also measured to be concentration independent from 5 μM to 20 μM. [c] The relative fluorescence yields reported are all ±0.01. [d] The solvents for the dimers contained 0.1% TFA to ensure the exclusive presence of the protonated amine. [e] Br-Oct in both solvents exhibited biexponential decay; the longer (and the major, >75%) component is given in the table.[21] The biexponential decay may be due to the presence of two conformers, which may differ in the side-chain torsional angles or have different backbone conformations. [f] In acetonitrile, the remote heavy atom effect enhances the intersystem-crossing rate constant by an additional $(26.6\ ns)^{-1} - (54.4\ ns)^{-1} = 2.0 \times 10^7\ s^{-1}$.

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(8) Solution-phase peptide synthesis techniques[14] were employed. Aib dimers were made by azirine[15] or oxazolone[12a] chemistry, and three such dimer blocks were coupled with the two appropriate guest amino acids by oxazolone or acid chloride[16] methods to produce the target octamers. Full synthetic details will be presented elsewhere.

(9) Short oligopeptides rich in α-aminoisobutyric acid (Aib) have been selected for the present work due to their proven ability[10] to form 3_{10} helices in solution[11] and crystalline[12] phases arising from the steric constraints of the *gem*-dimethyl groups of Aib.[13]

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within the sequence at positions 3 (Nap) and 6 (Bph in Br-Oct or Phe in C-Oct). The oligopeptides were characterized by ^{1}H NMR and FAB mass spectroscopy and purified by reverse-phase HPLC on a C_{18} column prior to all optical studies. ^{1}H NMR studies of the amide proton resonances of C-Oct showed a distinctive backbone hydrogen bonding pattern indicative of a 3_{10} helical conformation[18] in agreement with extensive similar studies of both pure Aib oligomers[11a] and Aib-rich polypeptides.[11c] Furthermore, circular dichroism spectra[19] of C-Oct measured in both methanol and acetonitrile indicated the presence of substantial helical secondary structure.[20]

Fluorescence. Both steady-state and time-resolved[21] fluorescence data have been obtained for C-Oct (no bromine) and Br-Oct (Table I). The fluorescence quantum yield of Br-Oct is observed to be a factor of 3 less than that of C-Oct in five solvents. The time-resolved data also unambiguously demonstrate fluorescence quenching by the remote bromine atom. It is especially interesting to compare the fluorescence quenching in the two brominated species, Br-Oct and Br-Dim. Remote heavy atom quenching is more than twice as effective in Br-Oct than in Br-Dim, even though the bromophenyl group is separated from the naphthalene by 13 σ bonds in Br-Oct and only seven σ bonds in Br-Dim (adjacent residues).[22] The addition of σ bonds would be expected to lower through-bond interactions. Despite the addition of six σ bonds, and the subsequent decrease in through-bond interactions, the octamer exhibits greater quenching than the dimer, demonstrating that noncovalent interactions exceed through-bond interactions in the helical octamer.[23]

Mechanism of Fluorescence Quenching. Intramolecular singlet exciplex formation can be ruled out as a mechanism of the quenching since the emission spectra of Br-Oct and C-Oct are identical in shape and the characteristic long-wavelength exciplex emission is absent.[24] Singlet–singlet energy transfer is also ruled out due to the lack of any absorption by bromobenzene at 290 nm or longer. The fluorescence yield of Br-Oct also exhibits a pronounced insensitivity to solvent dielectric constant (Table I), which is evidence against the possible involvement of a fast charge-transfer process in the mechanism of accelerated singlet-state decay.[25]

Nanosecond transient absorption spectroscopy of Br-Oct and C-Oct in acetonitrile demonstrated the formation of the Nap triplet state in both peptides. In addition, the transient spectrum of Br-Oct did not exhibit any absorption due to a Nap radical cation. The triplet yield of Br-Oct relative to C-Oct (Φ_T'/Φ_T) was measured to be (93 ± 3)%. This falls in between two simple limits: (i) Φ_T'/Φ_T = 32% (identical with the relative fluorescence yield, Table I) for a quenching mechanism that yields no additional triplets and (ii) $\Phi_T'/\Phi_T > 100\%$ for a mechanism that is exclusively enhanced intersystem crossing to the triplet. The triplet yield and biexponential time-resolved fluorescence data[21] taken together lead to a model with minor and major kinetic pathways. The Nap singlets of the minor components deactivate very quickly and not through accelerated intersystem crossing, whereas the quenching of the Nap singlets of the dominant component must be due to bromine-induced enhanced intersystem crossing ($S_1 \rightarrow T_1$). Triplet exciplex formation could be the minor pathway.[21] The dominant pathway is assigned to remote heavy atom induced intersystem crossing with a rate constant of 2.0×10^7 s^{-1} (see Table I).

Summary. Fluorescence studies on the bromine-containing peptides we have designed enable a new method for studying long-range electronic interactions in peptides. The results demonstrate that a remote heavy atom can effectively enhance the intersystem crossing in the naphthalene probe chromophore even when separated by 13 σ bonds and that it is possible for noncovalent interactions to play a dominant role in exchange interactions between aromatic residues within helical peptides. We are also currently engaged in parallel intramolecular electron transfer studies, based on the same helical Aib strategy, to further explore the electronic control of nonadiabatic reaction processes within molecular architectures.

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Supplementary Material Available: Detailed description and kinetic analysis of transient absorption and emission studies on C-Oct and Br-Oct (7 pages). Ordering information is given on any current masthead page.

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(17) The conformation in the figure is one of 10 locally minimized structures observed in preliminary energy minimizations performed with potentials kindly provided by Dr. M. Dudek in the laboratory of Dr. H. A. Scheraga.

(18) Solvent perturbation studies (DMSO-d_6 added to CD_3CN) on C-Oct unambiguously demonstrated seven intramolecular H bonded amide protons (out of nine) characteristic of a 3_{10} H-bonding pattern. For related NMR studies, see ref 10a.

(19) For CD studies of polypeptides containing Nap, see: Sisido, M.; Imanishi, Y. *Macromolecules* **1986**, *19*, 2187.

(20) CD measurements were performed on an AVIV (Model 60DS) machine in the laboratory of Dr. B. A. Wallace: 50 μM samples of C-Oct in CH_3OH and CH_3CN exhibited an intense band at 225 nm ($\sim -3.0 \times 10^4$ deg cm^2 dmol^{-1} of mean residue ellipticity) which indicates a high degree of helicity.

(21) See supplementary material for details.

(22) Extended conformations of the dimer are most probable, as confirmed by 10 minimized conformations obtained from CHARMm and the lack of bromo dimer exciplex emission.

(23) The interaction tunneling matrix element $\Delta = |TS + TB|$ is the result of through-bond (TB) interactions and through-space (TS) interactions, by which is meant here all nonbonded mechanisms of interaction. It is important to appreciate that both mechanisms may be simultaneously operative. From the data on the octamers and dimers we can conclude that $\Delta_8 > \Delta_2$; so $|TS8 + TB8| > |TS2 + TB2|$. Now if we assume that $|TB2| \gg |TS2|$ and define n such that $n|TB8| = |TB2|$, we have $|TS8 + TB8| > |TB2|$, from which it follows that $|TS8| > |TB2| - |TB8|$ and hence $|TS8| > (n-1)|TB8|$, where n may be estimated purely by a through-bond model.

(24) A dramatic long-wavelength singlet exciplex emission was readily observed in solutions of bromobenzene and α-methylnaphthalene.

(25) For an example and discussion of a solvent insensitive *intermolecular* heavy atom effect, see ref 2b.

Oxygen Insertion in the Ni(II) Complexes of Dioxopentaaza Macrocyclic Ligands

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This communication reports a new type of model monooxygenase-type reaction involving activation of dioxygen through metal–dioxygen complex intermediates. Analysis of the ligand degradation products formed from the dioxygen adducts of the Ni(II) complexes of the macrocyclic ligands 1,4,7,10,13-pentaazacyclohexadecane-14,16-dione (**1**), 15-ethyl-1,4,7,10,13-pentaazacyclohexadecane-14,16-dione (**2**), and 15-benzyl-1,4,7,10,13-pentaazacyclohexadecane-14,16-dione (**3**) shows conversion of these ligands in good yield (85 ± 5%) to the corresponding 15-hydroxylated derivatives, **4–6**. This is the first example of the hydroxylation of an aliphatic ligand by a metal dioxygen complex, since all previous examples involve oxygen insertion into aromatic rings. These include activation of dioxygen in tyrosinase model systems reported by Karlin et al.[1] and by others,[2-4] involving the formation of binuclear dioxygen adducts

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