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Molecular structure of the nucleoside analogue inosine using DFT methods: Conformational analysis, crystal simulations and possible behaviour

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HIGHLIGHTS

- The five tautomers of the nucleoside inosine were determined and optimized.
- In the most stable tautomer N1 were calculated and optimized 69 stable structures.
- The lack of the NH_2 group of guanosine reduces the negative charge on N3 and N1 atoms.
- X-ray crystal unit cell state was simulated through a pentamer form.

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ABSTRACT

Five tautomers of the nucleoside inosine were determined and optimized at the MP2 and B3LYP levels of theory. Several correlations were identified. A comprehensive conformational analysis was carried out on the most stable tautomer N1, and the whole conformational parameters (χ, β, γ, δ, ε, ε', P, ν_{max}) were studied as well as the NBO Natural atomic charges. The calculations were carried out with full relaxation of all geometrical parameters. The search located at least 69 stable structures, 3 of which are within a 1 kcal/mol electronic energy range of the global minimum, and 4 conformers are within a 1 kcal/mol Gibbs energy range. A lower reactivity in inosine than in the natural nucleoside guanosine appears in the N1 and N3 nitrogen atoms. The solid state was simulated through a pentamer form and the structural parameters were compared with the X-ray crystal data available. Several general conclusions were emphasized.

1. Introduction

Inosine (INO) and its derivatives are purine nucleosides of considerable interest because they are one of the components of nucleic acid's building blocks [1]. INO is formed when hypoxanthine is attached to a ribose ring (ribofuranose) via a β-N_9-glycosidic bond. It can be obtained from deamination of adenine by RNA editing [2]. As compared with guanosine, INO lacks the NH_2 group at the C2 position.

INO, whose structure appears in Scheme 1, is biologically found in *t*RNA. It is an adenosine deamination product in DNA, which must be repaired to maintain genomic fidelity [3]. It is observed in the first anti-codon position, which pairs with the third codon position on *m*RNA [1]. It is also an endogenous BDZ-receptor ligand. Administration of INO is very useful for its neuroprotective properties and, as other nucleosides, it plays a role in the pathophysiology of some neurodegeneratives and neuro psychiatric diseases [4]. It increases myocardial gross energy improving cardiac performance and preserving ATP ischemia [5–7]. It has been proposed for spinal cord injury [8] and it stimulates significant axonal reorganization after strokes, which leads to improve performance on several sensorimotor tasks [9].

INO's ability to act as an 'universal pairing base' was recognized soon after the sequences of many tRNA's became available [3,10]. Therefore, base pairs formed by INO play an important role in many physiological processes as well as in various DNA technologies. Examples include INO containing primers for the detection, isolation, and sequencing of genes and its use in DNA micro-array hybridization [11]. Thus, INO may be used to detect and to analyze the target of DNA strand containing ambiguities through binding with weak H-bond to any of the four natural DNA bases.

With the development of nucleoside chemistry, a number of INO analogues have been synthesized and their structural properties, stabilities, potential antitumor and antiviral activities have been studied [12–14]. One of these derivatives, 2′-deoxyinosine (2-dI), enhances the antitumoral activity of 5-fluorouracil (5-FU)

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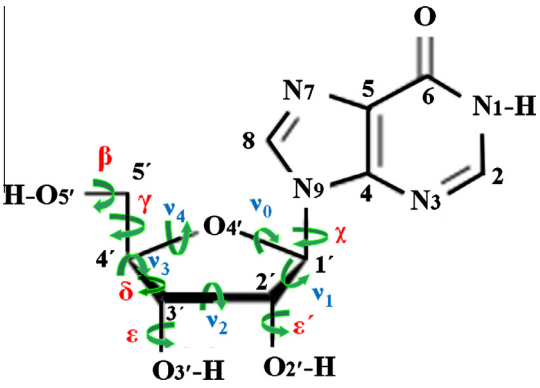

Scheme 1. Molecular structure and definition of the exocyclic and endocyclic torsional angles in tautomer N1 of inosine.

when it is combined with 5-FU in human xerografts [1]. Another derivative, Inosine 5′-Monophosphate Dehydrogenase (IMPDH) is involved in the GMP nucleotide biosynthesis pathway and its isoform type II is produced in a large amount by the cancer and tumour cells [15].

The conformational isomerism of DNA structural components has an intense interest today and many theoretical researches have been reported [16]. However, few studies appear on INO. As in other nucleosides, several tautomeric forms are possible in INO, although the vibrational IR and Raman spectra indicate that INO and its methylated analogues occur predominantly in the *keto* form in neutral aqueous solution [17]. The *enol* tautomer, if reasonably stable, could form base pairs of the Watson–Crick type with uridine [17]. Thus, spontaneous mutations may arise from mispairing due to the appearance of rare tautomers.

One of our aims is to study the possible tautomers of INO (Fig. 1), and focusing the attention on the most stable one, in the calculation of their different conformers. From our understanding would be interesting to analyze the different conformational possibilities of INO and its charge distribution, and to compare the results with the natural nucleoside guanosine (G). An accurate knowledge of the flexibility and conformeral properties of a nucleoside is an important help for the interpretation of their interactions. For this reason, different authors have analyzed previously the conformers of several natural and analogues nucleosides [16,18–24]. Now, an extensive theoretical study of the conformational preferences and intramolecular interactions in INO has been carried out with full relaxation of all geometric parameters. We will attempt to determine herein, if the various geometric features in INO are correlated or interact with one another. We are also interested in whether alternative forms of hydrogen bonding make significant contributions to the conformational behaviour of INO.

2. Computational details

Calculations were carried out by using the Becke exchange functional (B) [25a], Becke's three-parameter exchange functional (B3) [25b], Handy's OPTX modification of Becke's exchange functional (O) [25c,25d] and the extended (X3) [25e], in combination with the correlation functionals of Lee, Yang, and Parr (LYP) [25f], and Perdew and Wang's 1991 (PW91) [25g]. The Handy, Tozer and coworkers modification (B972) was also used [25h]. B3LYP Density Functional method (DFT) is the most used today, and for this reason the majority of the calculations were carried out with it.

All the methods appear implemented in the GAUSSIAN 03 program package [26]. The UNIX version with standard parameters of this package was used in the alpha computer of the Computational Centre from University Complutense of Madrid, in which all quantum chemical computations were performed. DFT methods provide adequate compromise between the desired accuracy and the heavy demands put on computer time and power. Different studies have shown that the data obtained with DFT methods are in good agreement with those obtained by expensive computational methods as MP2 [27]. Moreover, they have been used satisfactory in many studies on nucleosides and on drug design [19,23–25,28–32]. and they predict vibrational frequencies of DNA bases better than HF and MP2 methods [33–37]. Several basis set were selected but the 6-31G(d,p) represents a compromise between accuracy and computational cost and thus it was used in the majority of the calculations.

The 3D Potential Energy Surface (PES) of this molecule was determined by rotation of the exocyclic and endocyclic torsional angles χ (glycosidic bond), γ, β, ε and ε'. These dihedral angles were simultaneously hold fixed at values varying between 0° and 360° in steps of 60° in a first study. All other geometrical parameters were relaxed during these optimisations. 69 optimized geometries were obtained in this step by minimizing the energy with respect to all

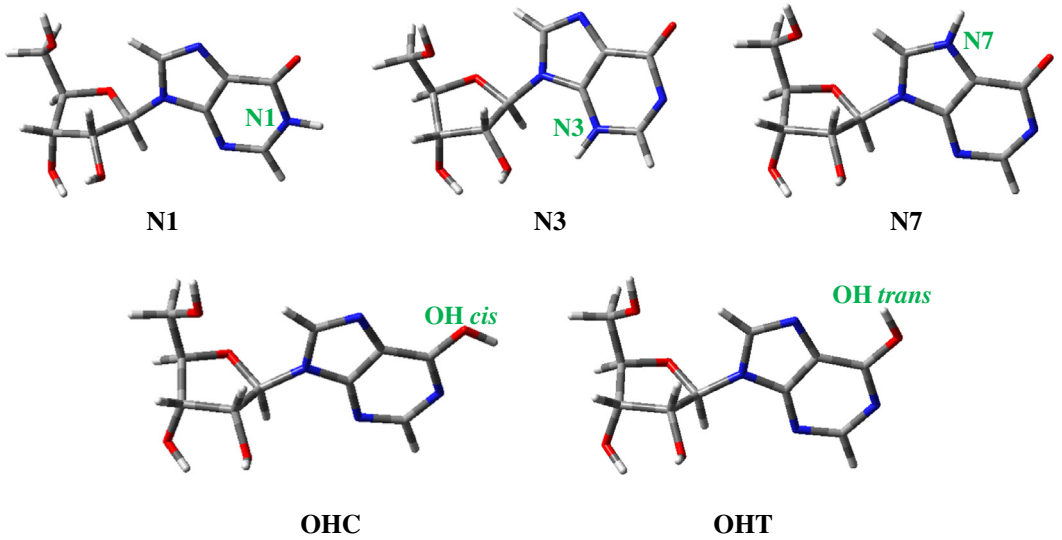

Fig. 1. Main tautomers of inosine molecule.