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A computational study of pyrazinamide: Tautomerism, acid–base properties, micro-solvation effects and acid hydrolysis mechanism

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ABSTRACT

Pyrazinamide (PZA) is a prodrug substance utilised in the treatment of tuberculosis related to mycobacterium strains. The activities of this compound are said to occur in acidic medium where it is converted to the active form of pyrazinoic acid. To better understand its molecular properties related to its ability to interact with other species in the body and to understand its reaction mechanism in acidic medium, the current study investigates conformational preferences, tautomerism, acid–base properties, interaction with explicit water solvent molecules as well as its hydrolysis in acidic medium. The study is performed utilising DFT/M06-2X, DFT/MPWB1K and the MP2 methods with several basis sets including 6-311+G(3df, 2p) and aug-cc-pVDZ. The results suggest that only the keto tautomeric form is stable and that its stability is strongly determined by the presence of intramolecular hydrogen bonds. The preferred site for protonation is the pyrazine nitrogen in *meta* position to the substituent group and the preferred site for deprotonation is the NH_2 group. The micro-solvated systems are stabilised by the interplay between intramolecular hydrogen bond and intermolecular hydrogen bonds. The acid hydrolysis mechanism is achieved through the protonation of the sp^2 O atom, the formation of the tetrahedral C atom, resulting in its pyramidalisation and eventual weakening of the C—N bond, protonation of the N atom in the NH_2 group, which is essential for the breaking of the C—N amide bond.

1. Introduction

Pyrazinamide (PZA, $C_5H_5N_3O$) is an anti-tuberculosis prodrug exhibiting anti-mycobacterial activity and utilised in the tuberculosis chemotherapy as a sterilizing drug [1,2]. PZA is also a prodrug of the pharmacologically active agent pyrazinoic acid, which is released through the acid catalysed hydrolysis of PZA [3,4]. The anti-mycobacterial activity of both PZA and pyrazinoic acid occurs preferentially in acidic medium [5,6]. Although PZA is utilised extensively as a prodrug, its mode of interaction with mycobacterium tuberculosis (MTB) strain and its acidic hydrolysis mechanism are not yet clearly understood [7].

The objectives of this study are to utilise computational approaches to investigate the interactions of PZA with selected solvent molecules to simulate the situation in the body and to elucidate the acid hydrolysis mechanism of PZA. The interaction of PZA with solvent molecules in the body may be understood by modelling its interaction with bulk solvent or with explicit solvent molecules. The energetics of the tautomers provides information on their stability. The geometric features of each optimised tautomer/conformer may help explain the factors accounting for the stability of the different tautomers or conformers. The acid–base properties such as gas phase basicity (GB) and proton affinity (PA) are important for understanding the variability of the biopharmaceutical properties of drug compounds [8]. PZA is known to absorb and eventual diffuse into the MTB strain, where it is converted into the active pyrazinoic acid [9]. Its conversion into the active pyrazinoic acid may be understood through the study of its acid hydrolysis mechanism.

The study is performed considering continuum medium in three solvents, differing in polarity, namely chloroform (chlrf, $\varepsilon = 4.71$), dichloromethane (DMSO, $\varepsilon = 46.83$) and water (aq, $\varepsilon = 78.36$). The selection is meant to mimic the non-polar and the polar media found in the body. The study in solution is performed through optimising the geometry obtained *in vacuo* with the objective of investigating the geometric relaxation features due to the electronic solute–solvent polarisation.

Micro-solvated studies of PZA with 1 and 2 water molecules were performed to investigate the specific interactions that PZA

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might form with water molecules, including the preferred sites for such interactions. The presence of C=O, NH_2 and other proton donor/acceptor groups implies that PZA has the ability to form both intramolecular hydrogen bonds (IHB) and intermolecular H-bonds with solvent molecules. Both H-bonds have significant roles in stabilising molecular conformations and in influencing biological activities [10–25]. In the isolate form, PZA may form only IHB, however, in the presence of the solvent molecules, it may form both IHBs and intermolecular H-bonds. In this study, the interplay of IHB and intermolecular H-bonds is also investigated to determine the possibility of their co-existence in solution. The study of micro-solvated systems is prompted by the fact that continuum models do not take into consideration the specific intermolecular H-bonds because such models consider the solvent in an average description [26,27].

The study is performed with the second order Möller Plesset perturbation theory (MP2) and with the Density functional theory (DFT) utilising the M06-2x functional. An increasing number of DFT functionals are reported to provide good estimate of thermochemical properties in good agreement with experimental values [28–31]. Moreover, many properties of amides, such as the acid–base properties, have been calculated by means of DFT methods and the results are in good agreement with higher-level calculations, such as CBS-QB3 and CCSD(T) [32–35]. DFT methods are also considered to provide good results for the study of chemical reaction mechanisms and for the study of H-bonds in micro-solvated systems [28,36–39]. In order to assess the performance of the selected functional, we also perform calculations with high level G2(MP2) and CB-QS3 methods.

2. Computational details

Geometry optimisations were performed utilising MP2 and the DFT using the M06-2x functional. The geometry optimisation for neutral species were performed the 6-311+G(d,p), 6-311+G(3df,2p) and cc-pVTZ basis sets. Acid–base analysis was performed using the 6-311+G(3df,2p) and the cc-pVTZ basis set; micro-solvation effects were studied using the 6-311+G(d,p) basis set; the hydrolysis phenomenon was investigated using the MPWB1 K/6-311+G(d,p) method, which is considered adequate for the study of chemical reaction [28]. The selected basis sets are considered to perform well in the estimation of gas phase acidity and basicity of organic compounds [32,34,35]. The inclusion of diffuse and polarisation functions also suggests that the selected methods are suitable for the study of the properties of IHB in PZA and H-bonds involving PZA and solvent molecules [11,13–15].

PA and GB values were estimated from the reaction involving the addition of a proton to the neutral molecule (B), $B_{(g)} + H^+_{(g)} \rightarrow BH^+_{(g)}$; the equation for the PA is written as;

$$\Delta H^{298}_{PA} = H^{298}(BH^+_{(g)}) - \left[H^{298}(B_{(g)}) + H^{298}(H^+_{(g)})\right] \quad (1)$$

where $H^{298}(BH^+_{(g)})$ is the sum of the electronic and thermal enthalpies for the protonated species, $H^{298}(B_{(g)})$ is the sum of electronic and thermal enthalpies for the isolated B and $H^{298}(H^+_{(g)}) = 2.5\ RT = 1.48$ kcal/mol. The equation for the gas phase basicity is written as

$$\Delta G^{298}_{PA} = G^{298}(BH^+_{(g)}) - \left[G^{298}(B_{(g)}) + H^{298}(H^+_{(g)})\right] \quad (2)$$

where $G^{298}(BH^+_{(g)})$ is the sum of the electronic and thermal free energies for the protonated species, $H^{298}(B_{(g)})$ is the sum of electronic and thermal free energies for the isolated B and $\Delta G^{298}(H^+_{(g)}) = 2.5\ RT - T\Delta S^{298} = 1.48–7.76 = -6.28$ kcal/mol at 298 K.

QTAIM was used to analyse topological properties of the adducts and the species involved in the acid hydrolysis mechanism. Molecular graphs and the properties related to the bond critical point data (i.e., the electron density (ρ) and the Laplacian of the electron density, $\nabla^2\rho$) were obtained using the Multiwfn program [40]. The H-bond energy for the different H-bonds in the PZA···-water clusters were estimated using the equation [41];

$$E_{HB} = \frac{V(\mathbf{r}bcp)}{2} \quad (3)$$

where $V(\mathbf{r}bcp)$ is the potential energy density V(r) at the bond critical point (*bcp*).

Natural bond order (NBO) analysis method [42] was utilised to determine the existence of IHB by analysing the interaction between a filled (bonding or lone pair) Lewis type NBO and an empty (antibonding) non-Lewis NBO (that act as an acceptor). In NBO, the strength of the delocalisation interactions, $E^{(2)}$ can be identified from the presence of off-diagonal elements of the Fock matrix and is estimated by second order perturbation theory.

Solvent effects were taken into account in optimisation calculations on the basis of the *in vacuo* M06-2x/6-311+G(d,p) geometries. The PCM model was selected for the study and the calculations were performed in chloroform, DMSO and in water solvents. The SMD solvation model was utilised for the calculation of the absolute solvation energies [43].

All calculations were performed with the Gaussian09 program [44]. The schematic representations of the structures were drawn using ChemOffice package in the UltraChem 2010 version and the optimised structures were drawn using the GaussView 5 program.

3. Results and discussions

3.1. Optimised geometries of the neutral tautomers

There are two tautomeric forms for PZA; calculations on each tautomeric form were performed to determine the relative stability *in vacuo* and in solutions. The optimised geometries and the schematic representation of PZA tautomeric isomers, together with atom numbering, are shown in Fig. 1. The isomers are named PZA-1 and PZA-2. There are two stable conformers for PZA-1, here denoted as PZA-1a and PZA-1b. The lowest-energy conformer (PZA-1a) has all the atoms lay on the plane of the pyrazine ring; it is stabilised by the presence of the N9—H13···N6 IHB. This IHB is confirmed by the NBO analysis, which shows that the interaction between the donor orbital (lone pair on N6) and the acceptor orbital, σ^* (N9—H14) antibond, results in stabilisation energy ($E^{(2)}$) of 1.07 kcal/mol. PZA-1b has the substituent at C1 off-plane with respect to the plane of the pyrazine ring and is 7.823 kcal/mol less stable than PZA-1a. The difference in the stability of the two conformers may be related to the presence of the IHB in PZA-1a, which is absent in PZA-1b. A scan of the rotation of the C1—C7 bond, is shown in Fig. S1 (Supplementary data), and it illustrates the preference for geometries in which the C2—C1—C7—O8 torsion angle (φ) is near 0° or 42°. The diagram shows two minima corresponding to $\varphi = 0°$ and 135°. The energy difference between the two structures is about 8.156 kcal/mol, which is close to the energy difference for PZA-1a and PZA-1b. PZA-2 has two conformers (PZA-2a and PZA-2b), each stabilised by IHB. PZA-2a is 1.117 kcal/mol more stable than PZA-2b. The difference in their stability may be related to the different H-bond donor/acceptor centres involved in the IHB.

A comparison of the energies of the lowest-energy conformer of PZA-1 and PZA-2 provides an indication of the relative stability of PZA tautomers. Table 1 reports the relative energy (ΔE) and Gibbs free energy difference between PZA-1a and PZA-2a tautomers. With all the methods, PZA-1a is more stable than PZA-2a *in vacuo*. The difference in the stability of the two isomers may be related to the different type of stabilising factors including, the difference in