Communication

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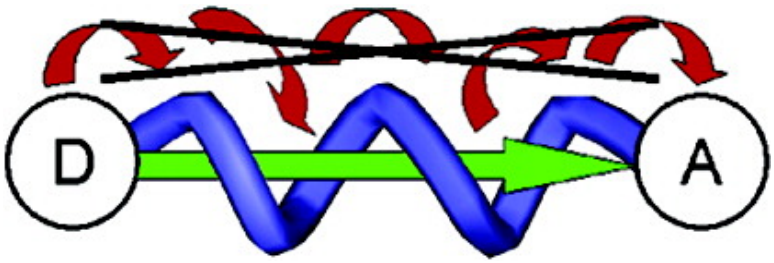

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Evidence Against the Hopping Mechanism as an Important Electron Transfer Pathway for Conformationally Constrained Oligopeptides

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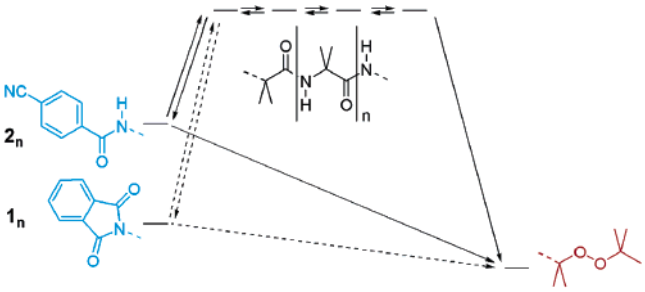
An electron transfer (ET) between a donor (D) and an acceptor (A) through a molecular bridge usually occurs by the superexchange mechanism where electron tunneling occurs without transient occupation of the states of the bridge.[1] The ET may also occur by an incoherent hopping mechanism where the bridge is more directly involved in the process.[2] This mechanism requires a thermally activated endergonic charge injection from D to the directly connected bridge unit. Afterward, the electron migrates through a set of coupled units of the bridge and then an exergonic ET to A occurs.[3] For short bridges, the hopping mechanism is less efficient than the superexchange pathway, but for longer bridges the latter may be overwhelmed by intrabridge electron hopping. The transition is detected by observing the onset of a weak distance dependence of the ET rate.[2,4,5] This view received considerable consensus for charge transfer across DNA strands.[2,4] Despite some intriguing results,[5] however, the issue of its applicability to peptides (and proteins), as proposed in recent studies,[2,6] is not yet settled. This communication is aimed at providing insight into this important issue.

We have recently[7] studied the electrochemically induced ET from a phthalimide radical anion donor (formal potential, $E° \sim -1.35$ V)[8] to a peroxide acceptor ($E° = -1.21$ V) across α-aminoisobutyric acid (Aib) homooligomers of different lengths (Scheme 1: $\mathbf{1}_n$). Aib oligopeptides are known to form 3_{10}-helices[9] and thus, for $\mathbf{1}_n$, n represents the number of the possible intramolecular C═O···H−N hydrogen bonds. The intramolecular ET rate was found to depend weakly on the number of Aib units and the related edge-to-edge D/A distance (d_{ee}); in fact, it was even found to increase in a certain peptide length range. Although an important bridge-length-dependent ET-mediating role of the intramolecular hydrogen bonds was proposed as being responsible for such unprecedented behavior,[7] a hopping mechanism also could be invoked. To address this problem, we designed another series of compounds, $\mathbf{2}_n$, aimed at assessing the feasibility of the latter mechanism across peptides.

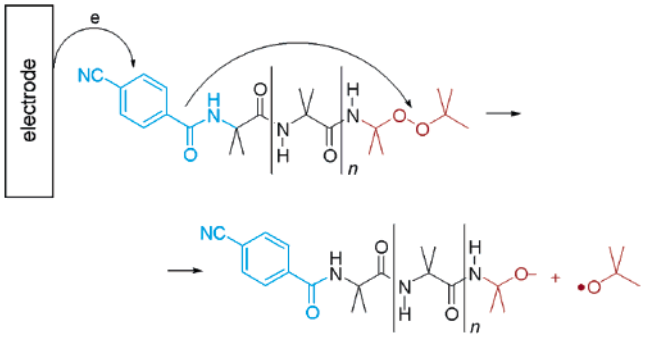
Compounds $\mathbf{2}_n$ differ from $\mathbf{1}_n$ only by the donor that is now the *p*-cyanobenzamide moiety ($E° \sim -1.70$ V).[8] This makes the ET free energy ($\Delta G°$) more negative by 0.35 eV. A more powerful donor was expected to cause two effects (Scheme 1): to increase the rate of the superexchange ET and, particularly important, to diminish the energy gap between the donor and the bridge, thereby favoring the hopping mechanism relative to compounds $\mathbf{1}_n$. Compounds $\mathbf{2}_n$ were synthesized and characterized along similar lines as reported for $\mathbf{1}_n$.[10] In keeping with the well-established propensity of Aib oligopeptides to form rigid 3_{10}-helices,[9] IR absorption and ^{1}H NMR spectral data revealed that the onset of a regular secondary structure in solution is already evident in the shortest peptides, resulting in conformers whose rigidity increases with *n*.

The ET between D and A was triggered electrochemically, as illustrated in Scheme 2. The peroxide group is a dissociative-type

Scheme 1



Scheme 2



acceptor, and thus the electron is trapped concertedly with O−O bond cleavage. While the reduction of peroxides has a large intrinsic barrier and thus is very slow,[11] ET to moieties such as the *p*-cyanobenzamide or phthalimide group undergoes fast electrode kinetics. Therefore, despite the unfavorable thermodynamics, the electrode process entails electron injection into the fast end of $\mathbf{2}_n$, D, followed by slow and irreversible intramolecular ET from D to the slow end of $\mathbf{2}_n$, A.

The intramolecular rate constant (k_{intra}) values were determined by cyclic voltammetry (CV) in *N*,*N*-dimethylformamide (DMF). By carrying out concentration studies and digital simulation of the CV curves obtained with peptides $\mathbf{2}_n$ and corresponding model molecules, the effect of the competitive intermolecular ET could be determined. Figure 1 shows the observed k_{intra} values, in comparison with the corresponding data of the $\mathbf{1}_n$ series.[7]

There are a number of features worth noting in Figure 1. In both cases, the rate is slightly dependent upon distance for $1 \leq n \leq 3$ (d_{ee} increases by 4.0 ± 0.5 Å). For example, from $\mathbf{2_1}$ to $\mathbf{2_3}$, log k_{intra} decreases by only 0.43 unit, much smaller than the ∼2.3 unit decrease expected for a simple exponential decay characterized by the scaling factor of α-helices, $\beta = 1.3$ Å^{-1}.[12] The relative rate increase (k_{intra} difference between the $\mathbf{2}_n$ and $\mathbf{1}_n$ series) is more marked for the shorter compounds. Finally, the rate decrease from $n = 3$ to $n = 4$ is similar for **1** and **2**, which provides support that the difference observed with the shortest peptides is real. To explain the latter, a more pronounced relevance of the superexchange

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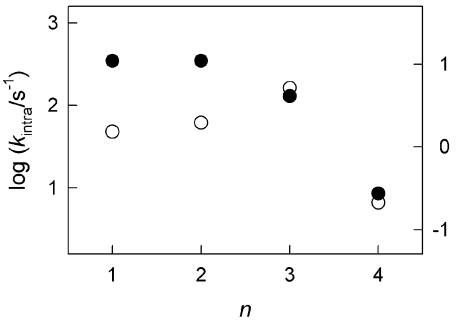

Figure 1. Dependence of the intramolecular ET rate constants for compounds $\mathbf{2}_n$ (●, left scale) and $\mathbf{1}_n$ (○, right scale) on the number of intramolecular hydrogen bonds. $T = 25$ °C.

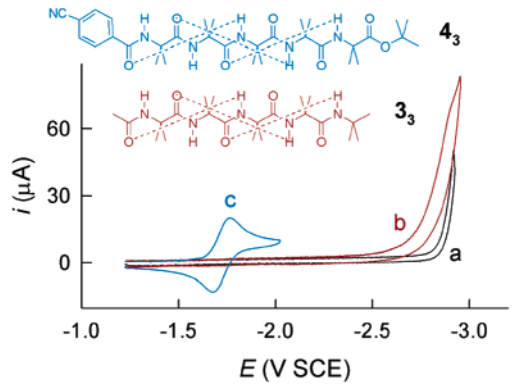

Figure 2. Cyclic voltammetries obtained in the absence (curve a) and presence of 3.83 mM $\mathbf{3}_3$ (curve b) or 1.08 mM $\mathbf{4}_3$ (curve c). Conditions: DMF/0.01 M Bu_4NClO_4, glassy carbon electrode, 25 °C.

mechanism brought about by the decrease of $\Delta G°$ can be invoked. But does the ET entail also some hopping component? To answer this, two quantities need to be determined or estimated: (i) the energy gap (ΔE) between D and the bridge and (ii) the ET activation energy (E_a), which for a hopping mechanism should be comparable to or even larger than ΔE.

We focused on $\mathbf{2}_3$ and synthesized a model bridge, peptide $\mathbf{3}_3$, which also contains three hydrogen bonds but lacks any specific electroactive function. Although electroreduction of simple alkyl amides is expected at potentials substantially negative to the solvent/electrolyte discharge, we found that $\mathbf{3}_3$ is indeed electroactive (Figure 2), which shows that intramolecular hydrogen-bond formation significantly lowers the energy of the lowest unoccupied molecular orbital (LUMO) of the peptide bridge. Although the reduction of $\mathbf{3}_3$ is irreversible, from the decomposition potentials of $\mathbf{3}_3$ and ester $\mathbf{4}_3$ we could estimate $\Delta E \sim 1.1$ eV. To evaluate E_a, we carried out a temperature study on $\mathbf{2}_3$ in the range from 37 to −39 °C. From the electrochemically determined k_{intra} values, we obtained an Arrhenius plot providing $E_a = 0.34$ eV (see Supporting Information). A similar value, 0.37 eV, was obtained for $\mathbf{2}_1$. By considering the outcome of these experiments and even by assuming a large error for ΔE, we can conclude that the thermal population of the bridge requires an energy that is at least twice the E_a value.

The peptides based on the Aib unit are constrained and stabilized by intramolecular hydrogen bonds, which lower the energy of the bridge and thus favor the hopping mechanism. Nevertheless, our results indicate that the latter mechanism can be discarded.[13] For peptides of similar lengths but composed of α-amino acids that either lack intramolecular hydrogen bonds (oligoprolines) or require longer main chains to originate a sufficiently stable secondary structure (oligopeptides based on other protein amino acids), the likeliness that a hopping mechanism would be operative appears to be even less probable. The smooth distance dependence that we observe with Aib oligopeptides is justified by the fact that while addition of a new α-amino acid unit increases d_{ee}, it also lowers the energy of the backbone and introduces new hydrogen-bond ET shortcuts. These effects increase the overall electronic coupling governing the ET reaction and thus would counteract the usual rate decrease expected for the distance dependence of a superexchange ET mechanism.

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Supporting Information Available: Electrochemical analysis and synthesis and characterization of the investigated peptides (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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