Amino Acid Conformers

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Structural Dynamics of Free Amino Acids in Diffraction**

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The photophysics and photochemistry of L-tryptophan in the condensed phase has been shown to vary greatly depending on the nature of the solvent as well as its local environment; for a review see reference [1]. The convoluted problem of understanding the excited-state interactions of a complex molecule in a complex environment can be approached, by first studying the photophysics and photochemistry of the molecule in the isolated environment. Time-resolved electron diffraction has the capability to determine the structure and dynamics of species formed after excitation and quantify their mole fractions.[2,3] Previously, we have investigated the structural dynamics of indole, the tryptophan chromophore, and have found that the involvement of a dark structure, formed on the picosecond time scale, played an important role in the nonradiative decay pathway of the initially excited state.[4]

Here, we report the first successful study of the amino acid tryptophan, free of solvent interactions, by combining laser desorption and electron diffraction methods. Unlike the indole chromophore, L-tryptophan has a "floppy" peptide moiety, which contains three torsional degrees of freedom (Figure 1); they allow for the formation of multiple conformers. The spectroscopic[5–12] as well as the quantum chemical calculations[13,14] of these conformers have been the subject of many studies. Indeed, multiple stable conformers have been identified in a jet-cooled molecular beam by the Levy group,[6–8] and in a later work, the fluorescence lifetime of photoexcited L-tryptophan was shown to be conformer-dependent.[15] For some specific conformers, the fluorescence emission showed a shorter lifetime, which hints at the existence of a nonradiative deactivation pathways of the excited state of some conformers. More recently, Tseng et al. studied the photolysis of L-tryptophan in a jet-cooled molecular beam using time-resolved mass spectrometry.[16] The direct homolytic C_α–C_β bond cleavage was reported to occur on the μs time scale, consistent with their Rice–Ramsperger–Kassel–Marcus (RRKM) calculations. This result led to the conclusion that the ground state may be involved in a barrier crossing process.

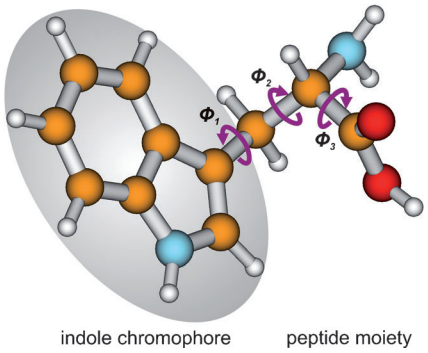

Figure 1. The structure of isolated, ground-state L-tryptophan. The molecule consists of a rigid indole chromophore and a "floppy" amino acid moiety. Through permissive rotation about the indicated dihedral angels (ϕ_1, ϕ_2, ϕ_3), a large distribution of conformational isomers can be populated in an ensemble of isolated molecules, especially at high temperatures.

The above studies do not determine the structure during the processes involved and diffraction of the isolated molecule is our method of choice. However, in this case conventional methods will not permit enough density to obtain diffraction patterns and for this reason we invoked laser desorption of tryptophan power to form the gaseous molecules prior to obtaining diffraction with a pulse of electrons. At elevated vibrational temperatures, the ground-state structure of L-tryptophan presents a challenge to gas-phase electron diffraction, because of the considerable conformational heterogeneity within the molecular ensemble. Detailed inspection of the theoretical molecular scattering function revealed that electron diffraction is primarily sensitive to the rigid portion of the molecule, namely the indole chromophore and the first- and second-order bond distances within the peptide moiety.[17] Information about internuclear distances affected by the large conformational heterogeneity produces a detectable interference signal exclusively at low scattering angles, where data collection is challenging.[17,18]

Here, we carried out the structural refinement of L-tryptophan by separating the two moieties, making the refinement of "rigid" internal coordinates unaffected by the small scattering contributions of the "floppy" parts of the molecule, at low scattering angles. Our quantum chemical calculations indeed showed that, for eleven identified stable ground-state conformers, the rigid parts within the indole moiety are identical to within 0.0025 Å for direct bond lengths, 0.55° for bond angles, and 0.65° for dihedral angles, providing further support for the validity of this separation.[17] The fit produced by the calculated structure could be further improved by fitting three orthogonal structural fitting parameters, which were identified by singular value decomposti-

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tion.[19] The refined structural coordinates for the ground state of L-tryptophan are listed in the Supporting Information, Table SI1. The corresponding fit of the experimental molecular scattering function, $sM(s)$, together with the radial distribution, $f(r)$, are shown in Figure 2.

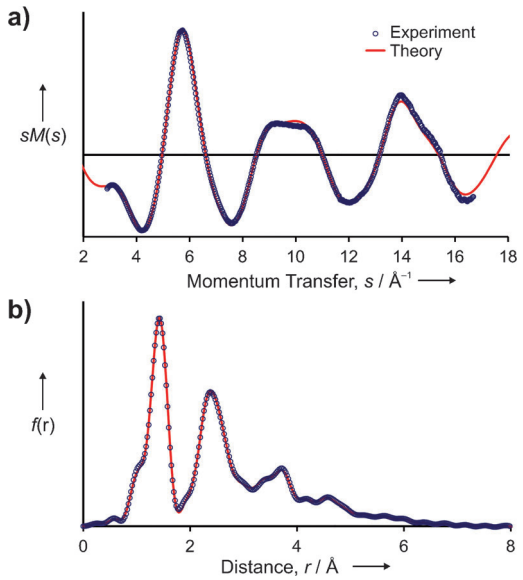
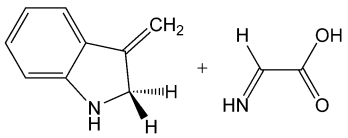


Figure 2. Diffraction results, experimental and theoretical, of the ground-state (GS) structure of isolated L-tryptophan. Shown are a) the molecular scattering function, $sM(s)$, and b) the radial distribution, $f(r)$, for the experimental (circles) and theoretical (line) curves.

To identify the reaction products following UV excitation, we recorded diffraction patterns at negative and positive times. Time-dependent diffraction intensities were obtained by the subtraction of a reference diffraction pattern recorded at -100 ns from the one recorded at $+100$ ns.[2,17] The resulting frame-referenced $\Delta sM(s)$ curve was fit with a linear combination of theoretically calculated $sM(s)$ curves corresponding to possible chemical species present in the ensemble of gaseous molecules, weighted by their fractional abundance; these include the parent L-tryptophan molecule in its lowest singlet and triplet electronic states, and the fragmentation channels considered by Tseng et al. (see Figure SI1 for chemical structures and energetics).[16] Some reaction channels result in fragmentation products with chemical structures differing only in the position of single hydrogen atom. The electronic configuration of these fragments is, however, distinct and the resulting difference in bond orders and, hence, difference in molecular structure is detectable with electron diffraction, as indicated by the pronounced difference in the simulated diffraction signal in Figure SI3.

The different linear combinations, including one, two, or three separate reaction channels, are ranked in order of their ability to reproduce the experimental data. We found that fitting the data with two reaction channels satisfactorily reproduces the experimental data (see Table SI2), while involving more products did not improve the quality of fit. The structures of the major fragmentation product pair were found to be:



with an average mole fraction of $67 \pm 20\,\%$ for the four best linear combinations. This pathway leads to products in S_0/S_0 and $S_0(T_1)/T_1(S_0)$ states. Because the structures obtained from quantum chemical calculations were satisfactorily used as the initial geometries, the quality of fits were improved by structural refinement, but the ranking order was not changed. The four best linear combinations of the "minor" channel indicate that the remaining $33 \pm 20\,\%$ of products originates from fragmentation/elimination (see Table SI2). 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 using the two fragmentation channels giving the overall best fit.

In our experiment, L-tryptophan was delivered into the gas phase using surface-assisted IR laser desorption, which

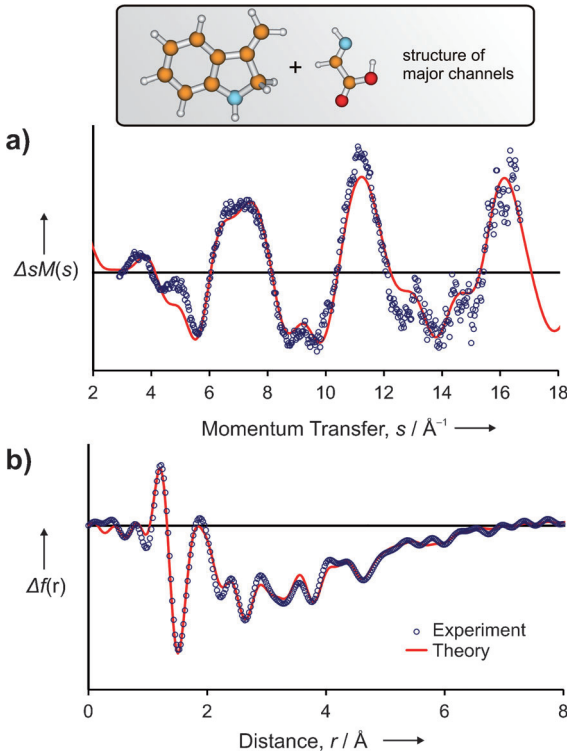


Figure 3. Diffraction results, experimental and theoretical, of the fragmentation product structures of isolated L-tryptophan, following ultraviolet excitation. Shown are a) frame-referenced molecular scattering function, $\Delta sM(s; t{=}{+}100\text{ ns}, t_{ref}{=}{-}100\text{ ns})$, and b) the frame-referenced radial distribution, $\Delta f(r; t{=}{+}100\text{ ns}, t_{ref}{=}{-}100\text{ ns})$, for the experimental (circles) and best fit linear combination (line) curves, as given in Table SI2. The dominant contribution ($67 \pm 20\,\%$) originates from indicated products; the minor product channels are discussed in the text.

led to average vibrational temperatures of 1000 K (fitted value) of the gaseous ground-state molecules, corresponding to 67 kcal mol^{-1} of initial vibrational energy. The total available energy (including the photon energy) provided in our experiment is 174 kcal mol^{-1}, which is higher than the available energy of 110 or 148 kcal mol^{-1} in the experiment of Tseng et al. The 1000 K initial vibrational temperature, in contrast to the very low temperature in a jet-cooled molecular beam, could allow for the population of conformers that are not accessible to the ultracold molecules, both on the ground state and, after excitation, the excited-state potential energy surface. The formation of a pair of radicals was suggested to be on S_0 (as photoproducts) after homolytic C_α–C_β bond cleavage; the excitation was at 193 nm and 212.6 nm L-tryptophan in a jet-cooled molecular beam.

Quantum chemical calculations by the Ni group (personal communication, unpublished) were able to identify a hydrogen migration pathway forming a stable biradical with a barrier height of 16.6 kcal mol^{-1} on the S_1 potential energy surface (130.7 kcal mol^{-1} from the S_0 origin). This biradical is the precursor of our observed fragmentation products, as shown in Figure 4. Energetically, the barrier to form the biradical on the excited state surface is accessible under our experimental condition as well as after 193 nm excitation in the work of Tseng et al.[16] However, they did not detect the corresponding fragmentation products. The same hydrogen migration pathway on the T_1 state was identified with a 16.8 kcal mol^{-1} barrier height in our quantum chemical calculation. Given the abovementioned differences in experimental conditions, the discrepancy of observed products in both works can be rationalized by the difference in the initial conformer distribution.

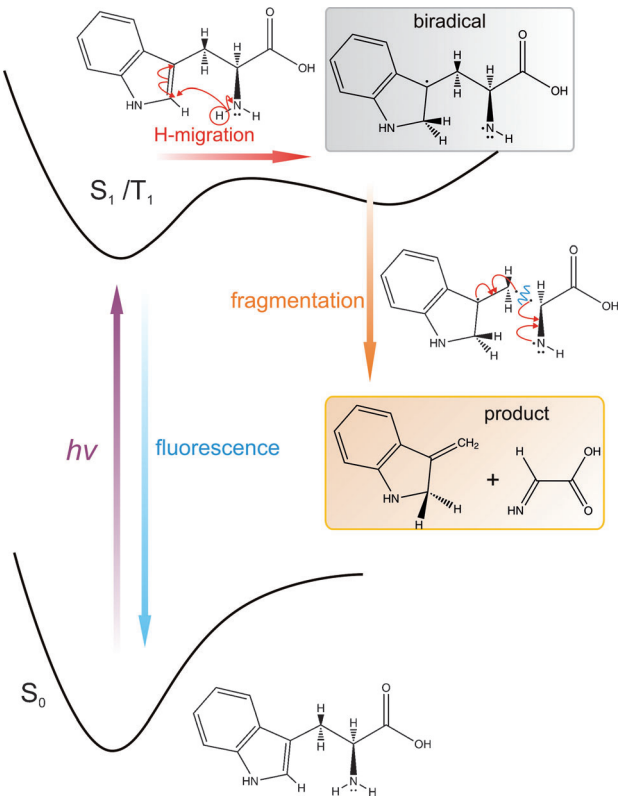


Figure 4. Structural dynamics and mechanism. Shown are excited, intermediate, and final-product structures.

In our work, the excitation of several different conformations generates a corresponding distribution on the excited state potential energy surface. As evident from the mechanism shown in Figure 4, intramolecular hydrogen atom migration requires a favorable geometry of the peptide moiety. Thus, the initial temperature plays an important role in determining the extent of populations and geometries reached upon excitation. In our case, the relatively high temperature permits the reach of the precursor biradical intermediate (see Figure 4), whereas in jet-cooled experiments this intermediate may not be reached as the initial geometry could be unfavorable at low temperatures. Thus, after laser desorption and broadband femtosecond laser excitation, the distribution of the initially prepared conformers in the excited state is the key to drive the reaction toward biradical formation and the resulting fragmentation products that we observe.

In summary, we have determined the structure of free amino acid L-tryptophan, as well as its major products after excitation at 267 nm. We show that the nonradiative pathway is a reactive process, and that the dominant reaction pathway of vibrationally hot molecules is not involving a prompt homolytic bond cleavage to produce two fragment radicals. The mechanism involves two steps, an intramolecular hydrogen atom migration followed by fragmentation to yield two closed-shell molecular structures. We determined an average branching ratio of $67 \pm 20\,\%$ for this fragmentation channel under our experimental condition. This combined laser desorption with electron diffraction study highlights the importance of determining the structures, and the abundance, and should now be applicable to other nonvolatile, biological specimens.

Experimental Section

Electron diffraction experiments were performed using high-intensity electron pulses (5×10^7 electrons/pulse, 60 keV, 1 kHz) in the UED4 apparatus.[3,17,20] The experimental camera distance (26.707 cm) and the instrumental point spread function[21] (18 pixel FWHM) were calibrated by fitting the measured diffraction data of CO_2 gas to its known structural parameters.[22] Finely ground L-tryptophan sample (Sigma Aldrich) was delivered into the gas phase as intact monomers by surface-assisted laser desorption using a ns-Nd:YAG laser. The vaporized sample was excited using the frequency-tripled output from a Ti:Sapphire femtosecond laser system (380 μJ/pulse at 266 nm) and subsequently interrogated by the electron pulses.

Theoretically calculated molecular scattering functions were obtained using the minimum energy molecular structures and vibrational frequencies from density functional theory calculations at the B3LYP/6-311G(d,p) level using the Gaussian 98 suite.[23] While the internal temperature of the ground state species could be estimated from the diffraction data, the internal temperature of the product species was calculated by considering the energy of the absorbed photons, the vibrational frequencies, and the difference in electronic energy between the product species and the ground state species. The most sensitive structural parameters for fitting were identified through singular value decomposition and are composed of linear combinations of the redundant internal coordinates that are used to

define the geometry of the molecules.[17,19] The orthogonal nature of these fitting parameters eliminates any correlations between them.

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