Excited-state dynamics of isolated nucleic acid bases and their clusters

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Abstract

The excited-state dynamics of the nucleic acid (NA) bases observed under isolated conditions is reviewed. The photophysical properties of the NA bases are very important for a mechanistic understanding of the UV photochemistry. These bases absorb strongly in the 200–300 nm region, but undergo fast internal conversion to the electronic ground state. The deactivation process is used to explain the apparent UV photochemical stability. The proposed mechanisms for the ultrafast decays are reviewed. Spectroscopic methods that are employed for isolation of the non-volatile bases in the gas phase and probing their excited-state dynamics are also summarized. Based on recent time-resolved spectroscopic results for the isolated NA bases, it is shown that the initial deactivation processes occur in the femtosecond range. To investigate the excited-state dynamics of the isolated NA bases, it is necessary to consider the existence of tautomers and structural isomers. Therefore, tautomerization and isomerization behavior of the individual bases and base pairs that can be obtained from analyses of their UV and IR spectra is discussed in detail.

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Keywords: Excited-state dynamics; Nucleic acid bases; Laser desorption

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1. Introduction

The photophysical and photochemical behavior of electronically excited states of nucleic acids (NA) is essential to understand the UV-induced damage to DNA [1]. When a DNA is irradiated by sunlight, the bases are the only nucleic acid components that can be electronically excited. However, they are apparently stable to photochemical reactions leading in principle to mutagenesis and carcinogenesis. The remarkable photostability of the DNA bases is usually ascribed to the ultrafast lifetime, which may be explained as being selected during a long period of molecular evolution. In fact, time-resolved spectroscopic studies of the isolated DNA bases in the condensed phase showed that their excited-state lifetimes are very short, on the time scale of subpicoseconds at room temperature [2,3]. The deactivation pathway of the NA bases could be affected by the sugar-phosphate backbone, hydrogen-bonding interaction, stacking interaction and hydration. As described below, the basic mechanism of the rapid excited-state relaxation has been the subject of controversy. The structures and ring numbering for the NA bases discussed in this review are shown in Fig. 1.

Experimental investigation of the excited-state dynamics of isolated NA bases in the condensed phase is complicated by several factors. Most of the studies were conducted in aqueous solution where it is not clear whether the short decay behavior is intrinsically intramolecular or it is due to the interaction with the solvent. It is likely that the photophysical behavior of DNA is dominated by the interplay of the sugar-phosphate backbone, base paring, and stacking interaction. Investigations of gas-phase clusters involving these bases will offer the possibility of separating these effects on the excited-state dynamics. Furthermore, upon isolation these bases can exist in a variety of tautomeric forms which are absent in DNA and RNA, and electronic structure and photophysical behavior can differ dramatically for individual tautomers [4]. For example, adenine (Ade) is known to exhibit a fluorescent tautomer in aqueous solution and it was identified as that of the 7H form (having hydrogen at the N7 position) [5]. It is therefore essential to determine what tautomers are actually present in a given experiment. When these bases are isolated in the gas phase, such tautomers will be separated and assigned by using relevant spectroscopic methods. It is also expected to reveal the role of the solvent in modifying the excited-state dynamics of the bases. More importantly, experimental results obtained in vacuo can be directly compared to theory.

2. Brief summary of condensed-phase results

A comprehensive review of excited-state dynamics of nucleic acids in condensed phase has been provided recently by Kohler and coworkers [3]. It covers recent photophysical results, especially those obtained by femtosecond laser spectroscopy.

The photophysical behavior of the natural bases is characterized by the very low fluorescence quantum yield $\Phi_F$, on the order of $10^{-4}$ in room temperature aqueous solution, as reviewed by Callis [4]. The weak fluorescence is a consequence of the shorter excited-state lifetimes, most of which are on the order of subpi-
coseconds [3]. However, tautomerism affects considerably the excited-state dynamics, which makes it difficult to obtain the decay lifetimes of individual tautomers. For example, a femtosecond transient absorption (TA) study [6] demonstrated that the amino-7H tautomer of Ade has a lifetime of ~8 ps, which is much longer than that of the natural amino-9H form (~0.2 ps). In this case, chemical substitution can be used to reduce the number of tautomers. Methylation of Ade at the N7 position, which blocks 9H–7H tautomerization, was found to increase the lifetime to 8 ps. This agrees well with the result obtained for 7H tautomer. The dramatic sensitivity of the excited-state lifetime to tautomerization and chemical substitution was ascribed to the proximity of the ππ* and ππ* excited states, leading to changes in coupling. A similar tautomerism was also inferred for guanine (Gua) in which ΦF increases upon methylation at the N7 position [5]. For other bases, tautomerism has not been extensively studied and only the canonical tautomers which are requisite for the Watson–Crick (WC) base pairing are believed to exist in aqueous solution.

Panzer et al. [7] measured the excited-state lifetimes of Ade and its ribonucleoside adenosine (Ado, Fig. 1) in aqueous solution by femtosecond fluorescence up-conversion (FU) spectroscopy. The biexponential decay curve observed for Ade was interpreted in terms of the presence of both 9H and 7H Ade tautomers. This result was found to be consistent with the observation that Ado exhibits only a short decay lifetime as short as 0.31 ps. They also found that the lifetime of the shorter decay component in Ade varies with the excitation energy, 0.67 ps at >269 nm and 0.34 ps at <260 nm. The sudden decrease in the fluorescence lifetime was interpreted as evidence for the opening of an additional decay pathway such as that involving a πσ* state. Non-exponential decay behavior was also observed for Thy in aqueous solution [8].

Solvent-assisted tautomerization from the 7H-enol to 7H-keto form of Gua was suggested to occur upon electronic excitation in bulk water [9]. Likewise, the low emission yields of Ade and Gua in aqueous solution were explained by excited-state tautomerization between the 9H and 7H forms [10,11]. Another study [12] showed that keto–enol tautomerization of Gua occurs with less efficiency in the excited singlet state.

Excited-state proton transfer in the DNA bases and base pairs is of particular interest because it may be involved in the mutation due to mispairing. Chou et al. [13] showed that a 1:1 complex formed between 9-cyclohexylmethyladenine and acetic acid in cyclohexane undergoes amino–imino double proton transfer upon electronic excitation. The complex was assigned to be of WC type in which acetic acid is incorporated at the N6 proton. Later, this reaction was suggested to occur from a complex of Hoogsteen type [14].

Effects of base pairing and stacking must also be considered in photophysical and photochemical behavior of UV-irradiated DNA. It is well known that pyrimidine dimers form upon UV radiation [1]. However, it appears to be difficult to study the effect of base pairing on the excited-state properties because isolated base pairs cannot be formed readily in condensed phases. These effects are normally investigated by using base assemblies in which two or more bases are covalently joined by phosphodiester linkages. For example, the fluorescence yield for double-stranded poly(deoxyguanylic–deoxycytidyllic acid), in which Gua–Cyt base pairs are expected to form between the strands, was found to be lower than for corresponding single-stranded species [15]. Base stacking effects can be examined using such base assemblies in which pairs of the bases are close enough to have substantial electronic coupling with one another. Many dinucleoside monophosphates were found to exhibit red-shifted emission with respect to the constituent mononucleotides. This observation was attributed to the formation of stacked excimer formed between the two neighboring bases in these species [16].

3. Experimental methods for studying excited-state dynamics of isolated bases and base clusters

3.1. Laser desorption

Supersonic jet spectroscopy can offer the opportunity of studying the intrinsic excited-state dynamics of the nucleic acid bases under isolated conditions. A major problem for applying this technique is that most of these molecules have very low vapor pressure and tend to decompose upon thermal heating. An attempt to entrain thermally vaporized uracil (Ura) into a supersonic jet was pioneered by Fujii et al. [17]. Although they reported well-resolved laser-induced fluorescence (LIF) spectrum which was assigned to that of Ura, the species was identified as an impurity formed upon heating [18]. Of all NA bases given in Fig. 1, Ade and its derivatives have been investigated extensively because they can be transferred into the gas phase by thermal heating. However, cytosine (Cyt) and Gua are not amenable to thermal vaporization without degradation. Pulsed laser desorption (LD) is an alternative means for isolating the NA bases in the gas phase. Moreover, this method can be easily incorporated into the supersonic jet technique for producing internally ultracold molecules, thus allowing for high resolution spectroscopy.

Li and Lubman [19] developed a pulsed LD technique to volatize various bases and their nucleosides with subsequent entrainment in a supersonic expansion. The LD is accomplished without using matrices, or with a glycerol matrix, by irradiating a sample deposited on a ceramic rod with pulsed CO2 laser. This type of LD source is referred to as open-type source. In this configuration, only partial rotational–vibrational cooling can be achieved. The desorbed neutral molecules are analyzed in a time-of-flight mass spectrometer by resonance-enhanced two-photon ionization (R2PI) at 266 nm. No fragmentation of the parent bases was observed when desorbed under mild ionization conditions. By increasing the ionization laser power, some fragmentation was found to occur. They also studied various nucleosides by this relatively soft ionization technique.

Nir et al. [20] succeeded in the formation of ultracold bases by LD and subsequent entrainment in a supersonic expansion, which enabled them to obtain well-resolved vibronic spectra of Gua and its base pairs by R2PI. The LD source is of open-type originally developed by Meijer et al. [21]. The sample consists of depositing neat powder material on a graphite surface, which
Fig. 2. Schematic diagram of the two LD sources: (a) open-type and (b) channel-type.

is placed directly under the orifice of a commercial pulsed valve (Fig. 2(a)). The LD is carried out at 1.06 μm by gradually moving the sample. This matrix-assisted LD method is also found to be effective in the isolation of base pairs involving Gua and Cyt [20], and some nucleosides of Gua [22]. A similar LD method was employed by Piuzzi et al. [23] for producing isolated Gua at a low temperature. In this method, the sample is mixed with a graphite matrix and vaporized at 532 nm. Although the combination of LD and jet-cooling techniques can be applied to the production of hydrated clusters of the NA bases, such reports are sparse. This is largely due to the characteristics of the open-type desorption sources that multiple collisions between desorbed molecules and solvent waters occur insufficiently to form larger hydrated clusters. Nevertheless, Crews et al. [24] were able to produce hydrated clusters of Gua by desorbing a thin layer of sample and ice from a graphite surface. A simple LD source called channel-type source (Fig. 2(b)) was developed in order to increase the hydration efficiency of desorbed molecules [25]. The principle of operation is similar to that of a standard laser vaporization source employed by Smalley [26]. The sample pellet is prepared by pressing a neat powder (i.e., matrix-free LD) of solid compounds in a hydraulic press. In some cases, the powder is mixed with graphite powder (5–30%) to increase the desorption efficiency (i.e., matrix-assisted LD) [27]. This LD is accomplished by pulsed laser at 532 nm onto the flat surface of the sample pellet through a quartz window. The window is used to hold the pressure inside the source, allowing for a greater number of collisions. The plume of desorbed molecules is directed through a narrow channel with a flow of argon gas, which quenches the desorbed molecules to condense into clusters. Efficient hydration was found to occur upon introduction of water by passing the carrier gas through a reservoir.

TOF mass spectra obtained for hydrated clusters of Gua generated by the two types of desorption sources are compared in Fig. 3. It is demonstrated that by using the channel-type desorption source, a variety of hydrated guanine clusters labeled \((\text{Gua})_m(\text{H}_2\text{O})_n\) are observed with higher abundances (Fig. 3(b)).

3.2. Structural characterization of tautomers and clusters

A major problem for studying the excited-state dynamics of isolated NA bases is the coexistence of several tautomers even at low temperatures. Double resonance spectroscopic methods are often employed for a separation of tautomers, and their vibrational and electronic spectra [28]. UV–UV hole-burning spectroscopy can be used to ascertain that a specific UV transition results from one isomer, whereas IR frequencies of each isomer are obtained by the IR–UV double resonance method. In the UV–UV double resonance method, UV radiation from the probe laser is fixed at the spectral feature of interest and the pump UV laser is scanned across the spectrum (Fig. 4(a)). When the pump laser transfers population out of the probe level, depletion of the probe signal (fluorescence or ion) is observed. Obviously, this scheme cannot be applied to the case where the selected vibronic band is overlapped with those of other species. The IR–UV method is an alternative approach to remove spectral congestion appearing in UV spectra. In this method, an IR laser is used to irradiate the species at its vibrational band in the ground electronic state while scanning the probe UV laser.

Fig. 3. Typical TOF mass spectra for hydrated Gua clusters \((\text{Gua})_m(\text{H}_2\text{O})_n\) generated with (a) open-type source and (b) channel-type source. In both cases, R2PI was carried out at 296.5 nm.

Fig. 4. Schemes for UV–UV and UV–IR double resonance spectroscopy.
Analyses of vibrational spectra provide qualitative structural information of the individual tautomers of the NA bases. In particular, vibrational frequencies of the O–H and N–H stretching modes are very sensitive to tautomerization and thus frequently used for structural assignment. In the case of the IR-UV double resonance method, the IR laser is scanned while recording dips in the probe UV signal (Fig. 4(b)). Comparison with the calculated IR frequencies allows for the identification of the tautomers. However, there are some vibrational modes which are less sensitive to tautomerization, which precludes unambiguous structural assignment. For example, it is difficult to distinguish between the 9H and 7H tautomers of the purine bases based on the N–H stretching frequency [29–31]. In addition, the frequency range currently available from conventional IR lasers is limited to the near IR region. The fingerprint region of IR spectra can be recorded by using free-electron lasers, which was demonstrated for the Gua–Cyt base pair [32].

3.3. Probing excited-state dynamics

Information on the excited-state dynamics of the NA bases can be obtained from pump-probe photoionization (PI) measurements. In this method also called two-color PI, excited-state species prepared by the pump laser are ionized by the probe laser. Mass-selected ion signal is obtained as a function of the time delay between the two laser pulses, thus giving rise to the time evolution of the excited-state population. Kang et al. [33] employed this technique in the femtosecond range to observe the transient ionization signal of the DNA bases prepared under effusive beam conditions. A similar PI measurement was carried out for Ade in the picosecond range [34]. Time-resolved photoelectron (PE) spectroscopy is also very powerful in studies of electronic relaxation dynamics of gas-phase molecules. Ullrich et al. [35,36] implemented this technique to probe the ultrafast excited-state decays of the DNA bases in the femtosecond range.

The time-resolved PI techniques can also be employed in the nanosecond range. With this method, long decay lifetimes of >10 ns were observed for jet-cooled thymine (Thy) and other pyrimidine bases [37]. Analogous nanosecond PI measurements suggested the existence of a long-lived excited state with a lifetime of 5 μs or longer for several purine bases [38]. It should therefore be reminded that different excited-state dynamics could be inferred depending on the time scale of the PI measurements.

Fluorescence detection is the most direct way of elucidating the excited-state dynamics of the DNA bases. Although the fluorescence yields are generally very low in condensed phases, some NA bases exhibit fluorescence under isolated conditions. For example, Kim et al. [39] were able to measure well-resolved LIF spectra of jet-cooled Ade. Evidence for the existence of different tautomers of Gua was obtained by LIF spectroscopy [23]. The fluorescence lifetimes were measured to be in the nanosecond range [23,40].

Dispersed fluorescence (DF) measurements also provide important information on the excited-state dynamics of the isolated bases. The DF spectra of Gua tautomers generated by LD revealed vibrationally resolved structure for lower energy tautomers [40]. For the highest energy isomer assigned to the 9H-enol form, only broad red-shifted emission was observed, which suggested the occurrence of a strong electronic coupling with a low-lying nπ* state.

4. Isolated NA bases

Essential aspects of studying the NA bases under isolated conditions are: (i) identification of various structural tautomers, (ii) characterization of the excited states (ππ*, nπ*, πσ*, triplet) involved in the deactivation process of each tautomer, and (iii) detailed comparison with theory and experiment. However, the theoretical investigations of these systems have outpaced the experiments so far.

Fig. 5. Proposed mechanisms for the excited-state deactivation of the NA bases: (a) proximity effect (according to Ref. [43]) and (b) conical intersection model (according to Ref. [46]).
4.1. Adenine and 2-aminopurine

The $\pi\pi^*$ transition of Ade (9H tautomer) was found to be the lowest one with the transition energy close to the first $\pi\pi^*$ transition [41,42]. The proximity between the two states will lead to frequency reduction of out-of-plane modes in the low-lying $\pi\pi^*$ state as a result of the vibronic coupling, as schematically shown in Fig. 5(a) [43]. This mechanism was used to explain the ultrafast internal conversion to the ground state. The canonical form (9H) was calculated to be the most stable tautomer [44].

Kim et al. [39] were the first to obtain well-resolved electronic spectra of thermally vaporized Ade in a supersonic jet by R2PI and LIF spectroscopy. The R2PI spectrum obtained with the LD method is given in Fig. 6. An intense transition at 36105 cm$^{-1}$ (around 277 nm) was assigned to the onset of the vibronic coupling, as shown in Fig. 5(a) [43]. This mechanism was used to explain the ultrafast internal conversion to the ground state. The canonical form (9H) was calculated to be the most stable tautomer [44].

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An analogous R2PI spectrum was observed for 9-methyladenine (m$^9$Ade) [34], as shown in Fig. 6, which supported that the $\pi\pi^*$ transition of Ade is that of the 9H tautomer. Another study [45] also revealed that the R2PI spectra of Ade and m$^9$Ade are very similar. Based on IR–UV double resonance measurements, the weak band at 35497 cm$^{-1}$ for Ade (band B in ref [39]) was assigned to that of the n$\pi^*$ state. In addition, the additional weak feature at 35824 cm$^{-1}$ (band B in ref [39]) was found to reveal an IR spectrum which differs from that of the $\pi\pi^*$ transition of the 9H tautomer [45]. This band was assigned to the 7H form of Ade based on comparison of the observed and calculated IR frequencies. Although 7-methyladenine (m$^7$Ade) is expected to give a similar electronic spectrum in this energy region, an attempt to measure its electronic spectrum was hampered by decomposition upon heating [45]. For these vibronic bands of Ade, no IR–UV double resonance signal was obtained even at zero time delay between the pump and probe lasers [45], indicating that the excited-state lifetimes are on the order of picoseconds.

Sobolewski and Domke [46,47] proposed that a very different type of excited state is involved in the nonradiative relaxation pathway of Ade (9H-keto form). The excited state is of $\pi\sigma^*$ type and associated with azine, amino, and enol groups. The $\pi\sigma^*$ state, nominally a 3s Rydberg state, is generally calculated to be dissociative and crossed not only by the $\pi\pi^*$ and n$\pi^*$ states but also by the ground state when the molecule is distorted along the stretching coordinate of the corresponding N–H and O–H bonds. The relevant potential energy surfaces are depicted in Fig. 5(b). This results in strong nonadiabatic interactions of the $\pi\pi^*$ and $\pi\sigma^*$ states and those of the $\pi\sigma^*$ and S$_0$ states, leading to ultrafast relaxation back to the ground state through the CIs. Later, they provided an alternative mechanism for the excess-energy dependent deactivation dynamics of the tautomer of Ade [48]. At lower excess energies of the initially excited $\pi\pi^*$ state, the deactivation process occurs via a CI with the n$\pi^*$ state, which can be accessed by out-of-plane deformation of the six-membered ring. As the excitation energy is increased, the hydrogen abstraction photochemistry via the repulsive $\pi\sigma^*$ state comes into play.

Direct evidence for the importance of the $\pi\sigma^*$ state in the deactivation process of Ade was obtained by detecting hydrogen atoms upon excitation into the broad, unstructured band region (243 nm) [49]. In order to identify the position for hydrogen atom detachment, analogous experiments were performed for m$^9$Ade and other methyl-substituted Ade compounds. The H-atom production yield was found to decrease substantially upon methylation, providing evidence for dissociation at the $\pi\sigma^*$ state. Time-resolved measurements on the formation of H-atoms were carried out to investigate the excited-state photochemistry and photochemistry of Ade and m$^9$Ade [50]. The result showed that dissociation of N9–H bond is responsible for the deactivation pathway in Ade, consistent with the scenario involving the $\pi\sigma^*$ state. It was found that some H-loss occurs on the methyl group of m$^9$Ade.

For Ade in the gas phase, a single-exponential decay lifetime of 1.0 ps at 267 nm, which was assigned to internal conversion to the ground state via the low-lying n$\pi^*$ [33]. Its mechanism was elucidated in terms of the effects of methyl-substitution on the excited-state decay. The lifetimes of these Ade derivatives were found to be nearly identical to that of Ade, which suggested that the n$\pi^*$ state, rather than the $\pi\sigma^*$ state, is responsible for the ultrafast decay. If the latter state were the dark state, the methyl-substitution at the N–H positions would affect the decay pathway by eliminating the stretching coordinates that could couple the $\pi\sigma^*$ state to the ground state. Likewise, the absence of noticeable deuterium isotope effects on the lifetimes was rationalized by this mechanism. The excited-state lifetime of Ade was also obtained following excitation at the $\pi\pi^*$ origin (277 nm, Fig. 6) by a picosecond PI technique [34]. The longer lifetime of 9 ps decay with respect to that observed at 267 nm [33] is consistent with the observation of the narrow absorption band. These lifetimes and others described in this review are summarized in Table 1.
A similar PI measurement was carried out to observe the excited-state dynamics of Ade and its methyl derivatives at an increased time resolution [51]. Two decay components with an ultrafast lifetime of 100 fs and a slow lifetime of 1.10 ps were observed for Ade excited at 267 nm. The lifetime of the fast component was found to increase significantly upon substitution of the amino group hydrogen atoms by the methyl groups, supporting that the early decay dynamics is facilitated by its inversion motion. This nuclear motion was explained to promote a state switch between the ππ* and nπ* states followed by internal conversion to the ground state. Another femtosecond PI measurement for Ade also demonstrated that the excited-state dynamics of Ade and its methyl derivatives at an excitation wavelength of 267 nm decays with two components of 100 fs and 1 ps, corresponding to the decays of the ππ* and nπ* states [52].

Time-resolved PE measurements were carried out to probe the deactivation pathway of the ππ* state in Ade [35,36]. Excitation into the ππ* band origin region was found to give lifetimes of several picoseconds. At higher excitation energies at 267 and 250 nm (the diffuse band region in Ref. [41]), the signal revealed two decays of an ultrafast lifetime of <50 fs and a slow lifetime of 0.75 ps. The former was assigned to internal conversion to the π0 state while the latter as the decay of the ππ* state.

The R2PI spectrum of N6,N6-dimethyladenine (mN6,N6 Ade) displayed in Fig. 6 appears to be red-shifted with respect to that of Ade. In contrast to Ade, it exhibits a low-frequency progression which is apparently similar to those of p-dimethylaminobenzonitrile (DMABN) and dimethylaniline [53]. This suggests that the progression arises from torsional motions of the dimethylamino group [54]. It is known that alkylation of the amino group of Ade results in an enhancement of its fluorescence yield in solution [55]. For mN6,N6 Ade and other alkylamino derivatives of Ade, dual fluorescence of distinctly separated bands is observed in solvents of different polarity [56]. The similarity of the fluorescence behavior to that of DMABN [57] suggests that intramolecular charge transfer occurs upon electronic excitation.

As compared to Ade, its isomer 2-aminopurine (2AP, see Fig. 1) exhibits a much longer excited-state lifetime and a larger fluorescence yield in aqueous solution. Thus, this base analogue is well suited to be incorporated as a fluorescent probe in DNA. As can be seen in Fig. 6, the first singlet ππ* absorption transition of 2AP is significantly red-shifted with respect to that of Ade. This observation suggests that the ππ* state is lower in this case as predicted by Broo [41], which is consistent with the enhanced fluorescence yield. However, a more recent calculation [31] has shown that the two states are almost degenerate. The R2PI spectrum of thermally vaporized, jet-cooled 2AP was assigned to that of the amino-9H form [31]. In contrast, a matrix IR study indicated that small amounts of 2AP are present as the 7H form although the majority is in the 9H form [58].

### 4.2. Guanine

The other purine base Gua has been investigated extensively in terms of keto–enol and 7H–9H tautomerism. Most calculations [59–61] indicate that there are four stable tautomeric forms which are depicted in Fig. 7. The most stable one is calculated to be of the 7H-keto form. In contrast to Ade, the first excited singlet state was found to be due to the ππ* state [62] while the second excited state was assigned to the nπ* state [63].

Nir et al. [20] reported the first vibrationally resolved electronic spectrum of jet-cooled Gua. This observation suggests that the excited-state lifetimes should not be shorter than a few picoseconds at lower excess energies. They also showed that the spectrum is composed of bands from three different tautomers based on UV–UV hole-burning and IR–UV double resonance measurements [29]. The R2PI spectrum of Gua obtained by using the channel-type source is shown in Fig. 8 (bottom spec-
The red-most band at 304.2 nm (band A) was assigned to the electronic origin of the 9H-enol tautomer [29], while the spectra built on the band origins at 0 + 404 cm\(^{-1}\) (band B) and 0 + 1044 cm\(^{-1}\) (band C) were assigned to the 9H-keto and 7H-keto forms, respectively.

Evidence for the existence of a fourth tautomer was provided by Mons and coworkers [30]. The electronic origin is located at +1891 cm\(^{-1}\) (band D) with respect to that of the red-most tautomer (band A). Based on IR–UV double resonance measurements and comparison with the results for methyl-substituted Gua, this tautomer was identified as the 9H-enol form. As opposed to the assignment of Nir et al. [29], the lowest excitation energy isomer A was associated with the 7H-enol form. Furthermore, the other isomers B and C were assigned to the 7H-keto and 9H-keto forms, respectively. This discrepancy apparently indicates that assignment of 7H–9H tautomerism based on IR–UV measurements is rather difficult.

Additional experiments were performed by using methylated species in which a particular tautomerism can be blocked. The R2PI spectra of the four methyl-derivatives, 1-methylguanine (m\(^1\)Gua), 6-methylguanine (m\(^{6}\)Gua), 7-methylguanine (m\(^7\)Gua), and 9-methylguanine (m\(^9\)Gua), are also shown in Fig. 8. For m\(^9\)Gua, it was found that it exists only as the enol form [64]. Furthermore, the methyl-substitution leads to a dramatic change in the electronic spectrum, which shows only a few spectral features with less Franck–Condon activity [30]. A similar spectrum was observed for the tautomer D of Gua and assigned to the 9H form. The origin of this tautomer appears to be significantly blue-shifted with respect to the other tautomers, and located outside of the UVB region (290–320 nm) of sunlight. This so-called 9\(-\)methylene effect was taken as evidence for the occurrence of characteristic excited-state dynamics only for the biologically relevant species. One possible reason for the absence of m\(^9\)Gua of the keto form is that its excited state is short-lived, resulting in less efficient ionization [62]. However, another theoretical study showed no indication of such dramatic effects [63]. Substantial blue shift of electronic spectra upon 9-

substitution was also observed for the more biologically relevant guanosine (Gua) and 2\(-\)deoxyguanosine [22,65], which can be seen in Fig. 8.

Choi and Miller [66] reported that Gua can be isolated in helium nanodroplets without noticeable decomposition. In this method, the sample was heated to 350 °C to vaporize in a pickup cell and deposited on a droplet. They identified the four stable tautomers (7H-keto, 9H-keto, and two rotational isomers of 9H-enol) based on high-resolution IR measurements. In contrast to the previous results obtained with LD, they could not observe the 7H-enol tautomer which is calculated to be the least stable one among the four tautomers in Fig. 7 [60]. The result was used to suggest that the possibility of decomposition products or the presence of other higher energy isomers generated upon LD.

Based on this new assignment, the tautomers B and C generated by LD was re-assigned to less stable imino forms [67]. More importantly, the failure to observe the amino–keto tautomers by R2PI was taken as an indication of a tautomer-dependent excited-state dynamical process. This assignment is also consistent with the observation of the long-lived fluorescence for the isomers A–C. Another evidence for the formation of rare tautomers upon LD can be seen in the electronic spectrum of Gua obtained the channel-type LD source (Fig. 8); the peaks corresponding to the tautomers B and C are nearly absent. This observation suggests that the tautomers B and C are less stable than A.

The excited-state dynamics of laser desorbed Gua was investigated by the fluorescence spectroscopy [40]. Fluorescence lifetimes were found to be 12, 22, 25, and 17 ns for the origins of the tautomers A, B, C, and D in Fig. 8, respectively. For the isomers A–C, resolved fluorescence bands superimposed on broad red-shifted spectra were observed in the DF spectra. In contrast, the isomer D exhibits only broad structureless features. The tautomer dependent fluorescence behavior was explained in terms of electronic coupling between the initially excited \(\pi\pi^*\) state and the low-lying \(\pi\sigma^*\) state. As listed in Table 1, a single exponential lifetime of 0.8 ps was obtained at 267 nm for an effusive beam Gua and assigned to the decay to the low-lying \(\pi\sigma^*\) state [33]. When a similar method was carried out with a higher time
resolution, two decay components with lifetimes of 148 fs and 0.36 ps were observed at the same excitation wavelength [51].

Excited-state dynamics of Gua in the 7H-enol form were investigated theoretically by Langer et al. [9]. The result pointed to the π π* scenario [46]. The initially excited π π* state undergoes transition to a π σ* state associated with the hydroxyl OH bond, leading either to hydrogen detachment or to internal conversion through a CI between the π σ* and S0 states. Deactivation pathways for the 9H-keto form of Gua were explored by the CAST2/CASSCF method [68]. The most probable relaxation was shown to occur to the minimum of the initially excited π π* state, followed by the decay to the ground state via a π π*/S0 CI with a small activation barrier. The result was found to be consistent with the experimentally observed decay behavior of Gua in the gas phase [51].

4.3. Pyrimidine bases

Nir et al. [69] reported well-resolved R2PI spectra for Cyt and its methylated compounds generated by the LD method described in Section 3.1. Two tautomeric forms, one keto and one enol were identified by UV–UV double resonance spectroscopy. They also employed two-color PI technique to measure the excited-state lifetimes for these bases. The result showed that the initially excited-state decays to a long-lived state having a lifetime of >300 ns, as opposed to the ultrafast decay lifetimes of 1.0 ps in solution [70] and 3.2 ps in the vapor phase [33]. This discrepancy may be explained by an assumption that the decay curve is characterized by a double exponential function and only the fast decay component is probed in the femtosecond measurements. The nanosecond result suggested that a triplet excited state populated by intersystem crossing is responsible for the long lifetime.

The R2PI spectra of Thy and Thy appear to be broad and diffuse even under extensively jet-cooled conditions [18]. The broadening was attributed either to mixing of electronic states or to a large geometry change upon excitation. Two-color PI measurements for Thy and methyl-substituted Ura (1,3-dimethyluracil) showed that the excited-state lifetime varies from tens to hundreds of nanoseconds, depending strongly on both the excitation and ionization wavelengths [37,71]. It was speculated that the initially excited-state decays rapidly to a long-lived dark state, assumed to be a low-lying π π* from which internal conversion occurs to the ground state. Therefore, much higher energies are required for ionization from the dark state.

The decay curve obtained for Thy using the femtosecond PI spectroscopy was found to be of double-exponential character, with a short component of 6.4 ps and a long one of >100 ps [33]. The slow component was attributed to a triplet state while the ultrafast decay of the initially excited state was interpreted by internal conversion to the ground state via the low-lying π π* state. A more recent PI measurement yielded two decay components, 105 fs and 5.1 ps, which were explained by a stepwise deactivation pathway [51]. The first step corresponds to relaxation driven by out-of-plane vibrations while the second step is to state switch between the π π* and nπ* states. Another PI measurement also indicated two decay components with lifetimes of 130 fs and 6.5 ps [52]. They were assigned to the decays of the initially excited π π* state and the low-lying nπ* state, respectively. The absence of π σ* signal was explained by the relative energy with respect to the nπ* state. The results of time-resolved PE measurements for the pyrimidine bases indicated two ultrafast decay components followed by a picosecond decay [36].

In the case of Cyt, a theoretical study [72] showed that the proximity of the lowest excited π π* state and the closely lying n0 π* state (excitation from the oxygen lone pair) is responsible for the ultrafast decay. It was explained that the two states undergo a switch through a CI followed by an n0 π*−S0 CI. A similar calculation suggested that the nonradiative decay of Cyt occurs via only one CI between the π π* and S0 states [73] or via a three-state CI [74]. The latter mechanism was also invoked for Ura and Ade [75]. Another study on Cyt [76] found the n0 π* state to be lower in energy than π π* state, suggesting the existence of CIs between the S1(n0 π*) and S2(π π*) states and between the S1(n0 π*) and S0 states.

5. Isolated base pairs

The WC base pairing in DNA occurs between the canonical forms of the NA bases, i.e., keto–amino tautomers. However, as shown in the previous section, each NA base can exist as several tautomers upon isolation in the gas phase. It is therefore essential to characterize the structures of isolated base pairs before elucidating their excited-state dynamics. This will enable us to understand the photophysical and photochemical processes relevant for the WC structure.

5.1. Adenine, thymine, and adenine–thymine

The first observation of a base pair of Ade was reported first by Plützer et al. [77]. Its structure was found to be different from the most stable hydrogen-bonded base pair. They showed that the vibronic spectrum of the dimer is also obtained by monitoring protonated Ade ions (Ade + H)+ which appear in addition to the parent mass. For m9Adm9Ad− heterodimer, its vibronic spectrum was observed exclusively at the (m9Ad+H)+ mass. Comparison of the IR spectrum with the calculated vibrational frequencies suggested that it possesses a stacked structure [78]. Later, it was shown that the observed protonated ions AdeH+ originate from AdeH+ radicals which are produced upon photodissociation of the neutral dimer [49]. This observation was regarded as evidence for the existence of a dissociative π σ* state.

The deactivation pathway in Ade and Thy dimers was probed by femtosecond PI spectroscopy [52,79]. The result for Ade dimer suggested the presence of another decay channel in competition with the π π* → n π* relaxation. The new channel was assigned to the π π* → π σ* relaxation and explained by substantial stabilization of the π σ* state upon dimer formation. The importance of the π σ* state in the photoinduced process of Ade dimer was supported by ab initio calculations [79]. In contrast, no evidence of such ultrafast relaxation was observed for Thy dimer [52].
Fig. 9. R2PI spectra of (Gua)2, (Gua)2(H2O)1, (Gua)2(H2O)2, and (Gua)1(H2O)1 recorded around 300 nm. All spectra were recorded using the channel-type. Two enol isomers for (Gua)1(H2O)1 are indicated as enol-1 and enol-2 according to Ref. [24].

The R2PI spectrum of an Ade–Thy dimer shows sharp features built on the prominent peak at 285.2 nm [80], which can be associated with local excitation of the Ade moiety. Its structure was assigned to be of non-WC type based on the IR–UV double resonance measurement. The Ade–Thy base pair was found to show similar ultrafast excited-state dynamics to those of the Ade and Thy homodimers when excited at 272 nm [52].

5.2. Guanine

Gua was found to form strongly stable dimers, as demonstrated first by Nir et al. [81]. The R2PI spectrum shown in Fig. 9 (bottom spectrum) reveals the existence of two structural isomers (denoted isomers 1 and 2) around 300 nm. Interestingly, both isomers were identified as hydrogen-bonded dimers consisting of two different keto tautomers, 7H-keto and 9H-keto, as derived from IR–UV double resonance measurements. The proposed dimer structures, which are given in Fig. 10, were found to be consistent with the results for methyl-substituted compounds [81]. However, the base pair having symmetric hydrogen bonding between the 9H-keto tautomers, which is calculated to be most stable (Fig. 10), or any other dimers involving enol tautomers could not be observed. It is also possible to explain that these dimers are composed of rare tautomers as explained above.

Although stacking interactions between the bases are known to play a role in the stabilization of the DNA structure, they are less well understood than the hydrogen bonding interactions. Calculations suggest that electron correlation is important in stabilizing stacks of the NA bases [82,83]. Although no spectroscopic evidence for stacked structure has been reported, one possible candidate is the observation of broad vibronic bands for dimers of methylated Gua [27]. In this case, vibronic spectra may exhibit broad features due to resonance interaction between the two chromophore planes. Broad excitation spectra arising from stacking interactions and excimer formation are commonly observed for dimers of aromatic molecules (e.g., naphthalene [84] and anthracene [85]).

5.3. Cytosine

Dimers of Cyt and its methyl derivatives were found to exhibit vibronic spectra with sharp features around 300 nm [69,86]. Only one isomer was observed for the Cyt dimer and Cyt-1-methylcytosine (m1Cyt) mixed dimer. IR–UV double resonance measurements showed that both isomers possess asymmetric dimer structures with both moieties in the keto form [86]. Analogous to the observation for Gua dimer, the most stable structure, which is calculated to have a symmetric hydrogen bonding, was not observed in this spectral region. Based on this finding, they suggested a possibility of ultrafast deactivation process for the symmetric dimer. In contrast, dimers of 5-methylcytosine (m5Cyt) dimer and Cyt–m5Cyt dimer were found to reveal three isomers, one of which being assigned to the symmetric hydrogen-bonded structure.

5.4. Guanine–cytosine

A number of theoretical studies have been performed for Gua–Cyt base pairs [87–91]. Experimentally, two structurally different isomers were identified for the Gua–Cyt pair [92,93]. The R2PI spectra were found to appear in the two spectral regions of 300 and 305 nm. The isomer appearing around 300 nm (isomer C in Ref. [93]) was assigned to a structure which is similar to the WC-type having a triple hydrogen-bonded structure but with the Cyt moiety as the enol form [92]. Further IR–UV double resonance experiments in the mid-IR region indicated a non-WC structure [32]. The isomer located around 305 nm (isomer B in Ref. [93]) was also assigned to a non-WC structure. In contrast, a dimer of 9-ethylguanine and m1Cyt was assigned to a base pair of the WC-type structure [93]. Based on the observation of
the broad vibronic spectrum, it was suggested that the excited-state lifetime of this base pair is on the order of $10^{-14}$ s. Similar spectral broadening was found for dimers of Gua–cytidine (Cyd) and Guo–Cyd and ascribed to a rapid internal conversion to the ground state.

Several theoretical results on the photophysical behavior of the WC base pairs were reported. Sobolewski and Domcke\cite{94} suggested the existence of a CI between the excited and ground state potential energy surfaces for the Gua–Cyt pair. This CI was inferred to occur by proton transfer from Gua to Cyt and subsequent out-of-plane deformation of Cyt in the excited state, leading to ultrafast excited-state deactivation. The Gua–Cyt base pair was found to be strongly nonplanar in the excited state and its nonplanarity was located at the excited-state Gua monomer\cite{95}. Excited-state properties of the Ade–Ura base pair were investigated by a theoretical calculation\cite{96}.

6. Hydrated clusters of NA bases

The DNA bases are surrounded by a shell of tightly bound water molecules with properties significantly different from those of bulk water. Single-crystal structural studies using X-ray and neutron diffraction have revealed ordered hydration pattern around the bases of DNA fragments.\cite{97–99} The presence of such specific hydration sites is considered to be essential to the nucleic acid structures. However, the precise nature of the interaction between the DNA bases and their hydration structures in the absence of the DNA backbone structure has not been well understood. It is also important to know how hydration affects the excited-state dynamics of the NA bases and base pairs.

6.1. Adenine and 2-aminopurine

Hydrated clusters of Ade can be prepared readily by thermal vaporization of the sample and subsequent jet-cooling. Kim et al.\cite{100} employed electron impact ionization to observe hydrated clusters of the DNA bases produced by supersonic expansion. Later, a striking anomaly was found in the mass spectrum of Ade generated upon R2PI, in which hydrated clusters of the Ade monomer (Ade)$_n$(H$_2$O)$_n$ ($n \geq 1$) were nearly absent\cite{101}. This behavior was ascribed to ultrafast relaxation into the low-lying $\pi\pi^*$ state and subsequent evaporation of all water molecules on the repulsive potential surface. Consistent with this interpretation, normal hydration pattern was obtained for the mass spectrum when excited by a femtosecond laser pulse\cite{101,102}. The lifetime of the initially excited state was measured to be 230 fs for (Ade)$_1$(H$_2$O)$_1$ and 210 fs for (Ade)$_2$(H$_2$O)$_2$\cite{102}. This dramatic decrease in lifetime was used to interpret the observation of different mass spectral pattern in going from nanosecond to femtosecond excitation.

For cluster hydrates of Ade [(Ade)$_m$(H$_2$O)$_n$ ($m \geq 2$)], it was explained that initial electronic excitation is localized on one adenine moiety and energy transfer to other chromophores is slow with respect to evaporation of water molecules\cite{101}. Thus, only the water molecules directly attached to the initially excited chromophore are subjected to evaporation. Another possible explanation for the normal hydration pattern of the cluster hydrates is that excimer formation takes place rapidly between a pair of chromophores and the excitation energy is trapped on the tightly bound dimeric state. If such excimer state is stabilized by a strong resonance interaction and located lower than the $\pi\pi^*$ state, then no dissociation is expected to occur for cluster hydrates.

Analogous anomaly in mass spectral pattern was observed for N6,9-dimethyladenine (m$^N$6,9Ade)\cite{54}, which is shown in Fig. 11(a). Mass peaks corresponding to monomer hydrates (m$^N$6,9Ade)$_1$(H$_2$O)$_n$ appear to be weak with respect to the cluster hydrates (m$^N$6,9Ade)$_m$(H$_2$O)$_n$ ($m \geq 2$). The result suggests that the $\pi\pi^*$ state associated with the N9–H bond is not responsible for the ultrafast excited-state dynamics. The ion signal due to the monomer hydrates of m$^N$6,9Ade can be observed as the excitation energy increases, as shown in Fig. 12. The excitation energy dependence is consistent with the observation for Ade\cite{101}. This result implies that excitation to higher electronic states does not lead to dissociation.
Femtosecond time-resolved PI measurements were carried out at a better time resolution to probe the deactivation pathway for hydrated Ade clusters \((\text{Ade})_n(\text{H}_2\text{O})_m (n=1\sim3)\) produced by supersonic expansions [79]. The measured lifetimes were 80, 70, and 60 fs for the mono-, di-, and trihydrated complexes, respectively, and assigned to the decay from the \(\pi\pi^*\) state to the \(\pi\sigma^*\) state. A theoretical calculation supported that the \(\pi\sigma^*\) state of Ade is stabilized substantially upon hydration [79].

Hydration behavior for laser-desorbed 2AP was investigated by the channel-type source. A typical mass spectrum exhibiting hydrated clusters \((2\text{AP})_n(\text{H}_2\text{O})_m\) is shown Fig. 11(b). It can be seen that the intense ion signal for hydrated clusters involving one monomer \((2\text{AP})_1(\text{H}_2\text{O})_n\) \((n=1\sim10)\) dominates [54]. This normal hydration pattern is very different from that of Ade, and ascribed to the presumption that the \(\pi\pi^*\) state is the lowest excited state. Ramaekers et al. [58] studied hydrated clusters of 2AP by matrix FT-IR spectroscopy and quantum chemical calculations. They identified four structural isomers for the monohydrate species. The most stable isomer was attributed to the amino-9H structure hydrogen-bonded water with a closed form N3-···H-O-···H-N9.

6.2. Guanine

The mass spectra obtained for hydrated clusters of Gua in Fig. 3(b) reveal that peaks due to monomer hydrates \((\text{Gua})_1(\text{H}_2\text{O})_n\) are nearly absent. This anomaly is apparently similar to that observed for Ade described above, which was explained by an ultrafast excited-state decay [25]. It turned out that this hydration behavior is associated with the formation of the dimers and larger clusters [27]. As described in Section 5.2, the strongly bound base pairs form between the keto tautomers, resulting in a low abundance of these monomer species. This is consistent with the observation that normal hydration behavior for the methyl-substituted Guas \((\text{m}^1\text{Gua}, \text{m}^6\text{Gua}, \text{m}^7\text{Gua}, \text{m}^9\text{Gua})\). No strongly bound base pairs are found to form for these methylated compounds. This in turn leads to the efficient hydration around the monomers.

The R2PI spectrum of monohydrate \((\text{Gua})_1(\text{H}_2\text{O})_1\) around 300 nm is shown in Fig. 9. The results of UV–UV and UV–IR double resonance measurements showed that the spectrum is dominated by isomers involving enol tautomers (labeled enol-1 and enol-2) [24]. Piuzzi et al. [23] also obtained similar spectra for this monohydrate and identified three conformational isomers. Possible structures for mono- and dihydrates of guanine were theoretically investigated for various tautomers [60], which suggested that the 7H-keto tautomer is the global minimum in the presence of the water molecule(s). The R2PI spectrum of \(\text{m}^9\text{Gua}\) with a single water molecule was found to be of an enol tautomer bound by water [64]. A theoretical calculation on the monohydrated cluster of Gua suggested that hydrogen transfer to the water molecule occurs along the OH dissociation coordinate, which is accompanied by the formation of a radical pair of Gua and \(\text{H}_2\text{O}\) [9].

The R2PI spectra for the mono- and dihydrates of the Gua dimer were reported [103]. In order to prepare these hydrates, a mixture of Gua and ice was employed for LD. Analogous spectra are obtained by the channel-type LD source, as shown in Fig. 9. The hydration structures were identified based on IR–UV double resonance measurements, which suggested that the water molecule(s) are attached to the isomer 1 of the base pair discussed in Section 5.2.

6.3. Pyrimidine bases

Studies on hydrated clusters of the pyrimidine bases are very limited. It was found that hydrated clusters of Thy monomer revealed excitation energy dependence of the mass spectral pattern which is similar to that observed for Ade [71]. The lifetime of the monohydrate cluster \((\text{Thy})_1(\text{H}_2\text{O})_1\) was found to be shorter than that of bare Thy \((12\text{ ns versus }22\text{ ns})\) by using the two-color R2PI method in the nanosecond range. Based on the reduced lifetime upon hydration, they concluded that the photo-stability of the pyrimidine bases is not an intrinsic property. For Ura, a calculation suggested that hydration causes a significant blue shift of \(\pi\pi^*\) states while leaving \(\pi\sigma^*\) states nearly unaffected [104].

7. Summary

In this review, the photophysical and photochemical properties of the NA bases observed under isolated conditions have been described in detail. The nanosecond laser spectroscopic studies show that several isomers exist for individual bases and base pairs when isolated in the gas phase. The vibronic spectra of these species reveal well-resolved spectral features at lower excitation energies, suggesting that the excited-state dynamics are not ultrafast. Some of the isomers are identified as the biologically irrelevant species which do not exist in the biological environment. This observation may be used to rationalize the possible ultrafast excited-state lifetimes associated with the biologically relevant species.

The excited-state dynamics of the NA bases appear to be strongly excitation energy dependent. The existence of ultrafast deactivation pathways, which are on the order of \(\approx\)100 fs at higher excitation energy energies, has been demonstrated by the femtosecond time-resolved studies. However, it is not clear what tautomers are actually probed by the time-resolved spectroscopy or whether the observed ultrafast dynamics are ascribed to those of the biologically relevant species. The basic mechanism for the deactivation process of the NA bases is still a controversial issue and has to be examined more extensively. Evidence for the formation of the WC base pairs has been obtained based on the nanosecond laser spectroscopic study, and the excited-state dynamics need to be examined by the time-resolved spectroscopy. It is also important to elucidate how hydration affects to the structure and excited-state dynamics of the NA bases and base pairs.

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