

Gas phase spectroscopy of capped tyrosine by laser desorption supersonic jet technique: Conformational dependence of excited state dynamics

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Introduction Electronically excited state dynamics of biomolecule sometimes changes between the bulk and in vivo. For instance, a chromophore of green fluorescence protein, which is a modified residue of tyrosine (Tyr), emits green fluorescence in the literature, however, does not in aqueous solution. This is explained by conformational change in the protein, i.e., the chromophore forms a stressful structure, which inhibits the non-radiative process, by engaged in the protein.¹ Another instance is DNA base pairs. In gas phase, not only the Watson-Crick's (WC) structures but also non-WC structures are observed. While the latter show sharp electronic spectra, the former give broad ones, which indicates the existence of rapid relaxation process in the electronic excited state. The facts that only the wild type structures, i.e. WC structures, show rapid relaxation process and DNA actually has low quantum yield of photo-chemical reaction imply that the living things may utilize that rapid relaxation process to avoid photo damages.

As these example, the living things may utilize excited state dynamics conveniently by controlling the conformations. So in this work, we took notice of one of aromatic amino-acids, Tyr, and peptides containing it. Recently Çarçabal and co-workers reported a resonance enhanced multiphoton ionization (REMPI) spectrum of capped Tyr (cTyr), in which N and C-termini are protected by acetyl group and methylamide group (Fig.1), respectively.² The REMPI spectrum shows a broad absorption with several sharp bands. They also measured UV-UV hole burning (HB) spectra and found that two conformers, one of which gives the broad absorption and another gives the sharp bands, contribute to the REMPI spectrum. This result indicates the excited state dynamics is different with each conformer. However, it is difficult to conclude whether other conformers exist or not because of low spectral quality. In this work, we re-measured REMPI and HB spectra of the cTyr and found tht it has at least 5 conformers. So we re-considered the structures of each conformer by IR spectra and quantum chemical calculations.

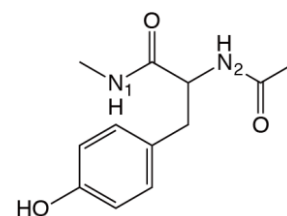


Fig. 1 Capped tyrosine

Experiment In the HB spectroscopy, two tunable UV lasers are employed (Fig. 2). The first UV laser, the probe laser, is fixed to a certain band observed in the REMPI spectrum, and ion current due to the probe laser is monitored, which is proportional to the population of a certain isomer at zero-vibrational level in S_0 . Before irradiation of the probe laser, the second UV laser, the burn laser, is introduced and scanned. If the burn laser is resonant to any electronic transitions of the certain isomer, which will be electronically excited, the population of the isomer in S_0 decreases, and thus the monitored ion current also decreases. Such depletions of ion current take place only when the specified isomer by the probe laser is excited by the burn laser. Therefore the isomer-selected electronic spectra can be measured as depletions of ion current. In addition, if a tunable IR laser is employed as the second UV laser, isomer-selected IR spectra can be measured (IR dip spectroscopy).

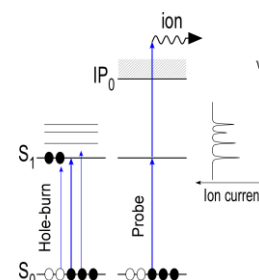


Fig. 2 The schematic for HB spectroscopy

Results and Discussion REMPI and HB spectra of cTyr is presented in Fig. 3. The burn laser was fixed at each transition indicated by dotted lines. In this work, we successfully measured 5 different HB spectra, not 2, thanks to high quality REMPI spectrum. Based on the patterns of vibronic bands, it is possible to make three groups, 1 and 2, 4 and 5, and 3, respectively. It implies that each group has similar structure except OH direction. Of course, it is highly supposed for conformer 3 to have a pair including the different OH direction. However, at this moment, it has been difficult to find it due to the small intensity of the peak. The most important feature of

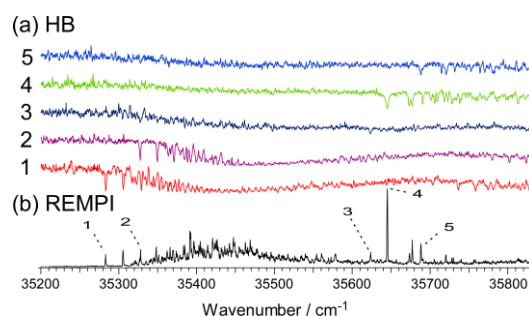


Fig. 3 REMPI and HB spectra of cTyr

REMPI spectrum is the broad absorption around 35450 cm^{-1} . According to HB spectra, the absorption arose from both conformer 1 and 2.

In order to figure the structures of each conformer out, IR dip spectroscopy was applied and the spectra are presented in Fig. 4 with simulated spectra which were calculated by CAM-B3LYP/cc-pVDZ. All spectra show a sharp peak around 3660 cm^{-1} which is assigned to a free phenolic OH stretch. Other bands are assigned to NH stretches. Based on the spectral features of the NH stretches, they could be classified to 3 groups, 1 and 2, 4 and 5, and 3, respectively, which corresponds to those by HB spectra. The first group shows two sharp bands at $3441, 3466\text{ cm}^{-1}$ for conformer 1 and $3436, 3468\text{ cm}^{-1}$ for conformer 2. On the other hand, in the second group, a sharp band at 3438 cm^{-1} for conformer 4, 3439 cm^{-1} for conformer 5 and a broad band around 3350 cm^{-1} are observed. In the third group, conformer 3, a slightly broad band is observed at 3438 cm^{-1} . According to the previous study,³ we can assign what kinds of hydrogen bond each NH group forms based on the frequency of the NH stretch vibration. The broad band observed around 3350 cm^{-1} is assigned to a NH stretch which forms C7-type intramolecular hydrogen bonds, in which the number of atoms between amine hydrogen and carbonyl oxygen getting involved in the hydrogen bond is seven. Similarly, NH stretch observed at $3436 \sim 3441\text{ cm}^{-1}$ is assigned to that forming C5-type intramolecular hydrogen bond. Thus, we tentatively assigned that the conformers 1 and 2 form C5-type hydrogen bond and the conformers 4 and 5 form C7-type.

In order to assign the structures more precisely, we compared the observed IR spectra with calculated ones. In the spectra of the first group, the IR transitions located in 3466 cm^{-1} for conformer 1 and 3468 cm^{-1} for conformer 2 correspond to N1H stretch indicated in Fig. 1 and the observed bands at 3441 cm^{-1} for conformer 1 and 3436 cm^{-1} correspond to N2H stretch. In the spectra of the second group, the IR transitions located in 3438 cm^{-1} for the conformer 4 and 3439 cm^{-1} for the conformer 5 correspond to N2H stretch and the broad IR transitions located around 3350 cm^{-1} correspond to hydrogen bonded N1H stretch. In the spectrum of conformer 3, two NH stretching vibrational modes are overlapped in 3438 cm^{-1} . According to the result about the relative energy among the conformers, the most stable one is the conformer including C7-type. It is more stable than conformers including C5-type, around 1 kcal/mol . It energetically supports the the intensity pattern of REMPI spectrum of cTyr, in which the strongest peak arose from the conformer including C7-type.

As mentioned above, conformers 1 and 2, which form the C5-type hydrogen bond, have broad electronic absorption, which may indicate a existence of a rapid relaxation process in the S_1 state. The relation between the relaxation process in S_1 state and conformational structure will be discussed in the presentation.

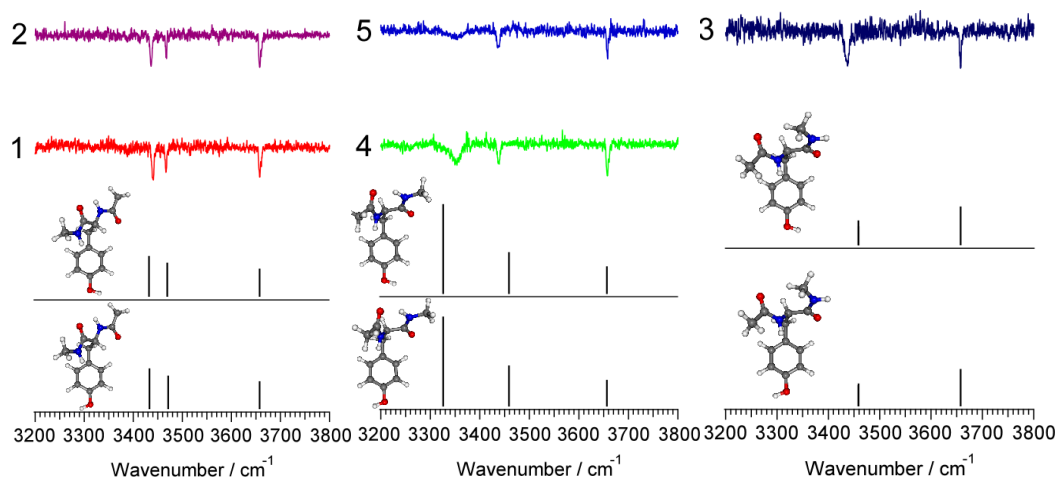


Fig. 4 IR dip spectra of each conformer with simulated spectra by CAM-B3LYP/cc-pVDZ

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