EPR and Density Functional Studies of Light-Induced Radical Pairs in a Single Crystal of a Hexaarylbiimidazolyl Derivative**

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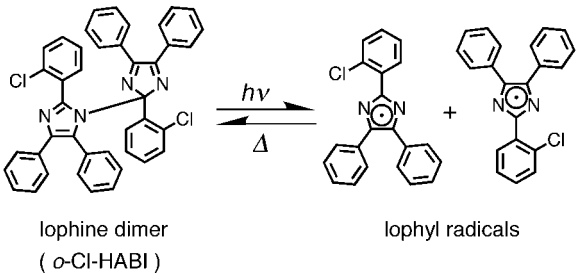
The intermolecular exchange coupling $2J$ is a measure of the electrostatic energy difference between the singlet and triplet states of a radical pair (RP), and is closely related to such important processes as electron and excitation transfer as well as bulk magnetism in both materials and biological systems.[1] The photolytic generation of RPs trapped in proximity in the solid state is a well-established phenomenon. Hexaarylbiimidazoles (HABIs) are readily cleaved, both thermally and photochemically, into a pair of triarylimidazolyl radicals called lophyl radicals that can recombine to form the lophine dimer (Scheme 1).[2] We have recently reported an unprecedented example of the in situ direct observation of a light-induced RP in a crystal of 2,2′-di(*ortho*-chlorophenyl)-4,4′,5,5′-tetraphenylbiimidazole (*o*-Cl-HABI) by X-ray diffraction at 103 K.[3] Radical pairs created by homolysis in single crystals present an opportunity to observe electron–electron through-space coupling between well-characterized species.[4] It enables one to explore systematically the chemistry of the radical pair, a process that is not easily probed by standard solution methods. We report here the first example of the measurement of intermolecular exchange coupling for the light-induced RP in a crystal of *o*-Cl-HABI (Figure 1) and describe the theoretical analyses based on density functional theory.



Scheme 1. Reversible photodissociation of *o*-Cl-HABI into two triarylimidazolyl radicals (lophyl radicals).

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Figure 1. Perspective views of *o*-Cl-HABI (gray line) and its RP (black line) as determined by X-ray crystallography.

Although the photochromic behavior of HABI and its derivatives in solution and solid matrices has been extensively studied by spectroscopic methods, including electron paramagnetic resonance (EPR), there is practically no knowledge of the intermolecular exchange coupling for the pair of lophyl radicals.[2b, e, f, 5] Moreover, no EPR studies have been reported on single crystals of HABIs. Although UV irradiation of a single crystal of *o*-Cl-HABI at room temperature leads to no photochemical reaction, the crystal turns from pale yellow to reddish brown on irradiation with UV light at low temperatures ($T \leq 160$ K). The EPR spectrum of the UV-irradiated powder sample of *o*-Cl-HABI at 3.6 K shows a randomly oriented triplet pattern with an overlapping doublet signal derived from a trace of free radicals. The detection of a forbidden weak transition ($\Delta M_s = 2$) at half-field indicates the presence of a triplet-state species, which is assigned to the light-induced triplet RP (pair A). The zero-field splitting (zfs) parameters for the triplet RP are estimated to be $|D/hc| = 0.0111\ \mathrm{cm}^{-1}$ and $|E/hc| \leq 0.0008\ \mathrm{cm}^{-1}$ by simulation of the spectral line shape by the eigenfield method.[6] The simulated and the experimental spectra are shown in Figure 2a for comparison. However, photolysis of the sample at 50 K gives a different EPR spectrum (Figure 2b) which is characterized by the zfs parameters $|D/hc| = 0.0103\ \mathrm{cm}^{-1}$ and $|E/hc| \leq 0.0007\ \mathrm{cm}^{-1}$ for another triplet state RP (pair B). The zfs parameters are an extremely sensitive probe of RPs since they are inversely proportional to the cube of the distance separating the radical centers. Pairs A and B are chemically identical, differing only in the spatial arrangement of the two lophyl radicals. The average distances between the two radical centers of pairs A and B, as derived from the point dipole approximation, correspond to 6.32 and 6.26 Å, respectively.[7]

The temperature dependence of the EPR spectral pattern shows that pair A converts irreversibly into pair B on increasing the temperature. The EPR measurement on the single crystal clearly demonstrates this conversion process

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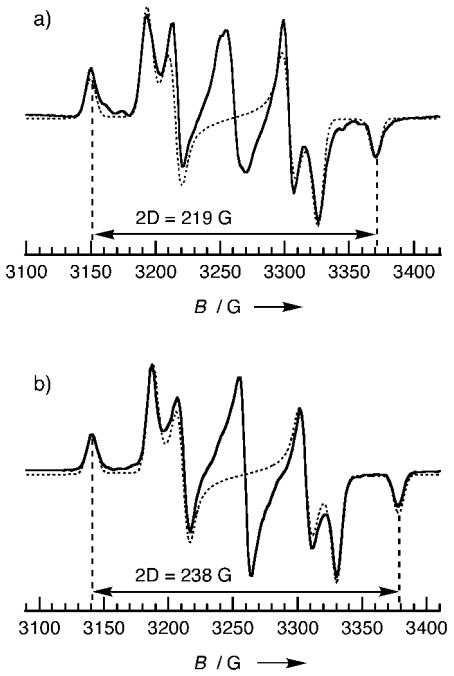

Figure 2. EPR spectra of a UV-irradiated powder sample of *o*-Cl-HABI at a) 3.6 K, and b) 50 K. Dotted curves show spectral simulations. Simulations used $g_{iso} = 2.003$ and Gaussian line shapes with varying line widths. The yellow single crystal of *o*-Cl-HABI was recrystallized from benzene/acetonitrile solution. The polycrystalline powder used in the powder-pattern EPR measurements was obtained by pulverizing the single crystals. The RP was generated at 2.0 K by the photolysis of the polycrystalline powder of *o*-Cl-HABI. Photolysis was carried out by 365 nm light from a 500 W high-pressure mercury lamp for 1 min. The powder-pattern EPR measurements were carried out at 3.6 and 50 K with a JEOL JES-RE3X spectrometer operating at the X-band.

(Figure 3a). The irradiated single crystal shows a typical triplet EPR signal pattern with doublet splitting arising from electron–electron dipolar interaction within closely spaced RPs. When the crystal irradiated at 2 K was warmed to 140 K, the EPR signal of pair A completely disappeared. In fact, the EPR signal of pair A did not reappear on cooling the sample from 140 to 20 K, but the EPR signal intensity of pair B at 20 K increased relative to that measured at 20 K before the warming process. This observation gives unambiguous evidence for the thermal conversion of pair A into pair B. It is remarkable that the doublet splitting for pair A is significantly larger than that for pair B. The dipole–dipole interaction between the lophyl radicals in pair A seems to have a different magnitude to that in pair B as a result of the difference in their spatial arrangement. Pair A is stable between 2–20 K and does not convert into pair B. Increasing the temperature above 170 K results in the disappearance of the EPR signal of pair B as a result of a reversible radical recombination to form the lophine dimer. Curie analyses were carried out on the intensities of the signals in the EPR spectra for each RP as functions of reciprocal temperatures (Figure 3b). The Curie plot for pair A gives a straight line between 6.1 and 20 K. A linear Curie plot does not prove absolutely that the spectrum observed belongs to a high-spin ground state, but strongly supports that the triplet state of pair A could be the ground state or is nearly degenerate with the singlet state. The Curie plot for pair B, however, shows a maximum at $T \approx 70$ K, which suggests that the observed triplet state is thermally populated and the singlet state is the ground state. By assuming thermal equilibrium between the ground singlet state and excited triplet state, the exchange coupling between the unpaired electrons ($2J$) was determined to be $-77\ cm^{-1}$ for pair B.[8] Thus the energy gap ΔE_{ST} between the ground singlet and the thermally excited triplet states for pair B was determined to be $218\ cal\ mol^{-1}$.

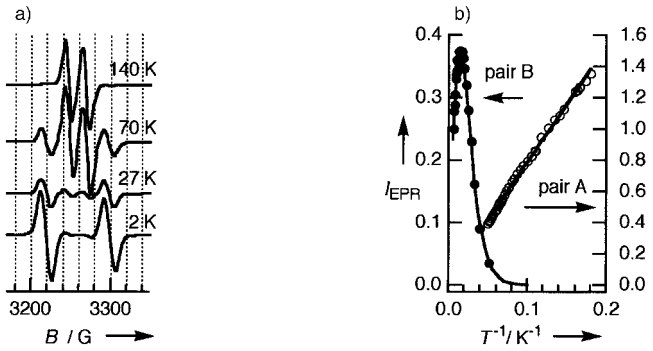

Figure 3. a) EPR spectra at 2 K for a single crystal of *o*-Cl-HABI after photolysis at 2 K and annealing it to the temperatures indicated above each trace. b) Curie plot of the EPR spectral intensity versus reciprocal absolute temperature for pairs A and B. The Curie plot for pair B was measured for the sample free of pair A by annealing to 145 K. The temperature dependence of the EPR spectra of the single crystal was observed between 2–140 K. The fitted curve for pair B was obtained by nonlinear least-squares analysis.

Our previous X-ray crystallographic study for a single crystal of *o*-Cl-HABI measured after UV irradiation at 103 K has revealed the molecular geometry of the light-induced RP for pair B. The success of the X-ray analysis makes it possible to explore theoretical analyses of the light-induced RPs of known structures. Density functional theory (DFT) is well-known to handle properly both open-shell and closed-shell structures, and the unrestricted Becke3LYP/6–31G* calculations provide excellent results for the ΔE_{ST} value of diradicals.[9] We then computed the zfs parameter and ΔE_{ST} for pair B by the spin-polarized DFT method.[10] The spin-up (α) and spin-down (β) electrons are calculated separately in spin-polarized DFT, which is critical for any system with unpaired spin density where local regions in the system can have different α and β electron densities. As can be seen from Figure 4, electrons with α and β spin can occupy different sets of spatial orbitals even in the singlet RP in order to stabilize the energy of the system. The spin density in pair B is mainly delocalized over each imidazolyl ring. The calculated $|D/hc|$ value of $0.0175\ cm^{-1}$ is in good agreement with the measured value of $0.0111\ cm^{-1}$, which indicates that the UB3LYP/6–31G* calculations appear to be promising for determining spin-density distributions in the light-induced RP. The DFT calculations yielded a lower singlet total energy with respect to that of the triplet, which reflects the EPR results. The total SCF energies and the eigenvalues of total spin operator $\boldsymbol{S}^2$ for the singlet and triplet RPs are summarized in Table 1. The unrestricted Kohn–Sham wave functions obtained from SCF calculations are eigenfunctions of $\boldsymbol{S}_z$ but are not eigenfunctions of $\boldsymbol{S}^2$. Therefore, we calculated the spin-projected energies for the triplet and singlet states for the RP in order to determine the value of the exchange coupling $2J$ from the

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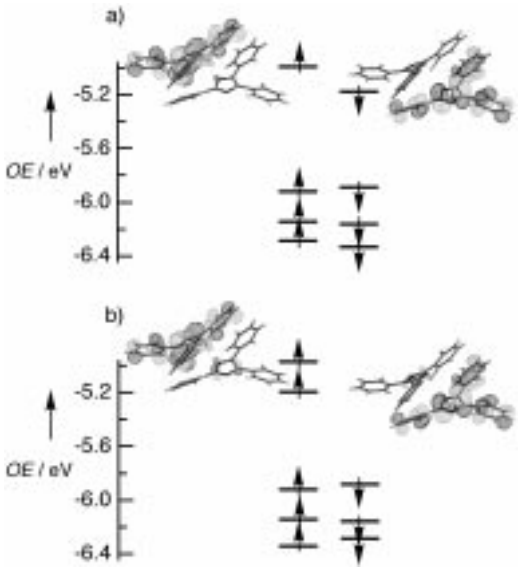

Figure 4. Molecular orbital energy level diagrams and singly occupied molecular orbitals (SOMOs) for a) the singlet state of pair B and b) the triplet state of pair B as obtained from the UB3LYP/6–31G* method. *OE* = orbital energy.

Table 1. Becke3LYP/6–31G* energies and $\langle \mathbf{S}^2 \rangle$ values for the singlet and triplet states for pair B.

Spin state	HF energy (UB + HF-LYP) [hartree]	$\langle \mathbf{S}^2 \rangle$
singlet	−2756.15237298	0.3394
triplet	−2756.15211899	2.0013

DFT calculations.[9a, d, 11] The coupling constant 2*J* was evaluated by calculating the spin-projected energy difference between the singlet and triplet states. The obtained 2*J* value of $-45\ cm^{-1}$ compares well with the experimental value of $-77\ cm^{-1}$.

In summary, we have succeeded for the first time in determining the intermolecular exchange coupling for the light-induced RP in a crystal of *o*-Cl-HABI. This study shows that DFT methods can provide an accurate description even for the through-space coupling in RPs. A combination study of X-ray diffraction and DFT calculations for the light-induced RPs in HABI derivatives will lead to a better understanding of radical-pair chemistry and solid-state reactions.

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[8] A fit of the plot for the thermal excited triplet state was performed with the following equation: $I = \text{const.} \times (1/T) \times \exp(\Delta E/RT)/[1 + 3 \times \exp(\Delta E/RT)]$, with I = EPR intensity, T = temperature, ΔE = energy difference, R = gas constant.

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[10] For the theoretical calculations, the molecular geometry of pair B was taken from the geometry determined in the X-ray diffraction study. The spin density of the triplet RP was calculated at the UB3LYP/6–31G* level. The zfs parameter D was derived from the following dipole–dipole equation: $|D/hc| = 3\beta g(\Sigma_i \Sigma_j \rho_i \rho_j / r_{ij}^3)$, where $3\beta = 1.299\ cm^{-1}Å^3$, g is the free electron g-value, β is the Bohr magneton value, ρ_i and ρ_j are interacting spin densities, and r_{ij} is the distance between them in angstroms.[9d] No interactions were allowed between densities that belong to the same lophyl radical.

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The First Direct Determination of a Ligand Binding Constant in Protein Crystals

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In the field of protein–ligand interactions there have been many recent attempts to predict protein–ligand dissociation constants by correlating solution binding measurements with structural features from series of protein–ligand crystal complexes.[1–6] Indeed the whole structure-based design approach relies on the assumption that the thermodynamics of binding and the structures of bound ligands in the crystal will be similar to those in the biological environment. The results presented herein provide the first evidence that this is indeed the case.

Cyclophilins provide a good template for the study of ligand binding as there is relatively little change in their backbone conformation on binding.[7] To date the only structural data on ligand complexes is for cyclosporin derivatives or proline-containing peptides.[8–10] The isoform cyclophilin 3 from *Caenorhabditis elegans* (ceCyp3) was selected as the template

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