ORGANIC LETTERS

2001
Vol. 3, No. 14
2197−2200

Neutral Peptide Biradicals Formed by Dissociative Electron Transfer

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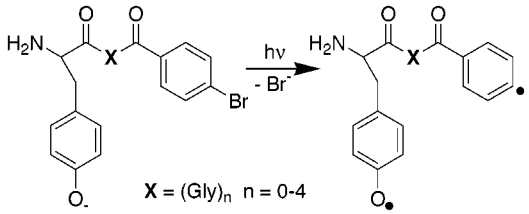
Received April 30, 2001

ABSTRACT



A new method for the generation of transient neutral biradicals in liquid solution is reported. Photoinduced electron transfer in aqueous solution of the structures shown above leads to neutral biradicals with peptide spacers. Exchange interactions were measured using time-resolved electron paramagnetic resonance (TREPR) and found to be large for the biradicals possessing two and three glycine residue spacers. These findings are compared to previous results from alkyl-spaced biradicals of similar chain length.

Electronic spin exchange couplings in biradicals have been under intense study for many years. In our laboratory[1,2] and others,[3,4] investigation of polymethylene chain biradicals using time-resolved magnetic resonance methods has led to a greater understanding of the connections between the spin physics, molecular dynamics, and chemical kinetics of such molecules. Quantifying the exchange interaction as a function of small systematic changes in the molecular structure has been possible over a wide range of values. Typically, biradicals with alkyl spacers are generated photolytically from cyclic molecules, most often via the familiar Norrish I cleavage reaction of ketones. As a means to better understand the electronic dependence of biological electron transfer and energy transfer processes,[5−11] we have recently developed a strategy for the investigation of exchange couplings through or across peptide spacers, shown schematically in the abstract graphic. Here the deprotonated amino acid tyrosine is photoexcited and ultimately transfers an electron to an aryl bromide acceptor. The acceptor then rapidly loses the bromide ion, leaving a neutral biradical consisting of a phenoxyl-type radical on the tyrosine and a para-substituted phenyl radical on the other side of the molecule.

The spacer X shown in the graphic can be any number of amino acids, with the stipulation that they do not also absorb light (e.g., tryptophan or another tyrosine). This route to peptide biradicals is appealing compared to the cleavage of a cyclic system for several reasons: (1) cyclic peptides are

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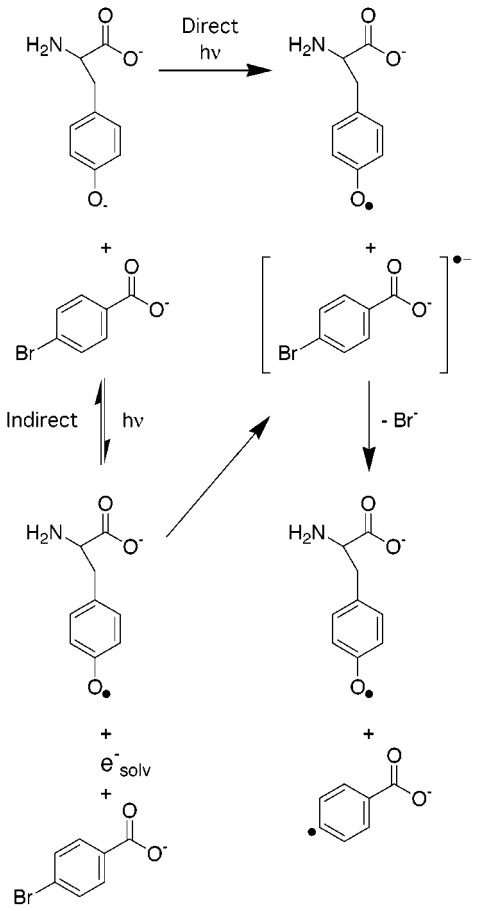
10.1021/ol0160452 CCC: $20.00
Published on Web 06/08/2001

synthetically challenging, (2) engineering into them a unique cleavage site is problematic, and (3) extrapolation of the method to larger systems, e.g. proteins, is not possible. Perhaps the largest advantage of this strategy is the absence of the possibility of back electron transfer because of the rapid rate of reductive dehalogenation.[12,13]

Before studying the formation and characterization of these peptide biradicals, it is desirable to have a complete understanding of the photochemistry and spin properties of the precursors and the corresponding monoradicals. In an earlier report from our laboratory it was established that photoionization of the tyrosine anion occurs predominantly from the excited triplet state, that it is monophotonic at low light intensities, and that the sign of the exchange interaction in the radical pair consisting of tyrosyl and the solvated electron (e^-_{solv}) is negative.[14] All of this information will be useful in our analysis of the biradicals reported here. However, we are using a different acceptor in this work, so prior to studying the biradicals the intermolecular electron-transfer process shown in Scheme 1 was investigated in detail

Scheme 1. Electron Transfer Pathways



by time-resolved electron paramagnetic resonance (TREPR) spectroscopy.[15]

An interesting question that arises upon consideration of the chemistry shown in both schemes is whether the initial electron transfer reaction is direct or indirect. Both pathways are illustrated in Scheme 1 for the intermolecular reaction. The two routes can be distinguished from the chemically induced electron spin polarization (CIDEP) patterns observed in their TREPR spectra. Figure 1A shows the direct photo-

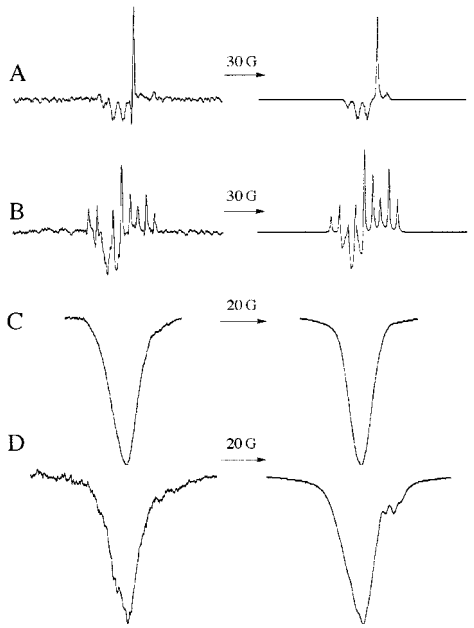


Figure 1. (A) TREPR spectrum (left) obtained from photolysis of 0.050 M L-tyrosine in 0.10 M NaOH solution at 0.3 μs, with simulation (right). (B) TREPR spectrum obtained from the photolysis of 0.031 M L-tyrosine and 0.029 M 4-bromobenzoic acid in 0.19 M NaOH solution at 0.3 μs, with simulation (right). (C) TREPR spectrum obtained from the photolysis of 0.036 M biradical precursor with $n = 2$ in 0.15 M NaOH solution at 1.0 μs, with simulation (right). (D) TREPR spectrum obtained by photolysis of 0.051 M biradical precursor with $n = 3$ in 0.17 M NaOH at 1.0 μs, with simulation (right). The excitation wavelength in all cases is 308 nm. All spectra were recorded on the X-band TREPR apparatus described in ref 15. See text for discussion of simulation parameters.

ionization of tyrosine anion in basic aqueous solution. The emissive transitions are assigned to the tyrosyl radical, while the strong absorptive single line is due to e^-_{solv}. The simulation on the right-hand side, which uses literature values for the hyperfine coupling constants and *g*-factors,[16,17] confirms this assignment. The emissive/absorptive (E/A) CIDEP pattern is consistent with a triplet precursor and negative exchange interaction in the radical pair. Figure 1B shows the TREPR spectrum obtained when *p*-bromobenzoic

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acid (BBA) is added as an acceptor. The emissive transitions of the tyrosyl radical are still present, but there are also many absorptive lines from another signal carrier. These transitions are assigned to a para-substitutued phenyl radical, which appears with all of its transitions in absorption. It can be concluded from this that the polarization actually comes from the solvated electron. The photionization pathway is therefore indirect, i.e., the electron is ejected into the solution first and is then trapped by the BBA and inherits the polarization generated in the original radical pair. If the electron transfer proceeded directly, each radical would appear with low-field lines emissive and high-field lines absorptive. Again, the simulation to the right conclusively identifies the radicals and the polarization pattern gives information as to the mechanism.

It is clear from the simulation that the intermolecular process leads to noninteracting monoradicals and confirms that our photochemical strategy for the production of linear peptide biradicals is feasible. Five biradical precursors have been synthesized in our laboratory using standard solution phase peptide coupling reactions. These precursors consist of the tyrosine donor, a BBA acceptor, and a number of glycine spacers in between. Figures 1C and 1D show TREPR spectra taken after photoexcitation of compounds with two and three glycine spacer units, respectively. Clearly, these spectra differ markedly in appearance from those in Figures 1A and 1B. There appears to be very little fine structure, and in both systems the spin polarization pattern is net emissive. Strong support for assignment of these spectra to the biradicals comes from observing that (1) the spectral widths of these signals are approximately half that of the monoradicals and (2) computer simulations (right-hand side, Figure 1), using our standard model for the prediction of the TREPR spectra of spin-correlated radical pairs (SCRPs)[18] with a large negative exchange interaction, reproduce at least the coarse features of the experimental data.

It is not possible to deduce directly from the spectra in Figure 1C and 1D whether the electron transfer reaction leading to biradicals is direct or indirect. The emissive polarization is present at the earliest possible delay times, and the light intensity dependence of the biradical signals shows monophotonic behavior. With the acceptor close to the donor at all times and strongly coupled to the donor via through-bond and through-solvent interactions, it seems more plausible that direct electron transfer takes place in the intramolecular reaction, as opposed to the indirect mechanism that leads to monoradicals. This is an interesting topic for further investigation as it allows comparison of the electronic coupling for the forward electron-transfer process with that for the spin exchange process in the biradicals.

The only other possible source of the strong net emission in Figures 1C and 1D is the triplet mechanism of CIDEP, but since very little net polarization was observed in the monoradical spectra, we rule this out as the triplet mechanism is unlikely to be significantly perturbed by covalent attachment to another nonphotochemically active moiety. The values for the exchange coupling obtained from the simulations are -1600 MHz for $n = 2$ and -1100 MHz for $n = 3$, respectively. In our SCRP model, when the exchange interaction, J, greatly exceeds the hyperfine couplings, the accuracy of the J values obtained is not high. The error in these numbers is on the order of 50%, but they are nonetheless informative in comparisons with other biradical systems in the literature, vide infra. One of the reasons for the lack of quantitative accuracy is that our model uses a perturbation treatment when J is large, and here the J values are approaching 20% of the Zeeman interaction. This may be the main reason for slight discrepancies in the fits of the simulations to the data.

Precursors with 0, 1, and 4 glycine spacer units showed no TREPR signals. In the case of $n = 4$, this was due to poor sensitivity as the solubility of the precursor was very limited. For $n = 0$ and $n = 1$, it is plausible that the exchange interaction is even larger than for $n = 2$ and $n = 3$. When J values become very large, the SCRP model predicts a much less intense TREPR spectrum because the spin state mixing processes leading to strong spin polarization are hampered by the large energy gap between the states.

The large J values are surprising when one compares the number of intervening bonds between the radical centers with other systems such as the alkyl spacers from our previous studies. From radical center to center, our biradicals have 18 and 21 intervening bonds, for $n = 2$ and $n = 3$, respectively. Alkyl biradicals with this many intervening bonds have average J values on the order of -200 MHz at room temperature.[19] These radicals are delocalized on both ends; however we have observed similarly delocalized bis-(benzylic) biradicals with alkyl spacers whose J values are not very different from the more localized alkyl counterparts.[2,20]

At present we cannot tell how much of the larger exchange interaction in our peptide biradicals is due to better through-bond coupling or if it is due to favorable conformations with larger through-solvent couplings. This will be investigated by changing the amino acid sequence in the spacer. For the present time we can conclude that we have developed a new method for the generation of neutral biradicals that should be applicable to both flexible and more rigid chemical systems and that the exchange couplings in peptide spacer are about 1 order of magnitude larger than their alkyl counterparts of similar chain length. These compounds also show significant promise for the study of the forward one-electron-transfer reaction and its dependence on electronic coupling through the same spacer in peptides and larger biological systems to which the two-electron spin exchange process can be compared.

Acknowledgment. The work reported here was funded by the National Science Foundation (Grant CHE-9820791) and the NSF National Young Investigator Program (M.D.E.F., CHE-9351708). C.S.B. is indebted to the Department of

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Education for a GAANN Fellowship (1995−1996). Mass spectra were obtained at the Mass Spectrometry Laboratory for Biotechnology, North Carolina State University, which is partially funded by the North Carolina Biotechnology Center and the National Science Foundation (Grant CHE-9111391).

Supporting Information Available: Complete descriptions of all synthetic and experimental procedures and characterization data for all biradical precursors. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0160452