Photostability of amino acids: Internal conversion versus dissociation

Ming-Fu Lin, Cheng-Ming Tzeng,a) Yuri A. Dyakov, and Chi-Kung Ni

Institute of Atomic and Molecular Sciences, Academia Sinica, P.O. Box 23-166, Taipei 10617, Taiwan

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Photodissociation dynamics for various tryptophan chromophores was studied at 193 or 248 nm using multimass ion imaging techniques. The competition between internal conversion to the ground electronic state and dissociation from the repulsive excited state reveals size-dependent photostability for these amino acid chromophores. As the size of chromophore increases, internal conversion to the ground state becomes the major nonradiative process. For tryptophan and larger chromophores, dissociation directly from the repulsive state is completely quenched. © 2007 American Institute of Physics.

One important photophysical characteristic of amino acids is their low fluorescence quantum yields upon excitation by ultraviolet (UV) light.1–4 Such behavior indicates the presence of fast nonradiative channel that efficiently quench the fluorescence. This nonradiative process is assumed to be the ultrafast internal conversions to the ground electronic state. The so-called “photostability” for these molecules can prevent undesirable photochemical reactions from initiating after UV irradiation.2,3 However, recent theoretical calculations5–9 suggest that the low fluorescence quantum yields for phenol, imidazole, and indole (the respective chromophores for the amino acids tyrosine, histidine, and tryptophan) are due to the dissociative characteristics of the excited electronic state potential energy surface, rather than the direct fast internal conversion to the ground electronic state.

Figure 1, taken from Ref. 7, shows a section through multidimensional diabatic potential energy surfaces for the ground and first excited $1\pi^*\sigma$ and $1\pi\pi^*$ states of indole molecules as plotted against N–H bond distances. The first excited $1\pi\pi^*$ state is bound with respect to N–H bond distance, but the $1\pi^*\sigma$ state is repulsive. The diabatic $1\pi^*\sigma$ potential correlates with the ground state products, C$_8$H$_{11}$N$_6$+H. Although the absorption of UV photons corresponds to excitation to the $1\pi\pi^*$ excited state, population of the $1\pi\pi^*$ state can be transferred to the $1\pi^*\sigma$ potential through a conical intersection. As a consequence, instead of direct internal conversion to the ground electronic state, dissociation from $1\pi^*\sigma$ provides an alternate explanation for the rapid fluorescence quenching. Similar repulsive potential energy surfaces (PESs) such as that of $1\pi^*\sigma$ were found for phenol along O–H bond and imidazole along N–H bond. The $1\pi^*\sigma$ potential minima in both indole and phenol lie below that of the $1\pi^*\sigma$ PES (in the area near equilibrium geometry at the ground electronic state), whereas in imidazole the $1\pi\pi^*$ state is lowest in energy and can be accessed optically. Dissociation from these repulsive excited states has been verified in recent molecular beam experiments.10–14 Because dissociation from an excited state having a repulsive potential energy surface is swift, quenching is incomplete even in the condensed phase. As a result, dissociation from the repulsive state and the reactions following generation of radicals from dissociation become a potential problem in amino acid photostability.

Tryptophan is the most photochemically active amino acid. Tryptophan has a greater capacity for absorbing UV photons than that for other aromatic amino acids, along with an ultraviolet spectrum, which extends to longer wavelengths. In this work, the photodissociation dynamics for various tryptophan chromophores, including indole, 3-methylindole, tryptamine, tryptophan, N-acetyl tryptophan methyl ester, and N-acetyl tryptophan ethyl ester was investigated. We report the competition between internal conversion to the ground electronic state and dissociation from the repulsive excited state in these chromophores and provide an explanation for the amino acid photostability.

The experimental techniques have been described in detail elsewhere.15,16 Basically molecules in a molecular beam were photodissociated by a pulsed UV laser beam at 193 or 248 nm, then photofragments were ionized by vacuum UV (VUV) laser at 157 nm (and 118 nm for indole, 3-methylindole, and tryptamine). The masses of fragments were identified along with their translational energy distributions using multimass ion imaging techniques. A homemade high temperature nozzle was used in this experiment. For 3-methylindole, tryptamine, n-acetyl tryptophan methyl ester, and n-acetyl tryptophan ethyl ester, a stainless steel oven maintained at 383 K was attached to the front port of a pulse valve. The inner surface of the oven was covered with glass and the plunger of the pulsed valve was extended to the exit port of the oven to control the opening of the oven. Compounds dissolved in methanol were sprayed on glass wool and dried by hot nitrogen gas before putting into the oven. For tryptophan, it was mixed with graphite powder and the oven was coated with graphite. Ultrapure He (or Ne) at a pressure of 400 Torr flowed through the oven and the sample/rare gas mixture was expanded through the exit port of the oven to form molecular beam. VUV photoionization

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a) Also at Department of Chemistry, National Taiwan University, Taipei, 10617, Taiwan.

b) Author to whom correspondence should be addressed. Also at Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan. Electronic mail: ckn@iams.sinica.edu.tw
H$_2$O cluster ions were observed in the molecular beam. In Fig. 1, the dissociation properties from a repulsive state, section and produce fragments in the ground state, as shown predictions derived from observation of H atom elimination from the excited state supports going into the potential well of the ground state. The observation of large kinetic energy release, may remain as long as molecules dissociate into fragments quickly and directly without going into the potential well of the ground state. The observation of H atom elimination from the excited state supports predictions derived from ab initio calculations. For the photolysis wavelength at 193 nm, the dissociation from the ground state can produce fragment in the excited state, or in the ground state after undergoing the conical intersection at long distance (1.8 Å). However, the 248 nm photon energy is only large enough to produce the fragment in the ground state. As we will show in the text below, fragment that ended in the excited state or the ground state is not important in the photostability of amino acids.

In the study of 3-methylindole, 3-methylindole ions ($m/e=131$) and a trace amount of dimer and 3-methylindole-H$_2$O cluster ions were observed in the molecular beam.

When the molecular beam was irradiated by 193 nm photons, fragment ions with $m/e=130$ and 116 were observed by 157 nm photoionization and $m/e=130$, 116, and 15 were observed by 118 nm photoionization. They correspond to the fragments of H atom and CH$_3$ elimination channels. The ion intensity ratio between $m/e=130$ and 116 is 60:1, indicating that the H atom elimination is the major channel. However, only H atom elimination was observed at 248 nm. The translational energy distributions are shown in Figs. 2(c)–2(e). Because the methyl group is not the absorption chromophore in this UV region, photoexcitation of 3-methylindole only correlates to the excitation of the indole ring ($\pi\pi^*$). However, the decay mechanism for the excited indole ring is different from indole. For 3-methylindole photodissociation at 193 nm, the relative intensity of the fast component decreases and that of the slow component increases, indicating that the cleavage of N–H or C–H (from CH$_3$) bonds from the ground state becomes more important. In addition, CH$_3$ elimination from the ground state was observed.

The content of tryptamine in the molecular beam checked by mass spectra shows tryptamine ion ($m/e=160$) and a small amount (<10%) of indole ions ($m/e=117$). The existence of indole is due to the thermal decomposition of tryptamine in the oven. Since we know the dissociation channels and fragment translational energy distributions of indole, we can subtract the interference from indole easily. The result shows that the photofragment ions of tryptamine include $m/e=130$ and 30. The momentum of line shape component of $m/e=130$ matches with the momentum of $m/e =30$, indicating the C–C bond cleavage: C$_8$H$_7$N$_2$H$_2$ → C$_6$H$_5$NH$_2$CH$_2$+CH$_2$NH$_2$. Because dissociation through a C–C bond generates two radicals, this type of dissociation on the ground electronic state does not feature an exit barrier. The small translational energy release, as shown in Figs. 2(f) and 2(g), indicates that this channel must occur from the ground electronic state after internal conversion. The other component of $m/e=130$ is a dislable image. The size of the disk changes with the pump-probe delay time, indicating that it is from heavy fragment cracking due to excess VUV photon energy. The most likely fragment is $m/e=159$, corresponding to H atom elimination. The photofragment translational energy distributions, as shown in Figs. 2(h) and 2(i), suggest that the dissociation occurs from the repulsive excited state. Since the amino group (NH$_3$) is also an absorption chromophore in this UV region, a large translational energy release can occur from amino group or NH group of the indole ring. The relative ion intensities of the dislable image and line shape image of $m/e=130$ are 3:1 and 1:1 at 248 and 193 nm, respectively. It suggests that the internal conversion to the ground state prior to dissociation is important in tryptamine.

Tryptamine (50%) was found in tryptophan molecular beam due to the thermal decomposition of tryptophan in the oven. Fragment ions with $m/e=130$, 74, and 30 were found at both 193 and 248 nm. Fragment ions with $m/e=30$ and part of $m/e=130$ were from dissociation of tryptamine. Fragment ions with $m/e=74$ and part of $m/e=130$ result from dissociation of tryptophan, corresponding to the reaction C$_6$H$_4$CH$_2$CHNH$_2$COOH → C$_6$H$_4$CH$_2$+CHNH$_2$COOH.
The photofragment translational energy distributions are shown in Figs. 2(j) and 2(k). In the investigation of n-acetyl tryptophan methyl ester (ATME), small amounts of 3-methylindole (4%) and indole (20%) were found in the molecular beam. Photodissociation of ATME at 193 nm results in fragment ions with m/e=259, 231, 201, 173, 144, 130, and 88. Images of m/e=130 are disklike, resulting from the dissociative ionization of excited parent molecules due to slow dissociation rate. The line shape images of m/e=130 are C₈H₆CH₂, corresponding to the dissociation channel:

\[
\text{C}_8\text{H}_6\text{CH}_2\text{CHNH(COCH}_3\text{)}\text{COOCH}_3 \rightarrow \text{C}_8\text{H}_6\text{CH}_2 \\
+ \text{CHNH(COCH}_3\text{)}\text{COOCH}_3.
\]

FIG. 2. Photofragment translational energy distributions and structural formulas of various chromophores. The left and right columns represent the proteolysis wavelength at 193 and 248 nm, respectively.
Dissociation must occur indirectly, either through coupling to the cited state that is stable with respect to dissociation. UV photoexcitation of the indole ring results in an ex- ample of nonradiative transitions, the transition from an electronic state dependent on the competition between the two nonradiative transitions present in the system. As the chromophores in our study vary from indole to large amino acid chromophores, the estimation of the density of states shows that the increase in the radiationless transition rate between $\pi^* \pi^*$ and ground electronic state due to the overlap of wave functions and energy mismatch is far greater than that between $\pi \pi^*$ and $\pi^* \sigma^*$. Meanwhile, internal conversion is very efficient through conical intersections. As the size of floppy side chain increases, it may introduce more conical intersections. These conical intersections not only increase both $\pi \pi^*$ to $S_0$ and $\pi^* \sigma^*$ to $\pi^* \pi^*$ radiationless transitions, but also increase the $\pi^* \sigma^*$ to $S_0$ radiationless transition if molecules are in the $\pi^* \pi^*$ state. If molecules go to the potential well of the ground state through some conical intersections between $\pi \pi^*$ and $S_0$, energy is randomized prior to dissociation. The dissociation rate or kinetic energy release are the same as those from direct internal conversion or through conical intersection from $\pi \pi^*$ to $S_0$. As a result, large amino acid chromophores are much easier to end up in the ground state through various internal conversion pathways and energy is randomized prior to dissociation. Dissociation from the excited state for these large chromophores is completely quenched. Similar side chain size-dependent dissociation properties have been found in the other smaller amino acid chromophores. Although under collisionless conditions, molecules upon absorbing UV photons eventually dissociate into fragments, either from an excited state or the ground state, dissociation on the ground state is slow and therefore it can be easily quenched in the condensed phase due to rapid intermolecular energy transfer. Our results show that large chromophores only dissociate on the ground state. This provides a mechanism for the photo-stability of amino acids in the condensed phase.

\[
C_8H_{16}CH_2CHNH(COCH)_3COOCH_3 \rightarrow C_8H_{16}CH_2CHNH(COCH)_3 + COOCH_3.
\]

Neither H atom elimination nor peptide bond cleavage was observed. Only the first dissociation channel was seen at 248 nm. The impurities in the molecular beam, the observed fragment ions, and dissociation channels of $n$-acetyl tryptophan ethyl ester (ATEE) are similar to those for ATME, except the ion $m/e=88$ observed in ATME changes to $m/e=102$ in ATEE. This change supports the fragment cracking pattern we proposed in the study of ATME. The translational energy distributions for these two molecules are illustrated in Figs. 2(l)–2(q). The ion intensity ratio between $m/e=130$ and 201 in ATME is 10:1, and the ratio of $m/e=130$ and 201 in ATEE is 30:1, suggesting that the second channel has a smaller branching ratio for these two molecules. For tryptophan, ATME, and ATEE, the translational energy distributions show that dissociation from the repulsive excited state is completely quenched. Only dissociation via cleavage of the weak chemical bonds following internal conversion to the ground electronic state occurs.

Comparison of these six amino acid chromophores reveals unique side chain size-dependent dissociation properties. UV photoexcitation of the indole ring results in an excited state that is stable with respect to dissociation. Dissociation must occur indirectly, either through coupling of the stable state with the repulsive state ($\pi \pi^*$) or after internal conversion from an excited electronic state to the ground electronic state. Branching ratios between dissociation on the repulsive potential and dissociation on the ground electronic state depend on the competition between the two nonradiative transitions, the transition from $\pi \pi^*$ to $\pi \sigma^*$ and from $\pi \pi^*$ to the ground state. The rate constant $k$ for radiationless transition between a pair of states depends on two factors. One is the overlap of wavefunctions and energy mismatch, which can be described by Fermi’s golden rule,

\[
k_{\Gamma \Gamma'} = \frac{2\pi}{\hbar} |\langle \Psi_\Gamma | H | \Psi_{\Gamma'} \rangle|^2 \rho_\Gamma.
\]

$H'$ is the perturbation operator, $\Psi_\Gamma$ and $\Psi_{\Gamma'}$ are the respective wave functions for the initial and final states, and $\rho_\Gamma$ is the density of states and is given by the number of energy levels per energy unit in the final state at the energy of the initial state. The other factor is the accessibility of conical intersections. As the chromophores in our study vary from indole to ATEE, the respective side chains for these molecules increase. Because floppy side chains contain a large number of low vibrational frequency modes, the density of states increases rapidly. The estimation of the density of states shows that the increase in the density of states for the ground state is larger than that for the repulsive state at the photoexcitation energy level. This is due to the large energy separation between the ground and repulsive states. As a consequence, the increase in the radiationless transition rate between $\pi \pi^*$ and ground electronic state due to the overlap of wave functions and energy mismatch is far greater than that between $\pi \pi^*$ and $\pi \sigma^*$. Meanwhile, internal conversion is very efficient through conical intersections. As the size of floppy side chain increases, it may introduce more conical intersections. These conical intersections not only increase both $\pi \pi^*$ to $S_0$ and $\pi^* \pi^*$ to $\pi^* \pi^*$ radiationless transitions, but also increase the $\pi^* \sigma^*$ to $S_0$ radiationless transition if molecules are in the $\pi^* \pi^*$ state. If molecules go to the potential well of the ground state through some conical intersections between $\pi \pi^*$ and $S_0$, energy is randomized prior to dissociation. The dissociation rate or kinetic energy release are the same as those from direct internal conversion or through conical intersection from $\pi \pi^*$ to $S_0$. As a result, large amino acid chromophores are much easier to end up in the ground state through various internal conversion pathways and energy is randomized prior to dissociation. Dissociation from the excited state for these large chromophores is completely quenched. Similar side chain size-dependent dissociation properties have been found in the other smaller amino acid chromophores. Although under collisionless conditions, molecules upon absorbing UV photons eventually dissociate into fragments, either from an excited state or the ground state, dissociation on the ground state is slow and therefore it can be easily quenched in the condensed phase due to rapid intermolecular energy transfer. Our results show that large chromophores only dissociate on the ground state. This provides a mechanism for the photo-stability of amino acids in the condensed phase.