



Spectroscopic evidence for a gas-phase librating G-quartet/ Na^+ complex

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Complete List of Authors:	Fraschetti, Caterina; Sapienza-University of Rome, Pharmaceutical Chemistry and Technologies Montagna, Maria; Sapienza-University of Rome, Pharmaceutical Chemistry and Technologies Guarcini, Laura; Sapienza-University of Rome, Pharmaceutical Chemistry and Technologies Guidoni, Leonardo; L'Aquila University, Chemistry, Chemical and Materials Engineering Filippi, Antonello; Sapienza-University of Rome, Pharmaceutical Chemistry and Technologies

COMMUNICATION

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C. Fraschetti^{*a}, M. Montagna^a, L. Guarcini^a, L. Guidoni^b, A. Filippi^a

The IRMPD spectrum of G-quartet/Na⁺ complex, in combination with an *ab initio* molecular dynamic simulation, revealed the presence of two metastable populations of conformers separated by a free energy barrier easily accessible at room temperature.

The capability of guanine to self-assemble in solution by forming the so-called G-quartet tetrads (G₄) has been observed for the first time by Gellert et al., which characterized its tetrameric arrangement (Figure 1) through crystallographic methods and studies of gel formation.¹

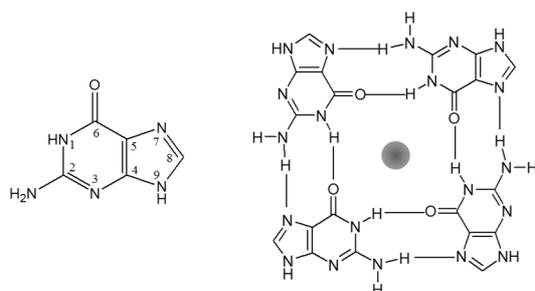


Figure 1. Neutral guanine structure and its tetrad aggregate. The grey sphere represents a sodium ion.

G-quadruplexes are more complicated supramolecules arising from stacking of G₄ moieties, which play a crucial role in several biochemical processes. The presence of guanosine rich motifs in telomeres, which are compact portions of DNA located in the terminal positions of chromosomes, has been widely studied in both their physico-chemical and biological properties.²⁻⁴ The lack of phosphate backbone in the guanine-tetrads allows to investigate the interactions stabilizing the single G₄ monolayer in different aggregation states. In

water solution the self-assembly of metal free G₄ is substantially entropy driven, meanwhile the presence of a template cation M⁺ (M = Li, Na, K) enthalpically stabilizes the G₄-structure in the order K⁺ > Na⁺ > Li⁺, by partially neutralizing the high negative electrostatic potential localized in the center of the cavity.⁵ In the gas-phase the stabilization due to alkaline metal ions follows the reverse order Li⁺ > Na⁺ > K⁺, and this inversion is usually ascribed to the decrease of the metal dehydration free energy moving down along the group of alkaline ions.⁵

The crystallographic studies point to a stacked arrangement stabilizing a G₄ dimer with a Na⁺ cation localized almost in the mean plane between the tetrads,³ meanwhile a multinuclear NMR study carried out on 5'-monophosphate quartets highlights the selective binding of metal ion exclusively to the channel cavity, and indicated contribution of peripheral backbone binding negligible.⁶ Despite the large body of evidences collected in both solid and solution phase, the gas phase structure of M⁺-G₄ aggregates has never been experimentally explored. The formation of M⁺-G₄ species (M = Li, Na, K) has been observed in electrosprayed CH₃OH/H₂O solutions containing guanine in presence of the relative salts of alkali ions.⁷⁻⁸ The theoretical investigations in the gas phase, performed at B3LYP/6-31+G(d) level of theory, revealed that the C₄ symmetry structure (which corresponds to an umbrella-shape structure with the alkali ion in the apical position) is a true minimum for K⁺-G₄ meanwhile smaller ions (*i.e.*, Li⁺) induce a tetrahedral coordination around the metal cation by forcing the structure to adopt a twisted S₄ symmetric structure.^{7,9} Concerning the Na⁺-G₄ system, some papers point to a fully planar C_{4h} symmetry structure,⁷ other publications indicate the coexistence of isoenergetic C_{4h} and S₄ symmetry structures,^{4,10} meanwhile a very recent monograph on G₄ aggregates and their derivatives indicates a quasi-planar structure as the preferred by Na⁺-G₄.¹¹

On the ground of the multi-faceted computational descriptions, herein the first spectroscopic investigation of gas phase Na⁺-G_n (n=2, 4) conformational landscape has been performed by means of ESI-

IRMPD technique, in the high frequency region (2800-3700 cm^{-1}) and in the low spectral region (900-2000 cm^{-1}). The computational support arises from a combination of an *ab initio* static and DFT molecular dynamic simulations, the latter performed with the aim to investigate both the effects of finite temperature and the anharmonicity on the vibrational properties of the mentioned adducts.¹²

In Figure 2a the IRMPD spectrum of $\text{Na}^+\text{-G}_2$ species is reported ($m/z = 325$ u). The Multi-Photon Induced Dissociation yields as the unique fragment the $\text{Na}^+\text{-G}$ ion ($m/z = 174$ u) with the loss of a neutral guanine, by exhibiting bands very similar to those observed in the spectrum of $\text{Na}^+\text{-A}_2$ complex (A = Adenine).¹³ In Table S1 the description of the calculated frequencies of 2b-d species is reported (the relative structures are reported in Figure S1). The profile of the optimized structures 2c-d does not match the experimental spectrum, meanwhile the structure 2b presents a good superimposition with the experiment, except for the position of the calculated peak at 3468 cm^{-1} ($\Delta\nu = 28$ cm^{-1}), which corresponds to the N9-H stretching. The same disagreement has been observed in the IRMPD spectrum of $\text{Na}^+\text{-A}_2$ complex, and discussed on the ground of the lack of anharmonicity term in the employed calculation.¹³

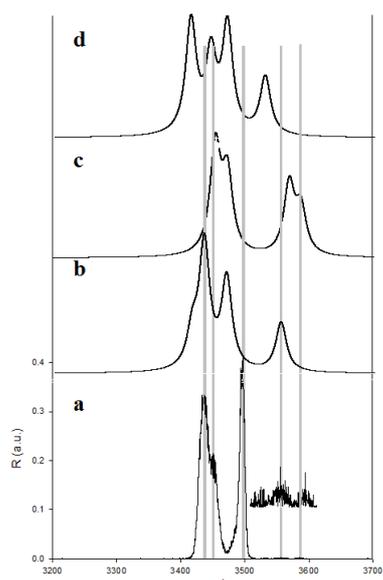


Figure 2. Experimental IRMPD spectrum (a) of $\text{Na}^+\text{-G}_2$ adduct compared with B3LYP/6-31G(d,p) calculation (b-d).

Structure 2b, which satisfactorily reproduces the experiment, involves a tetrahedral arrangement around the metal centre where both guanine molecules exist in the same tautomeric form, which lies 1.56 kJ mol^{-1} above the global minimum.¹⁴

Figure 3a shows the IRMPD spectrum of $\text{Na}^+\text{-G}_4$ aggregate ($m/z = 627$ u) measured in the 1000-2000 cm^{-1} and 2900-3600 cm^{-1} regions. The multi-photon dissociation yields the fragments $\text{Na}^+\text{-G}_3$ ($m/z = 476$ u), and the most abundant one $\text{Na}^+\text{-G}_2$ ($m/z = 325$ u). The high frequency domain exhibits four strong bands (3172, 3235, 3322, 3495 cm^{-1}), and two weakest absorptions (3050, 3510 cm^{-1}), meanwhile the low frequency region presents eight intense maxima.

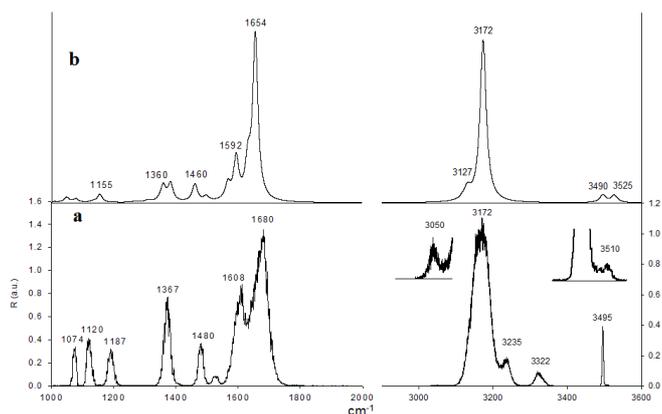


Figure 3. a) Experimental IRMPD spectrum of $\text{Na}^+\text{-G}_4$ adduct.; b) calculated spectrum of S_4 structure (B3LYP/6-311G(d,p)).

A DFT investigation on the arrangement of four guanine molecules around Na^+ ion has been performed. In particular different tautomeric combinations stabilized by Watson-Crick interactions have been optimized at the B3LYP/6-31G(d,p) level of theory (structures, energies, and spectra in Figure S2). Furthermore, the structures held by the Hoogsteen-type interactions, already observed in condensed phase, have been explored. In particular the C_{4h} , C_4 , and S_4 symmetric structures of $\text{Na}^+\text{-G}_4$ tetrad have been optimized in the harmonic approximation at the B3LYP/6-311G(d,p) level (S_4 has been optimized even at level B3LYP/6-31G(d,p)). The C_{4h} input structure does not change during the optimization, meanwhile the C_4 one converge to the C_{4h} , and finally the most stable S_4 structure slightly minimizes its twisted feature (structures, energies, and spectra in Figure S3). The significant energy difference between the most stable Hoogsteen and Watson-Crick-type $\text{Na}^+\text{-G}_4$ adducts ($\Delta G = 28.0$ kcal mol^{-1} in favour of S_4 species), and the spectral features of the calculated structures reported in Figure S2, allow to safely exclude the Watson-Crick type family from the discussion. Furthermore the spectra of C_{4h} and the S_4 species are almost superimposable, and similarly their structural differences are not significant, even though they belongs to different symmetry families. The comparison between vibrational modes of the optimized S_4 and measured spectrum is reported in Figure 3. The calculated low frequency spectrum satisfactorily reproduces the measured fingerprint region. On the contrary, although the comparison between the measured 2900-3600 cm^{-1} domain and the calculated spectra reveals a good matching of the bands at 3172 ($N2\text{-H}\cdots N7$ and $N1\text{-H}\cdots O6$ stretching), 3495 and 3510 (synchronous and asynchronous free $N2\text{-H}$ and $N9\text{-H}$ stretching), the absorptions observed at 3235 and 3322 cm^{-1} are completely absent in the calculated spectra. Those bands could be ascribed to overtones of the bands at 1608 and 1680 cm^{-1} , respectively. Alternately the unsatisfactory reproduction of IRMPD spectrum in the harmonic calculations could point to a substantial inadequacy of the static computational approach to non-covalent aggregates characterized by a particularly flexible supramolecular arrangement, which could be affected by temperature effects. In order to include the mentioned effects, the *ab initio* molecular dynamic (AIMD) simulation has been employed in the optimization of the guanine tetrads. The trajectory of AIMD simulation, starting from S_4 symmetric structure, suggests that at 300 K the $\text{Na}^+\text{-G}_4$ aggregate reversibly fluctuates between different conformations. Indeed Figure 4 reports the periodic evolution of ϕ dihedral angle ($\phi = O^I\text{-O}^{II}\text{-O}^{III}\text{-O}^{IV}$, where O^n are the carbonyl oxygen atoms of the four guanine molecules) which during the simulation assumes values included in the $\pm 40^\circ$ range. This behaviour indicates

that the potential energy surface at room temperature is flat enough to allow the structure to fluctuate substantially freely.

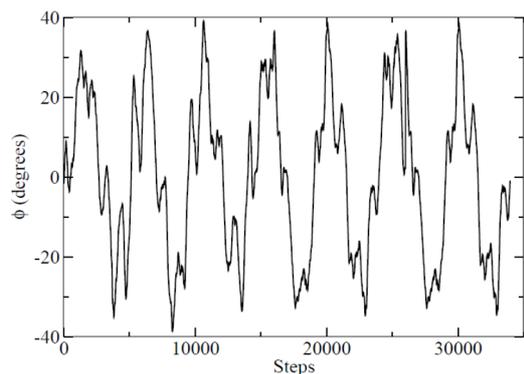


Figure 4. Values of ϕ dihedral angle during the AIMD simulation.

This result has been summarized in Figure 5, wherein the probability of occurrence of the ϕ angle is plotted versus the ϕ values. The histogram is characterized by three maxima which indicate the presence of three rapidly interconverting supramolecular conformers: the structures centered at about $\pm 30^\circ$ are equivalent and similar to a S_4 geometry, meanwhile the structures having a ϕ close to 0° remind the C_{4h} arrangement. The corresponding free energy plot is represented in Figure S4.

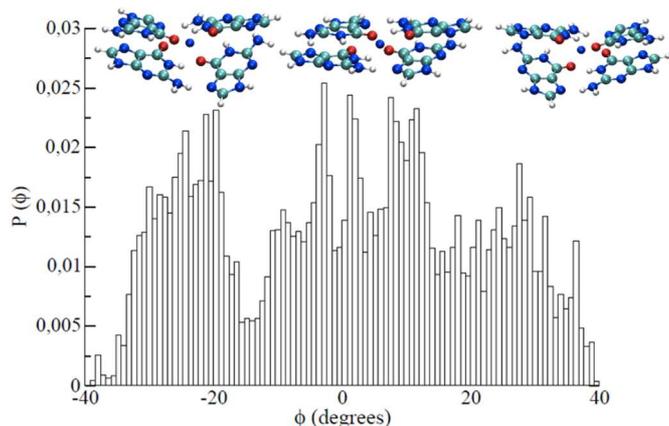


Figure 5. Probability of occurrence of a certain ϕ value vs ϕ values.

The adequacy of the dynamic description provided by the AIMD approach is supported by the good matching between the IR spectrum arising from the simulation and the experimental result (Figure 6). First of all, the calculated static and dynamic low frequency region are substantially identical (see the direct comparison in Figure S5). Nevertheless the missing bands in the $3200\text{--}3400\text{ cm}^{-1}$ domain are represented, and their deconvolution indicates that they lie on the tail of a broader band, attributable to both the $N2\text{--}H\cdots N7$ and $N1\text{--}H\cdots O6$ stretching modes (Figure S6). In other words, in the vibrational spectrum obtained from AIMD simulation the missing bands are observable and belong to a broader absorption ($2870\text{--}3400\text{ cm}^{-1}$ range, Figure S6). The broadening of the hydrogen bonded X-H ($X = N, O$) stretching is due to a temperature effect, and in particular to the dynamic coupling between the shared hydrogen and the ensemble of

vibrational modes which is critically modulated by the temperature.¹⁵ Furthermore at 300 K the internal energy of $\text{Na}^+\text{--G}_4$ adduct is high enough to let them completely free to fluctuate between transient quasi-isoenergetic structures, and those fluctuations continuously twist and deviate from the planarity the quartet, by consequently modifying the strength of hydrogen bond network. As a matter of fact the first hypothesis which attributes the 3235 and 3322 cm^{-1} bands to overtones cannot be completely excluded, but the satisfactory matching between the IR spectrum obtained from the AIMD simulation and the experiment is a safe evidence which points to a temperature effect on the broadening of specific absorptions.

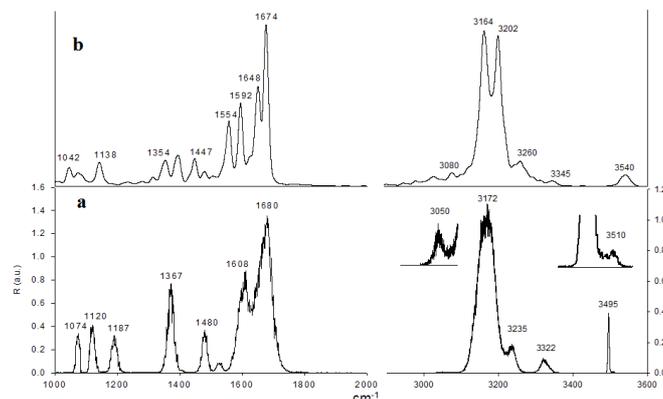


Figure 6. a) Experimental IRMPD spectrum of $\text{Na}^+\text{--G}_4$ adduct.; b) spectrum extracted from the AIMD simulation.

Conclusions

The features of the free energy surface of gaseous $\text{Na}^+\text{--G}_4$ self-aggregates indicate the coexistence of two metastable populations, divided by an activation barrier which lies below 1.0 kcal mol^{-1} (Figure S4), indicating that at room temperature they have enough energy to continuously and rapidly interconvert. The most abundant population corresponds to a slightly twisted structure, meanwhile the other exhibits a completely planar core. The librating nature of $\text{Na}^+\text{--G}_4$ adduct suggests that it is not correct to force the mentioned transient populations into a pre-defined symmetry, because they are too flexible to be described by a static modelling.

Notes and references

^a Pharmaceutical chemistry and technologies, Sapienza-University of Rome, Piazzale Aldo Moro, 5, 00185, Rome, Italy.

^b Chemistry, Chemical and Materials Engineering, L'Aquila University, Località Campo di Pile, 67100 L'Aquila.

† The $\text{Na}^+\text{--G}_n$ ($n = 1, 2$) ions have been generated by following the described procedure,⁷ mass-selected in a modified Bruker Esquire 6000 quadrupole ion trap, and then irradiated using the MS2 mode. IR spectroscopy in the $900\text{--}2000\text{ cm}^{-1}$ wavenumber range was performed using the CLIO FEL. The $2800\text{--}3800\text{ cm}^{-1}$ wavenumber range was explored using an IR optical parametric oscillator/amplifier (OPO/OPA) system of LaserVision, pumped by 10 Hz Nd:YAG laser.

Harmonic vibrational frequencies have been determined by using B3LYP/6-31G(d,p) (scaling factor: 0.95) and B3LYP/6-311G(d,p) (scaling factor: 0.96) exchange-correlation functional for G-dimer and G-quartet sodium

respectively, as implemented in the Gaussian09¹⁶ package. Exclusively G-quartet/Na⁺ complexes have been optimized with a SCF convergence criteria set as TightSCF. The AIMD simulation was carried out using the terachem package,¹⁷ and the trajectory was evolved according to the Born-Oppenheimer scheme. The electronic structure of the system was treated at the DFT level of theory with the BLYP/6-31G(d) exchange-correlation functional.¹⁸ The system was first equilibrated at 300 K for 4 ps using the Nosé-Hoover thermostat,^{19,20} and successively the production run was carried out in the NVE ensemble for 17 ps. Coordinates, velocities and dipole moments were stored each simulation step. The IR spectrum has been computed by performing the Fourier Transform of the autocorrelation function of total dipole moment.¹²

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