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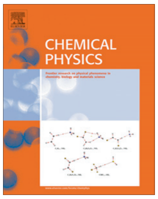

Stacked base-pair structures of adenine nucleosides stabilized by the formation of hydrogen-bonding network involving the two sugar groups

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ABSTRACT

We have employed a laser desorption technique combined with supersonic-jet cooling for producing base pairs of adenine nucleosides, adenosine (Ado) and N6,N6-dimethyladenosine (DMAdo) under low-temperature conditions. The resulting base pairs are then ionized through resonant two-photon ionization (R2PI) and analyzed by time-of-flight mass spectrometry. It is found that dimers of these adenine nucleosides are stable, especially in the case of DMAdo, with respect to those of the corresponding bases, i.e., adenine and N6,N6-dimethyladenine. Structural analysis of the DMAdo dimer is performed based on the IR–UV double resonance measurements and theoretical calculations. The result demonstrates that the dimer possesses a stacked structure being stabilized by the formation of hydrogen-bonding network involving the two sugar groups. The occurrence of the frequency shift and broadening is explained satisfactorily based on the anharmonic coupling of the OH stretching modes with specific bending modes and low-frequency modes of base and sugar moieties.

1. Introduction

The three-dimensional structure of DNA and RNA is determined largely by intermolecular associations between the nucleic acid bases, and the most important among them are hydrogen bonding and base stacking [1]. The former interaction can be considered as an electrostatic interaction, which leads to the formation of planar base-pair structures, as those of the Watson–Crick (WC) type. The base-stacking interaction is also recognized as crucial to the stabilization of nucleic acid structure, and occurs as a result of dispersion interactions between two adjacent bases linked by a sugar-phosphate group. Although a number of theoretical studies of base interactions have been carried out [2–10], there have been very few experimental studies determining the relative strength of the two contributions in the DNA and RNA [11].

Clusters of nucleic acid bases formed in the gas phase are expected to offer unique environments for elucidating their intermolecular association by separating stacking from hydrogen-bonding interactions. Experimentally, various dimeric species of the nucleic acid bases have been formed by utilizing supersonic-jet techniques and their structures have been examined by UV and IR spectroscopic methods, combined with quantum chemical calculations [12–21]. Most homodimers are found to possess planar hydrogen-bonded structures with a few exceptions of stacked structure; a heterodimer formed between adenine and 9-methyladenine is that of stacked structure [15]. Studies of guanine-cytosine [13,17,18] and adenine–thymine [16,19] dimers have shown that they also have planar base-pair structures. The hydrogen-bonded structure of the WC-type has been identified for the dimer of 9-ethylguanine and 1-methylcytosine, in which respective tautomerization pathways are blocked by the alkyl substitution [18]. Furthermore, a theoretical calculation has suggested that the WC base pairs undergo specific ultrafast excited-state decays to the ground, which prevent a sufficient amount of R2PI from the initially excited electronic states [22]. Numerous theoretical studies have been performed on the relative stability of hydrogen-bonded and stacked dimers of the nucleic acid bases [2–10]. Among them, it is particularly important to note that planar structures are generally more stable than stacked structures while for methylated bases, the stability of the latter structures dramatically increases [5].

In this work, we have studied the base-pair structure of adenine nucleoside in order to elucidate the effects of sugar substitution on the structural preference. In a previous study for dimers of bare adenine, a planar base-pair structure has been observed and assigned to that having two moieties doubly hydrogen bonded [15]. Here, we demonstrate that stacked structures are significantly stabilized for dimers of adenine nucleosides, in which the N9 hydrogen of adenine is substituted with a sugar group. Further-

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more, the preferential stabilization of stacked base pairing is found to be facilitated by the methylation of the amino group, i.e., DMAdo, in which the possibility of planar structures is substantially suppressed. The IR spectrum recorded for DMAdo dimer shows that the stacked structure is stabilized by the formation of hydrogen-bonding network involving the two sugar moieties. For guanine nucleosides, we have previously identified the existence of such networks formed via hydration to both base and sugar moieties [23,24].

The observed IR spectrum is characterized by three broad bands that can be associated with the OH stretching transitions of the sugar groups. In order to understand the IR spectrum, a high-level anharmonic vibrational structure calculation is performed by using the second-order vibrational quasi-degenerate perturbation theory (VQDPT2) based on the vibrational self-consistent field zeroth-order Hamiltonian [25]. The calculated IR frequencies are shown to be in good agreement with the experimental frequencies without scaling. The nature of the broad IR bands is also analyzed in terms of anharmonic mode–mode coupling of the OH stretching modes of the sugar groups with specific low-frequency vibrational modes.

2. Experimental and computational methods

The pulsed laser desorption source employed in this study has been described elsewhere [26]. The sample powder of Ado or DMAdo was mixed with graphite (10%) and pressed with a hydraulic press to make a pellet. The sample pellet was placed inside the source and irradiated by the second harmonics of a YAG laser. The plume of desorbed molecules was directed through a narrow channel (1 mm in diameter and 10 mm in length) with Ar carrier gas at 5 atm. The desorption laser was operated with typical fluences of <5 mJ/pulse, and focused to a spot size of 2 mm. The sample of DMAdo was synthesized from 2′,3′,5′-tri-*O*-acetylinosine according to the literature [27]. A tunable UV laser was employed to record the electronic spectra of mass-selected clusters by the R2PI method. The IR spectra were obtained in the frequency range 2850–3720 cm^{-1} based on the IR–UV double resonance scheme [23,24]. The IR beam (1–3 mJ/pulse) was provided with an OPO/OPA laser (LaserVision) pumped by a YAG laser.

Geometries of DMAdo dimer were optimized at the DFT/M06-2X level of theory with the 6-311G(d,p), and thereafter single-point calculations at the MP4(SDQ)/6-311G(d,p) level were carried out for the lower-energy structures to improve the accuracy of their energetic ordering. For relatively stable dimer geometries, harmonic vibrational frequencies were computed at the M06-2X/6-311G(d,p) level and scaled by a factor of 0.928. In this calculation, the 6-311++G(d,p) basis set was utilized for atoms (oxygen, nitrogen, and hydrogen) which are associated with the formation of hydrogen bonds.

Anharmonic vibrational structure calculations were carried out for the most stable dimer, in which the two adenine moieties are overlapped with each other (see Fig. 2a). In order to reduce the computational costs, this calculation was performed for Ado dimer with an analogous stacked geometry. Although the Ado dimer is still large, anharmonic vibrational analyses for such systems have recently become feasible by the method developed by one of the author. The potential energy surfaces (PES) were constructed by a multiresolution method [28] based on a combination of electronic structure methods (B3LYP, MP2, and MP4) with the cc-pVDZ and aug-cc-pVDZ basis sets. The level of electronic structure theory was deployed, depending upon the strength of mode coupling [29]. The vibrational energy levels and wave functions were obtained by VQDPT2 [25]. The details of the computational methods are described in the Supplementary data.

3. Results and discussion

3.1. Mass spectra of Ado and DMAdo

R2PI mass spectra recorded for the samples of Ado and DMAdo are compared in Fig. 1. The spectrum of Ado in Fig. 1a reveals mass peaks corresponding to dimers and larger clusters, with no discernible signal at the monomer mass channel. Additional mass peaks that can be assigned to the respective hydrates are observed though water vapor was not entrained in the Ar carrier gas. Furthermore, each mass peak is accompanied by a peak corresponding to its protonated species, and it is suggested that proton transfer reaction takes place upon R2PI. This mass spectral pattern is quite different from the results of previous reports, in which extensive fragmentation of Ado was found to occur upon laser desorption [30] or upon R2PI [31].

The DMAdo spectrum in Fig. 1b is dominated by a mass peak due to the dimer with a weak peak assignable to its monomer. Nevertheless, IR–UV double resonance measurements carried out by probing this ion signal reveal that it is produced upon fragmentation of the dimer following R2PI. Thus, R2PI signal associated with bare monomer is essentially absent in both spectra. This is in contrast to the cases of adenine [32] and N6,N6-dimethyladenine [26] where respective monomer peaks can be detected by the same R2PI method. Furthermore, the excited-state lifetimes of these adenine bases are found to be on the order of a few picoseconds when excited into the lower-energy region of the $\pi\pi^*$ state [32,33]. Therefore, the failure to observe monomer ion signal for both Ado and DMAdo using the nanosecond laser pulse (6 ns) suggests that their excited-state lifetimes are substantially shortened by the addition of the sugar group. This also implies that the lifetimes increase upon dimerization or cluster formation, presumably via excimer formation [34].

3.2. Stable structures of DMAdo dimer

Fig. 2 shows the two lowest-energy structures of DMAdo dimer, in which two DMAdo molecules are placed in parallel and anti-parallel with each other, respectively. In the parallel form of Fig. 2a, two adenine moieties are overlapped and their sugars are linked

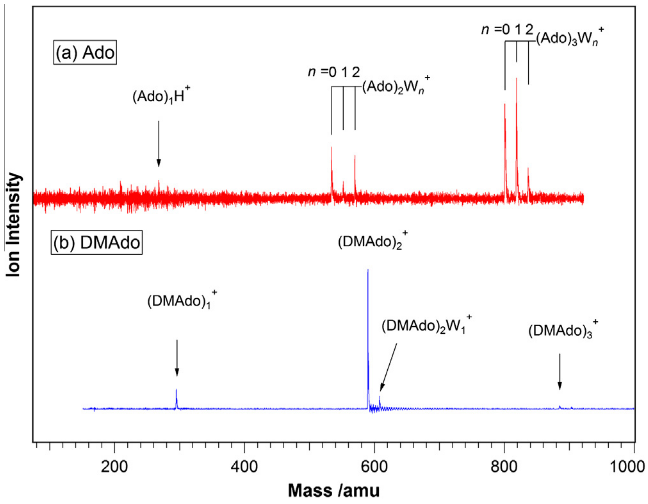

Fig. 1. R2PI mass spectra recorded following laser desorption for the samples of (a) Ado and (b) DMAdo. The UV excitation was carried out at 34188 cm^{-1}. Note that no peak is observed at the absent monomer mass channel in (a). The mass peaks corresponding to Ado clusters (dimer and trimer) and their hydrates are accompanied by those of respective protonated ions $[(\text{Ado})_m\text{W}_n\text{H}]^+$, thus appearing to be broad.