Conformational Analysis

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Direct Structural Determination of Conformations of Photoswitchable Molecules by Laser Desorption–Electron Diffraction**

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The reversible isomerization between the closed spiropyran (SP) and the open merocyanine (MC) forms of photoswitchable molecules, such as derivatives of 1,3,3-trimethylindolinobenzospiropyran (BIPS), have attracted considerable experimental and theoretical interest over the last decades. Because light of different wavelength can initiate both the forward and the reverse reaction in some BIPS derivatives, the photochromic pair of isomers presents interesting opportunities in holographic data storage[1] and as molecular switches to control material properties and biological function.[2]

The closed form consists of an indoline and a chromene subunit joined together at the central spiro-carbon, with the two fused ring systems forming two perpendicular planes. Absorption of a UV photon leads to cleavage of the spiro-carbon–oxygen bond and to subsequent rearrangement of the two subunits to form the extended open form(s) connected by a central bridge segment consisting of three bonds, labeled αβγ, as shown in Figure 1. Due to the larger conjugated π-electron system, the open forms have a strong $S_0 \rightarrow S_1$ absorption maximum that is red-shifted relative to the absorption of the closed form.

A large range of isomers is possible for the open forms, because each of the three ethylenic bonds can exhibit either the *cis* or the *trans* conformation. In addition, the involvement of a triplet-state mechanism has been discussed extensively for nitro-substituted BIPS derivatives.[3–9] Thus, a priori, $2 \cdot 2^3 = 16$ possible structures can exist for the open product(s). Quantum chemical calculations have shown that the isomers having the central bond in the *trans* configuration (designated TTT, CTT, TTC, CTC) are very similar in energy and are more stable than the *cis* isomers.[10–12]

In solution, time-resolved spectroscopic studies have concluded that the open photo-product is formed on the picosecond time scale.[13–18] Even though a distribution to different isomers has been proposed,[13,14,19,20] the number and identity of species formed are still subject of discussion.[7,16] Because the time scales of product formation are heavily dependent on the nature of the solvent,[6,16,18] it is difficult to deduce the molecular basis of the mechanism.[4–8,14,21] Using NMR spectroscopy, the TTC isomer, in rapid equilibrium with the TTT isomer, was identified as the thermally populated species of 6,8-dinitro-BIPS, which exists in its more stable open forms at room temperature;[22] similarly, the TTC and CTC isomers have been detected in the related compound spironaphtoxazine.[23] The TTT isomer of 6,8-dinitro-BIPS[22] and the TTC isomer of 6-nitro-8-bromo-BIPS[24] could be crystallized from acetone and water solutions, respectively, and identified using X-ray crystallography.

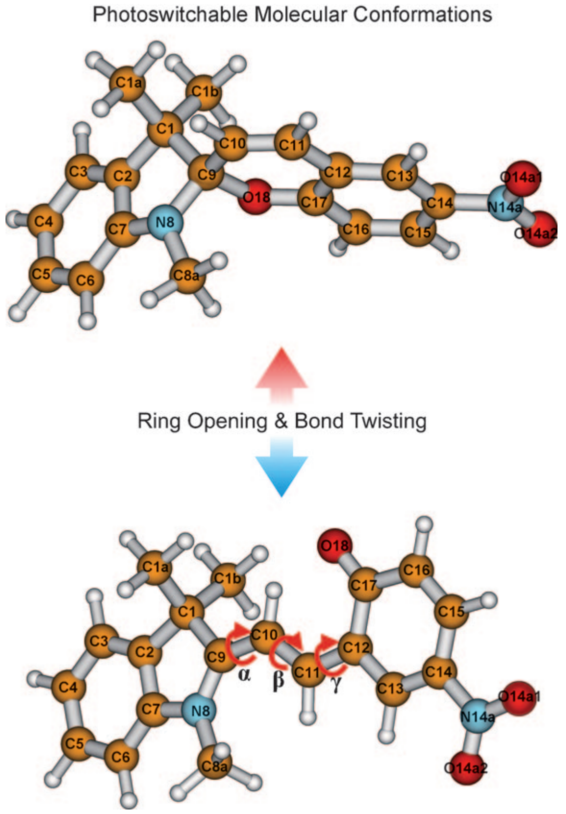

Figure 1. Structures of the photoswitchable closed and open *cis–trans–cis* form of 6-nitro-BIPS. The open structures, formed after photo-excitation, are labeled by the *cis* (C) or *trans* (T) configuration of the indicated dihedral angels ($\alpha\beta\gamma$) in the bridge segment.

Here, we report our study of the molecule 6-nitro-BIPS using laser desorption–electron diffraction in order to determine the nascent product structures upon photo-excitation.[25] In this study, the diffraction is from isolated molecules made into a plume with no perturbations from a solvent. While ambiguities continue to exist regarding the competition among reaction pathways, the various product yields, and

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the transient structures involved in solution, complete structural determination of all species involved in the absence of a perturbing solvent sheds light on the intrinsic chemistry of the molecule itself.

With electron diffraction we are able to determine both ground-state and product structures for excitation of 6-nitro-BIPS at 266 nm. For the ground-state structure, the experimental and the theoretical molecular scattering function, $sM(s)$, together with the radial distribution, $f(r)$, are shown in Figure 2. Starting with a calculated structure of the ground state, we refined seven orthogonal structural parameters, as well as the internal temperature of the molecule (fitted value: 510 K), to achieve satisfactory agreement between the experimental data and the theoretical model.[25] The refined structural parameters are listed in Table SI1 in the Supporting Information, together with values obtained from density functional theory (DFT) calculations; they have discrepancies of less than 0.056 Å and 0.33° for bond lengths and angles, respectively.

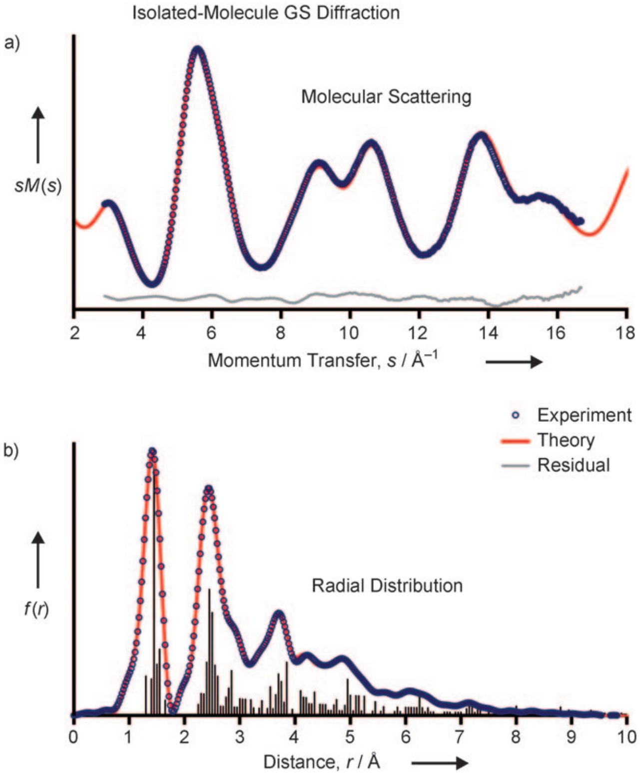

Figure 2. Diffraction results of the ground-state (GS) structure of 6-nitro-BIPS. Shown are a) the molecular scattering function, $sM(s)$, and b) the radial distribution, $f(r)$, for the experimental (circles) and theoretical curves (line). Also shown is a weighted histogram of all the internuclear distances measured in the diffraction experiment.

For studies of structural dynamics following UV excitation, we recorded time-resolved diffraction patterns at −100 ns and +100 ns. To identify the structures present and their abundance, the experimental frame-referenced $\Delta sM(s)$ curve,[26] obtained by subtracting the reference diffraction pattern recorded at −100 ns from the diffraction pattern recorded at +100 ns, is fit with a linear combination of theoretically calculated $sM(s)$ curves (see Experimental Section for structure and temperature calculations). The coefficients of this linear combination report directly on the fractional abundance of a given species.

In the initial screening for product structures, we considered all possible open isomers (except the TCC structure, for which no stationary point could be located)[11] as well as the closed forms in their lowest singlet and triplet electronic states (see Tables SI2 for calculated structure parameters and frequencies). The quality of the fit is quantified in the χ^2 value and the linear combinations are ranked in order of their ability to reproduce the experimental data. We found that fitting the data with less than two products is insufficient to produce a good fit. If three or more products are included, then ca. 70 % of the product species tend to be in a closed form while ca. 30 % are in an open form. Additionally, the linear combinations, which assign about equal abundance to the vibrationally hot ground state and to the closed form in its lowest triplet state, produce the best fits. Finally, the open structures, for which the central bridge segment is in the CTC or CTT conformation, are highly favored.

With the most plausible reaction products identified, we attempted to refine the product structures (with their temperatures constrained at calculated values) in each linear combination separately to further improve the quality of the fit. Table SI3 shows the resulting ranking of linear combinations after the attempt at structural refinement in each case. Only three of the combinations allowed for the fitting of four and five orthogonal parameters in each structure, while the rest produced unphysical geometries after only fitting one or two orthogonal parameters. The three combinations differ only in the identity of the open form, while the fractions remain similar. The χ^2 value of the combination including the CTC structure represents the global minimum (best fit) on the χ^2 hypersurface for the indicated number of orthogonal parameters. We thus conclude that, based on the quality of the fits, the following species are produced in the gas-phase: SP(S_0) at 1164 K, SP(T_1) at 843 K, and MC CTC(S_0) at 1132 K.

Using these product species, Figure 3 displays the fitted $\Delta sM(s)$ curve with the experimental data at the +100 ns time delay and the corresponding $\Delta f(r)$ curve; also shown are the theoretical curves, where the temperatures of all species are held at the initial temperature of 510 K, thus isolating the difference signal due to structural rearrangement only. However, because electron diffraction is recording vibrationally-averaged structures and is therefore sensitive to temperature, it is necessary to constrain the temperature of the product species to calculated values, accounting for photon absorption, when refining structural parameters (see Experimental Section).

Regardless, the depletion of the first, second, and higher order interatomic distances due to the ring opening and bond twisting manifest itself in the more prominent negative peaks in the $\Delta f(r)$ curve. The refined structural parameters are listed in Tables SI4, together with values obtained from DFT calculations. The successful identification of the product structure among the many candidates demonstrates the

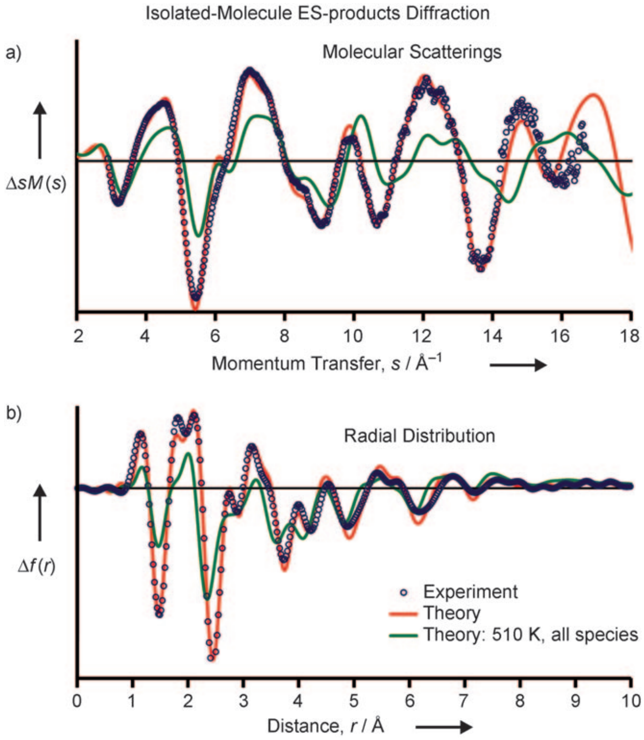

Figure 3. Diffraction results of the excited state(ES)-products structures of 6-nitro-BIPS, following UV excitation. Shown are a) frame-referenced molecular scattering function, $\Delta sM(s;\ t=+100$ ns, $t_{ref}=-100$ ns), and b) the frame-referenced radial distribution, $\Delta f(r;\ t=+100$ ns, $t_{ref}=-100$ ns), for the experimental (circles) and theoretical curves (line). Also shown in green are the difference curves, where all species are held at the temperature of the ground state (510 K), to highlight the contribution due to structural rearrangement.

ability of electron diffraction to solve a complex (nm-scale) molecular structure of low symmetry, even though the largest internuclear distances are beyond the present instrumental coherence length, which we estimate, given the different parameters involved in these experiments, to be 6–7 Å (88% visibility).[27]

The structural dynamics reported here support the involvement of a triplet-state mechanism in the overall photochemistry of 6-nitro-BIPS, because we detect a closed form in the T_1 state. However, the open CTC structure is detected in its electronic ground state and it is uncertain whether this product was formed through that triplet-state pathway or through a separate singlet-state pathway. Through theoretical means, the CTC structure has been found to be the first *trans* structure formed from the *cis* intermediate structure (CCC) after ring opening, due to the steric hindrance between the two methyl groups on the tetrahedral indoline carbon and the oxygen atom.[11–12] The quantum yield (30%) of the internally hot ground state determined here is comparable to the quantum yield (34%) found spectroscopically in trichloroethylene solution.[21] However, in our case, this species could have been formed either through internal conversion from the initially excited state, or through the thermal reverse reaction of ring-closing.

In a recent gas-phase study, 6-nitro-BIPS was also excited at 266 nm ($S_0 \rightarrow S_3$) and the results of the picosecond kinetics were accounted for using a sequential mechanism involving four distinguishable species. The authors detected a long-lived (> 500 ps) photo-product emerging with a formation time of 12 ps, which they argued to represent an open form.[28] However, as shown here, three long-lived product structures in different states at 100 ns have been identified. Efforts to determine whether these species are already present on the picosecond time scale will be the subject of a future contribution using ultrafast electron diffraction.[26]

In summary, we have determined the isolated-molecule structure of photochromic 6-nitro-BIPS, as well as its major photo-products after excitation at 266 nm. We were able to identify three different nascent species and their abundances: The closed form in the ground state (30%) and the lowest triplet state (39%) and the open form in the CTC conformation (31%) in the ground state. This study from electron diffraction combined with laser desorption elucidates the importance of identifying and following all major product structures, if the mechanism of this complex reaction in its entirety is to be established.

Experimental Section

Electron diffraction experiments were performed in our newly constructed UED-4 apparatus.[25] Briefly, electron pulses (5×10^7 electrons/pulse, 60 keV, 1 kHz) were generated using the 266 nm output from a *ns*-Nd:YAG laser. The camera distance (26.716 cm) and the instrumental point spread function[27] (13.5 pixel FWHM) were calibrated by fitting the measured diffraction data of CO_2 gas to its known structural parameters.[29]

The 6-nitro-BIPS sample powder (Sigma Aldrich, CAS#: 1498-88-0) was ground with a mortar and pestle to reduce the particle size and loaded into our desorption source. During the course of the experiment, the fine powder was continuously transferred onto the glassy carbon substrate by circular mechanical motion and subsequently desorbed into the gas-phase by a second *ns*-Nd:YAG laser. Once vaporized, the sample was photo-excited at 266 nm using the frequency-tripled output from a Ti:Sapphire femtosecond laser system and subsequently interrogated by the electron pulses.

Structural analysis was conducted using home-built software, as described previously.[26,30] Theoretically calculated curves were obtained using the starting geometries and vibrational frequencies from density functional theory calculations at the B3LYP/6-311G-(d,p) level using the Gaussian 98 suite,[31] and we calculated the internal temperature of the product species by considering the absorbed photon energy, the vibrational frequencies, and the difference in electronic energy relative to the ground-state species. Refinement of the (nonlinear) structural parameters was conducted using the method of singular value decomposition.[32] Thus, the orthogonal structural parameters that are fitted during refinement are composed of linear combinations of the redundant internal coordinates that are used to initially define the geometry of the molecules.

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