3P014

UV-UV hole burning and IR dip spectroscopy of hydrated adrenaline by laser desorption supersonic jet technique: Hydration effects on the conformation

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[Introduction] Neurotransmitters play an important role to propagate neural signals. When the neurotransmitter binds to a specific receptor protein, the signal is transferred over the neuro-system. Because of the high selectivity of the binding process, this molecular recognition is often likened to 'key' (neurotransmitter) and 'lock' (receptor). However, the neurotransmitters should have many stable conformations because they have many single bonds. The fact that such soft molecules act as elaborate keys is far from our common sense. Thus, to understand the molecular recognition mechanism in the molecular level, it should be the first step to know how many conformations exist and what kinds of structures they adopt. In the next step, the water molecule should be taken into account, because water can strongly interact with the neurotransmitters by intermolecular hydrogen bonds and it can influence their conformations. However, it is difficult to study the conformations in solution because many conformers cannot be distinguished due to the structural fluctuation and it is impossible to investigate each conformer individually. Gas phase spectroscopy, particularly, supersonic jet spectroscopy can be a very powerful tool to investigate their conformations because the fluctuation among several conformations can be frozen down and they can be distinguished as isomers by several double resonance laser spectroscopic techniques. In order to overcome a non-volatility of the neurotransmitters, a laser desorption method has been used instead of a general heating method. By measuring size-selected hydrated clusters of the neurotransmitters prepared by the laser desorption, we can investigate the solvent effects on the conformations step-by-step.

In this work, we studied adrenaline mono-hydrated cluster to understand hydration effect on the conformation by using laser desorption supersonic jet technique. Adrenaline is one of catecholamine neurotransmitters and the monomer has already been studied by Çarçabal et al.[1]. According to the former study, two conformers were identified by resonance enhanced multiphoton ionization (REMPI) and UV-UV hole burning (HB) spectra, and their structures were assigned by comparing observed and calculated IR spectra. However, some bands in REMPI spectrum were not found in any HB spectra, which indicate that some other conformers should exist than the observed ones. In addition, S/N ratio of the IR spectra was not high enough to assign their structures reliably. Thus we re-measured the adrenaline monomer by using high-sensitive and high-jet-cooling laser desorption source developed by ourselves. As a result, two more conformers were found and the structures of the total 4 conformers were assigned by IR dip spectroscopy together with quantum chemical calculations. To go to the second step, we expanded our scope to the hydrated adrenaline clusters. On the basis of structures of conformer-selected monomer, we investigated the hydration effect on the conformations.

[Experimental] In order to observe conformer selective UV spectra, we employed HB spectroscopy and the scheme is presented in Figure 1. The wavelength of the first UV laser (ν_P) was fixed at the transition of the target conformer and the second tunable UV laser (ν_B) was introduced 1 μs before the first UV laser to deplete the population of the target conformer. By scanning ν_B, the conformer-selected electronic spectrum can be measured as decreases of ion current signal. In order to measure conformer-selected IR spectra (IR dip spectra), the second UV laser was replaced by a tunable IR laser.

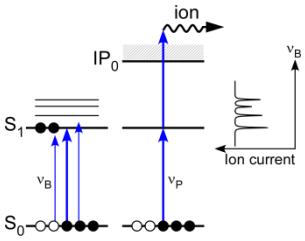

Fig.1 Scheme of HB spectroscopy

[Results and discussion] HB spectra of adrenaline $(H_2O)_1$ cluster measured by fixing the probe lasers at 35042 cm^{-1} for spectrum A and 34991 cm^{-1} for spectrum B are presented in Figure 2 together with REMPI spectrum of it. Because we found two different HB spectra, it clearly shows that at least two different conformers co-exist in the jet. We cannot exclude the possibility that more than two conformers exist because we could not probe very weak bands observed in the REMPI spectrum. However, it is also true that the population of the hydrated adrenaline are dominated by the two conformers.

In order to assign the observed spectra, it is necessary to explore possible conformations by using calculations. We tried to find several possible binding sites manually and optimized them by using density functional theory calculation (M06-2X/aug-cc-pVTZ level) and energetically favored three binding sites were selected (W1: catecholic OH – water, W2: OH in the chain – water, W3: OH in the chain – water – NH in the

chain). Four observed conformers in the former study of the adrenaline monomer were taken into account and the above mentioned three binding sites are considered together with them. In total, twelve conformers were chosen for the present study. The optimized structures of the twelve conformers are presented in Figure 3. In order to classify the geometry of adrenaline, we employed the similar notations used in synephrine in the former report.[2] Because synephrine has the same chain structure with adrenaline and includes phenol ring, it is possible to use similar notations for the chain structure (S-AG-OHN etc.). AG-OHN corresponds to the geometry of the chain and S in the head of the notation means the name of the molecule, synephrine. Thus, the notation for the molecular name should be changed from S to A (A-AG-OHN etc.). The way of the notations for the direction of the phenolic OH (c and t) is also changed because catechol ring has two more possible conformations (cR, cL, tR and tL). The observed four conformers of adrenaline were named PA-AG-OHN-cR and cL, A-AG-OHN-cR and A-GG-OHN-cR in accordance with the above mentioned notations.

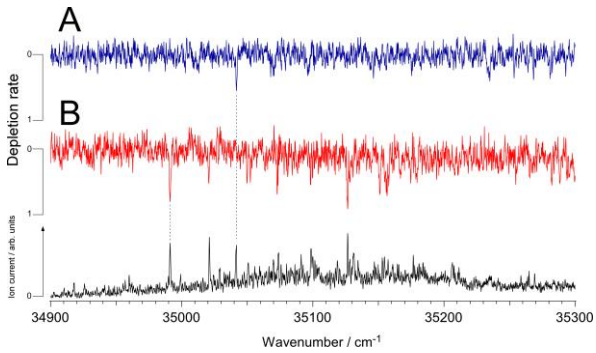

Fig.2 REMPI (lower) and HB spectra (upper) of adrenaline $(H_2O)_1$ cluster

Conformer-selected IR spectra were measured by fixing the probe laser at the same transitions used in the HB spectra and presented in Figure 4 together with calculated spectra obtained by the same level used in the conformational search. By comparing the observed IR spectra with the twelve simulated spectra, we assigned conformer A to A-GG-OHN-cR-W3 and conformer B to PA-AG-OHN-cR-W1. As can be seen in the figure, the water molecule is attached to the chain in conformer A (W3 form) and the geometry of the solute corresponds to A-GG-OHN-cR form as depicted in beside of the simulated spectrum. On the other hand, in conformer B, the water molecule is attached to the catecholic OH (W1 form) and the geometry of the solute corresponds to PA-AG-OHN-cR form. As can be seen in the figure, conformer A shows an interesting geometry, in which water is inserted between OH and NH in the chain. In order to generate this conformer, the intramolecular hydrogen bond should be broken and two intermolecular hydrogen bonds with water are made.

We found two main conformers of mono-hydrated adrenaline. Interestingly, the most stable conformer is also changed from PA-AG-OHN-cR to A-GG-OHN-cR by adding water. It suggests that conformations of adrenaline are changed by attaching the water molecule.

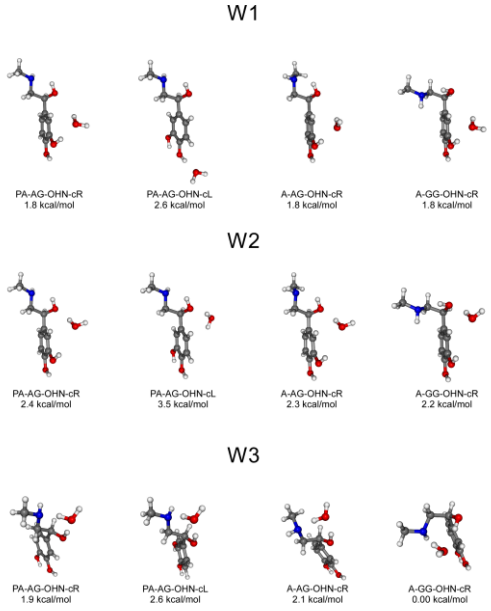

Fig.3 Twelve conformers of adrenaline $(H_2O)_1$ cluster

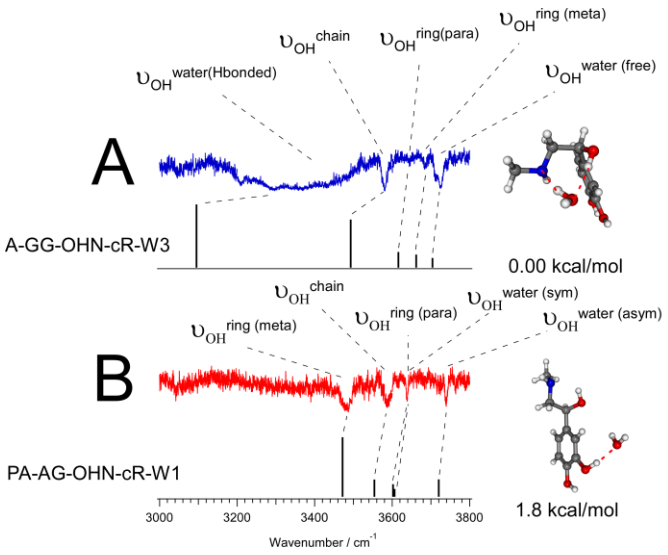

Fig.4 Conformer selective IR spectra of mono-hydrated adrenaline together with the simulated spectra

[References]

[1] P. Çarçabal, L.C. Snoek, T. Van Mourik, Mol. Phys. 103 (2005) 1633.
[2] S. Ishiuchi, T. Asakawa, H. Mitsuda, M. Miyazaki, S. Chakraborty, M. Fujii, J. Phys. Chem. A 115 (2011) 10363.