Structure Determination

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Nucleic Acid Bases in the Gas Phase**

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electron diffraction · laser ablation · nucleobases · rotational spectroscopy · supersonic expansion · tautomerism

Spectacular results have been obtained on the conformational/tautomeric properties of isolated biomolecules with the development and application of the following techniques: 1) combinations of two-color resonant two-photon ionization (2CR2PI), laser-induced fluorescence excitation (LIF), resonant ion-dip IR spectroscopy (RIDIRS), fluorescence-dip IR spectroscopy (FDIRS), and UV–UV hole-burning spectroscopy;[1] 2) rotationally resolved laser-induced fluorescence;[2] 3) a combination of mass-selected, conformer-specific UV and IR double-resonance hole-burning spectroscopy and ab initio quantum-chemical calculations;[3] 4) IR–UV double-resonance spectroscopy;[4,5] 5) chiral recognition by mass spectrometry;[6] 6) IR–UV hole-burning spectroscopy.[7] Helium nanodroplets have been used as a medium to investigate the vibrational spectra of biological molecules,[8] as this technique leads to simplification of the spectra; however, this oversimplification results in a loss of spectral content. Rotational spectroscopy was initially dedicated to the investigation of small molecules, even though quite broad studies of biomolecules have been performed on the basis of free-jet millimeter-wave (mmw) absorption.[9,10] More recently, the laser ablation technique has been combined with Fourier transform microwave (FTMW) spectroscopy[11] to unravel the conformational equilibria of several amino acids,[12] or to study the conformational/tautomeric forms of some nucleobases.[13,14] In parallel, electron diffraction (ED) studies of uracil,[15] cytosine,[16] and thymine[17] have been reported. For these studies, the nucleobases were vaporized by heating.

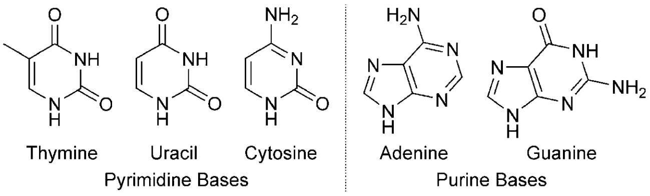
Microwave (MW) and ED techniques supply the most useful information on molecular structure and shape. However, large molecules, and in particular those of biological importance, readily undergo thermal reactions and degradation, which make them unsuitable for detailed structural and dynamic studies in interaction-free environments outside the condensed phase.

In two recent studies concerning tautomeric/conformational equilibria in nucleobases, the problem of thermal instability upon vaporization was overcome through the use of laser ablation or desorption. Gahlmann et al. reported the structure of isolated uracil and guanine, as determined by ultrafast electron diffraction (UED)–laser desorption,[18] whereas Alonso et al. reported the rotational spectral signatures of four tautomers of guanine, as determined by FTMW spectroscopy combined with laser ablation.[13]

The UED technique was developed to study structural dynamics in isolated molecules in the gas phase with combined spatial and temporal resolution.[19] It is combined with laser desorption in the UED-4 apparatus shown in Figure 1.

The supersonic-jet-expansion FTMW spectrometer used by Alonso et al.[13] consists of a Fabry–Pérot-type resonator with a coaxially oriented beam–resonator arrangement (COBRA).[20,21] It can reach a resolving power of a few kilohertz and a sensitivity several orders of magnitude higher than that of conventional MW spectroscopy.[22] The resonators and some details with respect to the laser ablation are shown in Figure 2.

These studies completed the structural characterization of the five nucleic acid bases in Scheme 1 (only the most stable forms are shown). The nucleic acid bases are divided into two groups: uracil, thymine, and cytosine have a pyrimidine nucleus, whereas adenine and guanine have a purine nucleus. However, all contain a pyrimidine ring and are subject to keto–enol equilibria between a form with a C_{ring}–OH or C_{ring}–NH_2 substituent and the corresponding form with a keto (C=O) or imino (C=NH) group. The prototype molecule for this kind of equilibrium is the system 2-hydroxypyridine/2-pyridinone, whereby 2-hydroxypyridine is more stable then the pyridinone by 3.2(4) kJ mol^{-1}.[23] However, in the system 4-hydroxypyrimidine/4-pyrimidinone, the keto form prevails by 2.0(9) kJ mol^{-1}; thus, the insertion of a second N/NH group in



Scheme 1. The most stable species of the five nucleic acid bases.

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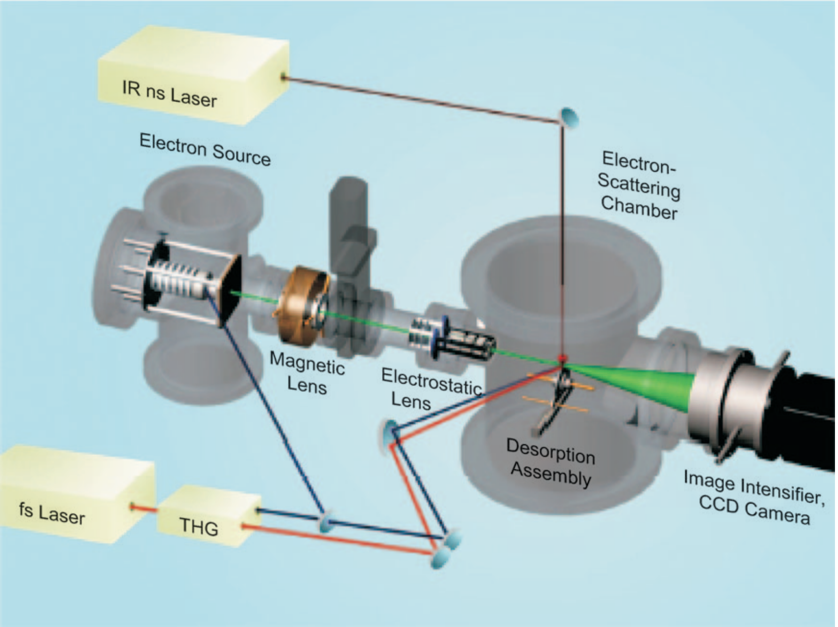

Figure 1. Schematic illustration of the electron diffraction–laser desorption (UED-4) apparatus used in the experiment in Ref. [18]. CCD = charge-coupled device, THG = third-harmonic generator.

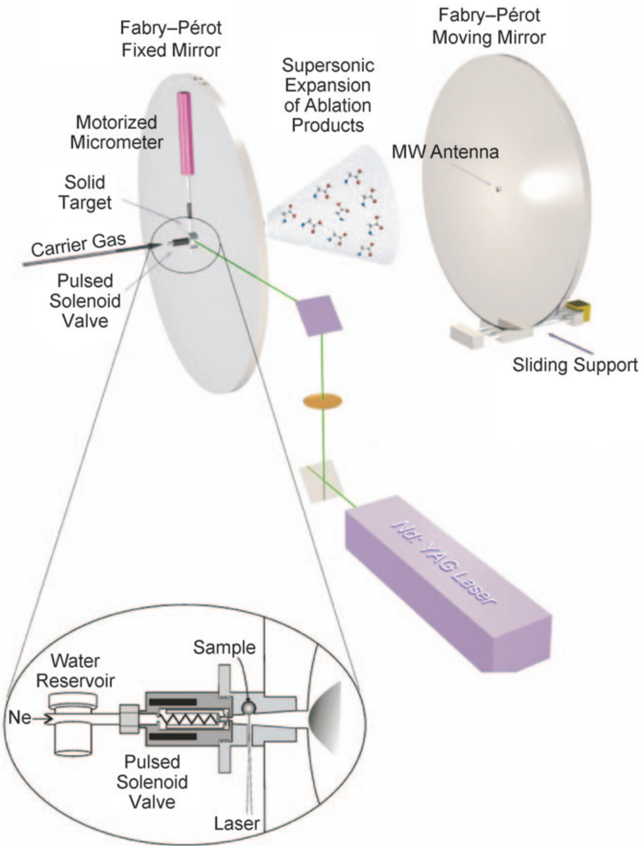

Figure 2. Schematic illustration of the COBRA supersonic-jet-expansion FTMW spectrometer used in the experiment in Ref. [13]. The Fabry–Pérot-type resonators and details concerning the laser ablation are shown.

the aromatic ring makes the keto form more stable.[24] The presence of an additional C_{ring}–OH/C=O group in the ring in uracil increases the stability of the keto forms even more, to the extent that only the diketo form has been observed by MW spectroscopy.[25]

As mentioned above, early MW spectroscopic studies of the three pyrimidine bases were performed by Brown et al. by free-jet mmw absorption spectroscopy.[9,25,26] As for uracil,[25] only the diketo tautomer of thymine was observed by rotational spectroscopy;[26] this tautomer is clearly considerably more stable than the monoketo forms. In contrast, cytosine is a multiform molecular system for which three species, a ketoamino, a ketoimino, and a hydroxyamino form, were detected.[9] The two keto forms are almost isoenergetic, and more stable than the hydroxyamino species by 6 kJ mol^{-1}.

These results were confirmed recently by two reports on the FTMW spectrum of uracil (vaporized by laser ablation) with precisely determined ^{14}N quadrupole coupling constants.[27,28] Brünken et al. provided improved spectroscopic data for a radioastronomical search,[27] whereas Vaquero et al.[28] supplied information on the structure through the study of several isotopologues. In both studies, only the diketo form was observed. An FTMW study was also performed for thymine to determine the structure, the ^{14}N nuclear quadrupole coupling constants, and the ν_3 barrier to internal rotation of the methyl group.[29] Brown et al. measured the rotational spectrum of adenine (6-aminopurine) in a seeded supersonic beam by continuous-wave millimeter wave spectroscopy and assigned the transitions to the N9H tautomer.[30] By using FTMW spectroscopy, López and co-workers extended the number of observed conformational/tautomeric cytosine species to five.[14] ED investigations of heated samples of uracil,[15] cytosine,[16] and thymine[17] with conventional instruments supplied the structure of the most stable species.

It has not yet been possible to assign the rotational spectrum of guanine or its ED molecular scattering owing to its thermal instability. The techniques developed by the research groups of Alonso and Zewail enabled the observation of guanine and have thus opened the way to the investigation of this class of molecules.

The newly constructed fourth-generation ultrafast electron diffraction (UED-4)–laser desorption apparatus described in Ref. [18] represents a technical innovation. It enabled the determination of the structures of the RNA nucleobase uracil and the DNA nucleobase guanine. However, it appears less effective than the laser ablation FTMW method for the determination of different conformational/tautomeric forms of nucleic acid bases. Explicitly, in the case of guanine, UED-4 enabled the determination of the structure of only one conformer, whereas it was possible with FTMW to characterize the four most stable forms individually.

Gahlmann et al.,[18] after the success of their UED-4 electron diffraction–laser desorption studies, have shown interest in exploring overall conformation changes and motifs in other isolated large biomolecules, including polypeptides and possibly functional proteins. This project may be challenging, if one considers that in the case of guanine, they were able to structurally characterize only one of the four species within the relative-energy range 0–400 cm^{-1}.

In contrast, the laser ablation FTMW approach enabled characterization of the vibrational ground states of the four most stable species of guanine,[13] and five species in the case of cytosine.[14] For this last base, it was possible to assign the different species on the basis of the different ^{14}N quadrupole coupling constants, which can serve as a fingerprint of each conformational/tautomeric form. This aspect is illustrated in a striking manner by the conformational study of several amino acids by Alonso and co-workers; for example, seven different conformers of serine were rotationally characterized.[12] Another advantage of MW-related techniques is that a specific rotational spectrum can be extracted from a mixture of hundreds of rotational spectra, whereas difficulties in discriminating between different chemical species are encountered with ED-based techniques.[18]

Finally, there has recently been a considerable breakthrough in field of rotational spectroscopy with the advent of broadband fast-passage excitation Fourier transform microwave spectrometers.[31] For example, with this technique it was possible to identify the rotational spectra of 15 different conformers of 1-octene.[32] Furthermore, by coupling molecular rotational spectroscopy with tunable laser excitation, it is possible to measure picosecond isomerization kinetics.[33] In combination with laser ablation, this technique should also be widely applicable to dynamic studies of radical intermediates, molecular complexes, and conformationally/tautomerically flexible biomolecules.[34]

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