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Infrared spectroscopy of isolated nucleotides 1. The cyclic 3′,5′-adenosine monophosphate anion

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Abstract

The intracellular second messenger deprotonated adenosine 3′,5′-cyclic monophosphate anion (cAMP-H)$^-$, generated as gaseous species by electrospray ionization (ESI) and stored in a Paul ion-trap mass spectrometer, has been investigated by mass-resolved infrared multiple photon dissociation (IRMPD) spectroscopy in the 900–1800 cm^{-1} fingerprint wavenumber range, exploiting the powerful and continuously tunable radiation from a free electron laser (FEL) at the Centre Laser Infrarouge d'Orsay (CLIO). The IRMPD features are interpreted by comparison with the IR spectra obtained by quantum chemical calculations for different low-lying conformers, allowing an assignment for the observed IRMPD bands. It is to be noted that the calculated IR spectra for the most stable conformers look all rather similar and do not allow an unambiguous structural assignment, based exclusively on the IRMPD spectrum. However, the positions and intensities of the IRMPD features of isolated (cAMP-H)$^-$ ions are consistent with a species deprotonated at the phosphate group and compatible with the main equilibrium structures lying within 18 kJ mol^{-1} from the lowest lying conformation, the *anti*-chair form with a C3′-*endo* sugar twist.

Keywords: cAMP; Nucleotide; IRMPD; Infrared spectroscopy; Mass spectrometry

1. Introduction

The cyclic nucleotide adenosine 3′,5′-cyclic monophosphate anion serves as an ubiquitous intracellular signal transductor. Its allosteric binding and release to modules highly conserved in all cells triggers long-range responses in many biological processes, including glycogenolysis and lipolysis [1]. There has been widespread interest in the analysis of the low-energy conformations involved in the recognition and binding of (cAMP-H)$^-$ to diverse enzymes [2–4]. Early experimental information about the structure of (cAMP-H)$^-$ was provided by X-ray crystallography [5,6] and by NMR spectroscopy [7–9], reporting an equilibrium mixture of two stable conformers, with adenine in an unspecified ratio of *syn*- or *anti*-orientation about the glycosidic bond. Accordingly, quantum chemical calculations predicted that isolated (cAMP-H)$^-$ prefers the *anti*-conformation [10–13], and successive addition of water molecules seems to further stabilize this *anti*-conformation [14].

Independent evidence gathered in the gas phase may provide a valuable contribution, particularly for those cases where the binding domain of the receptor locks the cyclic nucleotide via a stable hydrophobic core. In fact, the gaseous environment allows isolated species to be examined in the absence of interfering effects due to the presence of solvent or counterions.

A number of quantum chemical computational studies [15,16] and mass spectrometric investigations [17,18], relying on collision-induced dissociation (CID) [19], ion-mobility [20] and ion–molecule reactions [21–23], has been applied to characterize nucleotides and their derivatives. The dynamics of electron migration in mono- and oligonucleotides have been also investigated using photoelectron spectroscopy [24,25]. Recently, gas phase H/D exchange reactions have been exploited to char-

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acterize a series of biologically relevant (deoxy)nucleotides [21,22,26–28]. The structural dependence of these processes has been related to the key role of a flexible phosphate chain, confirmed by the absence of exchange in cyclic nucleotides, including $(cAMP\text{-}H)^-$, where the ribophosphate unit adopts a quite rigid conformation.

Among the methods employed to gather information on molecular ions under tandem mass spectrometric conditions, infrared spectroscopic methods based on mass-resolved photofragmentation [29,30] have recently been developed. These methods rely on recording the appearance of charged photofragments formed upon resonant absorption of infrared photons [31–41]. Two types of infrared light sources have been employed to investigate the vibrational features of gas phase ions [42]. For weakly bound systems, optical parametric oscillator/amplifier (OPO/OPA) laser systems can be used to derive infrared spectra in the 2500–4000 cm^{-1} energy range. Molecular ions solvated by few rare gas atoms [43,44], solvated clusters [45], solvated transition metal complexes [46], and solvated amino acids [47] have been studied. More recently, infrared free electron lasers at FELIX [33] and CLIO [34] have been shown to provide sufficient intensity in the mid-infrared region for inducing the fragmentation of strongly bound molecular ion following an infrared multiple photon dissociation mechanism. These FEL sources, tunable in the 500–2500 cm^{-1} spectral range, have been coupled to tandem mass spectrometers, thus allowing an access to the highly structure-informative fingerprint region [48–53]. The fundamental frequencies of vibrational modes, determined experimentally by IRMPD spectroscopy, are usually complemented by high level theoretical calculations, which afford the low-energy equilibrium structures, together with their IR spectra, for plausible candidates for the species being sampled.

In the present contribution, the first IR spectroscopic assay of a bare nucleotide ion is reported in the 900–1800 cm^{-1} region. The species is deprotonated adenosine 3′,5′-cyclic monophosphate, the stable form at physiological pH, formed by electrospray ionization and stored in a Paul type ion trap. The IRMPD study was possible by using the IR FEL radiation source made available at the Centre Laser Infrarouge d'Orsay. The investigation was aimed at gathering the IR signatures and properties for the preferred conformations of this cyclic nucleotide, unaffected by solvent or other environmental effects.

2. Experimental and computational section

IRMPD spectra of deprotonated adenosine 3′,5′-cyclic monophosphate were recorded using the tunable IR radiation from the CLIO FEL, coupled with a modified Bruker Esquire 3000 plus Paul ion-trap mass spectrometer [54]. A conical hole drilled in the ring electrode allowed optical access to the center of the trap. The CLIO IR FEL is based on a 10–48 MeV electron linear accelerator. It delivers 8 μs macropulses (25 Hz), each containing 500 micropulses (few picoseconds long). The laser frequency profile was monitored with a monochromator associated with a pyroelectric array detector, and the bandwidth was about 0.5% of the central wavelength. In the present work, the electron energy was set to 45 MeV providing a continuously tunable radiation between 900 and 1800 cm^{-1}. Analyte solutions (1.0×10^{-5} M of adenosine 3′,5′-cyclic monophosphate in pure methanol), infused through a fused-silica capillary to the ESI source at a rate of 100 $\mu L\,h^{-1}$ by a syringe pump, were typically submitted to a spray voltage of 3800 V. The ion population, desolvated by a heated (380 K) N_2 countercurrent drying gas, was mass-analyzed yielding a prominent species at m/z 328 assigned to $(cAMP\text{-}H)^-$.

Deprotonated $(cAMP\text{-}H)^-$ ions were isolated from other ionic species in the ion trap, allowed to relax through multiple collisions with helium at a pressure of $\sim 10^{-2}$ mbar in the ion trap for 100 ms and exposed to 10 macropulses of the IR FEL light. For each wavelength, the mass spectrum was derived from the accumulation over 10 scans. The IRMPD spectrum is obtained by plotting the photodissociation efficiency, evaluated as $R = -\ln[I_{parent}/(I_{parent} + I_{fragments})]$, as a function of the radiation wavenumber. No corrections were applied, although the laser power did not remain constant throughout the frequency range. However, the spectral dependence of the laser intensity as a function of the wavenumber is shown along with the experimental IRMPD spectrum.

Molecular structures and relative energies for isomeric $(cAMP\text{-}H)^-$ species were calculated using the B3LYP hybrid density functional [55,56], as implemented in the Gaussian 98 set of programs [57]. Full structure optimizations, relative energies, and harmonic vibrational frequencies were calculated using the 6-311+G** basis set. The calculated vibrational stick spectra are convoluted by a 20 cm^{-1} wide Lorentzian function, to be comparable with the experimental bandwidth.

3. Results and discussion

Electrospray ionization of a methanol solution of adenosine 3′,5′-cyclic monophosphate yields gaseous anion with the negative charge conceivably carried by the phosphate group. Depending on the orientation of the base unit about the glycosidic bond and on the conformation of the phosphate ring, gaseous $(cAMP\text{-}H)^-$ may be depicted in several conformational isomers. Four low-energy conformers have been obtained with adenine rotated either *anti* (**1**, **2**) or *syn* (**3**, **4**) with respect to the ribose ring and the cyclic phosphate either in a chair (**1**, **3**) or in a twist boat (**2**, **4**) conformation. Fig. 1 displays the optimized geometries for these four lower-energy conformers (**1–4**) along with their relative energies calculated at the hybrid density functional level of theory (DFT) B3LYP/6-311+G** level. Obviously, other higher energy conformers may be considered besides **1–4**. In particular, it may be interesting to comment another structure (**5**), in all respects similar to 1, differing chiefly for the C3′-C2′-O-H dihedral angle that changes from −28.4° to −176.3° (Fig. 1). All species **1–5** bear a negative charge on the exocyclic phosphate oxygen and present the adenine base above the plane of the sugar, which is twisted into a major C3′-*endo* and a minor C4′-*exo* conformation [12]. At variance with nucleosides and acyclic nucleotides whose C3′- and C2′-*endo* forms are found quite close in energy, any search of possible C2′-*endo* conformers for $(cAMP\text{-}H)^-$ failed [15]. Consistent with early findings [10–13], for bare $(cAMP\text{-}H)^-$ the *anti*-

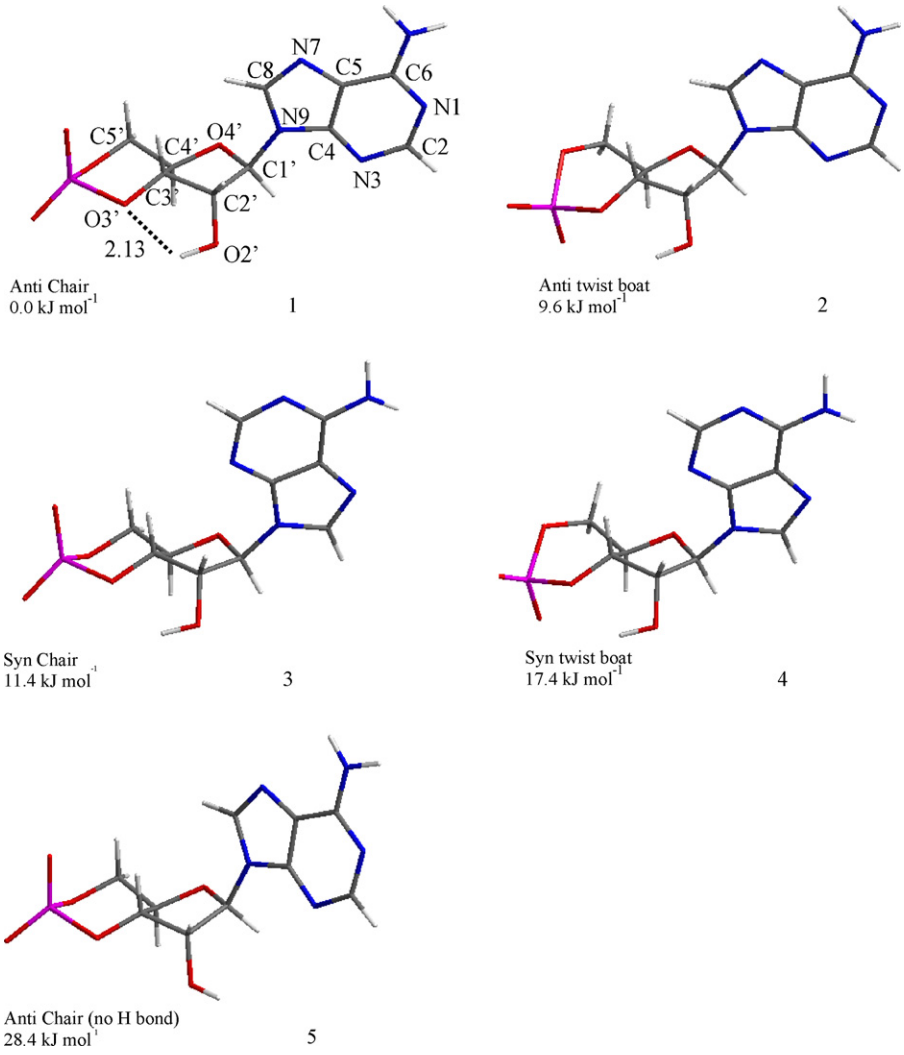

Fig. 1. Optimized structures and relative 0 K enthalpies (kJ mol^{-1}) calculated at the B3LYP/6-311+G** level of the (cAMP-H)$^-$ conformers **1**–**5**. Included is the atom labelling used in this paper. The indicated O3′–H distance in **1** is expressed in Å.

chair conformation (**1**) is found to be the most stable, with the *anti*-twist boat form (**2**) lying 9.6 kJ mol^{-1} higher in energy. Easy chair → twist interconversion of a nucleoside cyclic 3′,5′-monophosphate was suggested to accompany the binding within the enzyme cavity [58]. Compared to (**1**), the *syn*-chair structure (**3**) and the *syn*-twist boat (**4**) are found to be less stable by 11.4 and 17.4 kJ mol^{-1}, respectively, at the B3LYP/6-311+G** level of theory.

In each conformer, the base expands rather apart from the cyclic phosphate ring. As a result, a comparable energy difference for the chair versus the twist boat is found in the *anti*-form (9.6 kJ mol^{-1}) and in the *syn*-form (6.0 kJ mol^{-1}), as already found at a lower level of theory (STO-3G//AM1 level) [10]. Interestingly, each structure **1**–**4** may benefit from an intramolecular stabilization, as suggested by the orientation of the C2′OH group pulled towards the phosphodiester axial oxygen atom at a short distance (∼2.1 Å), approximately independent of the sugar ring conformation. In contrast, any intramolecular H-bond is hampered in the case of **5**, by the rotation of the hydroxyl group, placing this conformer substantially higher in energy (28.4 kJ mol^{-1}) with respect to the ground state species **1**.

In the explored photon energy range, which spans from 900 to 1800 cm^{-1}, mass-selected electrosprayed (cAMP-H)$^-$ ions dissociate upon resonant IR excitation exclusively via a single photofragmentation channel, presumably leading to adenilyl negative ion (*m*/*z* 134) by cleavage of the sugar-base bond. Fig. 2 shows exemplary mass spectra recorded upon mass selection of the parent ion before (lower trace) and after (upper trace) exposure to the FEL IR radiation. Both spectra appear notably clean, as consistently found in the explored spectral range. Interestingly, using multiple collisions-induced dissociation, also leading to a slow heating of the sampled ions, the same species is formed as the major fragment ion upon activation of (cAMP-H)$^-$ [59]. The potential energy profile for the dissociation process of (cAMP-H)$^-$ is not known; however, it is likely that the energy associated to the cleavage of (cAMP-H)$^-$ forming the adenilyl negative ion is quite high. In this respect, one may note that the activation energy values of

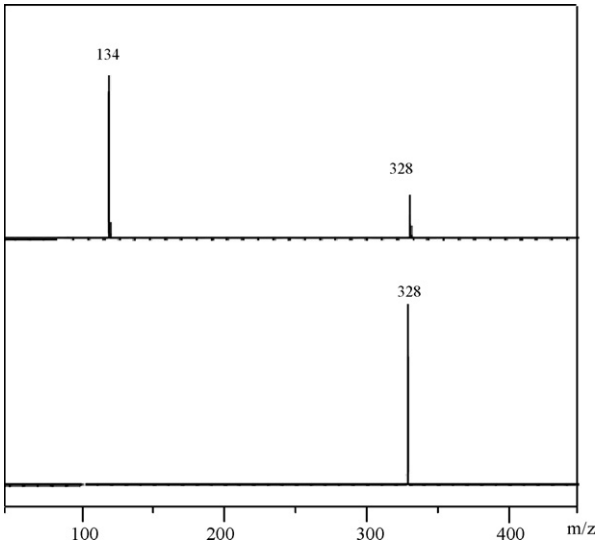

Fig. 2. Mass spectra obtained after mass selection of $(cAMP\text{-}H)^-$ (*m*/*z* 328) in a Bruker Esquire Paul trap mass spectrometer when the laser is turned off (bottom trace) and upon irradiation in correspondence with an IR active mode ($1340\,cm^{-1}$) triggering the photodissociation process leading to ions at *m*/*z* 134 (upper trace).

150 and $163\,kJ\,mol^{-1}$ for the formation of deprotonated adenine have been determined experimentally from the CID of the 3′- and 5′-adenosine monophosphate anion, respectively [60]. These experiments were interpreted by a dissociation initiated by the attack by the phosphate chain to the C2′ hydrogens, an interaction that is hampered by the rigid cyclic phosphate unit in $(cAMP\text{-}H)^-$.

The experimental IRMPD spectrum of mass-selected $(cAMP\text{-}H)^-$ is displayed in the lowest section of Fig. 3. When the IR photon energy is on resonance with an active mode, a photodissociation process is observed which may be highly efficient, with a depletion of the parent ion signal as large as 60% when using 10 macropulses at $1105\,cm^{-1}$. The ensuing IRMPD spectrum as obtained by monitoring the appearance of the photofragment at *m*/*z* 134 as a function of wavelength and normalizing it with respect to the total ion population yields a background-free infrared spectrum of the species [38].

For comparison purposes, Fig. 3 shows the linear IR absorption spectra for conformers **1**–**5** obtained by convolution with a $20\,cm^{-1}$ wide Lorentzian profile.

The IRMPD spectrum displays four major pronounced features at 1022, 1105, 1340 and $1624\,cm^{-1}$, along with several other peaks of weaker intensity at 976, 1146, 1214, 1247, 1412, 1472 and $1582\,cm^{-1}$. As previously reported, the experimental bands appear in general slightly red-shifted with respect to the calculated frequencies, an effect that may be ascribed to the inherently complex and anharmonic nature of the present photodissociation spectroscopy [33]. However, probably due to the incoherent nature of this process, the IRMPD features are found to be in fair agreement when examined against the calculated IR absorption spectra. Throughout the experimental spectrum, the full width at half-maximum (FWHM) is about 25–$30\,cm^{-1}$, as already reported for mid-size species probed with a similar experimental procedure [40,48,54,61].

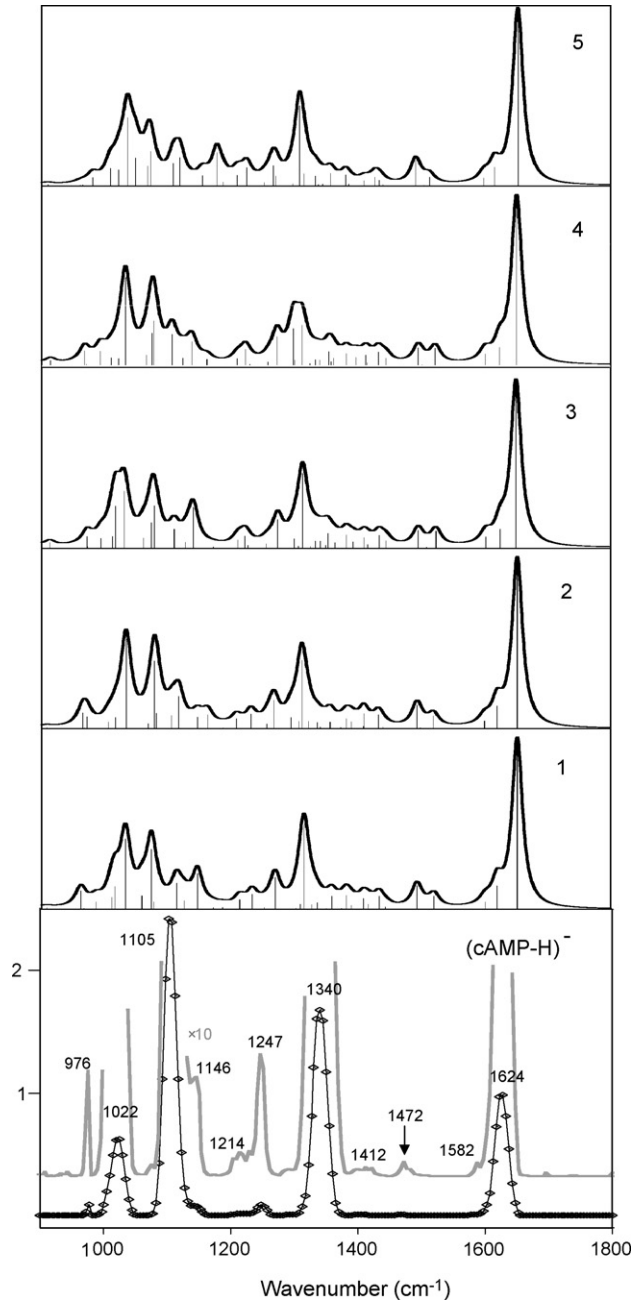

Fig. 3. Comparison of the IRMPD spectrum ($R = -\ln[I_{parent}/(I_{parent} + I_{fragments})]$) of $(cAMP\text{-}H)^-$ (black line), expanded by a factor of 10 (grey line), with linear IR absorption spectra ($km\,mol^{-1}$) of conformers **1**–**5**, at the B3LYP/6-311+G** level of theory.

The relatively narrow bandwidths observed for species sampled in a Paul trap have been ascribed to the efficient thermal equilibration by collisional cooling of the ion with the helium buffer gas [40,54]. Mass-selected molecular ions can thus be estimated to be at room temperature [62]. On the basis of the relative energies calculated at the B3LYP/6-311+G**, 97% of the ions should thus present the lowest energy conformation (**1**) assuming a Boltzmann population of the different conformers. An assignment of the observed IRMPD features to specific vibrational modes may thus be proposed, based on the comparison with the computed frequencies for the ground state conformer **1**.

Because of the noted similarity between the IR spectra calculated for **1**–**5**, the ensuing discussion holds a fair degree of generality. To this end, the band positions and relative intensities of the main experimental resonances are provided along with calculated frequencies and IR intensities, besides a concise description of the associated vibrational modes (Table 1). Despite the multiple photonic character of the IRMPD process, the experimental spectrum is rather well predicted above 1200 cm^{-1}, where even some weak absorption features are nicely reproduced, while in the long wavelength region (900–1200 cm^{-1}) deviations between calculated and observed line intensities and positions are observed.

The prominent IRMPD band at 1624 cm^{-1} can be assigned to the NH_2 scissoring mode predicted by computations at 1654 cm^{-1}. On the red side of the peak, the shoulder located at 1582 cm^{-1} matches with the vibrational mode calculated at 1621 cm^{-1} associated to an in-plane ring-deformation of adenine. One can notice that the IRMPD efficiency observed on resonance with these strongly IR active bands is relatively low (Fig. 3). However, this spectral region suffers from a somewhat lower IR laser intensity and the activity of these features may be consequently undervalued. The spectral dependence of the laser power as a function of the IR wavenumber is shown in Fig. 4 along with a profile of the experimental IRMPD spectrum.

In correspondence with the strongly IR active asymmetric PO stretch expected at 1315 cm^{-1}, the IRMPD spectrum exhibits a strong band at 1340 cm^{-1}. As for the prominent experimental band at 1105 cm^{-1}, it likely results from the overlapping

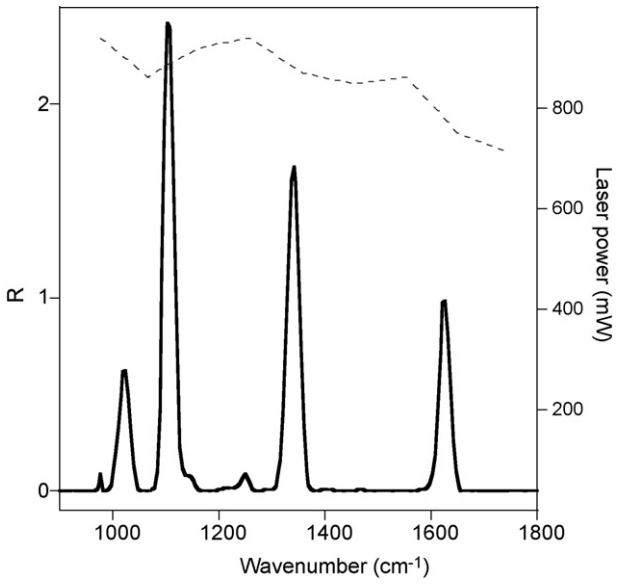

Fig. 4. IRMPD spectrum and corresponding laser power profile.

contribution of two IR active modes calculated at 1074 and 1113 cm^{-1}, associated mainly to highly active symmetric P–O and C2′–OH stretches, respectively. As the spacing between the calculated bands is about as large as the experimental bandwidth (FWHM ≈ 25–30 cm^{-1}), the coalescence observed in the experimental spectrum probably derives from a shift of about 30 cm^{-1} to the blue of the intense and well-localized P–O

Table 1
Experimental and calculated vibrational modes of $(cAMP\text{-}H)^-$ (conformer **1**) in the 900–1800 cm^{-1} region

Wavenumber		Vibrational mode[a]		
Experimental[b]	Calculated[c]	Adenine	Ribose	Phosphate
976 (0.030)	962 (73)		In-phase: σ C1′–O, σ C′2–C′3	
	1011 (47)	β NH_2	Out-of-phase: σ C1′–O, σ C′2–C′3	
	1015 (89)	β NH_2	σ C′1–C′2	
1022 (0.265)	**1033** (**274**)			σ C5′–O
	1059 (53)	β NH_2	σ C4′–O	
1105 (1.000)	**1074** (**233**)	ip β NH_2, β C8H		sym σ PO
	1113 (**102**)		σ C2′–O	ip δ C5′
1146 (0.037)	1146 (139)		σ C2′-C3′	σ C3′–O
1214 (0.0075)	**1234** (**58**)	ip β C8H		oop δ C5′
1247 (0.036)	**1269** (**125**)	σ C1′–N9		
1340 (0.69)	**1315** (**327**)			asym σ PO
	1359 (52)	β NH_2	β C3′H	
	1382 (55)	β C2H	*δ*	
1412 (0.0029)	**1409** (**42**)		β C2′O2′H	
	1435 (52)	oop δ		
1472 (0.0032)	**1495** (**92**)	ip δ		
	1521 (51)	ip δ		
1582 (0.0031)	**1621** (**92**)	ip δ		
1624 (0.41)	**1654** (**657**)	α NH_2		

[a] β = bend; α = scissor; σ = stretch; δ = deformation; ip = in-plane; oop = out-of-plane.
[b] The relative intensities (arbitrary units) of experimental IRMPD features are given in parentheses.
[c] Calculated vibrational modes for conformer **1** at B3LYP/6-311+G** level of theory. Calculated intensities given in parentheses are in km mol^{-1}. Bands with intensity <20 km mol^{-1} are not included. The vibrational modes that may be assigned to the experimental IRMPD features are highlighted in bold.

stretch predicted at 1074 cm^{-1}. Blue-shift effects on asymmetric stretch modes combining with red-shifted bend modes in this spectral region have already been described [63]. At lower wavenumber values, the IRMPD spectrum reveals a distinct feature at 1022 cm^{-1}, matching the C5′–O stretching calculated at 1033 cm^{-1}. The observed line positions and relative intensities of the weak features centered at 1214, 1247, 1412 and 1472 cm^{-1} may be assigned to the modes predicted at 1234, 1269, 1409 and 1495 cm^{-1}, respectively, as proposed in Table 1. These vibrations are associated to CH, 2′OH and NH_2 bends, adenine deformations and C1′–N9 stretch.

As noted above, the comparative examination of Fig. 3 indicates that the calculated IR absorption spectra are almost indistinguishable in several vibrational modes of the **1**–**4** species, thus rendering an absolute assignment of the actual conformational isomers being sampled difficult, if based only on the analysis of the IR transitions. Nonetheless, several investigations have revealed that, for species formed under the mild experimental conditions of the present study, the lowest energy isomer is by far the major species accounting for the detected IR features [40,54]. In the case of protonated cytosine, however, two competing structures differ in energy by only 0.3 kJ mol^{-1}, and the analysis of the observed IRMPD spectrum seems to indicate that both tautomers are present in the ion trap [64]. Based on these premises and relying on the relative energies reported in Fig. 1, the *anti*-chair conformer **1** is expected to account for the major contribution to the present IRMPD spectrum of the species present in the Paul ion trap at about 310 K [62]. The attainment of a conformationally equilibrated ion mixture should be ensured by the low activation energies involved in the interconversion process. For example, the barrier for the isomerization process between the *anti* and the *syn* conformers of neutral cAMP is calculated to be only 15.5 kJ mol^{-1} [13].

It is worthy to note that, in the calculated IR spectra the C3′O stretching mode moves from 1177 cm^{-1} for conformer **5**, which lacks the intramolecular H–bond, to 1146 cm^{-1} for conformer **1**. The latter value entirely matches with the IRMPD feature observed at 1146 cm^{-1}. The observed red shift for the C3′O stretching mode may well represent a probe for the occurrence of an intramolecular H-bond in nucleotide ions, as already reported in other systems, including protonated amino acids and peptides [40,41,54,61].

4. Conclusions

The most stable structures of $(cAMP\text{-}H)^-$ have been characterized in the gas phase and their mid-infrared spectroscopy is reported based on the tunable IR radiation from the FEL facility at CLIO. The experimental IRMPD spectrum in the fingerprint range (900–1800 cm^{-1}) presents several characteristic bands which are accounted for by the detailed features of the IR absorption spectra calculated at the B3LYP/6-311+G** level for the lower-lying equilibrium structures. Given the nonlinearity of the multiple photon absorption process and the ensuing problems in predicting relative intensities, the agreement between the experimental IRMPD spectrum and calculated IR spectra above 1200 cm^{-1} is fair.

The sampled $(cAMP\text{-}H)^-$ ions, formed by ESI and stored in a Paul ion trap at internal energies close to room temperature, display features consistent with a ribose unit twisted in a C3′-*endo* conformation, the adenine moiety above the plane of the sugar and the presence of an intramolecular H bond between the C2′OH group and the phosphate function. The four low-lying conformers **1**–**4**, which differ in energy by 9.6–17.4 kJ mol^{-1}, display very similar IR spectra according to calculations. In agreement with previous evidence, pointing to a by far prevailing occurrence of the most stable isomer under comparable experimental conditions and on the computed relative stabilities, the lowest energy *anti*-chair rotamer **1** may conceivably account for a major contribution to the IRMPD spectrum presently reported.

Finally, the present investigation is a novel addition to a varied array of significant ionic species, including σ- and π-complexes [36,48–50,65,66], charged nucleobases and peptides [61,64,67], and transition metal ion complexes [52,53,68,69], characterized in an isolated state by means of the powerful combination of FEL IR "action spectroscopy" with theoretical calculations. This same approach proves to be valuable in revealing inherent properties of mid-size charged species of biological relevance, related to the equilibrium conformations that play a role in molecular docking to effector proteins.

Acknowledgements

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